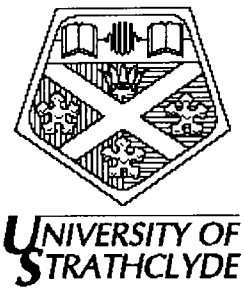

PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON SOME SPECIES OF ANNONACEAE

Thesis submitted by

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for the degree of

DOCTOR of PHILOSOPHY

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January 1999

Dedicated to my parents,

Acknowledgements

I wish to express my sincere thanks to *Professor P. G. Waterman* for his supervision, expert guidance, constructive criticism and for allowing me to present my work at the Young Scientists Symposium on Future Trends in Phytochemistry in The Netherlands in May 1998. I also acknowledge my French supervisor *Professor F. Bailleul* of the University of Lille II for his interest, encouragement and support throughout this research work and for providing useful references.

I wish to thank *Professor P. G. Waterman* for the collection of plant materials. I am grateful to *Dr. T.C. Fleischer* for providing photographs. I am also thankful to *Professor P. G. Waterman*, *Dr. T.C. Fleischer* and *Dr. Z. Latif* for running some of my NMR samples at the early stage of my project. I also appreciate the kind assistance of members of the Department of Chemistry and the Department of Pharmaceutical Sciences of the University of Strathclyde who provided technical support; *Dr. P. Dennison, Ms C. Bates, Mrs A. B.. Bhasin, Mr B. Morton.* I acknowledge *Mrs C. Clements* from the Strathclyde Institute for Drug Research for carrying out the bioassays and *Professor A. Harvey* for help with the interpretation of results.

I am grateful to *Dr. A. I. Gray* for helpful discussions, *Professor R. D. Waigh* and *Mrs. Sally Waigh* for their support, along with all my colleagues of the Phytochemistry and Medicinal Chemistry laboratories for their friendship and help.

This work would not have been possible without the financial backing of the Stevenson Foundation and the University of Strathclyde, to which I am greatly indebted.

Finally, I also wish to express my deep gratitude to my parents, my brothers and sisters for their patience and support.

Abstract

This thesis describes the phytochemical investigation of five species of Annonaceae, *Cleistopholis glauca*, *Cleistopholis patens*, *Piptostigma fasciculata*, *Goniothalamus thwaitesii* and *Goniothalamus gardneri*. Extracts of specific parts of each plant were fractionated using standard chromatographic techniques and pure compounds isolated identified on the basis of their physical and spectral data (IR, UV, MS, 1D and 2D NMR).

The stem bark of *Cleistopholis glauca* yielded fourteen compounds. These included the tri- and tetrarhamnosides, cleistri- and cleistetrosides with cleistetroside-6 and -7 as new compounds, farnesane sesquiterpenes including the new methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate, flavonoids, simple aromatic compounds, the azaanthracene alkaloid cleistopholine and the neolignan *rel*-(2α,3β)-7-*O*-methylcedrusin. This represents the first report of *rel*-(2α,3β)-7-*O*-methylcedrusin in the family, of flavonoids in the genus and of cleistopholine in the species.

The leaves of *Cleistopholis patens* afforded seven compounds. These were six cleistri- and cleistetrosides, with cleistrioside-2, -3, -4 and cleistetroside-6 being new, and shikimic acid. This is the first report of shikimic acid in the genus and of cleistri- and cleistetrosides in the species.

The leaves of *Piptostigma fasciculata* yielded three compounds, which included the sesquiterpene β-caryophyllene-4,5-oxide, the diterpene (2*E*,7ξ,11ξ)-phytol and the flavonol glycoside nicotiflorin. This is the first report of (2*E*,7ξ,11ξ)-phytol in the family and of all three compounds in the genus.

The aerial parts of *Goniothalamus thwaitesii* afforded five compounds. These were the triterpenes friedelinol, friedelin and betulinic acid, the flavonol glycoside mearnsitrin and the flavonol-3-*O*-methyl ether annulatin. This is the first report of friedelinol, betulinic acid, mearnsitrin and annulatin in the family and of all compounds in the genus.

The aerial parts of *Goniothalamus gardneri* afforded nine compounds. These included eight flavonoids and the new acetogenin, *rel*-5α-hydroxymethyl-3β-eicosa-19'-en-11'-yn-tetrahydrofuran-2-one (goniothalamusin). Among the flavonoids, 2',4'-dihydroxy-4,6'-dimethoxychalcone and dihydroflavokawain A are reported for the first time as natural products. All compounds are isolated for the first time in the family.

A bioassay-guided search for new bradykinin antagonists in the crude extracts of the two *Cleistopholis* species was carried out using a radioligand binding assay on bradykinin receptors. The compounds showing affinity for the receptors were identified as the cleistri- and cleistetrosides.

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Chapter 2: Materials and Methods

Chapter 3: Phytochemical Results and Discussion

Chapter 4: Pharmacological Results and Discussion

List of Abbreviations

Phytochemical

Acetone-d_6	Deuterated acetone
$AlCl_3$	Aluminium chloride
Anisaldehyde-H_2SO_4 (AS)	Anisaldehyde sulphuric acid reagent
ATP	Attached proton test
BIRD	BIlinear Rotation Decoupling
C_5D_5N	Deuterated pyridine
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
COSY	^{1}H-^{1}H COrrelation SpectroscopY
LR-COSY	^{1}H-^{1}H Long Range COrrelation SpectroscopY
D_2O	Deuterated water
DBE	Double Bond Equivalents
DG	Dragendorff's reagent
DMSO	Dimethylsulfoxide
EtOAc	Ethyl acetate
FABMS	Fast Atom Bombardment Mass Spectrometry
FC	Flash Chromatography
GF	Gel Filtration
H_3BO_3	Boric acid
H	Heating
HC-COBI	^{1}H-^{13}C heteronuclear COrrelation using a BIrd pulse
HCl	Hydrochloric acid
HMBC	^{1}H-^{13}C Heteronuclear Multiple Bond Connectivity
HREIMS	High Resolution Electron Impact Mass Spectrometry
IR	Infra Red light
MeOD	Deuterated methanol
MeOH	Methanol
Mp	Melting point
NaOAc	Sodium acetate
NaOMe	Sodium methoxide
n-BuOH	*n*-butanol
NMR	Nuclear Magnetic Resonance
NOESY	^{1}H-^{1}H Nuclear Overhauser Effect SpectroscopY
Petrol	Light petroleum ether bp 40-60°
Short/long-wave UV	λ = 254/366 nm Ultra Violet light
TLC	Thin Layer Chromatography
TOCSY	^{1}H-^{1}H TOtal COrrelation SpectroscopY
Vanillin-H_2SO_4 (VS)	Vanillin sulphuric acid reagent
VLC	Vacuum Liquid Chromatography

Pharmacological

BK	Bradykinin
BSA	Bovine serum albumin
TES	Trimethylaminoethanesulfonic acid

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List of Schemes

List of Spectra

List of Tables

List of TLC solvent systems

A	*n*-hexane/EtOAc	(9:1)
A^2	*n*-hexane/EtOAc	(9:1) developed twice
B	*n*-hexane/EtOAc	(1:1)
C	$CHCl_3$/EtOAc	(9:1)
D	$CHCl_3$/MeOH	(9:1)
D^2	$CHCl_3$/MeOH	(9:1) developed twice
E	$CHCl_3$/MeOH/H_2O	(5:4:1)
F	$CHCl_3$/EtOAc/MeOH	(85:7.5:7.5)
G	$CHCl_3$/EtOAc/MeOH	(78:11:11)

Chapter 1:
Introduction

1.1 General considerations

The structural diversity of molecules from natural sources (micro-organisms, plants, marine organisms, insects, animal venoms) can provide a unique supply of novel lead compounds with potential therapeutic interest. Plants have been used for their medicinal properties since ancient times and in the past years, progress in the search for bioactive natural products from plants has been intensified considerably. This is attributed to the availability of modern isolation and characterisation techniques and the development of new screening methods for a range of disease targets.

Despite this, today less than 5% of the world's flora is estimated to have been chemically investigated and very few plants have been screened using all the assays now available. Therefore, many plant species still remain to be investigated for their potential medicinal interest. This is particularly true for those of the tropical rain forests, which are the richest source of plant chemical diversity (Waterman, 1993; Fellows and Scofield, 1995; Aylward, 1995).

1.2 Background of the study

This project stemmed from a collaboration between the Strathclyde Institute for Drug Research (SIDR) and the Phytochemistry Research Laboratories. The area of interest was the search for natural products which could prove useful for the development of novel drugs to treat various inflammatory conditions. The assessment of the potential anti-inflammatory activity of a high number of plant extracts, randomly selected, had been carried out by the pharmacologists of SIDR using a radioligand binding assay on bradykinin receptors. Of the plants tested, extracts from two species of Annonaceae displayed receptor-binding affinity worthy of further investigation.

Furthermore, the on-going chemical investigation of species of Annonaceae within the Phytochemistry Research Group (Pootakahm, 1978; Hasan, 1981; Muhammad, 1984; Etse, 1986; Fleischer, 1997) was at the origin of the second part of the study in which the area of interest was the thorough chemical investigation of species previously unexplored.

1.3 The family Annonaceae A. L. de Jussieu

The Annonaceae is a large family of flowering plants, which is placed in the order Annonales. It is made up of two distinct sub-families, Annonoideae and Monodoroideae. The Annonoideae is further subdivided into the tribes Uvarieae, Miliuseae and Unoneae, which includes the sub-tribes Xylopiineae and Annonineae (Hutchinson, 1964).

On the basis of its morphology and habitat, the Annonaceae is a very uniform family. It comprises *ca.* 2300 species distributed among 120 genera, predominantly occurring at low elevations in humid dense forests of tropical and sub-tropical regions of Australia, Asia, Africa and the Americas. Annonaceous plants are characterised by many extremely primitive morphological features and often described as "living fossils" (Hutchinson, 1964; Takhtajan, 1969; Dassanayake and Fosberg, 1985).

They occur as trees, shrubs or climbers, usually evergreen and aromatic. The leaves are alternate, entire, exstipulate. The flowers are usually green, yellowish or purple, terminal, leaf-opposed, extra-axillary or axillary, solitary or sometimes in fascicles or panicles on the older wood. They are bisexual, rarely unisexual, and mostly trimerous. The sepals, usually three, are valvate or imbricate. The petals, generally six in two series, are valvate or imbricate. Their aestivation, shape and size separates the sub-family Annonoideae into three tribes.

The stamens are numerous, free, spirally arranged in several series around the carpels. Their filaments are thick, very short and the connective is often truncate. The carpels are numerous, free or united into a many or unilocular ovary. They have short and thick styles, one or more ovules, and are inserted on a flat or conical receptacle. The fruits consist of free monocarps, sessile or stipitate, mostly indehiscent, woody or succulent, one to many-seeded. Alternatively they may be pseudo-syncarpous with numerous 1-seeded coalescent monocarps in an edible fleshy receptacle or even syncarpous, unilocular, many-seeded. The type of fruiting carpels separates the family into two sub-families and the tribe Unoneae into two sub-tribes. The seeds are mostly ovate, glabrous, with a copious ruminate endosperm and a small embryo (Hutchinson, 1964).

The family is of appreciable economic importance. Species like *Annona squamosa* (sweetsop), *A. muricata* (soursop), *A. reticulata* (West Indian custard apple), *A. senegalensis*, *A. cherimola* (cherimoya), *Asimina triloba* (American pawpaw) and some species of *Hexalobus* are grown for their edible fruits. Seeds of some *Xylopia* and *Monodora* species are used as condiments. Petals of *Artabotrys uncinatus* and *Cananga odorata* yield the volatile oil of "ylang-ylang" used for its fragrance in perfumery. The wood of many species is locally employed for timbers and inner layers of the bark are a source of fibres used for cordage (Hutchinson, 1964; Le Thomas, 1969; Burkhill, 1985; Vivien and Faure, 1996).

Many annonaceous species are employed as medicinal plants by local peoples. Their therapeutic values in the treatment of a variety of disorders in Asian and West tropical African populations have been exhaustively compiled respectively by Perry (1980) and Burkhill (1985). They are most commonly used for broncho-pneumonial and skin affections, diarrhoea, stomach-aches, oedema, rheumatism and febrile conditions as well as for their insecticidal and vermicidal properties (see Sections 1.4.1, 1.4.2 & 1.6.1). The widespread use of annonaceous species in local folk medicine has led to several phytochemical investigations in search for bioactive substances (see Sections 1.4.4 & 1.6.4).

1.3.1 Previous phytochemical investigations

The chemistry of the Annonaceae was extensively reviewed by Leboeuf *et al.* (1982) and Waterman (1986). The family is a major source of alkaloids, mostly of the isoquinoline-type (Leboeuf *et al.*, 1982; Cavé, 1985; Guineaudeau *et al.*, 1988). It also produces other types of alkaloids as well as a whole range of non-alkaloidal constituents. This has been demonstrated particularly in previous studies within the Phytochemistry Research Group (see Section 1.2). In recent years the variety of structures discovered has been greatly enhanced.

The purpose of this brief introduction to the chemistry of the family is not to provide a complete review but rather to focus on classes of compounds encountered in the course of the study.

1.3.1.1 Alkaloids

a) Isoquinoline alkaloids

The isoquinoline alkaloids most commonly isolated include (Figure 1.1):

i) benzyltetrahydroisoquinolines

ii) bisbenzyltetrahydroisoquinolines of various structural types. Common modes of linkage between units include diaryl carbon-carbon or diaryl ether bridges, tail-to-tail **(1)**, head-to-head and tail-to-tail **(2)**, head-to-tail and tail-to-head **(3)**

iii) proto-**(4)** and tetrahydroprotoberberines **(5)**

iv) aporphinoids including aporphines *sensu stricto* [aporphines (R_1 = Me, R_2 = H) and noraporphines (R_1, R_2 = H) **(6)**], dehydroaporphines, 7-oxygenated aporphines, oxoaporphines **(7)** and phenanthrenes

v) bisaporphinoids **(8)** and lactams **(9)**

b) Non-isoquinoline alkaloids

Among the non-isoquinoline compounds isolated are indole-derived alkaloids; naphthyridine alkaloids **(10, 11, 12)**; copyrine alkaloids **(13)** (Rao *et al.*, 1986; Liu *et al.*, 1990); azaanthracenes **(14)** and azafluorenones **(15)** (Laprévote *et al.*, 1988; Bou-abdallah *et al.*, 1989; Achenbach and Schwinn, 1995) (Figure 1.2).

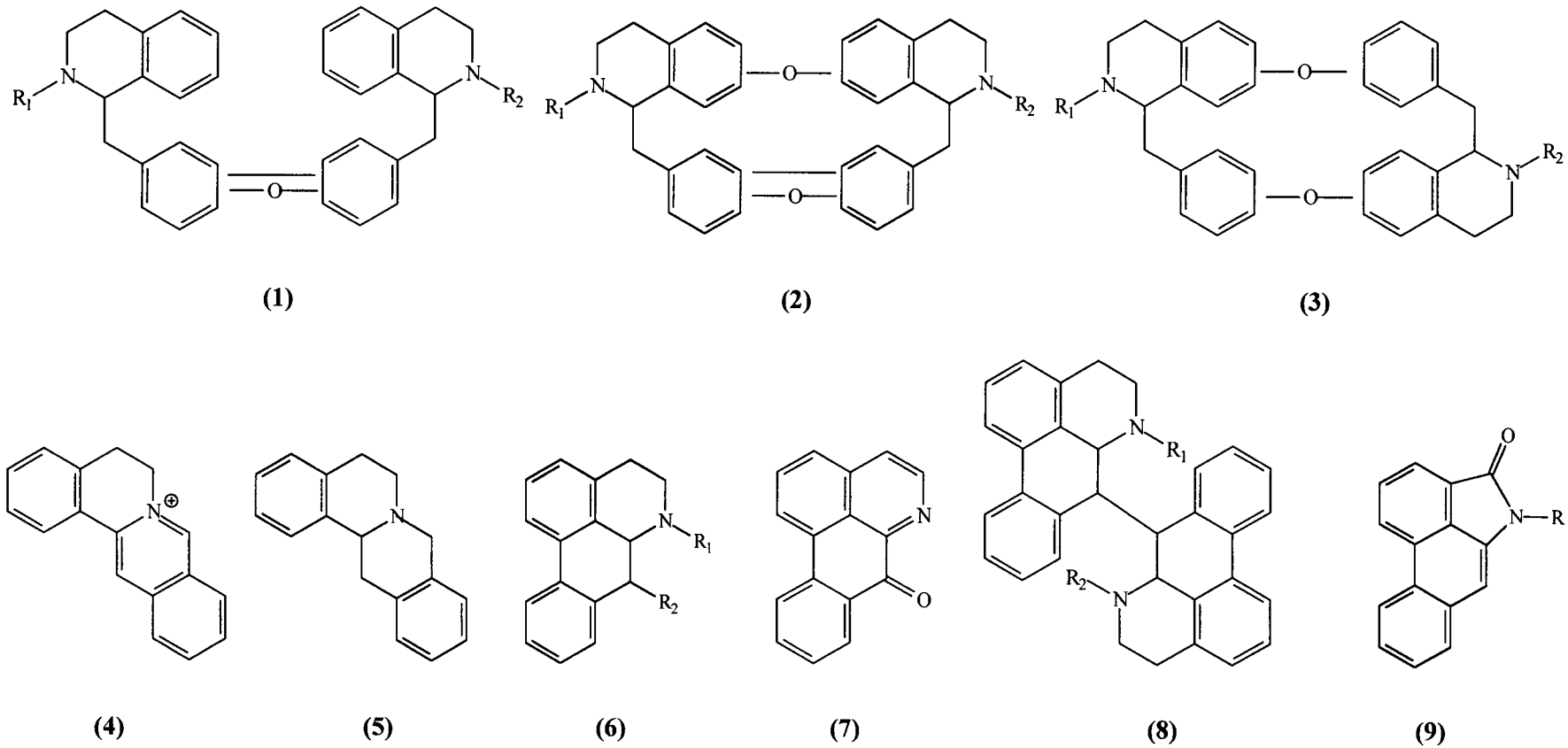
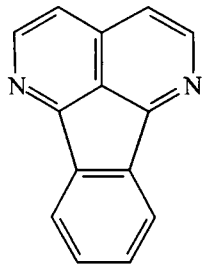


Figure 1.1: Skeletal types of some isoquinoline alkaloids in the Annonaceae



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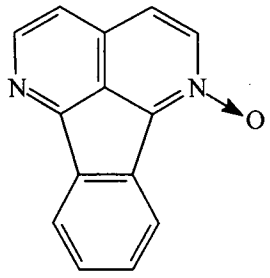

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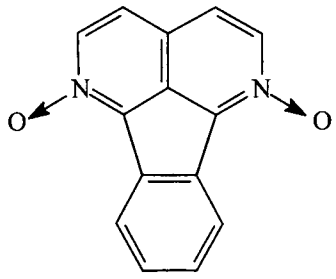

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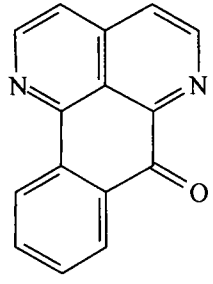

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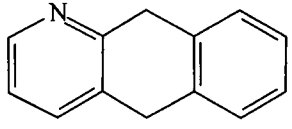

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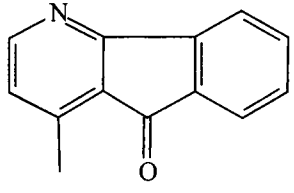


(15)

Figure 1.2: Skeletal types of some non-isoquinoline alkaloids in the Annonaceae

1.3.1.2 Non-alkaloidal compounds

Classes of compounds include terpenoids, flavonoids, acetogenins and miscellaneous structures.

a) Terpenoids

Monoterpenes of various skeletal types are frequently encountered as constituents of essential oils (Ekundayo *et al.*, 1988).

Sesquiterpenes are common in the family and are often also present in essential oils. Compounds isolated include some cadinane (Ekundayo and Ogutimein, 1987), farnesane (Etse *et al.*, 1988), caryophyllane (Etse, 1986; Fleischer, 1997), bisabolane (Fleischer, 1997) and guaiane derivatives (Achenbach and Schwinn, 1995; Fleischer, 1997).

Diterpenes are also quite widespread and kaurane derivatives have been predominantly isolated. Other main skeletal types include clerodane and kolavane (Hasan *et al.*, 1994a; Hasan *et al.*, 1995b/c), labdane and trachylobane.

Only a few triterpenes and phytosterols have been isolated. Triterpenes include friedelane, taraxerane, glutinane, lanostane and cycloartane skeletons while among phytosterols, mainly sitosterol, stigmasterol and their derivatives occur (Achenbach and Schwinn, 1995).

b) Flavonoids

Flavonoids of various structural types have been reported but very rarely as glycosides. Aglycones isolated include a flavonol, a flavonol-3-*O*-methyl ether, chalcones, flavanones and flavones variously methoxylated or hydroxylated. Other structures of interest include *C*-benzylated dihydrochalcones and flavanones, *C*-methylated and *C*-formylated flavones and flavanones, and also *C*-methylated flavones and flavanones (Fleischer, 1997). A common feature among the flavonoids isolated is the absence of B-ring substitution. A retrochalcone and a

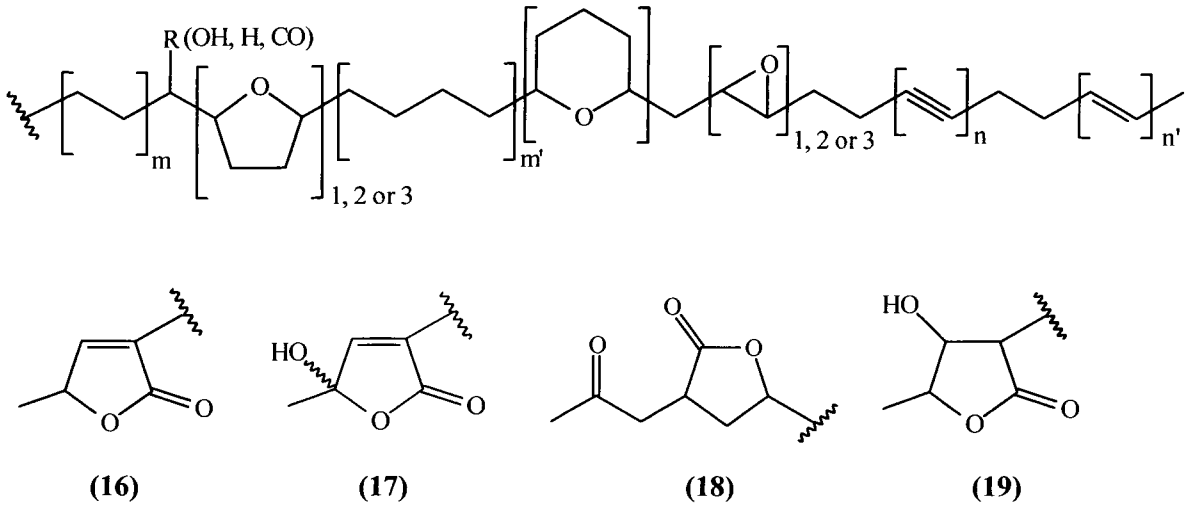
retrodihydrochalcone have been reported recently (Colegate *et al.*, 1992; Fleischer, 1997).

c) Acetogenins

Several reviews on annonaceous acetogenins have been published (Rupprecht *et al.*, 1990; Cavé *et al.*, 1993; Cavé *et al.*, 1997; Zafra-polo *et al.*, 1998) as an increasing number of these particular compounds, which appear exclusive to the family, have been isolated in recent years. A total of *ca.* 250 different structures have been identified so far.

Acetogenins are a series of compounds derived from fatty acids. They are characterised by a long alkyl chain (usually C_{35} or C_{37}) bearing a terminal γ-methyl-γ-lactone moiety. The chain can be linear or have a varying number (one, two or three) of tetrahydrofuran rings or epoxides, sometimes a tetrahydrofuran/ tetrahydropyran system. It can be substituted by oxygenated substituents (hydroxyl, acetoxyl, ketonic) in different positions or in some cases can be unsaturated (double and/or triple bonds).

Classification of acetogenins in various types and sub-types is based on the characteristics of the chain (linear, epoxy, mono-, bis-, tri-tetrahydrofuran or tetrahydrofuran/tetrahydropyran acetogenins) and of the γ-methyl-γ-lactone moiety present [unsaturated **(16)**, unsaturated γ-hydroxy-substituted **(17)**, saturated α-acetonyl-substituted **(18)**, saturated β-hydroxy-substituted **(19)**] (Figure 1.3).



(16) **(17)** **(18)** **(19)**

Figure 1.3: Skeletal types of acetogenins in the Annonaceae

d) Miscellaneous

Some complex oligosaccharide derivatives have been reported in the family. They are tri- or tetra-α-L-rhamnosides, linked (1→3) or (1→4), substituted by an alkyl ether group in C-1 on the first rhamnose unit and partially esterified in different positions (see Sections 1.4.3 & 3.6.1).

Other structures of interest include styryl-lactones (see Section 1.6.3), 2-aryldihydrobenzofuran neolignans (see Section 3.6.1), simple aromatic compounds, shikimate-derived metabolites (Nkunya *et al.*, 1987; Liang *et al.*, 1988) and non-alkaloidal nitrogen-containing compounds (see Section 1.5.2).

1.4 The genus *Cleistopholis* Pierre ex Engl.

The genus *Cleistopholis* is placed in the sub-family Annonoideae, tribe Uvarieae. It comprises 6 species found mainly in West tropical Africa (Hutchinson, 1964). One species occurs in East tropical Africa (Verdcourt, 1971).

Cleistopholis species are trees; leaves oblong, usually glabrous and shiny; flowers bisexual, axillary, in fascicles or pseudo-umbelliferous inflorescences; sepals 3, small, free and valvate; petals 6, free, in 2 series, outer spreading, valvate, elliptic-oblong or linear, inner imbricate, ovate, much shorter and concave, closely appressed over the stamens and carpels; stamens numerous, cuneiform, connective truncate; carpels numerous, oblong, stigma sessile, ovules 1-2; fruits subglobose or ellipsoid, thick pedicels, subsessile; seeds with smooth or tuberculate testa (Hutchinson, 1964; Le Thomas, 1969; Verdcourt, 1971).

1.4.1 *Cleistopholis glauca* Pierre ex Engl. & Diels

Synonyms: *C. grandiflora* De Wild., *C. bequaerti* De Wild.

C. glauca is a tree (10-35m high) found in humid dense forests of Cameroon, Gabon, Central Africa Republic, Congo and Angola, especially along rivers. The bark is greyish, fibrous and fragrant. The leaves are elliptic-oblong to oblanceolate, glabrous, shiny on the upper surface, mat on the inner surface, with lateral nerves (8-15 pairs). The flowers are small, greenish yellow, in axillary, pseudo-umbelliferous 2 to 8-flowered inflorescences. The outer petals are elliptic-oblong. There are *ca.* 12 to 24 carpels. The fruit is ellipsoid, dark violet when ripe, subsessile, with a smooth pericarp. The seeds have a smooth or very slightly tuberculate testa (Figure 1.4).

Throughout West tropical Africa, it is used for timbers and to make canoes. The fibres serve as a useful material for making ropes, baskets and hut-walls. In Congo, the sap of the root is part of an arrow hunting poison mixture. The species is also employed for medicinal purposes. Thus, in Congo the sap of the bark or the bark decoction is drunk for tuberculosis and bronchial diseases, the crushed bark is part of a poultice applied to treat oedema and ulcers, the bark decoction is prescribed for stomach pain and diarrhoea. In Gabon the bark decoction is used as an emetic and the macerate of the bark is rubbed in for scabies. In Central Africa Republic people drink the macerate of twig and root barks against intestinal worms (Le Thomas, 1969; Raponda-Walker and Sillans, 1995; Neuwinger, 1996).

1. Flowering branch × 2/3; **2**. Inflorescence × 1.5; **3**. Flower × 3; **4**. Inner petals × 5; **5**. Flower without petals × 4; **6**. Stamens × 12; **7**. Carpels × 12; **8**. Fruits; **9**. Seed.

Figure 1.4: ***Cleistopholis glauca*** **Pierre ex Engl. & Diels after Le Thomas (1969)**

1.4.2 *Cleistopholis patens* (Benth.) Engl. & Diels

Synonyms: *Oxymitra patens* Benth., C. *klaineana* Pierre ex Engl. & Diels, *C. pynaertii* De Wild., *C. verschuereni* De Wild., *C. lucens* De Wild., *C. brevipetala* Exell, C. *patens* var. *klaineana* Pellegrin

C. patens is a tree (up to 30m high) common in disturbed forest lands, rapidly colonising abandoned areas, especially in wet places, along rivers and swamps in humid dense forests from Sierra Leone eastwards into Congo and Uganda. It has a spreading crown and is light-loving and fast growing. The bark is thick, greyish black, smooth or vertically furrowed, fibrous, moist and fragrant when fresh. The sap is reddish and like palm-oil with a salty taste, hence the Ghanaian name "salt-and-oil tree". The leaves are oblong to oblong-lanceolate, slightly falcate, shiny and glabrous on the upper surface, with lateral nerves (10-24 pairs). The flowers are greenish yellow in 2 to 9 flowered fascicles. The outer petals are oblong to obovate-oblong. There are *ca.*10 carpels. The fruit is subglobose with a tuberculate pericarp and a thick stipe. The seeds have a tuberculate testa (Figures 1.5 & 1.6).

The stem bark is used for its wood and fibres as *C. glauca* (see Section 1.4.1). *C. patens* is also widely employed in folk medicine. In Sierra Leone and in Gabon a leaf infusion is drunk against fever. In the Ivory Coast a leaf decoction is drunk against sleeping sickness and leaves are also employed as a vermifuge. In Ghana leaf or stem bark infusions are drunk for infective hepatitis. In Congo the bark decoction is taken for tuberculosis and bronchial affections while the crushed bark is part of a poultice applied to treat oedema. In Sierra Leone the bark is used as a purgative and in Gabon and Congo it is said to soothe colic. In the Ivory-Coast the bark decoction enters into a general treatment in washes and topical friction for rachitic children and the sap is used as a nasal instillation for headaches. In Ghana the roots are used as a vermifuge (Irvine, 1961; Burkhill, 1985; Raponda-Walker and Sillans, 1995).

A. Flowering branch × 1/2; **B**. Flower × 2; **C**. Section of flower × 4;
D. Stamen, × 10; **E**. Carpel, longitudinal section × 10; **F**. Fruits × 1/2;
G. Seed, 1-seeded monocarp; **H**. Seed, 2-seeded monocarp × 1/2.

Figure 1.5: *Cleistopholis patens* (Benth.) Engl. & Diels

after Verdcourt (1971)

Figure 1.6: ***Cleistopholis patens*** **(Benth.) Engl. & Diels (taken in Ghana, 1998)**

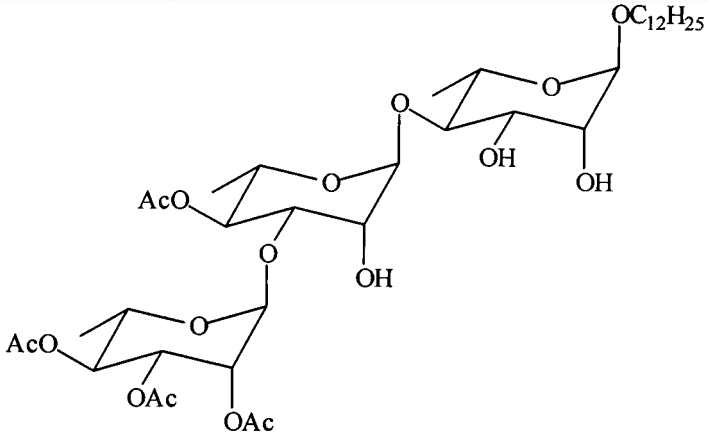
Figure 1.6: ***Cleistopholis patens*** **(Benth.) Engl. & Diels (taken in Ghana, 1998)**

1.4.3 Previous phytochemical investigations

Previous studies on *Cleistopholis* have revealed the presence of lipids in *C. glauca* (Ngiefu *et al.*, 1976) and of saponins and polyphenols in *C. patens* (Odebiyi and Sofowora, 1978). The isolation of a volatile oil from the latter species has also been reported (Ekundayo and Oguntimein, 1987; Ekundayo *et al.*, 1988). *Cleistopholis* species have also yielded some oligosaccharides, terpenoids, alkaloids and miscellaneous compounds (Tables 1.1 to 1.4; Figures 1.7 to 1.10).

Table 1.1: Oligosaccharides previously isolated from *Cleistopholis*

Isolated oligosaccharides	Species (references)
1-*O*-dodecanyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside **(20)**	*C. glauca* (Tané *et al.*, 1988b; Woods, 1989)
1-*O*-dodecanyl 3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside **(21)**	*C. glauca* (Tané *et al.*, 1988b; Woods, 1989)
1-*O*-dodecanyl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside **(22)**	*C. glauca* (Tané *et al.*, 1988b; Woods, 1989)
1-*O*-dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside **(23)**	*C. glauca* (Tané *et al.*, 1988b; Woods, 1989)
1-*O*-dodecanyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside **(24)**	*C. glauca* (Tané *et al.*, 1988b; Woods, 1989)
1-*O*-dodecanyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside **(25)**	*C. glauca* (Tané *et al.*, 1988b; Woods, 1989)



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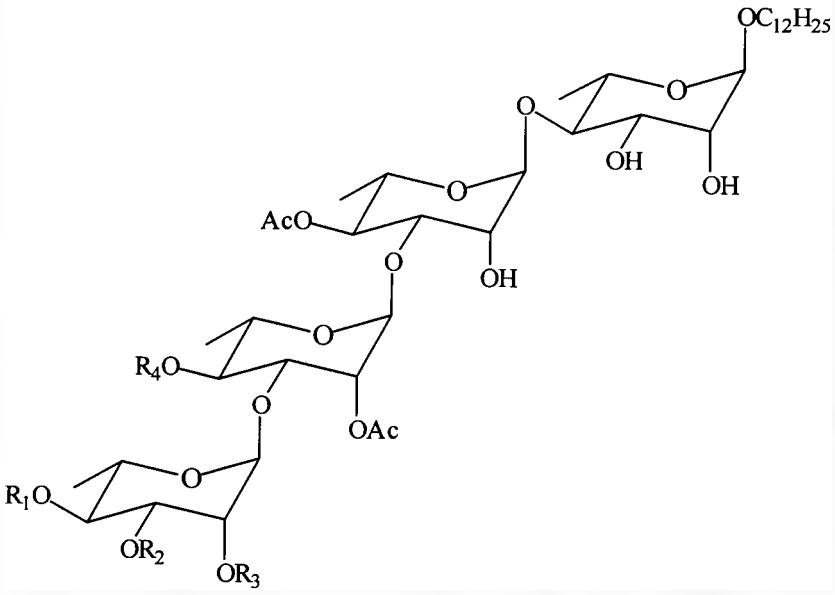
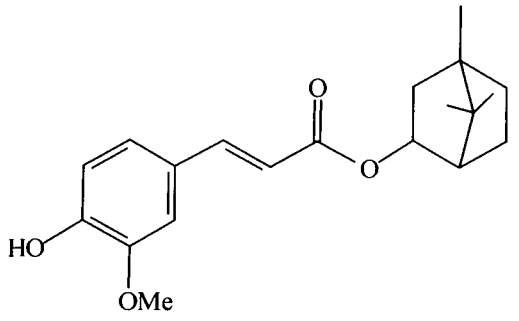


Compound	R_1	R_2	R_3	R_4
(21)	Ac	Ac	H	Ac
(22)	H	H	Ac	Ac
(23)	Ac	H	H	Ac
(24)	H	H	H	Ac
(25)	H	H	H	H

Figure 1.7: Structures of the oligosaccharides from *Cleistopholis*

Table 1.2: Terpenoids previously isolated from *Cleistopholis*

Isolated terpenoids	Species (references)
Cleistophostaudin **(26)**	*C. staudtii* (Tané *et al.*, 1988a)
Methyl (+)-(2*E*,6*E*,10ξ)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate **(27)**	*C. patens* (Waterman and Muhammad, 1985) *C. glauca* (Etse *et al.*, 1988) *C. staudtii* (Tané *et al.*, 1988a)
Methyl (+)-(2*E*,6*E*,10ξ)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate **(28)**	*C. glauca* (Etse *et al.*, 1988)
Methyl (+)-(1'ξ,2*E*,3'ξ,6'ξ)-3-methyl-5-(3',6'-dihydroxy-2',2',6'- trimethylcyclohex-1'-yl)-pent-2-enoate **(29)**	*C. glauca* (Etse *et al.*, 1988)
Methyl (1'ξ,2E,3'ξ)-3-methyl-5-(3'-hydroxy-2',2'-dimethyl-6'-methylenecyclohex-6'-en-1'-yl)-pent-2-enoate **(30)**	*C. patens* (Waterman and Muhammad, 1985) *C. glauca* (Etse *et al.*, 1988)
Methyl (1'ξ,2*E*,3'ξ)-3-methyl-5-(3'-hydroxy-2',2',6'- trimethylcyclohex-5'-en-1'-yl)-pent-2-enoate **(31)**	*C. patens* (Waterman and Muhammad, 1985) *C. glauca* (Etse *et al.*, 1988)
Methyl (2*E*,3'ξ)-3-methyl-5-(3'-hydroxy-2',2',6'- trimethylcyclohex-6'-en-1'-yl)-pent-2-enoate **(32)**	*C. glauca* (Etse *et al.*, 1988)



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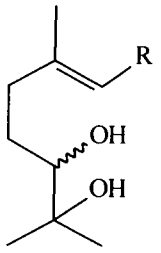

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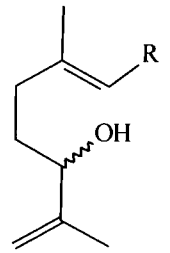

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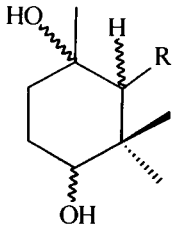

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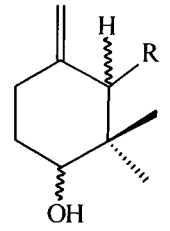

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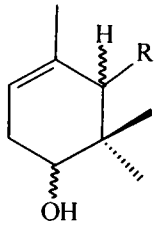

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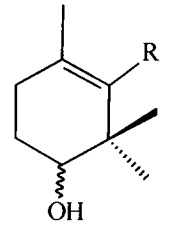

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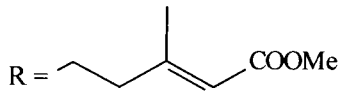
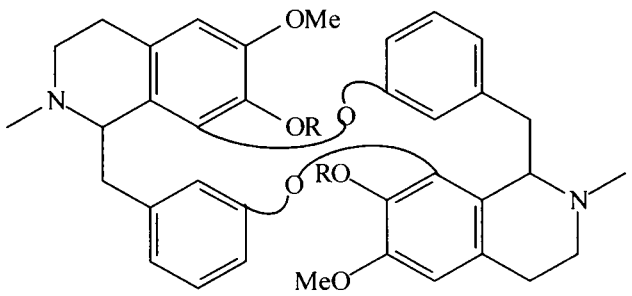


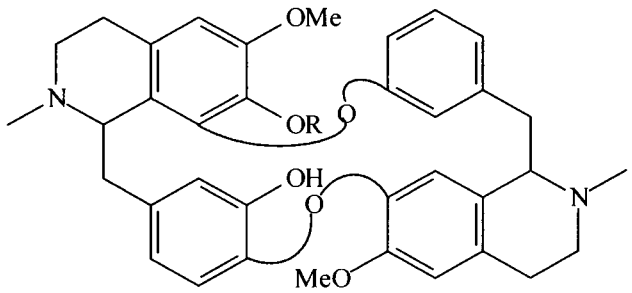
Figure 1.8: Structures of the terpenoids from *Cleistopholis*

Table 1.3: Alkaloids previously isolated from *Cleistopholis*

Isolated alkaloids	Species (references)
(R,R)-(-,-)-cycleanine **(33)**	*C. staudtii* (Waterman and Muhammad, 1984)
(R,R)-(-,-)-isochondodendrine **(34)**	*C. staudtii* (Waterman and Muhammad, 1984)
(R,R)-(-,-)-chondrofoline **(35)**	*C. staudtii* (Waterman and Muhammad, 1984)
(R,R)-(-,-)-curine **(36)**	*C. staudtii* (Waterman and Muhammad, 1984)
Liriodenine **(37)**	*C. patens* (Abd-el-Ati *et al.*, 1982)
	C. patens (Waterman and Muhammad, 1985)
	C. patens (Liu *et al.*, 1990)
Isomoschatoline **(38)**	*C. patens* (Abd-el-Ati *et al.*, 1982)
Eupolauridine **(39)**	*C. patens* (Waterman and Muhammad, 1985)
	C. patens (Hufford *et al.*, 1987)
	C. patens (Liu *et al.*, 1990)
Eupolauridine *N*-oxide **(40)**	*C. patens* (Waterman and Muhammad, 1985)
	C. patens (Liu *et al.*, 1990)
Eupolauridine di *N*-oxide **(41)**	*C. patens* (Waterman and Muhammad, 1985)
Cleistopholine **(42)**	*C. patens* (Waterman and Muhammad, 1985)
Onychine **(43)**	*C. patens* (Waterman and Muhammad, 1985)
3-Methoxysampangine **(44)**	*C. patens* (Liu *et al.*, 1990)

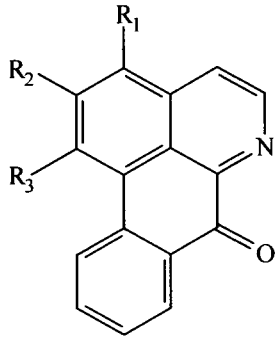


Compound	R
(33)	Me
(34)	H

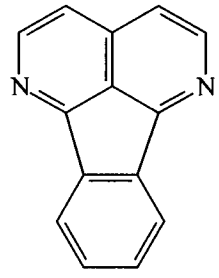


Compound	R
(35)	Me
(36)	H

Figure 1.9: Structures of the alkaloids from *Cleistopholis*



Compound	R_1	R_2	R_3
(37)	H	OCH_2O	
(38)	OH	OMe	OMe



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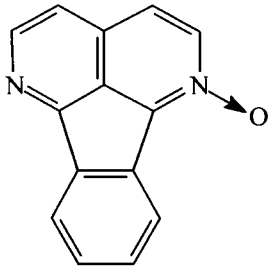
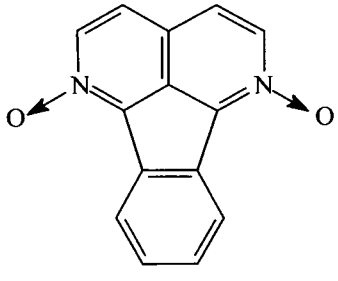


(40)



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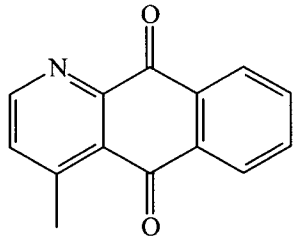

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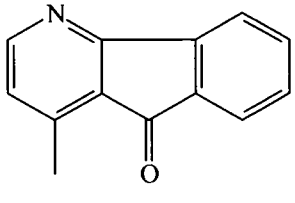

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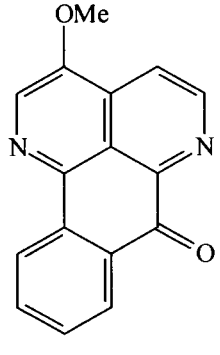
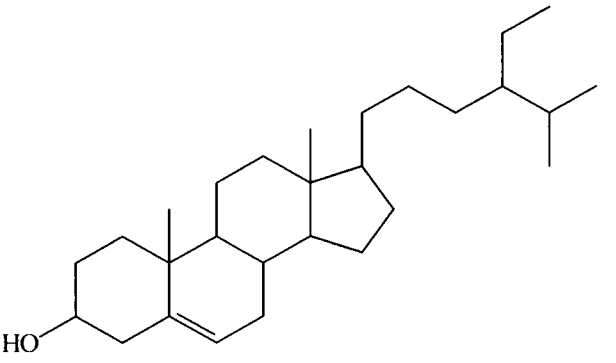


(44)

Figure 1.9 (cont.): Structures of the alkaloids from *Cleistopholis*

Table 1.4: Miscellaneous compounds previously isolated from *Cleistopholis*

Isolated compounds	Species (references)
β-Sitosterol **(45)**	*C. patens* (Waterman and Muhammad, 1985)
	C. glauca (Etse *et al.*, 1988)
2'-*E*-4-hydroxy-3-methoxy-cinnamaldehyde (ferulaldehyde) **(46)**	*C. glauca* (Etse *et al.*, 1988)
5-Hydroxymethyl-2-furaldehyde **(47)**	*C. glauca* (Etse *et al.*, 1988)



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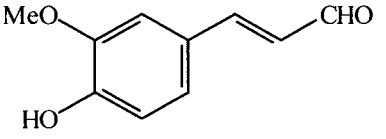
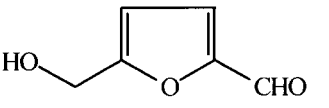


(46)



(47)

Figure 1.10: Structures of the miscellaneous compounds from *Cleistopholis*

1.4.4 Previous pharmacological investigations

Pharmacological studies have only been carried out on *C. patens* so far. An ethanolic fraction of the root bark showed a significant *in vitro* antifungal activity against strains of *Candida albicans*. Bioassay-guided fractionation led to the isolation of the naphthyridine alkaloid eupolauridine **(39)** as a promising potential new antifungal drug. The azafluorenone alkaloid onychine **(43)** also exhibited significant *in vitro* (Hufford *et al.*, 1987) and *in vivo* (Hufford and Clark, 1989) antifungal activity.

Further examination of the active ethanolic extract of the root bark led to the isolation of the copyrine alkaloid 3-methoxysampangine **(44)**, which demonstrated significant *in vitro* antifungal activity against strains of *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus* (Liu *et al.*, 1990).

1.5 The genus *Piptostigma* Oliv.

The genus *Piptostigma* is placed in the sub-family Annonoideae, tribe Miliuseae. It comprises *ca.* 15 species, which occur in West tropical Africa (Hutchinson, 1964).

Piptostigma species are shrubs or small trees; branches shortly tomentose; leaves subsessiles; nerves lateral, ascending, small nerves parallels; flowers bisexual, in panicles, cymes or fascicles or solitary on woody twigs; sepals 3, small, valvate; petals 6, in 2 series, valvate, outer much shorter than inner and sepals like, inner long, lanceolate to linear, concave at base; stamens numerous, cuneiform, oblong, connective dilated-truncate; carpels 3-14, oblong, hairy, stigmas sessile, subglobose; ovules 6-20, biseriate; fruits ellipsoid, oblong, sessile; seeds ellipsoid, in 2 series (Hutchinson, 1954; Le Thomas, 1969).

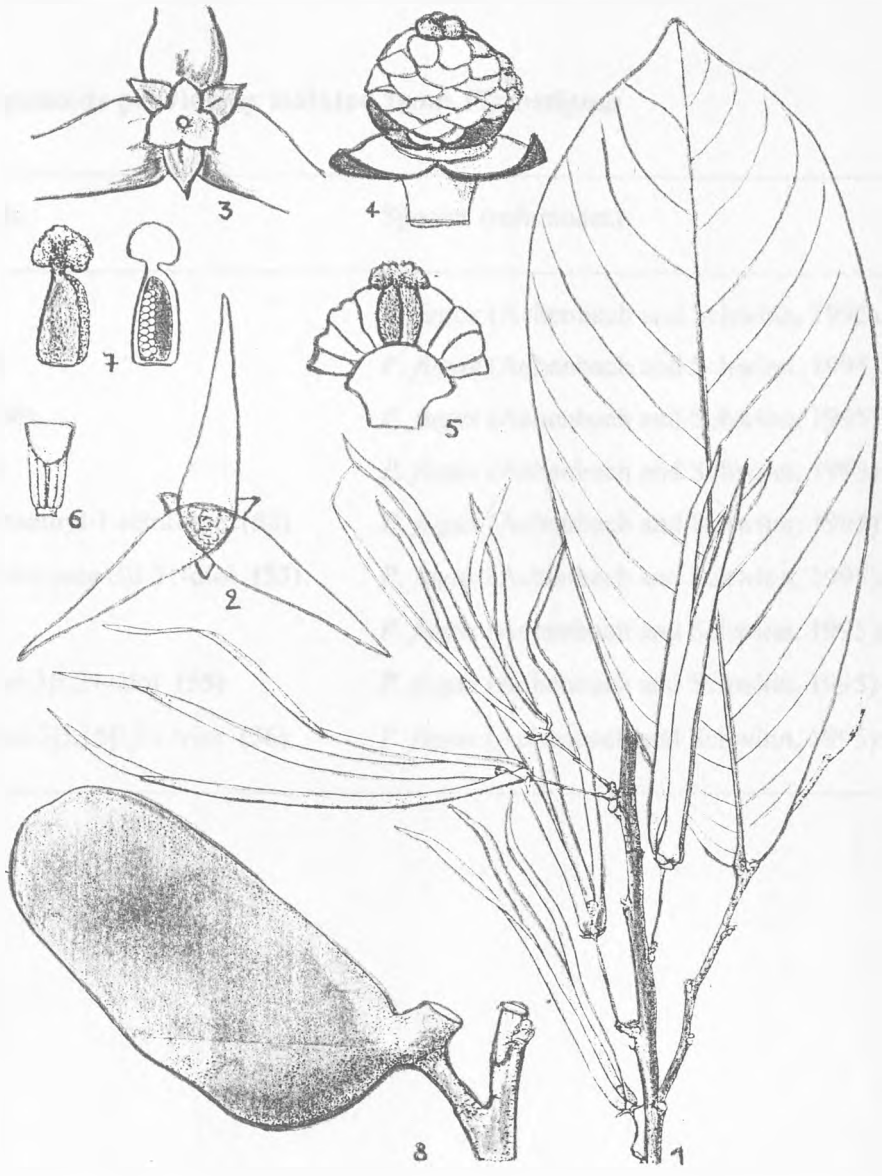
1.5.1 *Piptostigma fasciculata* (De Wild.) Boutique

Synonyms: *Brieya fasciculata* De Wild., *Piptostigma aubrevillei* Ghesq. ex Aubr.

P. fasciculata is a tree (20m high) of the dense rain forests of the Ivory Coast, Ghana, Cameroon, Gabon, Congo and Angola. The branchlets are hairy, glabrous at length. The leaves are obovate-oblong, rounded to subcordate at the base and rounded to broadly acuminate, scarcely emarginate, at the apex. They are glabrous when mature with a shiny upper surface and ascending lateral nerves (15-20 pairs), prominent beneath. The flowers are bisexual, green, solitary or in 2-3 fascicles on woody twigs. The pedicels are thin and tomentose. The 3 sepals are short, broadly triangular-ovate. The 6 petals are biseriately valvate. The outer 3 are short, brownish and resembling sepals. The inner 3 are much longer, elongated and linear, greenish yellow. The stamens are numerous with a dilated-truncate connective. There are 4-5 carpels, subglobose, scarcely lobed, deciduous, densely hairy, with stigmas shortly

stipitate and 18-20 ovules. The fruit is yellowish orange, ellipsoid-oblong to subglobose, berry-like, glabrous and with a thick pedicel (Figure 1.11).

To our knowledge neither reports of use in folk medicine or pharmacological studies have been reported on any *Piptostigma* species.



1. Flowering branch × 2/3; **2.** Flower, above view × 2; **3.** Flower, beneath view × 2; **4.** Flower without petals × 4; **5.** Section of receptacle × 4; **6.** Stamen × 6; **7.** Carpels × 6; **8.** Fruit × 2/3.

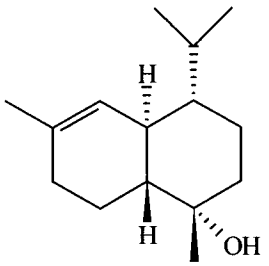
Figure 1.11: ***Piptostigma fasciculata*** **(De Wild.) Boutique after Le Thomas (1969)**

1.5.2 Previous phytochemical investigations

Studies on the chemistry of the genus *Piptostigma* have been restricted to the species *P. fugax*. Phytochemical investigation of the root and stem bark of the species collected in Ghana yielded terpenoids, phytosterols, alkaloids and some miscellaneous compounds (Tables 1.5 to 1.8; Figures 1.12 to 1.16).

Table 1.5: Terpenoids previously isolated from *Piptostigma*

Isolated terpenoids	Species (references)
T-cadinol **(48)**	*P. fugax* (Achenbach and Schwinn, 1995)
Cyperenone **(49)**	*P. fugax* (Achenbach and Schwinn, 1995)
Patchoulenone **(50)**	*P. fugax* (Achenbach and Schwinn, 1995)
Spathulenol **(51)**	*P. fugax* (Achenbach and Schwinn, 1995)
4-Hydroxy-4,7-dimethyl-1-tetralone **(52)**	*P. fugax* (Achenbach and Schwinn, 1995)
Lanosta-7,9(11),24-triene-3β,21-diol **(53)**	*P. fugax* (Achenbach and Schwinn, 1995)
Polycarpol **(54)**	*P. fugax* (Achenbach and Schwinn, 1995)
Lanosta-8,24-diene-3β,21-diol **(55)**	*P. fugax* (Achenbach and Schwinn, 1995)
5α-Cycloart-24-ene-3β,16β,21-triol **(56)**	*P. fugax* (Achenbach and Schwinn, 1995)



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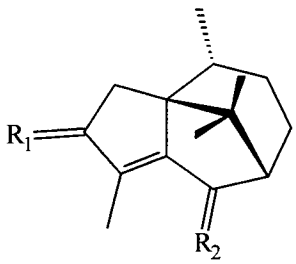
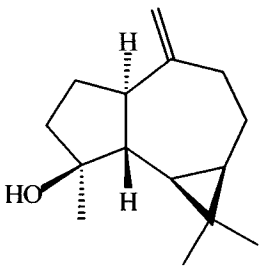


Compound	R_1	R_2
(49)	O	H_2
(50)	H_2	O



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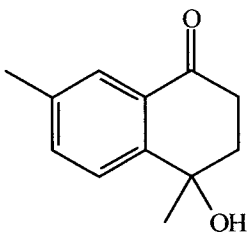

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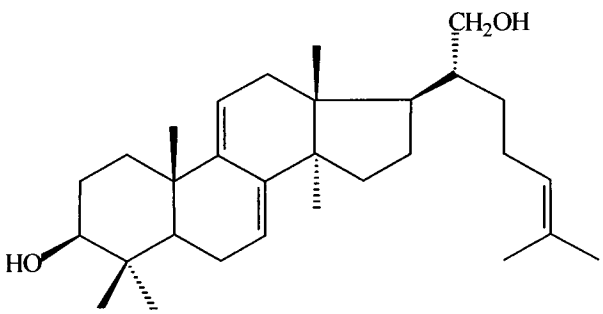
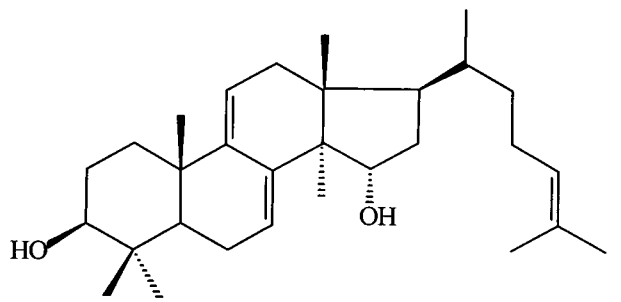


(53)

Figure 1.12: Structures of the terpenoids from *Piptostigma*



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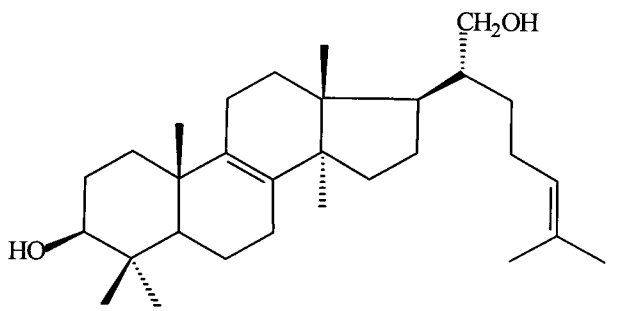

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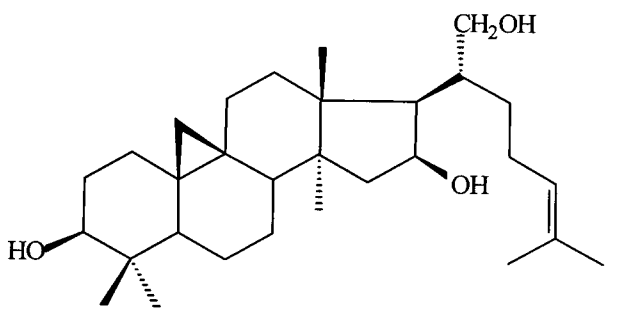
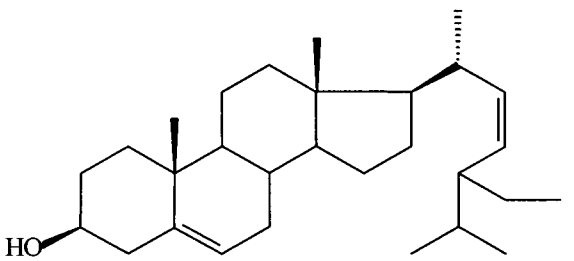


(56)

Figure 1.12 (cont.): Structures of the terpenoids from *Piptostigma*

Table 1.6: Phytosterols previously isolated from *Piptostigma*

Isolated phytosterols	Species (references)
β-Sitosterol **(45)**	*P. fugax* (Achenbach and Schwinn, 1995)
Stigmasterol **(57)**	*P. fugax* (Achenbach and Schwinn, 1995)
β-Sitosterol-*O*-β-D-glucoside **(58)**	*P. fugax* (Achenbach and Schwinn, 1995)
Stigmasterol-*O*-β-D-glucoside **(59)**	*P. fugax* (Achenbach and Schwinn, 1995)
3β-Hydroxystigmast-5-en-7-one **(60)**	*P. fugax* (Achenbach and Schwinn, 1995)
3β-Hydroxystigmasta-5,22-dien-7-one **(61)**	*P. fugax* (Achenbach and Schwinn, 1995)



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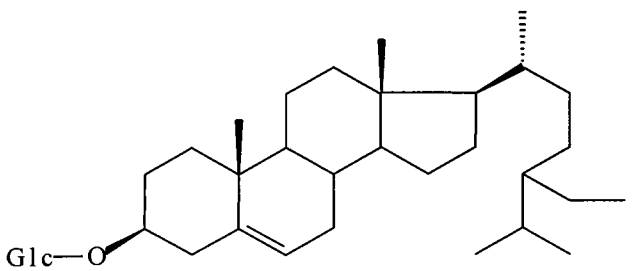
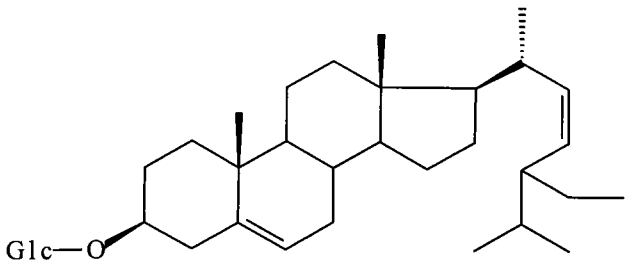


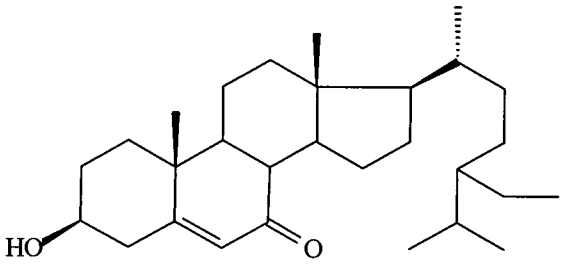
(58)

Figure 1.13: Structures of the phytosterols from *Piptostigma*

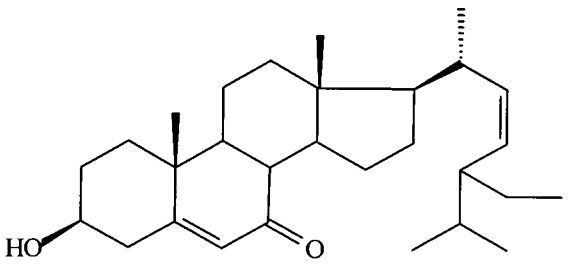
Glc = Glucose



(59)



(60)



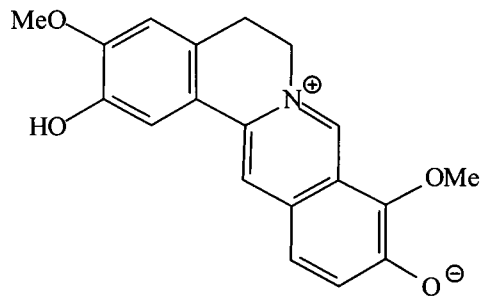
(61)

Figure 1.13 (cont.): Structures of the phytosterols from *Piptostigma*

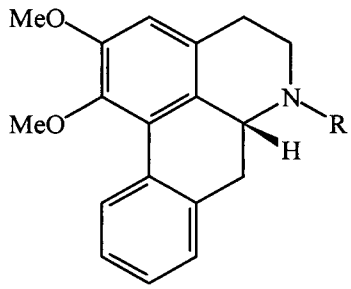
Glc = Glucose

Table 1.7: Alkaloids and other nitrogen compounds previously isolated from *Piptostigma*

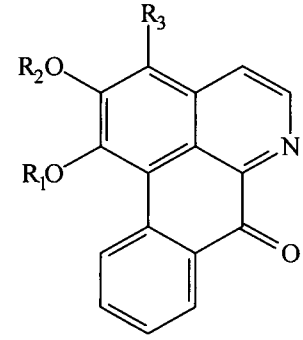
Isolated alkaloids	Species (references)
Stepharanine **(62)**	*P. fugax* (Achenbach and Schwinn, 1995)
N-Formylnomuciferine **(63)**	*P. fugax* (Achenbach and Schwinn, 1995)
Nomuciferine **(64)**	*P. fugax* (Achenbach and Schwinn, 1995)
Liriodenine **(65)**	*P. fugax* (Achenbach and Schwinn, 1995)
Lysicamine **(66)**	*P. fugax* (Achenbach and Schwinn, 1995)
O-Methylmoschatoline **(67)**	*P. fugax* (Achenbach and Schwinn, 1995)
N-*N*'-Dimethylurabaine **(68)**	*P. fugax* (Achenbach and Schwinn, 1995)
N-Methylurabaine **(69)**	*P. fugax* (Achenbach and Schwinn, 1995)
Urabaine **(70)**	*P. fugax* (Achenbach and Schwinn, 1995)
Heteropsine **(71)**	*P. fugax* (Achenbach and Schwinn, 1995)
N-Methylheteropsine **(72)**	*P. fugax* (Achenbach and Schwinn, 1995)
Trivalvone **(73)**	*P. fugax* (Achenbach and Schwinn, 1995)
2,7-Dihydroxyonychine **(74)**	*P. fugax* (Achenbach and Schwinn, 1995)
7-Hydroxy-2-methoxyonychine **(75)**	*P. fugax* (Achenbach and Schwinn, 1995)
7-Hydroxy-2,8-dimethoxyonychine **(76)**	*P. fugax* (Achenbach and Schwinn, 1995)
Indole-3-carbaldehyde **(77)**	*P. fugax* (Achenbach and Schwinn, 1995)
N-Methyltryptamine **(78)**	*P. fugax* (Achenbach and Schwinn, 1995)
N-Carbamoyl-2-methoxypyrrolidine **(79)**	*P. fugax* (Achenbach and Schwinn, 1995)
Trans-feruloyltyramine **(80)**	*P. fugax* (Achenbach and Schwinn, 1995)



(62)

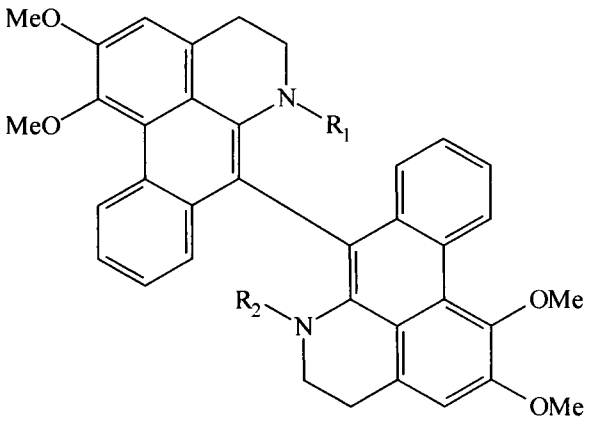


Compound	R
(63)	CHO
(64)	H

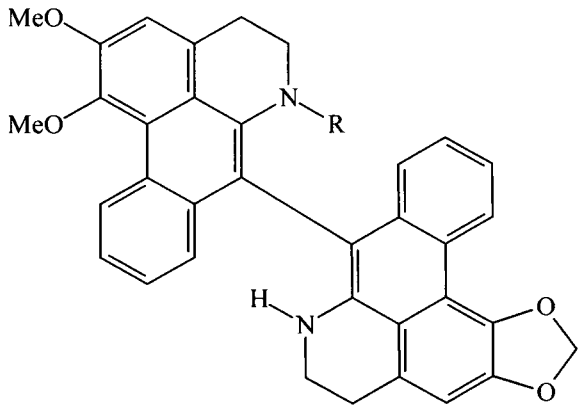


Compound	R_1	R_2	R_3
(65)	-CH_2-		H
(66)	Me	Me	H
(67)	Me	Me	OMe

Figure 1.14: Structures of the alkaloids from *Piptostigma*

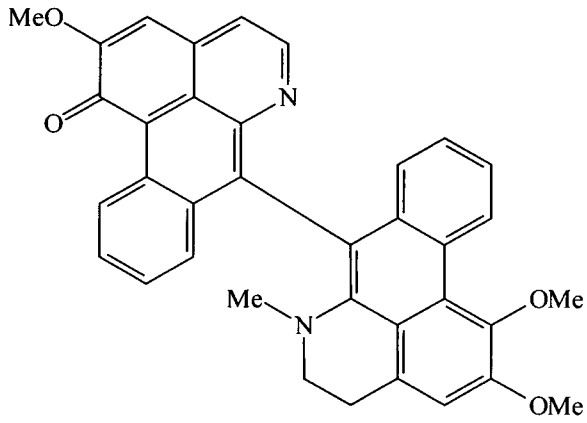


Compound	R_1	R_2
(68)	Me	Me
(69)	Me	H
(70)	H	H

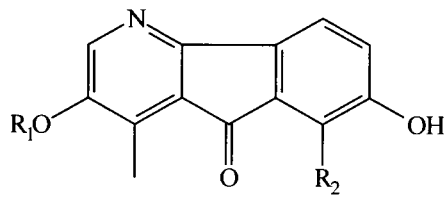


Compound	R
(71)	H
(72)	Me

Figure 1.14 (cont.): Structures of the alkaloids from *Piptostigma*

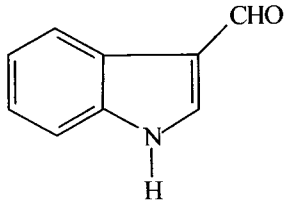


(73)

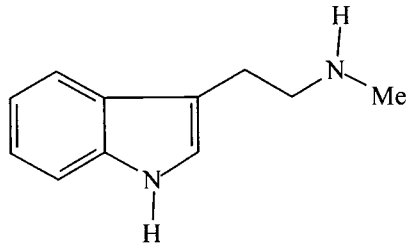


Compound	R_1	R_2
(74)	H	H
(75)	Me	H
(76)	Me	OMe

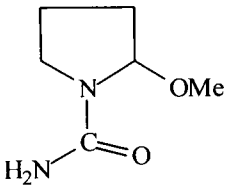
Figure 1.14 (cont.): Structures of the alkaloids from *Piptostigma*



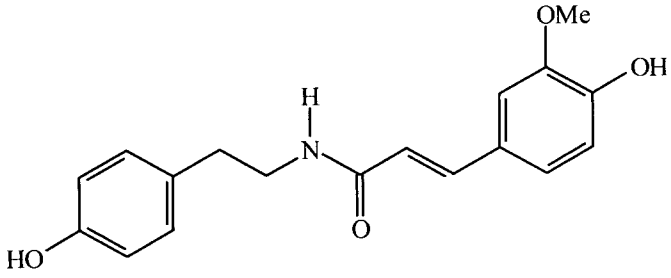
(77)



(78)



(79)

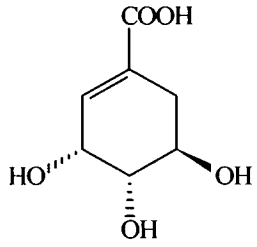


(80)

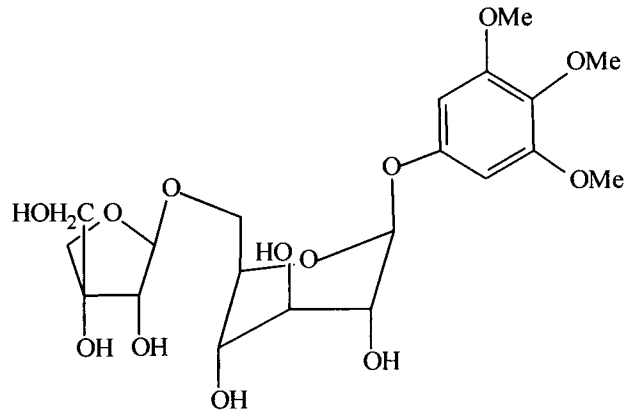
Figure 1.15: Structures of the nitrogen compounds from *Piptostigma*

Table 1.8: Miscellaneous compounds previously isolated from *Piptostigma*

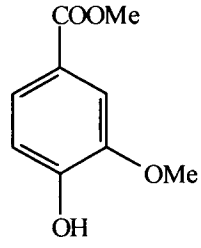
Isolated compounds	Species (references)
Shikimic acid **(81)**	*P. fugax* (Achenbach and Schwinn, 1995)
3,4,5-Trimethoxyphenyl β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside **(82)**	*P. fugax* (Achenbach and Schwinn, 1995)
Vanillic acid methyl ester **(83)**	*P. fugax* (Achenbach and Schwinn, 1995)



(81)



(82)



(83)

Figure 1.16: Structures of the miscellaneous compounds from *Piptostigma*

1.6 The genus *Goniothalamus* Hook. f. & Thoms.

The genus *Goniothalamus* is placed in the sub-family Annonoideae, tribe Unoneae, sub-tribe Xylopiineae (Hutchinson, 1964). It comprises 50 to 114 species ranging from India to Polynesia (Dassanayake and Fosberg, 1985).

Goniothalamus species are small to medium-sized trees; young branches puberulous, glabrous with age; leaves shortly decurrent, often acuminate, coriaceous or firmly membranous, almost glabrous or with scattered blackish bristles, nerves lateral (7-20 pairs); flowers green, yellowish-green or yellowish-brown, with an acid smell, solitary or paired from the leaf axils or fused for a short distance with the axis above the subtending leaf, or terminal on a short, one or 2-leaved branch; sepals 3, valvate in bud, distinct or shortly connate at base; petals 6, in 2 series, valvate in bud, outer petals ovate-lanceolate, spreading, distinct, coriaceous, flat or nearly so, inner petals about one-third or half the length of the outer ones, lozenge-shaped or obovate, distinct and clawed at base, coherent in a vaulted cap over the stamens and carpels; stamens numerous, connective broad and truncate; carpels numerous, ovules 1-2; fruits ovoid, short-stipitate, mostly glabrate, baccate, orange or reddish, indehiscent with 1-2 seeds (Thwaites, 1864; Hooker, 1875; Dassanayake and Fosberg, 1985).

1.6.1 *Goniothalamus thwaitesii* Hook. f. & Thoms.

G. thwaitesii is a tree (6-20m high) widespread in the mid-mountain forests (800 to 1400m alt.) of Sri-Lanka. The young branches are pubescent becoming glabrate with age. The leaves are elliptical or obovate in shape, obtusely acuminate, thinly coriaceous, shiny on the upper surface, glabrous at maturity and with lateral veins (8-12 pairs). The sepals are broadly ovate and persistent. The flowers are bright green in colour, turning yellowish with age. They are solitary, rarely paired. The inner petals are broadly clawed forming an obtuse cone. The fruits are shortly stipitate, one-seeded (Figure 1.17).

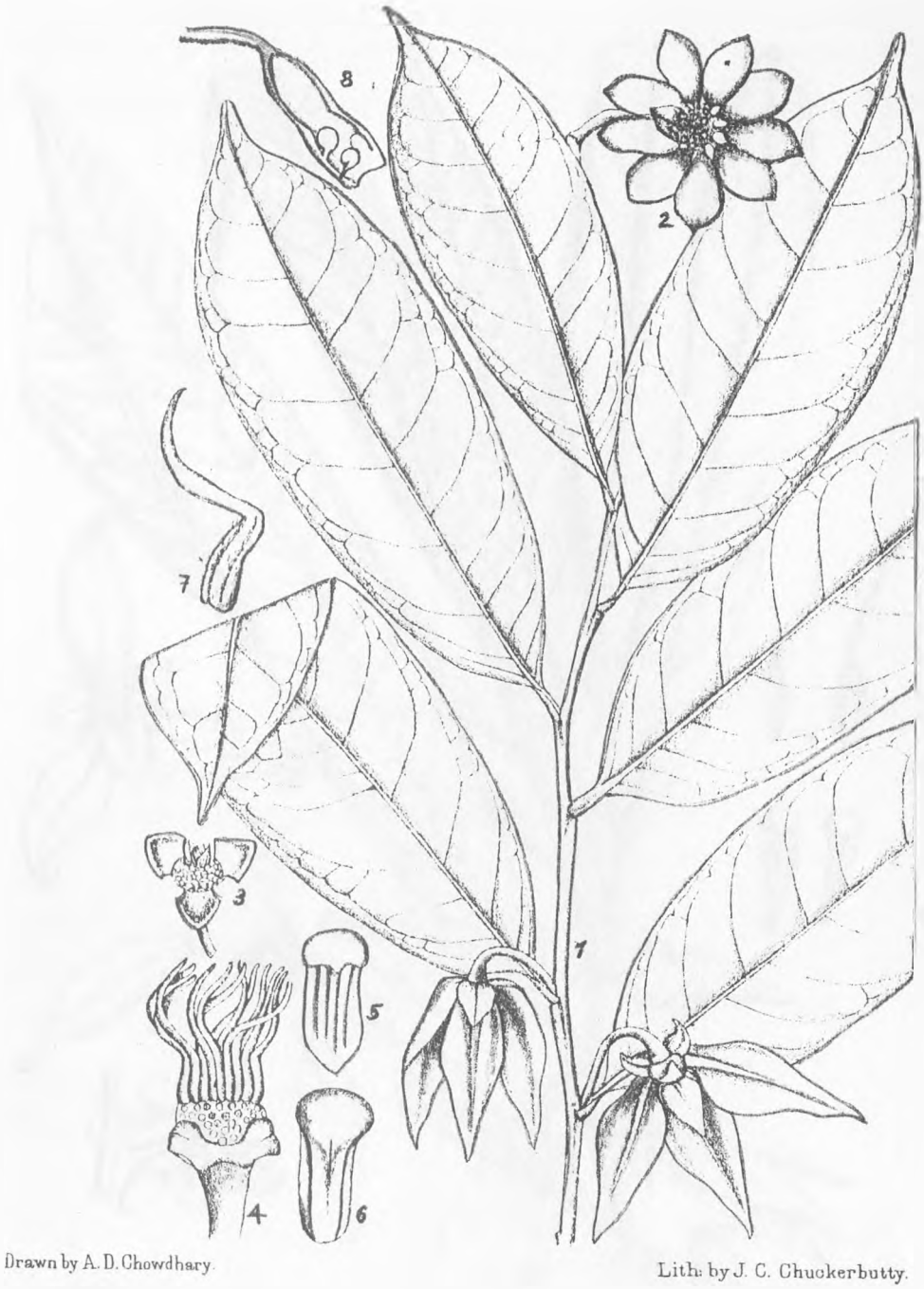
Although there is no apparent use of this species on record, many of the species in the genus *Goniothalamus* are employed throughout Asia for their medicinal value. A decoction, especially of the roots or leaves of *G. macrophyllus, G. scortechinii, G. malayamus, G. tapis* (Malaysia) and *G. sesquipedalis* (Bangladesh, India) alone or as part of a herbal mixture, is given during labour pain and as a post-partum protective medicine. It may also be used to induce abortion in the early months of pregnancy.

The fragrance emitted from the burnt stem bark of *G. dolichocarpus* (Malaysia) and *G. tapis* (Indonesia) or from the burnt leaves of *G. malayanus, G. macrophyllus* (Malaysia) and *G. sesquipedalis* (Bangladesh, India) is an effective mosquito repellent. In Indonesia a root infusion of *G. macrophyllus* is used against typhoid fever. In the Philippines the seeds of *G. amuyon*, cooked with oil, serve as a liniment to treat rheumatism and the fruit is eaten for its stomachic properties. Similarly the seeds extracts is used for the treatment of oedema and rheumatism in Taiwan (Perry, 1980; Talapatra *et al.*, 1985a; Lu *et al.*, 1985; Mat Salleh, 1988; Goh *et al.*, 1995a/b; Hasan *et al.*, 1996).

1.6.2 *Goniothalamus gardneri* Hook. f. & Thoms.

G. gardneri is a shrub (2-5m high) endemic to the rain forests of Sri-Lanka (300 to 900m alt.). The young branches are pubescent becoming glabrous when mature. The leaves are narrowly elliptical or obovate-oblong in shape, acuminate, coriaceous, not shiny on the upper surface, glabrous when mature with lateral nerves (14-20 pairs). The sepals are distinct and persistent. The flowers are green turning yellow in colour, mostly solitary, axillary. The inner petals form an acute cone. The fruits are short, stipitate, orange-yellow in colour, smooth and glabrous, 2-seeded (Figure 1.18).

As for *G. thwaitesii*, no apparent use for this species have been reported (see Section 1.6.1).



1. Flowering branch; **2**. Fruits; **3**. Sepals, carpels and stamens × 1; **4**. Receptacle and carpels; **5, 6**. Stamens; **7**. Carpel; **8**. Carpel, longitudinal section.

Figure 1.17: *Goniothalamus thwaitesii* Hook. f. & Thoms. after King (1893)

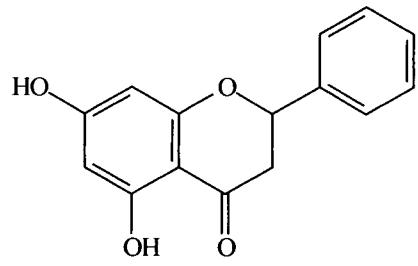
1. Flowering branch; 2. Fruits; 3. Flower, front view;
4. Flower without petals × 1; 5. Stamens; 6. Carpels.

Figure 1.18: *Goniothalamus gardneri* Hook. f. & Thoms.

after King (1893)

1.6.3 Previous phytochemical investigations

Extensive phytochemical studies on the genus *Goniothalamus* have revealed the presence of alkaloids, acetogenins, styryl-lactones and miscellaneous compounds. One flavonoid has also been reported from *G. griffithii* (Talapatra *et al.*, 1985b) (Tables 1.9 to 1.12; Figures 1.19 to 1.23).



(84)

Figure 1.19: Structure of the flavonoid (pinocembrin) from *Goniothalamus*

Table 1.9: Acetogenins previously isolated from *Goniothalamus*

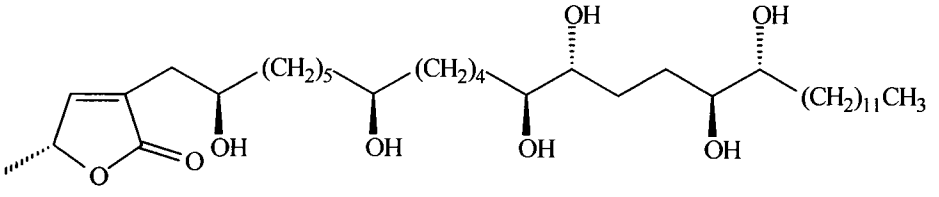
Isolated acetogenins	Species (references)
Donhexocin **(85)**	*G. donnaiensis* (Jiang and Yu, 1997b)
Giganin **(86)**	*G. giganteus* (Fang *et al.*, 1993b)
Murisolin **(87)**	*G. howii* (Zhang *et al.*, 1993)
	G. donnaiensis (Jiang and Yu, 1997a)
Corossolin **(88)**	*G. amuyon* (Li and Chang, 1996)
	G. howii (Zhang *et al.*, 1993)
Longifolicin **(89)**	*G. giganteus* (Alali *et al.*, 1997)
Longicoricin **(90)**	*G. giganteus* (Alali *et al.*, 1997)
4-Deoxyannomontacin **(91)**	*G. giganteus* (Alali *et al.*, 1997)

Table 1.9 (cont.): Acetogenins previously isolated from *Goniothalamus*

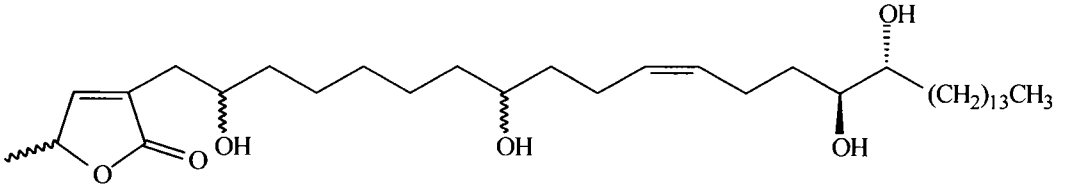
Isolated acetogenins	Species (references)
Donnaienin **(92)**	*G. donnaiensis* (Jiang *et al.*, 1998)
Giganenin **(93)**	*G. giganteus* (Fang *et al.*, 1992b)
Annonacin **(94)**	*G. amuyon* (Li and Chang, 1996)
	G. giganteus (Alkofahi *et al.*, 1988)
	G. *dolichocarpus* (Goh *et al.*, 1995b)
	G. howii (Zhang *et al.*, 1993)
	G. donnaiensis (Jiang and Yu, 1997a)
Goniothalamicin **(95)**	*G. giganteus* (Alkofahi *et al.*, 1988)
	G. howii (Zhang *et al.*, 1993)
	G. donnaiensis (Jiang and Yu, 1997a)
Annomontacin **(96)**	*G. giganteus* (Fang *et al.*, 1992a)
Xylomaticin **(97)**	*G. giganteus* (Alali *et al.*, 1997)
Gonionenin **(98)**	*G. giganteus* (Gu *et al.*, 1994b)
Gigantransenin-A **(99)**	*G. giganteus* (Zeng *et al.*, 1996a)
Gigantransenin-B **(100)**	*G. giganteus* (Zeng *et al.*, 1996a)
Gigantransenin-C **(101)**	*G. giganteus* (Zeng *et al.*, 1996a)
Gigantetrocin-A **(102)**	*G. giganteus* (Fang *et al.*, 1991c)
	G. howii (Yang *et al.*, 1994)
	G. sesquipedalis (Hasan *et al.*, 1996)
4-Acetylgigantetrocin-A **(103)**	*G. giganteus* (Zeng *et al.*, 1996b)
Muricatetrocin-A **(104)**	*G. howii* (Yang *et al.*, 1994)
Gigantetronenin **(105)**	*G. giganteus* (Fang *et al.*, 1992a)
Gigantriocin **(106)**	*G. giganteus* (Fang *et al.*, 1991c)
	G. amuyon (Li and Chang, 1996)
	G. howii (Yang *et al.*, 1994)

Table 1.9 (cont.): Acetogenins previously isolated from *Goniothalamus*

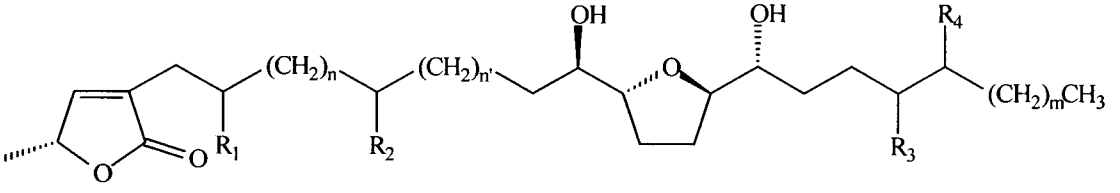
Isolated acetogenins	Species (references)
Cis-gigantrionenin **(107)**	*G. giganteus* (Zeng *et al.*, 1996b)
Gigantrionenin **(108)**	*G. giganteus* (Fang *et al.*, 1992a)
Isoannonacin **(109)**	*G. donnaiensis* (Jiang and Yu, 1997a)
Annomontacinone **(110)**	*G. giganteus* (Alali *et al.*, 1997)
Gigantetrocinone **(111)**	*G. giganteus* (Alali *et al.*, 1997)
	G. donnaiensis (Jiang *et al.*, 1997)
Gigantetroneninone **(112)**	*G. giganteus* (Alali *et al.*, 1997)
Donnaienin-A/34-*epi*-donnaienin-A **(113)**	*G. donnaiensis* (Jiang and Yu, 1997a)
Goniodonin/34-*epi*-goniodonin **(114)**	*G. donnaiensis* (Jiang *et al.*, 1997)
Cis-goniodonin/34-*epi-cis*-goniodonin **(115)**	*G. donnaiensis* (Jiang *et al.*, 1997)
Donnaienin-B/34-*epi*-donnaienin-B **(116)**	*G. donnaiensis* (Jiang and Yu, 1997a)
Asimilobin **(117)**	*G. giganteus* (Zhang *et al.*, 1995)
Goniodenin **(118)**	*G. giganteus* (Zhang *et al.*, 1995)
Gigantecin **(119)**	*G. giganteus* (Alkofahi *et al.*, 1990)
4-Deoxygigantecin **(120)**	*G. giganteus* (Fang *et al.*, 1992b)
Goniocin **(121)**	*G. giganteus* (Gu *et al.*, 1994a)



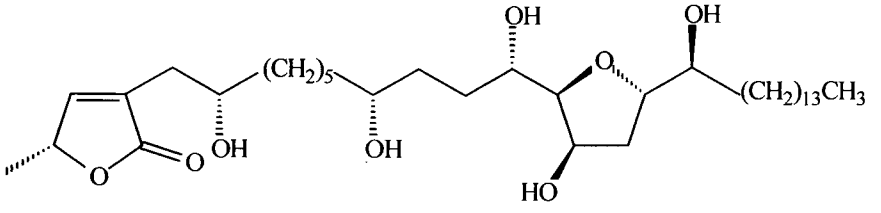
(85)



(86)

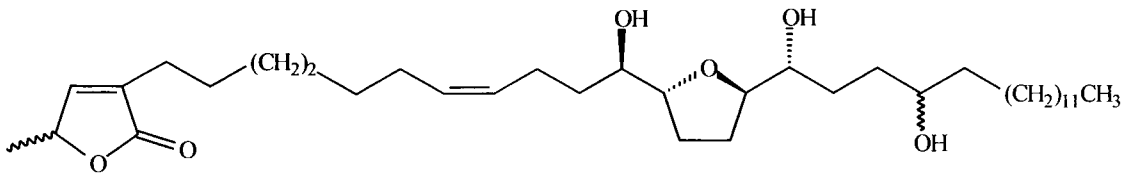


Compound	R_1	R_2	R_3	R_4	n	n'	m
(87)	OH	H	H	H	5	3	7
(88)	H	OH	H	H	5	3	7
(89)	H	OH	H	H	5	1	9
(90)	H	OH	H	H	5	3	9
(91)	H	OH	H	H	5	5	7

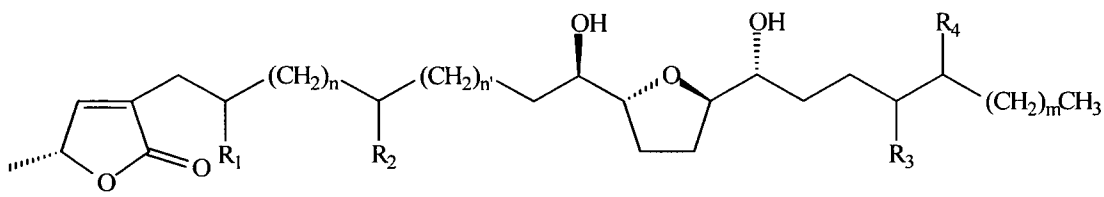


(92)

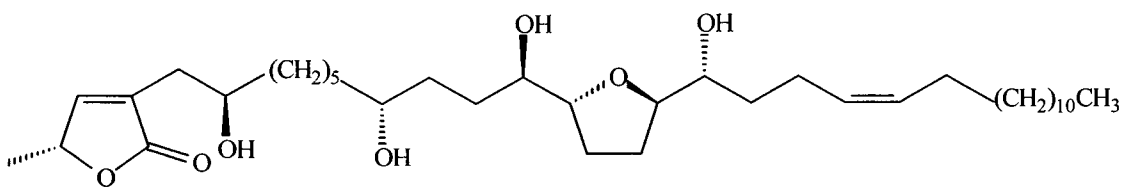
Figure 1.20: Structures of the acetogenins from *Goniothalamus*



(93)

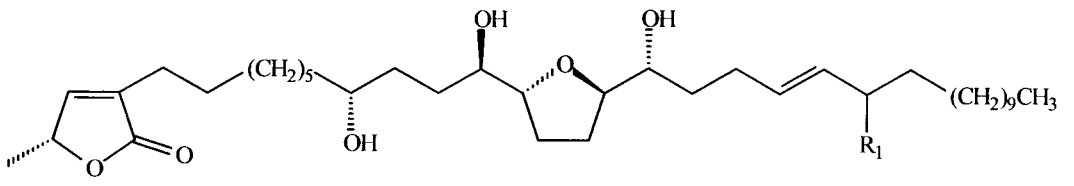


Compound	R_1	R_2	R_3	R_4	n	n'	m
(94)	OH ····· OH		H	H	5	3	7
(95)	OH ····· OH		H	H	5	1	9
(96)	OH	OH	H	H	5	5	7
(97)	OH	OH	H	H	5	3	9

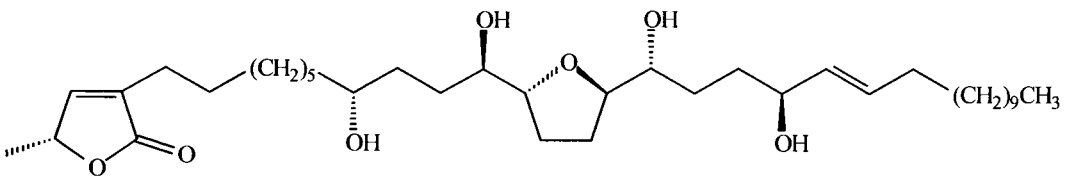


(98)

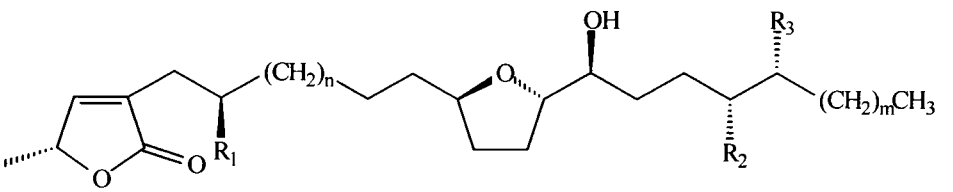
Figure 1.20 (cont.): Structures of the acetogenins from *Goniothalamus*



Compound	R_1
(99)	OH
(100)	OH

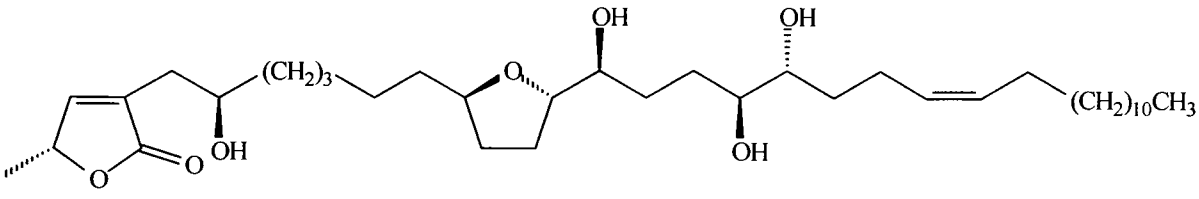


(101)

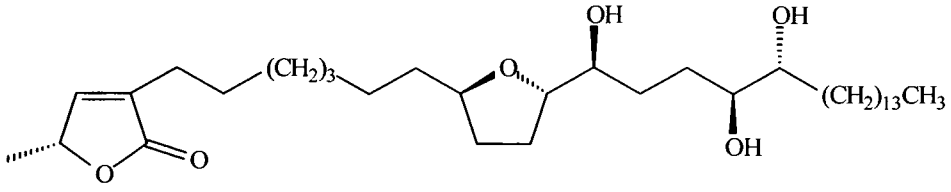


Compound	R_1	R_2	R_3	n	m
(102)	OH	OH	OH	3	13
(103)	OAc	OH	OH	3	13
(104)	OH	OH	OH	5	11

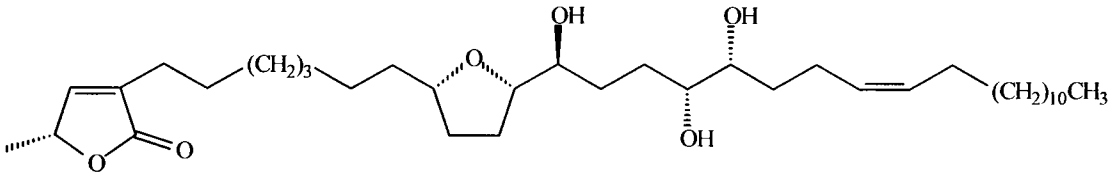
Figure 1.20 (cont.): Structures of the acetogenins from *Goniothalamus*



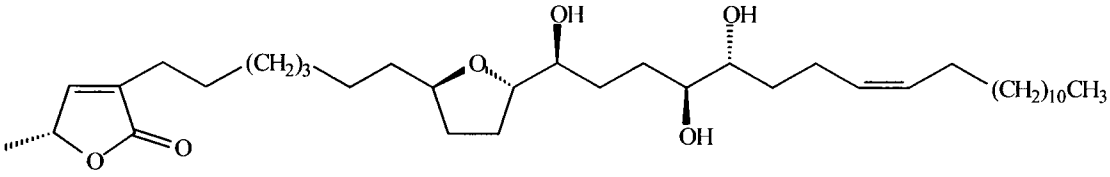
(105)



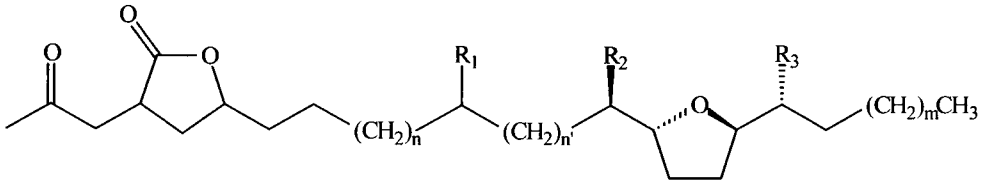
(106)



(107)

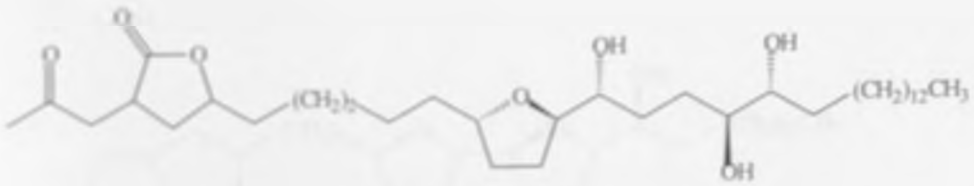


(108)

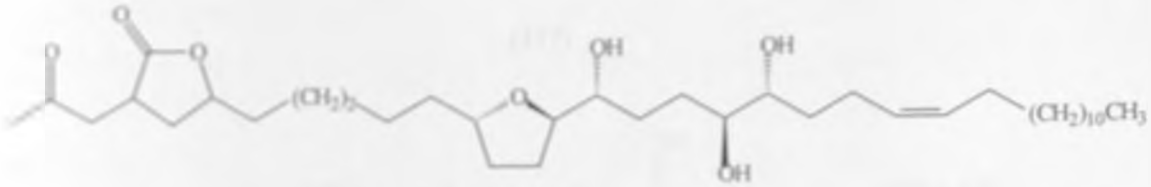


Compound	R_1	R_2	R_3	n	n'	m
(109)	~~~ OH	OH	OH	3	4	10
(110)	····· OH	OH	OH	3	6	11

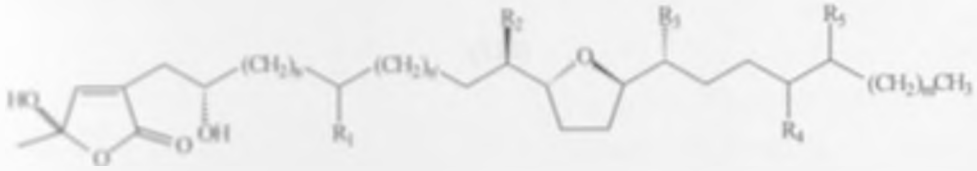
Figure 1.20 (cont.): Structures of the acetogenins from *Goniothalamus*



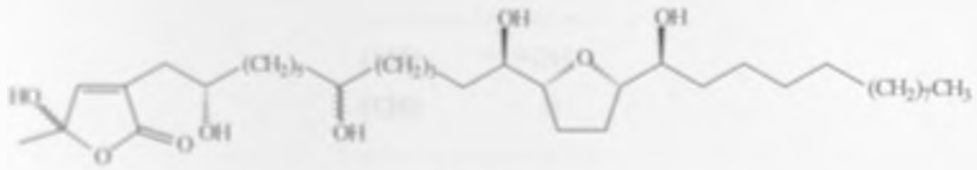
(111)



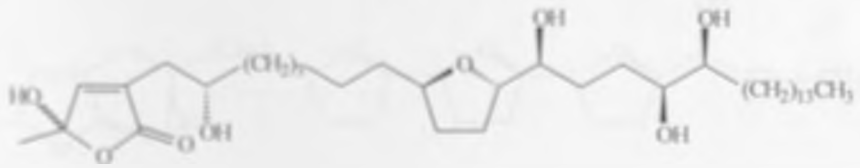
(112)



Compound	R_1	R_2	R_3	R_4	R_5	n	n'	m
(113)	H	OH	OH	H	H	5	3	7
(114)	~~~~ OH	OH	OH	H	H	5	3	7

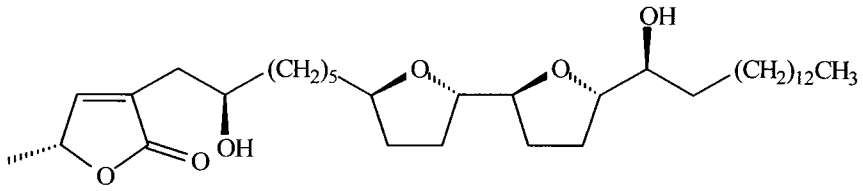


(115)

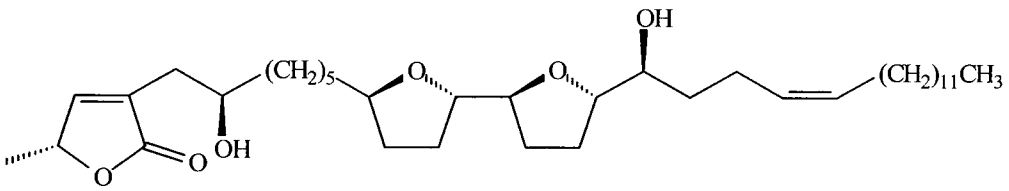


(116)

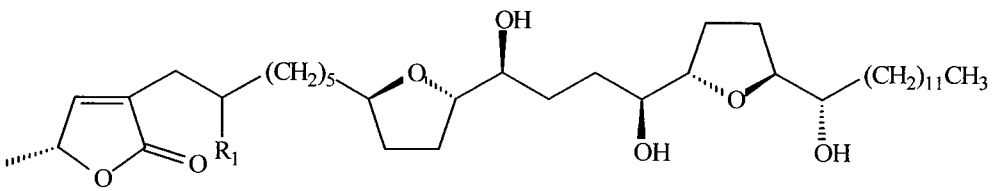
Figure 1.28 (cont.): Structures of the acetogenins from *Goniothalamus*



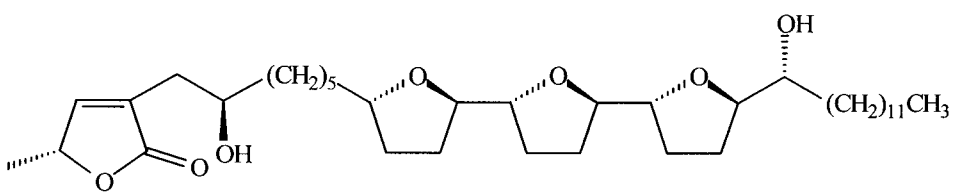
(117)



(118)



Compound	R_1
(119)	OH
(120)	H

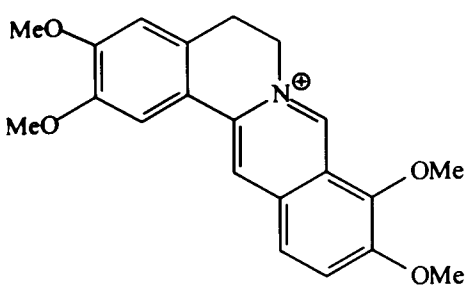


(121)

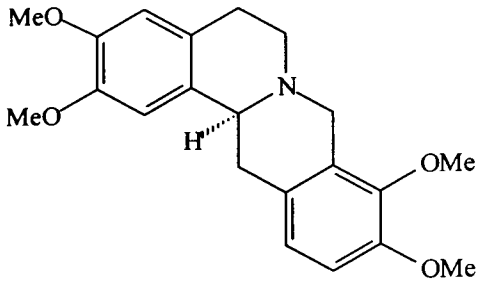
Figure 1.20 (cont.) : Structures of the acetogenins from *Goniothalamus*

Table 1.10: Alkaloids previously isolated from *Goniothalamus*

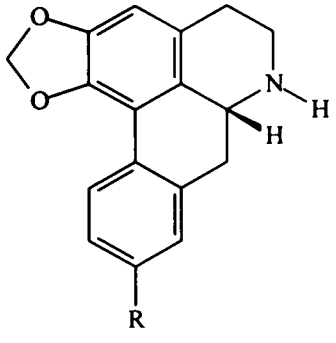
Isolated alkaloids	Species (references)
Palmatine **(122)**	*G. amuyon* (Lu *et al.*, 1985)
(-)-Tetrahydropalmatine **(123)**	*G. amuyon* (Lu *et al.*, 1985)
(-)-Anolobine **(124)**	*G. amuyon* (Lu *et al.*, 1985)
(-)-Anonaine **(125)**	*G. amuyon* (Lu *et al.*, 1985)
Liriodenine **(126)**	*G. amuyon* (Lu *et al.*, 1985)
	G. tapis (Bin Zakaria *et al.*, 1989)
	G. scortechinii (Bin Zakaria *et al.*, 1989)
Oxostephanine **(127)**	*G. tapis* (Bin Zakaria *et al.*, 1989)
	G. scortechinii (Bin Zakaria *et al.*, 1989)
Scorazanone **(128)**	*G. scortechinii* (Din *et al.*, 1990)
Goniopedaline **(129)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)
Aristolactam-AII **(130)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)
N,O-Diacetyl aristolactam-AII **(131)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)
Taliscanine **(132)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)
Velutinam **(133)**	*G. velutinus* (Omar *et al.*, 1992)
Aristolactam-BII **(134)**	*G. velutinus* (Omar *et al.*, 1992)



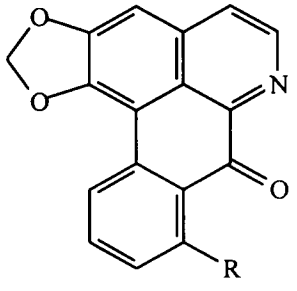
(122)



(123)

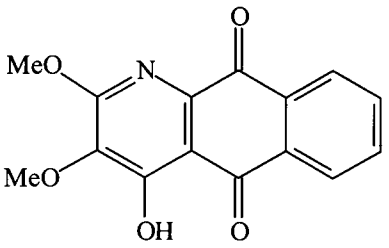


Compound	R
(124)	OH
(125)	H

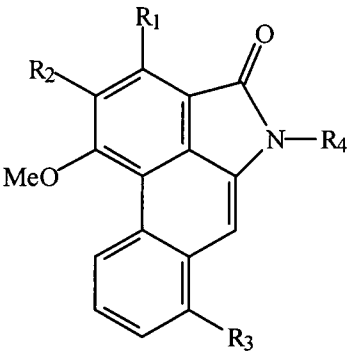


Compound	R
(126)	H
(127)	OMe

Figure 1.21: Structures of the alkaloids from *Goniothalamus*



(128)



Compound	R_1	R_2	R_3	R_4
(129)	OMe	OH	H	H
(130)	H	OH	H	H
(131)	H	OAc	H	Ac
(132)	H	OMe	OMe	H
(133)	H	OMe	OH	H
(134)	H	OMe	H	H

Figure 1.21 (cont.): Structures of the alkaloids from *Goniothalamus*

Table 1.11: Styryl-lactones previously isolated from *Goniothalamus*

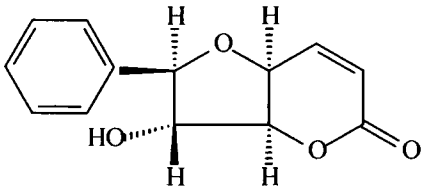
Isolated styryl-lactones	Species (references)
(+)-Altholactone (Goniothalenol) **(135)**	*G. giganteus* (Elzayat *et al.*, 1985)
	G. arvensis (Bermejo *et al.*, 1995)
(+)-Isoaltholactone **(136)**	*G. malayanus* (Colegate *et al.*, 1990)
	G. montanus (Colegate *et al.*, 1990)
	G. tapis (Colegate *et al.*, 1990)
	G. arvensis (Bermejo *et al.*, 1995)
(-)-Goniofupyrone **(137)**	*G. giganteus* (Fang *et al.*, 1991a)
(+)-Goniotharvensin **(138)**	*G. arvensis* (Bermejo *et al.*, 1995)
(-)-Etharvensin **(139)**	*G. arvensis* (Bermejo *et al.*, 1997)
	G. arvensis (Bermejo *et al.*, 1998b)
(+)-Goniofufurone **(140)**	*G. giganteus* (Fang *et al.*, 1990)
	G. arvensis (Bermejo *et al.*, 1998a)
(+)-7-*epi*-Goniofufurone **(141)**	*G. giganteus* (Fang *et al.*, 1991b)
(+)-Goniopypyrone **(142)**	*G. giganteus* (Fang *et al.*, 1990)
(+)-9-Deoxygoniopypyrone **(143)**	*G. giganteus* (Fang *et al.*, 1991b)
(-)-Iso-9-deoxygoniopypyrone **(144)**	*G. dolichocarpus* (Goh *et al.*, 1995b)
(-)-Leiocarpin A **(145)**	*G. leiocarpus* (Mu *et al.*, 1996)
(+)-Goniobutenolide-A **(146)**	*G. giganteus* (Fang *et al.*, 1991a)
(-)-Goniobutenolide-B **(147)**	*G. giganteus* (Fang *et al.*, 1991a)
(+)-Almuheptolide-A **(148)**	*G. arvensis* (Bermejo *et al.*, 1998b)
(+)-Gonioheptolide-A **(149)**	*G. giganteus* (Fang *et al.*, 1993a)
(+)-Gonioheptolide-B **(150)**	*G. giganteus* (Fang *et al.*, 1993a)

Table 1.11 (cont.): Styryl-lactones previously isolated from *Goniothalamus*

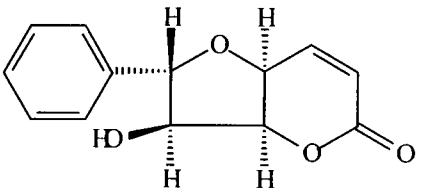
Isolated styryl-lactones	Species (references)
(+)-Goniothalamin **(151)**	*G. tapis* (Azimahtol Hawariah *et al.*, 1994)
	G. tapis (Bin Zakaria *et al.*, 1989)
	G. giganteus (Elzayat *et al.*, 1985)
	G. uvaroides (Ahmad *et al.*, 1991)
	G. macrophyllus (Sam *et al.*, 1987)
	G. macrophyllus (Jewers *et al.*, 1972)
	G. dolichocarpus (Goh *et al.*, 1995b)
	G. andersonii (Jewers *et al.*, 1972)
	G. malayanus (Jewers *et al.*, 1972)
	G. sesquipedalis (Hasan *et al.*, 1995a)
	G. scortechinii (Bin Zakaria *et al.*, 1989)
	G. fulvus (Bin Zakaria *et al.*, 1989)
	G. howii (Zhang *et al.*, 1993)
(-)-5-Acetylgoniothalamin **(152)**	*G. uvaroides* (Ahmad *et al.*, 1991)
(+)-5β-Hydroxygoniothalamin **(153)**	*G. dolichocarpus* (Goh *et al.*, 1995a)
(+)-Goniothalamin oxide **(154)**	*G. macrophyllus* (Sam *et al.*, 1987)
	G. dolichocarpus (Goh *et al.*, 1995b)
(+)-5-Acetylisogoniothalamin oxide **(155)**	*G. sesquipedalis* (Hasan *et al.*, 1994b)

Table 1.11 (cont.): Styryl-lactones previously isolated from *Goniothalamus*

Isolated styryl-lactones	Species(references)
(+)-Goniodiol **(156)**	*G. dolichocarpus* (Goh *et al.*, 1995b)
	G. giganteus (Fang *et al.*, 1991b)
	G. sesquipedalis (Talapatra *et al.*, 1985a)
	G. grifithii (Talapatra *et al.*, 1985a)
(+)-Goniodiol-7-monoacetate **(157)**	*G. sesquipedalis* (Talapatra *et al.*, 1985a)
	G. grifithii (Talapatra *et al.*, 1985a)
	G. amuyon (Wu *et al.*, 1991)
(+)-Goniodiol-8-monoacetate **(158)**	*G. amuyon* (Wu *et al.*, 1992)
(+)-Goniodiol-7,8-diacetate **(159)**	*G. sesquipedalis* (Talapatra *et al.*, 1985a)
	G. grifithii (Talapatra *et al.*, 1985a)
(+)-Goniotriol **(160)**	*G. giganteus* (Alkofahi *et al.*, 1989)
	G. amuyon (Wu *et al.*, 1992)
	G. sesquipedalis (Talapatra *et al.*, 1985a)
(+)-8-Acetylgoniotriol **(161)**	*G. giganteus* (Fang *et al.*, 1990)
(+)-Etharvendiol **(162)**	*G. arvensis* (Bermejo *et al.*, 1998a)
(+)-Garvensintriol **(163)**	*G. arvensis* (Bermejo *et al.*, 1998a)



(135)



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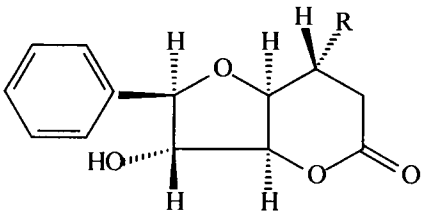
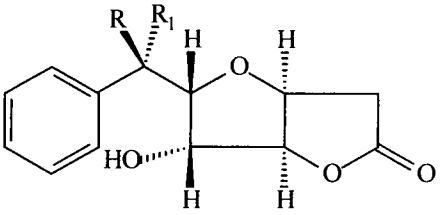
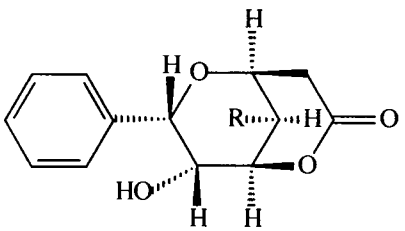


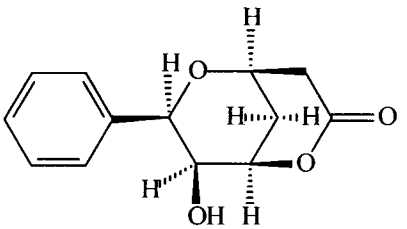
Compound	R
(137)	OH
(138)	H
(139)	OEt



Compound	R	R_1
(140)	H	OH
(141)	OH	H

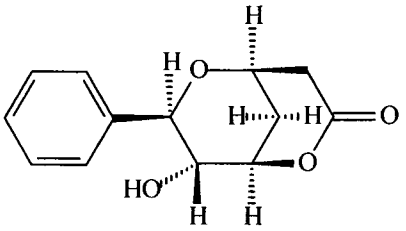


Compound	R
(142)	OH
(143)	H

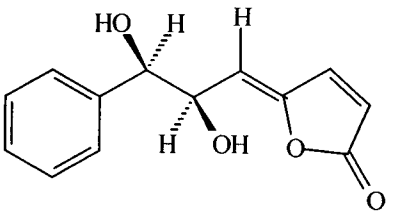


(144)

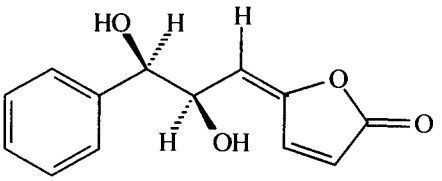
Figure 1.22: Structures of the styryl-lactones from *Goniothalamus*



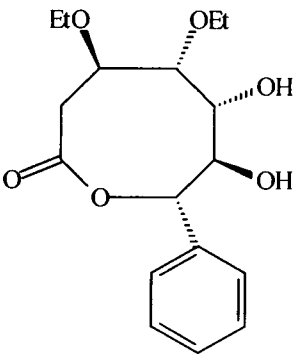
(145)



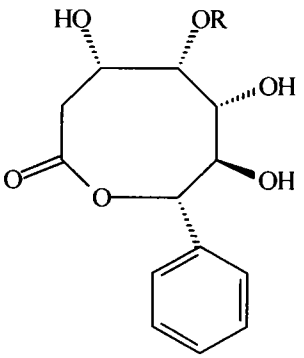
(146)



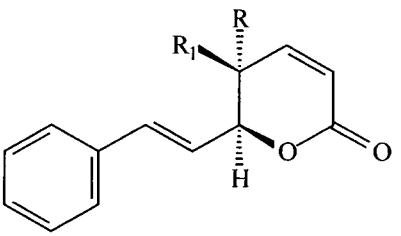
(147)



(148)

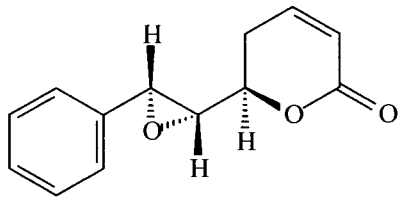


Compound	R
(149)	Me
(150)	Et

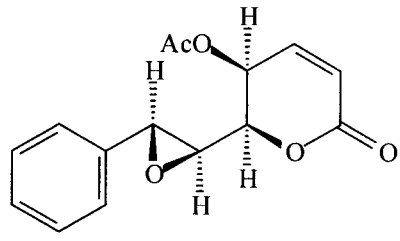


Compound	R	R_1
(151)	H	H
(152)	H	OAc
(153)	H	OH

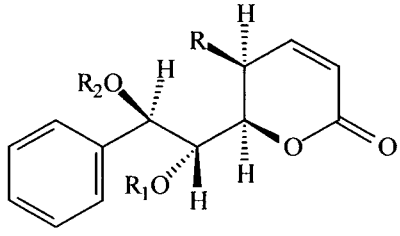
Figure 1.22 (cont.): Structures of the styryl-lactones from *Goniothalamus*

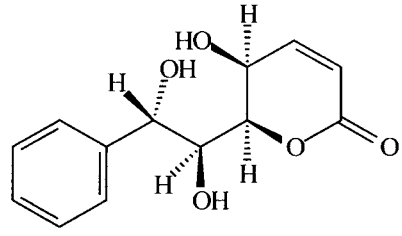


(154)



(155)





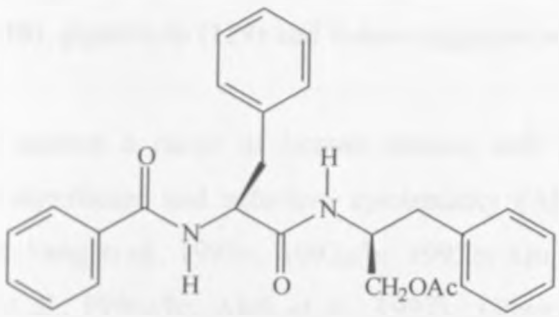
(163)

Compound	R	R_1	R_2
(156)	H	H	H
(157)	H	Ac	H
(158)	H	H	Ac
(159)	H	Ac	Ac
(160)	OH	H	H
(161)	OH	H	Ac
(162)	OEt	H	H

Figure 1.22 (cont.): Structures of the styryl-lactones from *Goniothalamus*

Table 1.12: Miscellaneous compounds previously isolated from *Goniothalamus*

Isolated compounds	Species (references)
Aurantiamide acetate **(164)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)
β-Sitosterol **(45)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)
β-Sitosterol-*O*-β-D-glucoside **(58)**	*G. sesquipedalis* (Talapatra *et al.*, 1988)



(164)

Figure 1.23: Structure of the miscellaneous compound (aurantiamide acetate) from *Goniothalamus*

1.4.4 Previous pharmacological investigations

A number of pharmacological studies have been carried out on *Goniothalamus*, particularly on *G. giganteus*. Bioassay-guided fractionation of an ethanolic extract of the stem bark, using mostly the brine shrimp lethality test, resulted in the isolation of the acetogenins giganin (**86**), 4-deoxyannomontacin (**91**), giganonin (**93**), goniothalamicin (**95**), annomontacin (**96**), gonionenin (**98**), gigantrancinin-A (**99**), B (**100**) and C (**101**), gigantetrocin-A (**102**), 4-acetyl-gigantetrocin-A (**103**), gigantetronenin (**105**), gigantriocin (**106**), *cis*-gigantrionenin (**107**), gigantrionenin (**108**), annomontacinone (**110**), asimilobin (**117**), goniodenin (**118**), gigantecin (**119**) and 4-deoxygigantecin (**120**).

When tested against a range of human tumour cell lines *in vitro*, these compounds showed significant and selective cytotoxicity (Alkofahi *et al.*, 1988; Alkofahi *et al.*, 1990; Fang *et al.*, 1991c; 1992a/b; 1993b; Gu *et al.*, 1994b; Zhang *et al.*, 1995; Zeng *et al.*, 1996a/b; Alali *et al.*, 1997). Other acetogenins isolated were 4-deoxyannomontacin (**91**), annonacin (**94**), goniothalamicin (**95**) and annomontacinone (**110**), which exhibited insecticidal properties against blowfly and/or mosquito larvae (Alali *et al.*, 1997; Alkofahi *et al.*, 1988).

Another group of active compounds from *G. giganteus*, which showed significant and selective cytotoxicity, were the styryl-lactones 7-*epi*-goniofufurone (**141**), goniopypyrone (**142**), 9-deoxygoniopypyrone (**143**) and goniodiol (**156**) (Fang *et al.*, 1990, 1991b).

Similarly, investigation of the stem bark of *G. dolichocarpus* afforded the styryl-lactones goniothalamin (**151**), goniothalamin oxide (**154**), iso-9-deoxygoniopypyrone (**144**), goniodiol (**156**) and the acetogenin annonacin (**94**), which were cytotoxic to mosquito larvae (Goh *et al.*, 1995b).

From the roots of *G. donnaiensis*, the acetogenins donhexocin (**85**), donnaienin (**92**), donnaienin-A/34-*epi*-donnaienin-A (**113**), goniodonin/34-*epi*-

goniodonin **(114)**, *cis*-goniodonin/34-*epi*-*cis*-goniodonin **(115)** and donnaienin-B/ 34-*epi*-donnaienin-B **(116)** showed potent cytotoxicity against human tumour cell lines (Jiang and Yu, 1997a/b; Jiang *et al.,* 1997/1998).

The styryl-lactones goniodiol-7-monoacetate **(157)** and goniodiol-8-monoacetate **(158)**, from the leaves, and the acetogenins corossolin **(88),** annonacin **(94)** and gigantriocin **(106)**, from the seeds of *G. amuyon*, exhibited cytotoxicity against selected human tumour cell lines in culture (Wu *et al.*, 1991/1992; Li and Chang, 1996).

Investigation of the roots of *G. macrophyllus* led to the isolation of the stryryl-lactones goniothalamin **(151)** and goniothalamin oxide **(154)** as teratogenic and embryotoxic compounds in mice (Sam *et al.,* 1987). Similarly, investigation of the stem bark, leaves and roots of *G. tapis* gave the styryl-lactones goniothalamin **(151)**, which showed significant abortifacient effects in mice (Azimahtol Hawariah *et al.,* 1994).

Studies on the stem bark of *G. sesquipedalis* led to the isolation of the styryl-lactones 5-acetylisogoniothalamin oxide **(155)**, which demonstrated strong *in vitro* antibacterial activity against a selected range of Gram $\pm$ bacteria (Hasan *et al.*, 1993). Likewise, the lactam alkaloids velutinam **(133)** and aristolactam BII **(134)**, isolated from the stem bark of *G. velutinus*, showed selective *in vitro* antibacterial activity against Gram + bacteria. They also showed significant cytotoxicity against human cervical carcinoma and against some murine leukemia cell lines (Omar *et al.,* 1992).

1.7 Pharmacological screenings

Among the five species described in this study, two had previously been identified as active in screens for their potential anti-inflammatory activity using bradykinin receptor affinity studies.

1.7.1 General considerations

A variety of natural products have been shown to interfere with different stages of the inflammation process. These substances are of interest as potential leads in the development of new anti-inflammatory drugs for a whole range of inflammatory-related diseases which lack satisfactory treatments (Duwiejua and Zeitlin, 1993). In this study, the action of kinins, represented by bradykinin, an important peptide mediator implicated in the pathogenesis of inflammation, was targeted with the search for bradykinin antagonists from plant sources.

1.7.1.1 Bradykinin

a) Metabolism

Bradykinin is a nonapeptide, which is generated as a result of the activity of kallikreins, proteolytic enzymes in the plasma and tissues, on kininogens. Kallikreins are the activated form of the inactive precursors prekallikreins. The conversion of prekallikreins into kallikreins is induced by the activated Hageman factor.

In response to tissue trauma (injuries, invasion of micro-organisms), the vasoactive amines released induce an increase in vascular permeability. As a result the Hageman factor leaks out of the vessels and is activated in contact with negatively charged surfaces (collagen, bacterial lipopolysaccharides). This triggers the formation of bradykinin. Bradykinin and other kinins are inactivated by several kininases and peptidases which cleave the sequence of amino-acids in different sites on the peptides (Figure 1.24) (Regoli and Barabé, 1980; Ward, 1991).

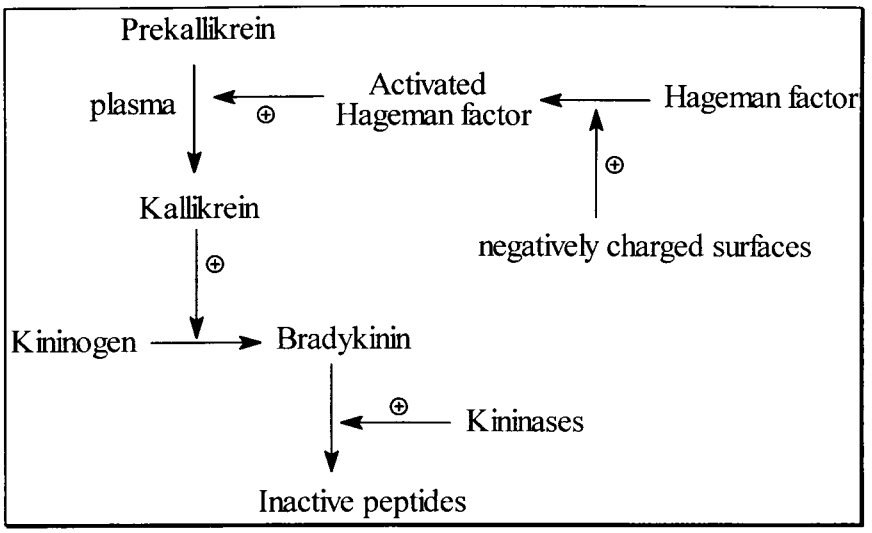


Figure 1.24: The formation and inactivation of bradykinin

b) Bradykinin receptors

The effects of bradykinin and other kinins involve the activation of specific cell surface receptors, which have been characterised by binding studies and pharmacological experiments. At least two subtypes, B_1 and B_2, are currently recognised. The B_2 receptors, predominantly encountered in mammalian systems, mediate most of the effects of bradykinin (Regoli and Barabé, 1980; Farmer and Burch, 1991; Burch and Kyle, 1992).

c) Pharmacological actions

The activation of kinin-receptors leads to a wide range of pharmacological effects on several organ systems. Bradykinin elicits slow sustained contraction of bronchial, uterine and intestinal smooth muscle. It increases the vascular permeability and produces a potent arteriolar vasodilatation. In some cases, this action is mediated by stimulating the formation of vasodilating prostaglandins. Bradykinin causes pain by stimulating nociceptive nerve terminals. Moreover, bradykinin activates ion transport and fluid secretion by various epithelia.

Bradykinin is implicated in a number of pathological conditions characterised by inflammation. These include arthritis, pancreatitis, asthma, rhinitis, inflammatory bowel disease and allergic reactions. Its potent bronchoconstrictor effect is related to

the pathogenesis of airways inflammation. Its algesic effect is involved in some pain conditions including anginal pain, pain following tissue anoxia, pain associated with inflammation and rheumatoid diseases. Its vasodilating properties, leading to hypotension, are implicated in shock syndromes (Regoli and Barabé, 1980; Farmer, 1991; Pongracic *et al.*, 1991; Steranka and Burch, 1991; Gavras and Gavras, 1991).

1.7.1.2 Bradykinin antagonists

Because of bradykinin's potent pharmacological actions, the availability of compounds that block its effects may be of therapeutical utility. Various substances, synthesised or isolated from natural sources, have been shown to antagonise *in vitro* and/or *in vivo* the activity of BK on different tissues.

a) Synthetic products

A whole range of peptide analogues, synthesised by modifying the amino-acid sequence of bradykinin, have been characterised as specific B_2 antagonists (Stewart and Vavrek, 1991). Further improvement in their structures led to the development of new peptide analogues metabolically stable (Lembeck *et al.*, 1991; Hock *et al.*, 1991; Wirth *et al.*, 1991). Bradykinin receptor antagonists of non-peptide nature have also been developed (Calixto *et al.*, 1991). Recent structures synthesised include selective and potent B_2 antagonists (Pruneau *et al.*, 1998).

b) Natural products

Several compounds from natural sources have been screened for BK antagonism. The activities observed were functional antagonisms of the effects induced by BK and have not been related directly to an interaction with the receptors. Natural products with selective anti-BK activity include glycoside steroids from *Mandevilla velutina* (Apocynaceae) (Calixto *et al.*, 1991). Other compounds showing anti-BK activity but with a limited selectivity include flavonoids from *Scutellaria baicalensis* (Labiateae) (Yun-Choi *et al.*, 1992), biflavonoids from *Gingko biloba* (Gingkoaceae) and *Cupressus tarubosa* (Cupressaceae), flavone and flavonol glycosides (vitexin, apiin, astilbin), flavonols (quercetin, rhamnetin), dihydroflavones (hesperitin,

homoeriodictyol), coumarins (khellin, esculin), a saponin (aescin) and an aminoglycoside (streptomycin) (Calixto *et al.*, 1991). Finally, a glycoprotein from *Aloe arborescens* var. *natalensis* (Liliaceae) exhibited anti-BK activity due to its proteolytic action (*i.e* degradation of bradykinin) (Yagi *et al.*, 1986).

1.7.2 Bradykinin receptor affinity studies

1.7.2.1 Aim and principle of the assay

The purpose of BK receptor affinity tests is to screen for substances with the capacity to bind to specific BK receptors. This is studied on the basis of a competition between a radiolabelled ligand and a tested substance for binding to the receptors. In this study an *in-vitro* radioligand binding assay was used to test the affinity of plant extracts/fractions and isolated compounds to BK receptors of the B_2 subtype. The aim was to identify compounds worthy of further pharmacological investigations for the development of a non-peptide antagonist of natural origin.

The technique consisted of incubating B_2 receptor-bearing membranes from guinea-pig ileal tissues with radiolabelled BK, first in the presence of an unlabelled ligand to measure the non-specific binding, then in the presence of a plant extract/fraction or isolated compound. This was followed by the separation of free and receptor-bound radiolabelled BK by filtration and measurement of bound radiolabelled BK retained on the filter paper by radioactivity counting.

If a component in the plant extract exhibited affinity for the receptors, the displaced radiolabelled BK passed through the filters and this resulted in a decrease in the amount of remaining bound radiolabelled BK, accounting for low values of specific binding.

1.7.2.2 Bioassay-guided fractionation

Bioassay-guided fractionation was used to isolate pure active components from the plant extracts, by monitoring systematically their presence using the bradykinin assay. For a crude extract showing activity, fractionation led to a number of fractions which were in turn tested for activity. Further purification of active fractions was carried out until pure active compounds were isolated.

1.8 Hypothesis and objectives

Plants are a rich source of a wide range of compounds which are interesting chemically and which may be of potential medicinal value (see Section 1.1). In the search for therapeutic agents from plants, chemical investigation of the family Annonaceae has already yielded many novel lead compounds exhibiting different types of bioactivity (see Sections 1.4.4 & 1.6.4). However, many annonaceous species remain to be investigated for their chemical and biological potential. Targeting research on anti-inflammatory drugs from natural sources, a number of active compounds have already been isolated from plant extracts (see Section 1.7). Therefore, it is possible that:

i) among the range of metabolites produced by annonaceous species, some compounds may be leads to novel drugs in the treatment of inflammatory diseases.

ii) the phytochemical investigation of unexplored annonaceous species may lead to the isolation of constituents of chemical diversity and of possibly new structure which may also exhibit specific types of bioactivity.

Thus, the objectives of the study were:

i) to carry out bioassay-guided fractionation on the two selected crude extracts exhibiting activity in the bradykinin assay to identify bioactive compounds.

ii) to isolate and identify a high number of compounds from non-active fractions of the two species tested, as well as investigate thoroughly three unexplored species of Annonaceae to provide an update review on the chemistry of the family and build up a "library" of compounds.

2.1 Plant materials

The species investigated were selected by Prof. P. G. Waterman from his personal collection and the Strathclyde Institute for Drug Research (SIDR) collection. Plant parts were either collected in their natural habitat or obtained directly from a commercial supplier. They were attributed code numbers (Table 2.1) and voucher specimens were deposited (Table 2.2).

Table 2.1: Plant parts, sources and code numbers of the species investigated

Species	Plant parts	Sources	Code numbers
Cleistopholis glauca	SB	Korup National Park, Cameroon	SIDR 763
Cleistopholis patens	L	Korup National Park, Cameroon	SIDR 728
Piptostigma fasciculata	L	Tarkwa road, Dompin, Ghana	Ann 26
Goniothalamus thwaitesii	AP	Plantation of Lalani Botanicals, Sri-Lanka	SIDR 738
Goniothalamus gardneri	AP	Plantation of Lalani Botanicals, Sri-Lanka	SIDR 737

SB = Stem bark, L = Leaves, AP = Aerial parts

Table 2.2: Voucher details of the species investigated

Species	Voucher details
Cleistopholis glauca	Herbarium of the Missouri Botanic Gardens, (Thomas 2561)
Cleistopholis patens	National Herbarium of Cameroon, (n° 3938) (Figure 2.1)
Piptostigma fasciculata	Herbarium of the Botanic Garden, Edinburgh, (A. Enti FE-1298)
Goniothalamus thwaitesii	Held by Lalani Botanicals
Goniothalamus gardneri	Held by Lalani Botanicals

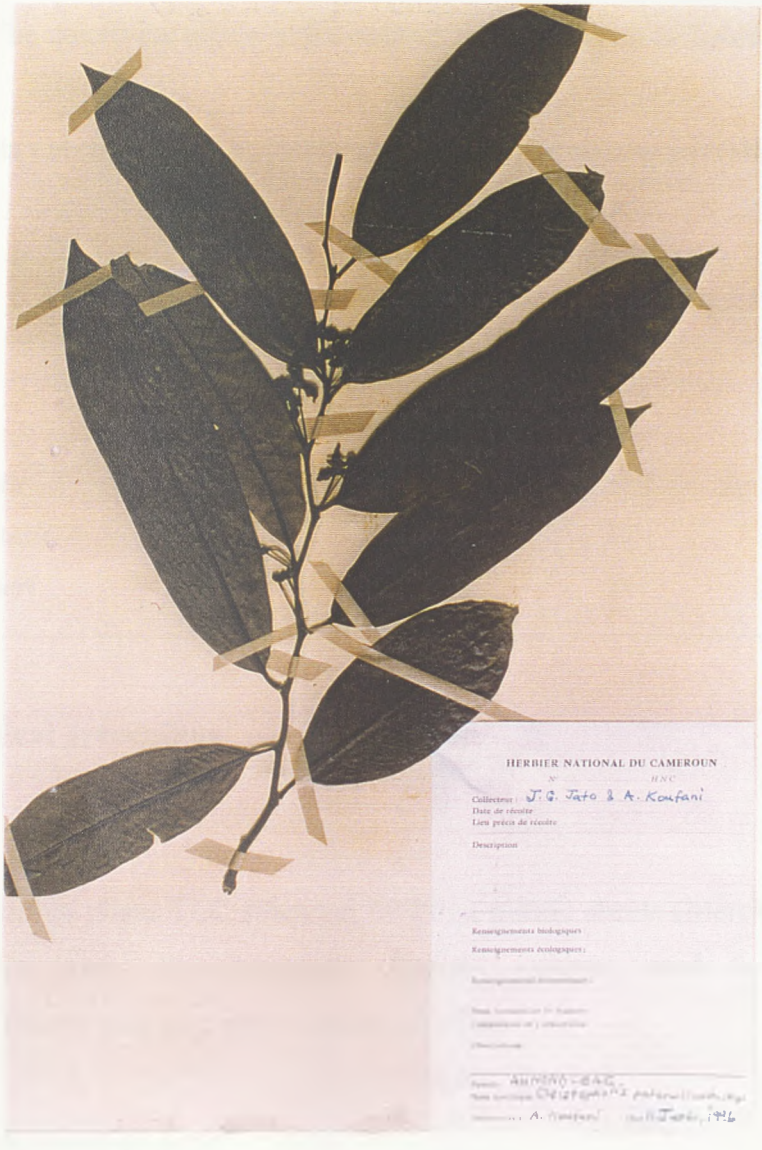


Figure 2.1: Voucher specimen of *Cleistopholis patens* (Benth.) Engl. & Diels (collected in 1996, deposited in the National Herbarium of Cameroon)

2.2 Extraction techniques

Dried plant parts were ground into coarse powders, using a hammer mill, and then extracted sequentially with solvents of increasing polarity, typically petrol or *n*-hexane, followed by EtOAc and MeOH, using a large Soxhlet apparatus. The subsequent extracts were filtered and concentrated to dryness *in vacuo*, using a rotary evaporator. The quantities obtained for each extract are shown in Table 2.3.

Table 2.3: Amounts extracted and extract quantities of the species investigated

Species	Amounts extracted (g)	Extract quantities (g)		
		petrol/*n*-hexane	EtOAc	MeOH
Cleistopholis glauca	500	11/	30	40
Cleistopholis patens	250	/9	25	40
Piptostigma fasciculata	440	/8	9	25
Goniothalamus thwaitesii	495	/9.5	13	28
Goniothalamus gardneri	510	12/	21	25

2.3 Phytochemical screenings

2.3.1 General

Pre-coated normal phase TLC silica gel 60 PF_{254} plastic sheets (Merck 5735) were used for thin layer chromatography. Solvent systems used for TLC (see Page xxi) were freshly prepared with distilled solvents.

2.3.2 Thin layer chromatography

The initial screenings of the crude extracts (*i.e* examination of chemical profiles) were carried out by thin layer chromatography. Each extract was spotted on TLC plates, which were placed in tanks saturated with a suitable mobile phase, to allow the best migration and separation of the compounds. Various solvent systems were employed to separate non-polar, semi-polar and polar compounds. In some

cases, multiple development was used to improve separation. The plates were initially visualised under a UV light and then revealed chemically by spray reagents as coloured spots.

TLC was a useful tool to assess the complexity of mixtures, to optimise the conditions of the separation (*i.e* determine the most appropriate solvent system) and to check the purity of pure compounds.

2.4 Separation and detection of compounds

A range of chromatographic techniques were employed to fractionate the crude extracts and to separate mixtures in the subsequent fractions until a pure compound was obtained. Crystallisation was another alternative, usually used as a final purification step for compounds present in less complex mixtures.

2.4.1 General

2.4.1.1 Solvents and stationary phases

All solvent systems used for chromatography were freshly prepared with distilled solvents. Silica gel 60 H (*Merck* 7736) and silica gel 60 (70-230 mesh) (Merck 7734) were respectively used as stationary phases for vacuum liquid chromatography and open column chromatography. The latter was also employed to obtain samples in a powdered form. *KP-Sil*® silica (32-63 μm) in pre-packed polyethylene cartridges was used for flash chromatography with the *Flash 40*® system. Pressurisation was obtained with nitrogen (*ca.* 0.5 bar) for an average flow rate of 25 ml/min. *Sephadex*® LH-20-100 (Sigma) was used for gel filtration.

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® *Sephadex* is a registered trademark of Pharmacia, Inc.

2.4.1.2 Spray reagents

Vanillin-H_2SO_4 was prepared by dissolving 1g of vanillin in 80ml of ethanol and 20ml of concentrated sulphuric acid. Chromatograms were heated at 120° until maximum coloration was attained.

Anisaldehyde-H_2SO_4 was prepared by mixing 0.5ml of anisaldehyde in 10ml of glacial acetic acid, 85ml of MeOH and 5ml of concentrated sulphuric acid. Chromatograms were heated at 100-105° until maximum coloration was attained.

Dragendorff's reagent (acc. to Munier and Macheboeuf) was prepared by mixing equal parts of a solution A and a solution B (10ml of each) to 20ml of glacial acetic acid and 100ml of water. Solution A was made up of a mixture of 2.125g of bismuth subnitrate, 25ml of glacial acetic acid and 100ml of water. Solution B was made up by dissolving 40g of potassium iodide in 100ml of water (Merck, 1980).

2.4.2 Separation techniques

2.4.2.1 Partition

This allowed the separation of compounds according to their partition coefficients in two immiscible liquid phases of varying polarities. The phases were shaken in a separating funnel, left to settle by gravity, and then collected separately and reduced to dryness *in vacuo*. The subsequent fractions obtained were screened by TLC and subjected to further purification.

Partition proved to be an efficient method systematically used for the direct fractionation of polar crude extracts. MeOH extracts were re-dissolved in water and shaken first with EtOAc. The EtOAc fraction was collected and the aqueous layer shaken with *n*-BuOH. The *n*-BuOH fraction was collected and all aqueous phases recombined to afford three fractions of different polarities.

2.4.2.2 Vacuum liquid chromatography

VLC was used as described by Pelletier *et al.* (1986) and Coll and Bowden (1986). A sintered-glass column was loaded with silica. As the vacuum was applied, the silica was compressed to a hard layer (*ca.* 6-8cm deep), leaving enough space at the top of the column for the eluent. The packed column was washed with a solvent to check its uniformity and allowed to dry completely before the vacuum was released.

Dried crude extracts or fractions, re-dissolved in a suitable solvent, were mixed with a sufficient amount of silica and concentrated to dryness to afford powdered samples. These were placed as a thin layer on the top of the column, covered with cotton wool. As the vacuum was applied, the elution was performed isocratic or stepwise with solvent mixtures of increasing polarity. In the case of crude extracts, the elution started with petrol or *n*-hexane and was followed with increasing amounts of EtOAc and then MeOH, up to 20% MeOH in EtOAc. The fractions collected were concentrated *in vacuo* and their constituents screened by TLC to allow further re-combination.

VLC proved to be a very efficient technique systematically used for the direct fractionation of non polar and semi-polar crude extracts.

2.4.2.3 Open column chromatography

A slurry of silica in the required mobile phase was loaded into an open glass column with a sintered glass base or plugged with a cotton wool. Excess solvent was allowed to flow out and the packed column left to settle undisturbed. The samples were applied as concentrated solutions to the top of the column and the elution was carried out under gravity, isocratic or gradient with solvents of increasing polarity. Eluates collected were screened by TLC and bulked as appropriate (Salituro and Dufresne, 1998).

Direct or successive open column chromatography was extensively used to purify fractions.

2.4.2.4 Flash chromatography

The samples dissolved in a suitable mobile phase were directly injected as concentrated solutions onto the head of a dry silica cartridge. The elution was carried out under pressure, isocratic with solvent systems optimised for the best separation. Eluates collected were screened by TLC and bulked as appropriate.

The technique used was fast, safe and efficient for purifying fractions which could not be separated by open column chromatography.

2.4.2.5 Gel filtration

Gel filtration or size exclusion chromatography was carried out for the separation of compounds according to their molecular sizes (*i.e* larger molecules eluting first), eluting with organic solvent systems and using a hydrophilic/lipophilic dextran gel as a stationary phase (Joustra *et al.*, 1967). Dried beads of *Sephadex*® were allowed to swell in a chosen solvent for a few hours, then poured as an homogeneous slurry into an open glass column with a sintered glass base or plugged with cotton wool. Excess solvent was allowed to flow out and the packed column left to settle undisturbed.

The samples, re-dissolved as a very concentrated solution in the same chosen solvent, were applied gently and uniformly to the surface of the column. Elution was carried out isocratic or with solvents of increasing polarity. Different solvent systems were used, depending on the polarity of the samples:

i) non polar samples were eluted starting with $CHCl_3$ or 5% petrol or *n*-hexane in $CHCl_3$ followed by straight $CHCl_3$.

ii) semi-polar samples were eluted starting with $CHCl_3$ followed by 5, 10% and 20% MeOH in $CHCl_3$.

iii) polar samples were eluted starting with 10%, 30%, 50% MeOH in $CHCl_3$ followed by straight MeOH or starting with acetone followed by 5, 10 and 20% EtOH in acetone.

The fractions collected were screened by TLC and later bulked as appropriate.

Gel filtration proved to be a rapid technique, mainly used to purify fractions and sometimes afford compounds pure enough to be identified by spectroscopic methods. It was also extensively used for the removal of plant pigments from leaves and aerial parts extracts.

2.4.2.6 Crystallisation

A few compounds, usually present in non complex mixtures, were purified directly by crystallisation. A solvent was added to the sample, placed in a conical flask, to give a saturated solution. With a suitable solvent, dissolution occurred on warming up and the solution was left undisturbed at room temperature for a few hours. On cooling down, pure compounds formed crystals at the bottom of the flask, which were separated by pipetting from the supernatant containing impurities.

Another technique used involved adding a suitable solvent in a minimum quantity to dissolve the sample and then adding dropwise another solvent, until cloudiness developed in the solution. The saturated solution was left undisturbed and eventually formed crystals on cooling down.

Crystallisation was accelerated when required by scraping the edge of the flask with a glass rod, or cooling down the temperature even more by placing the sample in the fridge.

2.4.3 Detection

2.4.3.1 UV detection

The visualisation of compounds on the TLC plates was initially achieved by viewing chromatograms under short and long-wave UV light, respectively to mark the presence of quenching or fluorescent spots. This was followed by chemical detection.

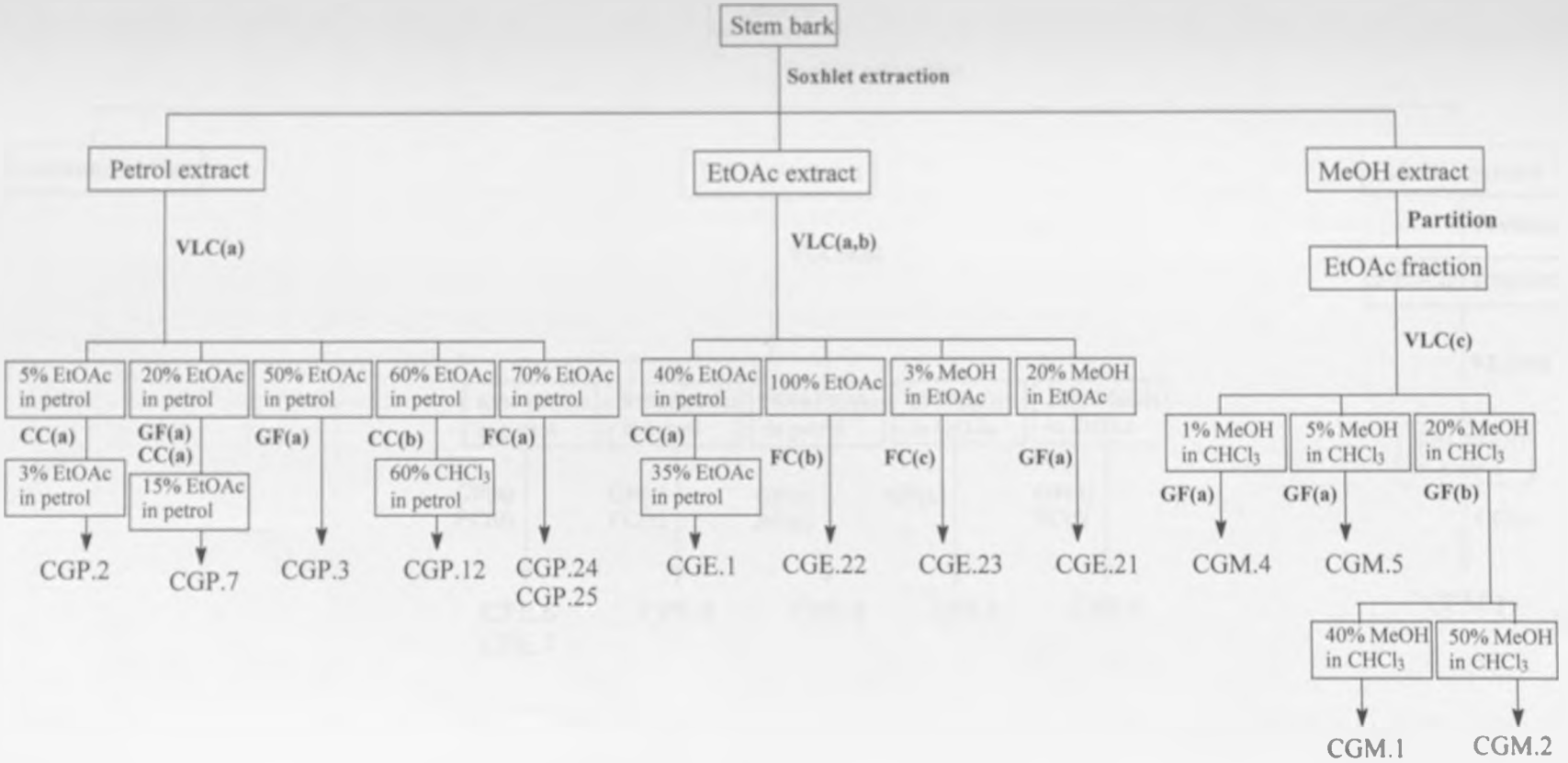
2.4.3.2 Chemical detection

Chemical detection was achieved by spraying the plates with vanillin- or anisaldehyde-H_2SO_4 reagents, followed by heating in an oven for 3 to 5 minutes until coloration appeared. This enabled a general detection of many compounds, especially terpenes (pink and purple spots), sugars (yellow-greenish-brown spots) and phenols (red, orange or yellow spots).

Dragendorff's reagent was employed for the detection of alkaloidal compounds which displayed an orange colour. Chromatograms did not require to be heated.

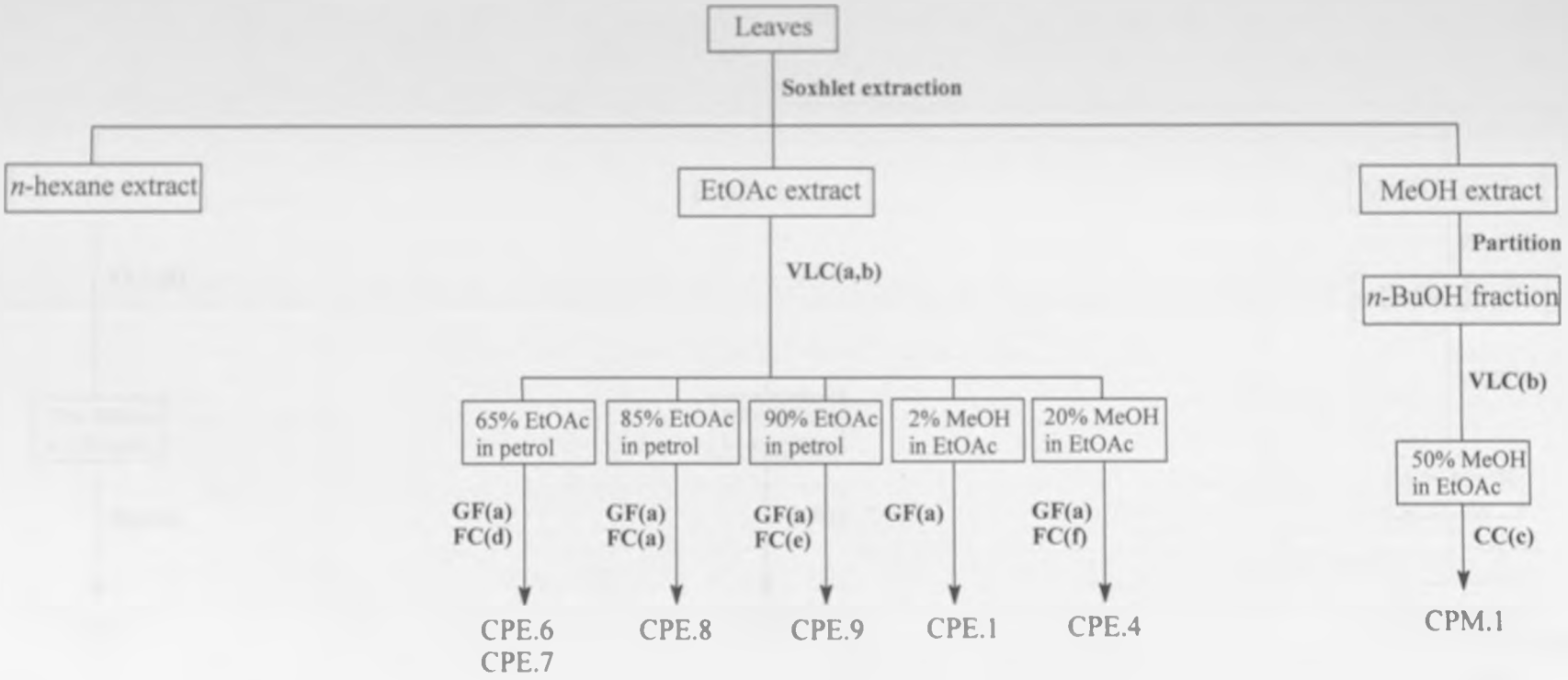
2.4.4 Compounds isolated from the species studied

The procedures adopted for the isolation of compounds from the species studied are shown in Schemes 2.1 to 2.5. Pure compounds were coded according to their sources (first initials of genus, species and polarities of extracts) and their order of discovery. Amounts obtained and TLC characteristics are given in Tables 2.4 to 2.8.



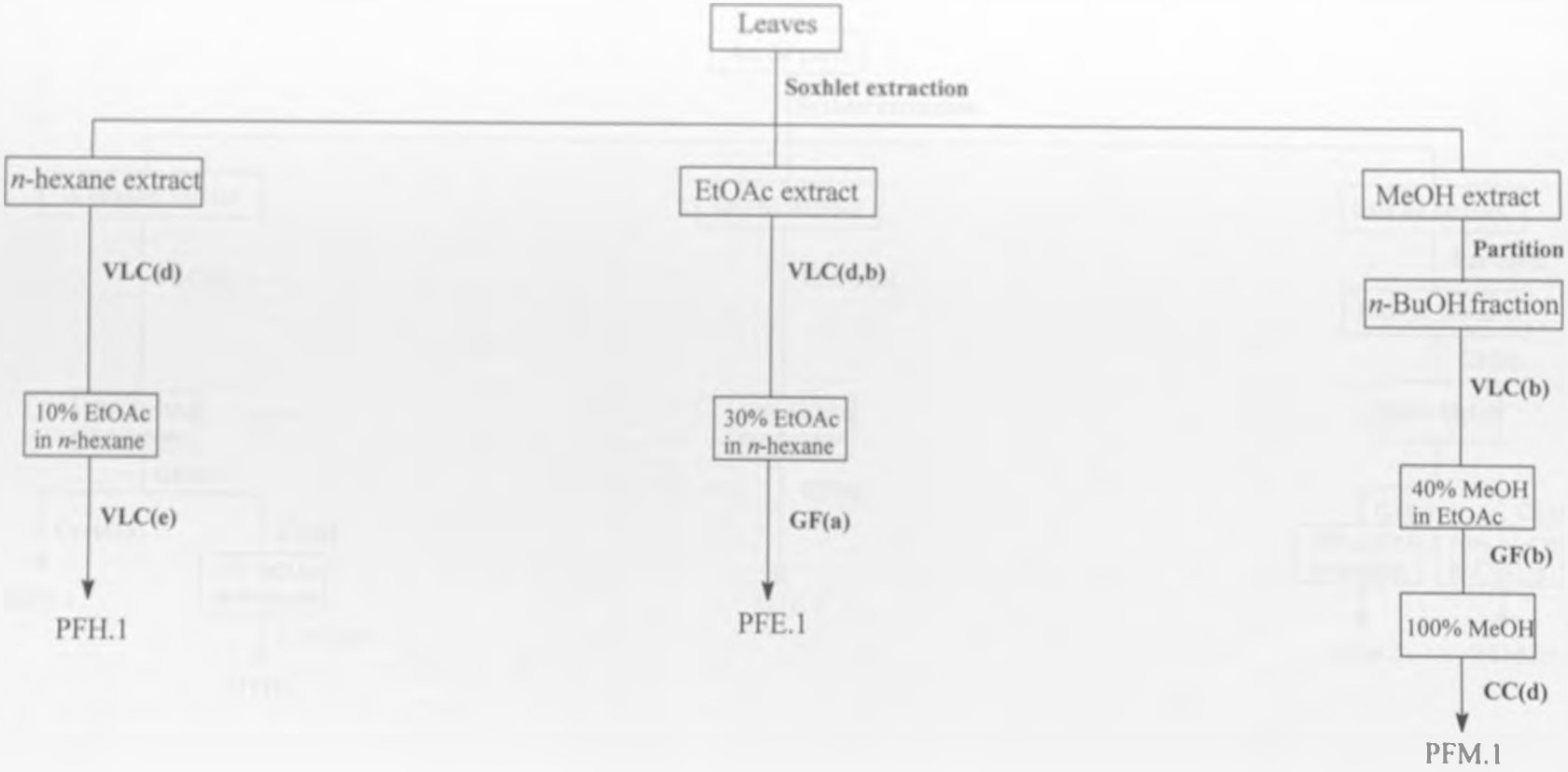
Scheme 2.1: Isolation of compounds from *Cleistopholis glauca*

CC eluting with **(a)** petrol, EtOAc **(b)** petrol, $CHCl_3$ mixtures of increasing polarity.
FC eluting with **(a)** $CHCl_3$/EtOAc/MeOH (85:7.5:7.5) **(b)** $CHCl_3$/EtOAc/MeOH (82:9:9) **(c)** $CHCl_3$/EtOAc/MeOH (78:11:11).
GF eluting with **(a)** $CHCl_3$ **(b)** $CHCl_3$, MeOH mixtures of increasing polarity.
VLC eluting with **(a)** petrol, EtOAc **(b)** EtOAc, MeOH **(c)** $CHCl_3$, MeOH mixtures of increasing polarity.



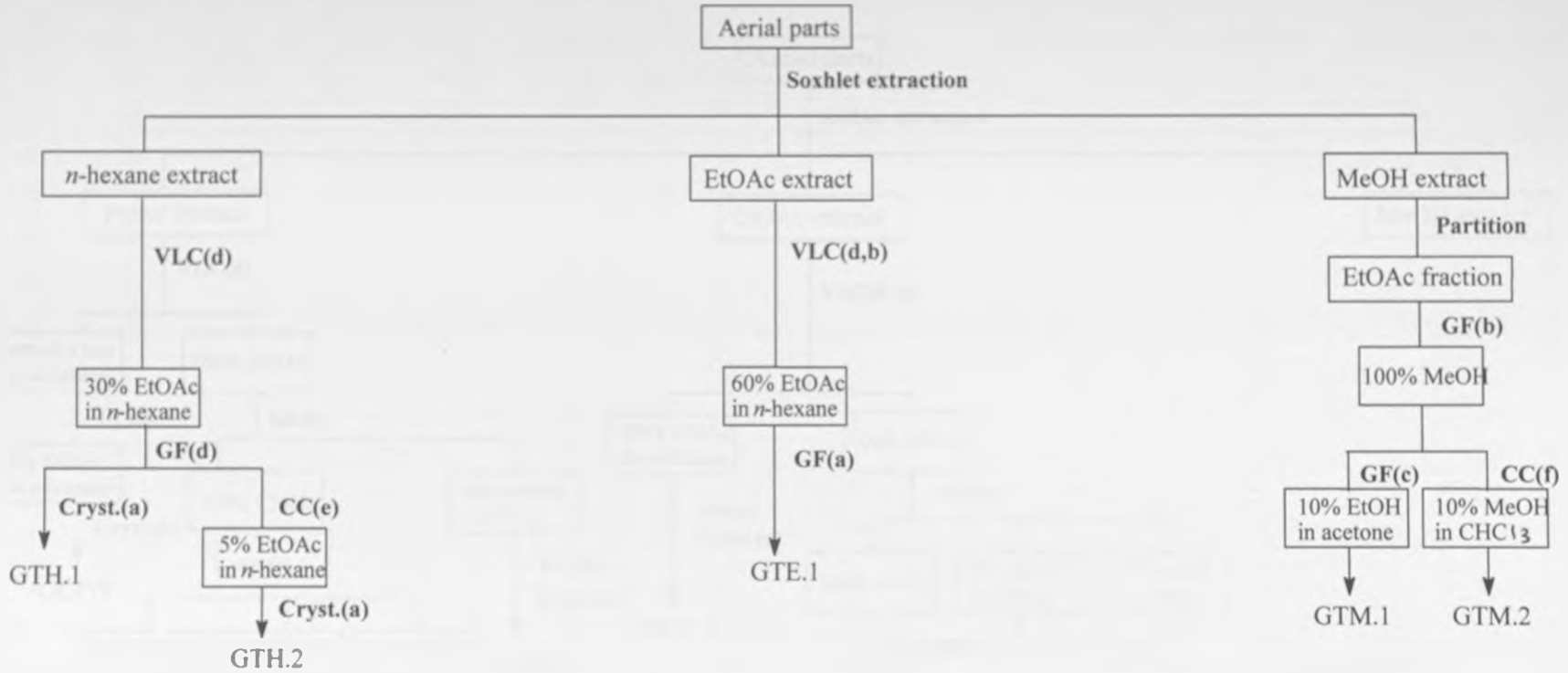
Scheme 2.2: Isolation of compounds from *Cleistopholis patens*

CC eluting with **(c)** $CHCl_3$/MeOH/H_2O (5:4:1).
FC eluting with **(a)** $CHCl_3$/EtOAc/MeOH (85:7.5:7.5) **(d)** $CHCl_3$/EtOAc/MeOH (91:4.5:4.5) **(e)** $CHCl_3$/EtOAc/MeOH (83:8.5:8.5) **(f)** $CHCl_3$/MeOH (9:1).
GF eluting with **(a)** $CHCl_3$.
VLC eluting with **(a)** petrol, EtOAc **(b)** EtOAc, MeOH mixtures of increasing polarity.



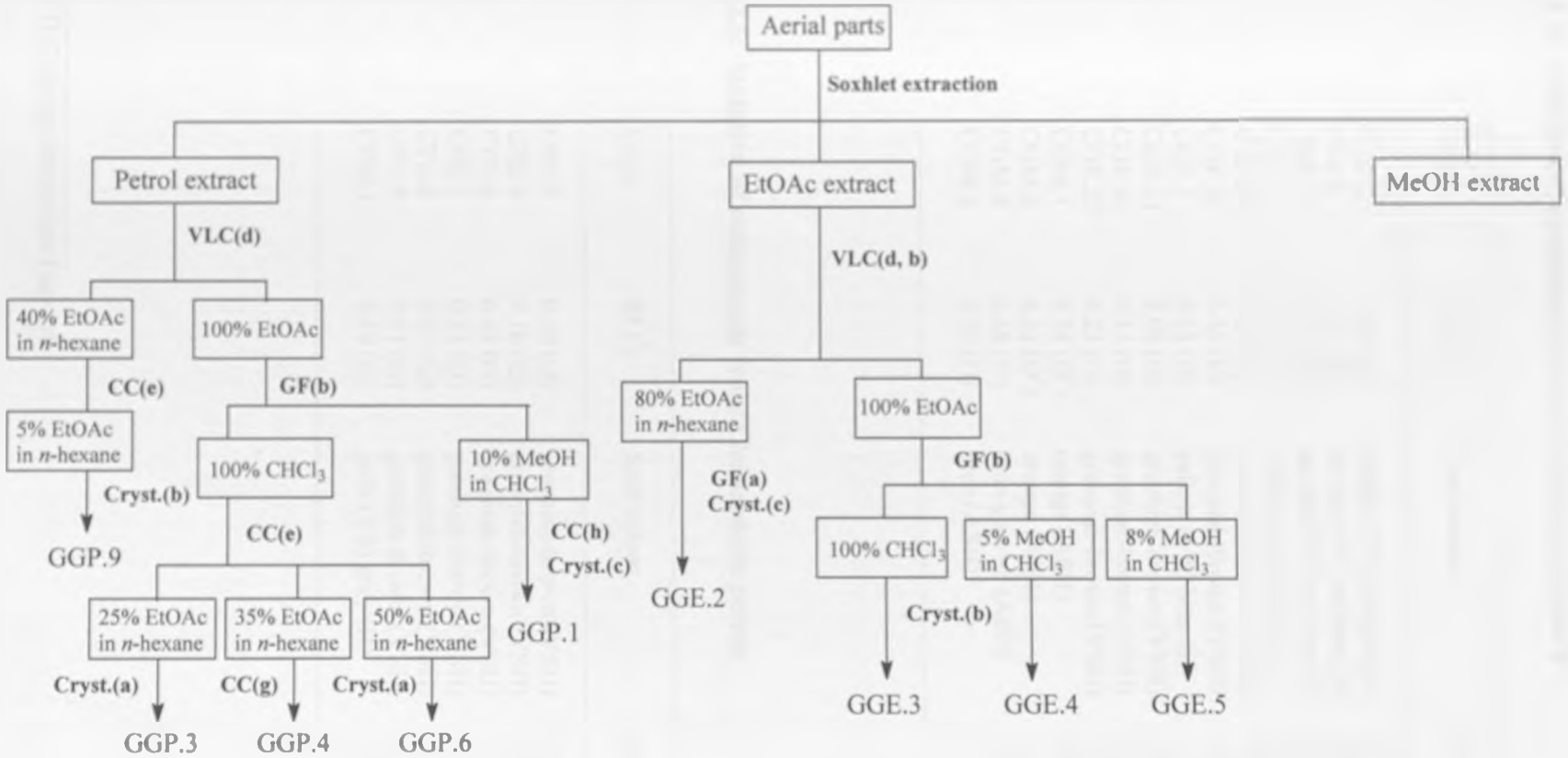
Scheme 2.3: Isolation of compounds from *Piptostigma fasciculata*

CC eluting with **(d)** $CHCl_3$/MeOH/H_2O (14:6:1).
GF eluting with **(a)** $CHCl_3$ **(b)** $CHCl_3$, MeOH mixtures of increasing polarity.
VLC eluting with **(b)** EtOAc, MeOH **(d)** *n*-hexane, EtOAc mixtures of increasing polarity **(e)** $CHCl_3$/*n*-hexane (7:3).



Scheme 2.4: Isolation of compounds from *Goniothalamus thwaitesii*

CC eluting with **(e)** *n*-hexane, EtOAc **(f)** $CHCl_3$, MeOH mixtures of increasing polarity.
Crystallisation in **(a)** *n*-hexane/$CHCl_3$.
GF eluting with **(a)** $CHCl_3$ **(b)** $CHCl_3$, MeOH **(c)** acetone, EtOH mixtures of increasing polarity **(d)** $CHCl_3$/*n*-hexane (95:5).
VLC eluting with **(b)** EtOAc, MeOH **(d)** *n*-hexane, EtOAc mixtures of increasing polarity.



Scheme 2.5: Isolation of compounds from *Goniothalamus gardneri*

CC eluting with **(e)** *n*-hexane/EtOAc mixtures of increasing polarity **(g)** $CHCl_3$, EtOAc (9:1) **(h)** petrol/EtOAc/EtOH (14:5:1).
Crystallisation in **(b)** EtOH **(c)** MeOH.
GF eluting with **(a)** $CHCl_3$ **(b)** $CHCl_3$, MeOH mixtures of increasing polarity.
VLC eluting with **(b)** EtOAc, MeOH **(d)** *n*-hexane, EtOAc mixtures of increasing polarity.

Table 2.4: Isolation of compounds from *Cleistopholis glauca*

Code	Rf ([1])	Spot colour	Amount (mg)
CGP.2	0.26 (A)	purple (VS) yellow (H)	6.3
CGP.3	0.50 (D)	purple (VS) yellow (H)	91.4
CGP.7	0.42 (D)	purple (VS) yellow (H)	12.5
CGP.12	0.28 (C)	yellow-orange (DG)	6.7
CGP.24	0.27 (F)	greenish-brown (VSH)	293
CGP.25	0.30 (F)	greenish-brown (VSH)	44.1
CGE.1	0.55 (D)	yellow (VS) blue (H)	5.4
CGE.21	0.05 (G)	greenish-brown (VSH)	335.7
CGE.22	0.31 (G)	greenish-brown (VSH)	72.2
CGE.23	0.23 (G)	greenish-brown (VSH)	38
CGM.1	0.34 (D^2)	orange (ASH)	267.5
CGM.2	0.22 (D^2)	orange (ASH)	4.4
CGM.4	0.46 (D)	blue-greyish (ASH)	9.8
CGM.5	0.33 (D)	blue (ASH)	11.1

Table 2.5: Isolation of compounds from *Cleistopholis patens*

Code	Rf ([1])	Spot colour	Amount (mg)
CPE.1	0.20 (G)	greenish-brown (VSH)	2500
CPE.4	0.10 (G)	greenish-brown (VSH)	224.5
CPE.6	0.45 (G)	greenish-brown (VSH)	158.6
CPE.7	0.51 (G)	greenish-brown (VSH)	40.6
CPE.8	0.33 (G)	greenish-brown (VSH)	36.3
CPE.9	0.31 (G)	greenish-brown (VSH)	87.2
CPM.1	0.19 (E)	pink (VS) grey (H)	69.7

[1] List of TLC solvent systems (see Page xxi)

Table 2.6: Isolation of compounds from *Piptostigma fasciculata*

Code	Rf ([1])	Spot colour	Amount (mg)
PFH.1	0.65 (C)	pink (ASH)	5.5
PFE.1	0.44 (C)	purple (ASH)	14.6
PFM.1	0.42 (E)	greenish-brown (ASH)	7.5

Table 2.7: Isolation of compounds from *Goniothalamus thwaitesii*

Code	Rf ([1])	Spot colour	Amount (mg)
GTH.1	0.33 (A^2)	pink (ASH)	17.7
GTH.2	0.52 (A^2)	pink (ASH)	17.6
GTE.1	0.58 (D)	pink (ASH)	40.1
GTM.1	0.44 (D)	orange (ASH)	18.8
GTM.2	0.22 (D)	greenish-orange (ASH)	30.7

Table 2.8: Isolation of compounds from *Goniothalamus gardneri*

Code	Rf ([1])	Spot colour	Amount (mg)
GGP.1	0.40 (B)	orange (ASH)	31.4
GGP.3	0.49 (B)	orange (ASH)	9.5
GGP.4	0.32 (B)	greenish yellow (ASH)	93.9
GGP.6	0.17 (B)	red (ASH)	101.6
GGP.9	0.54 (B)	orange (ASH)	32.9
GGE.2	0.33 (B)	orange (ASH)	104.1
GGE.3	0.11 (B)	red (ASH)	105.5
GGE.4	0.10 (B)	orange (ASH)	17.4
GGE.5	0.27 (B)	orange (ASH)	41.5

[1] List of TLC solvent systems (see Page xxi)

2.5 Structure elucidation

The purity of the compounds isolated, presumed initially by TLC (*i.e* single spot in different solvent systems), was established by running routine ^{1}H NMR spectra. Their structures were identified by comparison of the physical and spectral data with those of the literature in case of known compounds. Unknown structures were elucidated mainly by NMR spectroscopy.

2.5.1 General

2.5.1.1 Physical data

Melting points (mp) were determined using a *Gallenkamp* melting point apparatus and are uncorrected. Optical rotations ($[\alpha]_D$) were measured using a *Bellingham & Stanley model ADP 220* polarimeter in various solvents.

2.5.1.2 Spectral data

Infra-red (IR) spectra were obtained using a *Mattson Galaxy 5000 FTIR* spectrometer with samples as pressed potassium bromide (KBr) discs or as liquid film (film) on sodium chloride discs.

Ultra-violet (UV) spectra were recorded in MeOH or EtOH using a *Unicam UV 4-100 UV/Visible* spectrophotometer. Shift reagents were powdered NaOAc and H_3BO_3, and $AlCl_3$, NaOMe and HCl solutions prepared according to Mabry *et al.* (1970). NaOH solution was prepared according to Harborne (1984).

High resolution electron impact (HREI) and Fast atom bombardment (FAB) mass spectra (MS) were run on a *JEOL JMS-AX505HA*. HREIMS were obtained using a direct probe insert at 70 eV. Positive-ion FABMS were obtained using a nitrobenzoyl alcohol matrix.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a *Bruker AMX-400* (400 MHz) spectrometer in various deuterated solvents, using the residual solvent peaks as references (Table 2.9). Chemical shifts are expressed in parts per million (ppm) as δ values. Multiplicities are abbreviated as; (obsc) obscur; (s) singlet; (brs) broad singlet; (d) doublet; (dd) doublet of doublets; (ddd) doublet of doublets of doublets; (dt) doublet of triplets; (ddt) doublet of doublets of triplets; (t) triplet; (td) triplet of doublets; (tt) triplet of triplets; (m) multiplet. Coupling constants (*J*) are expressed in Hertz (Hz). Two dimensional NMR experiments were carried out using the standard microprogramms.

Table 2.9: Chemical shifts of the deuterated NMR solvents used

Deuterated NMR solvents	$^{\delta}H$	$^{\delta}C$
Acetone-d_6	2.05	29.92, 206.68
$CDCl_3$	7.27	77.23
D_2O	4.80	
CD_3OD	3.31, 4.87	49.15
C_5D_5N	7.22, 7.58, 8.74	123.87, 135.91, 150.35

2.5.2 NMR spectroscopy

The structures of simple and known compounds were directly established by one-dimensional ^{1}H and *J*-modulated ^{13}C NMR experiments. For complex and new molecules, these techniques only revealed key structural fragments, requiring the use of two-dimensional NMR or correlation spectroscopy for complete and unambiguous structure elucidation.

2.5.2.1 One dimensional NMR

a) ^{1}H NMR

^{1}H NMR spectra were routinely acquired for all isolated compounds to check their purity and to predict their structures. Observation of chemical shifts, intensities, multiplicities and coupling constants provided direct information about the type of protons present and their chemical environments (Williams and Fleming, 1995).

b) J-modulated ^{13}C NMR

Carbon resonances were distinguished according to their proton attachments (C, CH, CH_2, CH_3), using the APT pulse sequence. The *J*-modulated ^{13}C NMR spectra displayed the CH_3 and CH resonances (arbitrarily pointing down) inverted with respect to the CH_2 and the quaternary carbons (arbitrarily pointing up) (Sanders and Hunter, 1987).

c) Proton-coupled ^{13}C NMR

Carbon resonances were distinguished according to their splitting patterns. Signals for C, CH, CH_2 and CH_3 were respectively identified as singlets, doublets, triplets and quartets (Williams and Fleming, 1995). Proton-coupled experiments were successfully applied to establish the relative configuration of sugars by measuring $^1J_{C,H}$ coupling constant values for anomeric positions (see Section 3.1.1.1).

2.5.2.2 Two dimensional NMR

Two-dimensional NMR or correlation spectroscopy was used to confirm the established structures, to elucidate structures of unknown compounds and to identify unambiguously ^1H and ^{13}C NMR assignments. Spectral data obtained in one time domain were plotted against data obtained in another, generating a correlation map. According to the experiment used, interactions were seen among nuclei of the same species (homonuclear) or between two different nuclei (heteronuclear) in the molecule.

A) Two dimensional homonuclear correlation spectroscopy

In the two dimensional homonuclear NMR experiments, the ^1H NMR spectra were displayed along both axes and as a contour plot along the diagonal. Correlations were seen as cross-peaks symmetrically placed about the diagonal.

a) ^1H-^1H COrrelation SpectroscopY

Cross-peaks in COSY experiments represented trough-bond ^2J (geminal) and ^3J (vicinal) connectivities. Cross-peaks in LR-COSY or delayed COSY represented trough-bond long-range ^4J (W pathway) and ^5J (zig-zag pathway) connectivities (Williams and Fleming, 1995). LR-COSY was useful in determining relative configuration of sugars (see Section 3.1.1.1).

b) ^1H-^1H TOtal COrrelation SpectroscopY

Cross-peaks in TOCSY experiments represented through-bond connectivities for groups of protons part of the same spin system which were serially linked in the molecule, thus helping to assign overlapping resonances (Williams and Fleming, 1995). TOCSY was used extensively to assign proton resonances of sugars (see Section 3.1.1.1).

c) ^1H-^1H Nuclear Overhauser Effect SpectroscopY

Cross-peaks in NOESY experiments represented through-space interactions between protons in the molecule which were in spatial proximity (nuclear Overhauser effect) (Williams and Fleming, 1995). Interpretation of NOESY spectra were made by comparison with COSY to reveal only spatial couplings, as scalar couplings were often seen in NOESY. Nuclear Overhauser effect spectroscopy was useful when positioning methoxylation sites and establishing relative stereochemistry of compounds.

B) Two dimensional heteronuclear correlation spectroscopy

In two dimensional heteronuclear NMR, ^1H NMR spectra were displayed along one axis and ^{13}C NMR spectra along the other. Cross-peaks revealed interactions between the two different nuclei.

Cross-peaks in the HC-COBI experiments (^{13}C-detecting technique, using a BIRD pulse sequence) represented direct (^1J) ^{13}C-^1H connectivities (Sanders and Hunter, 1987) whilst the HMBC experiments (^1H-detecting technique) were

optimised to show long range (^2J, ^3J) ^1H-^{13}C correlations (Williams and Fleming, 1995). The combination of both experiments in the current study provided considerable structural information, allowing the unambiguous structure elucidation of samples in small amounts.

2.6 Pharmacological screenings

The two selected extracts identified as having a binding affinity for bradykinin receptors were those of *Cleistopholis glauca* and *C. patens*.

2.6.1 General

2.6.1.1 Tissues and instrumentation

Ileal smooth muscle tissues were obtained from adult male *Hartley* guinea pigs and kept ice cold throughout the assay. Homogenisation was carried out using an *Ultra-Turrax T25* instrument. Filtration was done using a *Brandel* cell harvester through *Whatman GF/B* glass fibre filters. Radioactivity was measured in a *Packard* liquid scintillation spectrometer.

2.6.1.2 Solvents, reagents and buffers

DMSO and MeOH were double distilled solvents. ^{3}[H] BK was purchased from Du-Pont New England Nuclear with a specific activity of 106 Ci/mmol.

Buffer 1, 25 mM TES (pH 6.8), was prepared the day before the assay and stored chilled overnight. Other buffers were prepared on the day of the assay and kept ice cold.

Buffer 2 was made up with buffer 1 and 1 mM 1,10-phenanthroline.

Buffer 3 was made up with buffer 2 and 1 μg/ml zinc free bacitracin, 0.1% BSA and 1 μM captopril.

Buffer 4 was 0.1% aqueous polyethyleneimine.

2.6.2 The assay procedure

The binding assay used followed the method of Manning *et al.* (1986), with minor modifications.

2.6.2.1 Preparation of plant extracts/fractions and compounds

Prior to testing, all polar extracts were detannified to exclude the interaction of tannins with receptors (proteins) often known to cause false-positive results (Silva *et al.*, 1998). This was done by passing polar extracts (dissolved in alcohol) through a sintered funnel, packed with polyvinylpyrrolidone. The detannified solutions were concentrated to dryness *in vacuo*, placed in a vacuum oven at 40° for 2-3 days and freeze-dried.

Stock solutions of extracts/fractions were prepared in MeOH or if non-soluble, in DMSO and buffer 1 (concentration in mg/ml). Stock solutions of pure compounds were prepared in either of the two solvents (concentration in mol/l).

2.6.2.2 Membrane preparation

Ileal tissues (2-3g) were homogenised in buffer 2 for 10 sec and the suspension was centrifuged at 20 000 rpm for 10 min at 4°C. The pellet was re-homogenised in fresh buffer 2 and centrifuged again. The final pellet was re-suspended in buffer 3 to make a 10 mg/ml membrane suspension, which was re-homogenised and used on the same day of its preparation.

2.6.2.3 Incubation

Bradykinin B_2 receptors were labelled by incubating, at 25°C for 2 hours, the membrane preparation (final concentration of 1 mg/ml) with ^{3}[H] BK (final concentration of 1 nM) and:

i) buffer 3 to measure the total binding for the control.

ii) unlabelled Lys-BK (final concentration of 1-5 μM) to measure the non-specific binding.

iii) an extract/fraction (final concentration of 0.5 mg/ml) or a pure compound (range of six final concentrations in mol/l) to measure residual specific binding.

Each determination was performed in triplicate and in two separate experiments.

2.6.2.4 Filtration and radioactivity counting

The contents of each tube was filtered under reduced pressure through glass fibre filter paper, pre-treated with cold buffer 4. Filters were rinsed with 3×4 ml of cold buffer 1 and placed in vials with some scintillation fluid. Radioactivity was counted by liquid scintillation spectrometry after at least 2 hours.

2.6.3 Expression of results

Results were the average of triplicate determinations from a representative experiment that was repeated twice. Specific binding was calculated by subtracting the non-specific binding from total binding observed. Specific binding in the presence of an extract/fraction or compound was expressed as a percentage of the control.

Competition binding data in the presence of compounds were analysed using the non-linear regression program *RADLIG®*. Percentage of specific binding was plotted against concentration (in mol/l, on a log scale). IC_{50} values were determined graphically from the fitted inhibition curves. These represented the concentration of the substance reducing specific binding by 50% (see Figure 4.1).

B *RADLIG (version 4 of KINETIC, EBDA, LIGAND, LOWRY for IBM-PC, a collection of programs for the analysis of radioligand binding experiments by Dr. G. A. Mc Pherson)* is a registered trademark of BIOSOFT, Inc.

Chapter 3:

Phytochemical Results and Discussion

3.1 Oligosaccharide derivatives

3.1.1 Partially acetylated 1-*O*-dodecanyl oligorhamnosides

Ten oligosaccharide derivatives, characterised as partially acetylated α-L-oligorhamnosides with a dodecanyl ether chain linked to C-1 of the first rhamnose unit, were obtained from *Cleistopholis glauca* and *Cleistopholis patens*. Individual compounds differed in whether interglycosidic links were (1→3) or (1→4), in the number and positions of the acetoxyl substituents and in the number of rhamnose units present.

Thus, they include four trirhamnosides and six tetrarhamnosides, which have been given the trivial names cleistri- and cleistetrosides respectively. New isolated compounds and known ones, previously reported from *C. glauca* (Tané *et al.*, 1988b; Woods, 1989), were numbered from 1 upward in each series, giving the first numbers to previously known compounds.

3.1.1.1 General characterisation of isolated partially acetylated 1-O-dodecanyl oligorhamnosides

On TLC analysis, the oligosaccharide derivatives were only revealed on spraying with vanillin-H_2SO_4 reagent, followed by heating for a few minutes, when they displayed greenish-brown spots. IR spectra showed absorption bands typical of hydroxyl groups (*ca.* 3465 cm^{-1}) and ester carbonyl functions (*ca.* 1745 and 1230 cm^{-1}) (Williams and Fleming, 1995).

The ^1H NMR spectra each displayed:

i) signals for protons of either three or four rhamnopyranose units, with the anomeric H-1 (d, *J ca.* 2 Hz), H-2 (dd, *J ca.* 2, 3.5 Hz), H-3 (dd, *J ca.* 3.5, 10 Hz), H-4 (t, *J ca.* 10 Hz), H-5 (m, *J ca.* 10, 6.3 Hz) and Me-6 (d, *J ca.* 6.3 Hz).

ii) signals for the ether as two multiplets (*ca.* δ 3.75/3.45) for non equivalent oxymethylene protons, two broad multiplets (*ca.* δ 1.55 and *ca.* δ 1.30), a broad

singlet (*ca.* δ 1.18) for methylene groups and a triplet (*ca.* δ 0.80) for a methyl resonance.

iii) from three to six sharp singlets assignable to acetoxymethyl substituents (δ 2.29-1.87).

The most deshielded resonances on the ^{1}H NMR spectra could be attributed to the protons attached to the acetylated positions on the rhamnose units. However, in some cases, the relevant signals overlapped with the solvent peak. Therefore, a series of COSY, in combination with TOCSY experiments, were run to allow the unambiguous assignment of all esterified positions and ^{1}H chemical shift values for each rhamnose unit (Powell *et al.,* 1990). They also assigned ^{1}H resonances for the alkyl ether side chain, with the non equivalent oxymethylene protons (Ha/a'), showing couplings to two distinctive methylene groups, identified respectively as the methylenes in β (*ca.* δ 1.55) and γ position (*ca.* δ 1.30), as well as to the methylene envelope (*ca.* δ 1.18). The latter, coupling to a terminal methyl resonance, established the linearity of the side chain.

The *J*-modulated ^{13}C NMR, with resonances assigned by ^{1}H-^{13}C HC-COBI, revealed:

i) either three or four anomeric methines (*ca.* δ 100) and the same number of Me-6 (*ca.* δ 18), thus confirming the number of rhamnose units present.

ii) the oxymethylene for the ether group (*ca.* δ 68.5), the aliphatic side chain methylenes (δ 32 to 23), including the methylenes in β (*ca.* δ 30.4) and γ (*ca.* δ 27.1) positions, the terminal methyl (δ *ca.* 15).

iii) from three to six carbonyls (*ca.* δ 170) and the same number of acetoxymethyls (*ca.* δ 21).

The location of interglycosidic links, the position of the ether side chain and of the acetoxyl substituents were unambiguously established on the basis of direct and detailed long-range heteronuclear correlations using the HMBC experiment. Key features were:

i) a series of ^{3}J interactions between the oxymethine H-3 or H-4 of one rhamnose unit and the anomeric carbon C-1 of another, thus establishing (1→3) or (1→4) interglycosidic links.

ii) a ^{3}J interaction between the non equivalent oxymethylene protons Ha/a' and the anomeric C-1 of one rhamnose unit. On this basis, this unit (bearing the ether side chain) was named unit A and accordingly the following linked units were named B, C and D in the case of tetrarhamnosides and A, B and C in the case of trirhamnosides.

iii) a ^{2}J interaction between each acetoxymethyl proton resonances and their corresponding acetyl carbonyl and a ^{3}J interaction between each oxymethine protons adjacent to a position of esterification and their corresponding acetyl carbonyl.

Positive-ion FABMS confirmed the number of rhamnose units and acetoxyl substituents present. It also established the exact length of the side chain, which was deduced to be a dodecanyl ether group since the difference between the molecular weight of each compound and the molecular weight calculated for each partially acetylated oligorhamnoside was 169, *i.e* $C_{12}H_{25}$.

A series of proton-coupled ^{13}C NMR studies (see Section 2.5.2.1) determined the α-configuration for the rhamnose units through $^{1}J_{C\text{-}1,H\text{-}1}$ values which were of *ca.* 170 Hz. β-glycosides would have given values *ca.* 10 Hz lower (Bock *et al.*, 1973; Bock and Pedersen, 1974). Results are exemplified for **OS-3** in Spectrum 3.7.

The equatorial position of the anomeric protons in α-rhamnose was further confirmed with:

i) the observation of a long-range ^{5}J coupling, following a zig-zag pathway, between the anomeric H-1 and the Me-6 protons in LR-COSY experiments (see Section 2.5.2.2) (Massiot *et al.*, 1990). Results are exemplified for **OS-7** in Spectrum 3.12.

ii) the absence of NOE interactions between H-1/H-3 and H-1/H-5 (Tané *et al.*, 1988b; Woods, 1989).

A) Partially acetylated 1-O-dodecanyl trirhamnosides

Four partially acetylated 1-*O*-dodecanyl trirhamnosides were isolated from *Cleistopholis glauca* and *C. patens* (see Section 2.4.4). They were coded:

OS-1 = (CPE.6)

OS-2 = (CPE.7)

OS-3 = (CPE.8)

OS-4 = (CGP.24)

and identified respectively as :

1-*O*-dodecanyl 3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L- rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L- rhamnopyranoside (cleistrioside-2)

1-*O*-dodecanyl 2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L- rhamnopyranoside (cleistrioside-3)

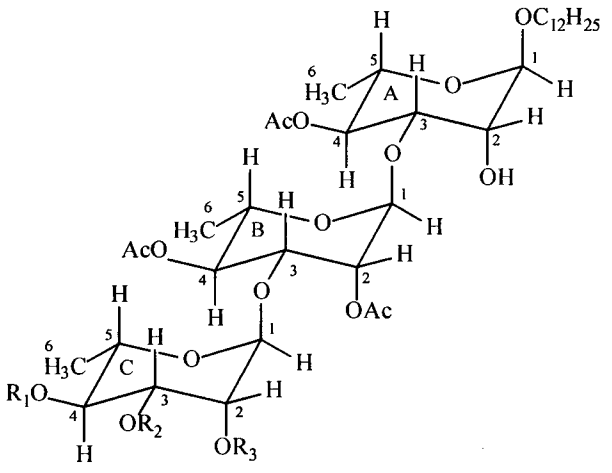
1-*O*-dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside (cleistrioside-4)

1-*O*-dodecanyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistrioside-1) (Figure 3.1).

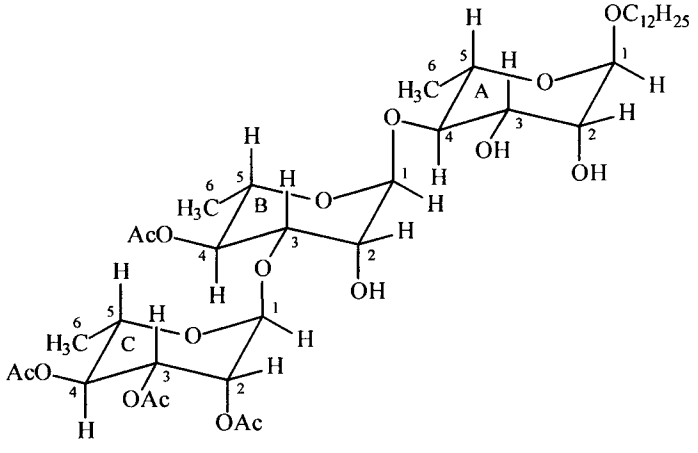
Characteristic features in the ^{1}H and ^{13}C NMR (Tables 3.1 & 3.2) included:

i) signals for three rhamnose units at δ *ca.* 5.76-4.70 (1H each, d, J = *ca.* 1.2 Hz) and *ca.* 1.08-1.41 (3H each, d, J = *ca.* 6.2 Hz) for three anomeric H-1 and three Me-6 substituents respectively.

ii) four or five acetoxymethyl resonances (δ 2.24-1.87).



Compound	R_1	R_2	R_3
OS-1	Ac	Ac	H
OS-2	Ac	H	Ac
OS-3	Ac	H	H



OS-4

Figure 3.1: Structures of OS-1 to OS-4

Table 3.1: ^{1}H NMR (400 MHz) spectral data of OS-1 to OS-4

Position	OS-1	OS-2	OS-3	OS-4
1A	5.05 (d, 1.2)	5.04 (d, 1.2)	4.99 (d, 1.5)	4.93 (brs)
2A	4.34 (brs)	4.35 (dd, 1.2, 3.1)	4.28 (dd)	4.11 (dd)
3A	4.22 (dd, 3.1, 9.8)	4.21 (dd, 3.1, 9.8)	4.16 (dd, 2.9,9.9)	4.16 (dd, obsc, 8.6)
4A	5.61 (t, 9.8)	5.61 (t, 9.8)	5.55 (t, 9.8)	3.96 (t, 8.8)
5A	3.99 (m)	3.99 (m)	3.94 (m)	3.88 (m)
Me-6A	1.27 (d, 6.2)	1.26 (d, 6.2)	1.24 (d, 6.2)	1.41 (d, 5.9)
1B	5.15 (d, 1.2)	5.11 (d, 1.2)	5.08 (d, 1.5)	5.76 (brs)
2B	5.44 (obsc)	5.33 (dd, 1.2, 3.6)	5.34 (dd, 1.6, obsc)	4.57 (brs)
3B	4.33 (dd)	4.26 (dd, 3.7, 9.9)	4.27 (dd)	4.21 (dd, obsc, 9.8)
4B	5.32 (t, 9.8)	5.29 (t, 10)	5.24 (t, 9.9)	5.56 (t, 9.6)
5B	4.35 (m)	4.33 (m)	4.31 (m)	4.08 (m)
Me-6B	1.20 (d)	1.19 (d)	1.17 (obsc)	1.15 (d, 7)
1C	4.96 (d, 1.2)	4.70 (d, 1.2)	4.93 (d)	5.11 (brs)
2C	4.29 (dd)	5.21 (dd, 1.2, obsc)	4.08 (dd)	5.40 (brs)
3C	5.33 (dd, 3.2, 10)	4.26 (dd, 3.7, 9.9)	4.10 (dd, 3.1, obsc)	5.45 (dd, 3.3, 10)
4C	5.57 (t, 10)	5.30 (t, 10)	5.40 (obsc)	5.22 (t, 10)
5C	4.13 (m)	4.02 (m)	3.96 (m)	4.33 (m)
Me-6C	1.31 (d, 6.2)	1.35 (d, 6.2)	1.28 (d, 6.2)	1.08 (d, 6.2)
*Me*CO	2.24 (s)	2.24 (s)	2.21 (s)	2.14 (s)
	2.01 (s)	2.11 (s)	1.96 (s)	1.98 (s)
	2.00 (s)	1.96 (s)	1.94 (s)	1.92 (s)
	1.98 (s)	1.94 (s)	1.91 (s)	1.87 (s)
	1.88 (s)	1.93 (s)	-	-
OCH_2	3.74/3.45 (m)	3.73/3.45 (m)	3.71/3.43 (m)	3.67/3.36 (m)
$CH_2\beta$	1.58 (m)	1.58 (m)	1.55 (m)	1.52 (m)
$CH_2\gamma$	1.31 (m)	1.32 (m)	1.32 (m)	1.26 (m)
$(CH_2)_8$	1.19 (brs)	1.18 (brs)	1.16 (brs)	1.17 (brs)
Me	0.80 (t, 7)	0.81 (t, 6.7)	0.80 (t, 6.8)	0.80 (t, 6.2)

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
Non equivalent oxymethylene signals are listed as Ha/a' in each column.

Table 3.2: ^{13}C NMR (100 MHz) spectral data of OS-1 to OS-4

Position	OS-1	OS-2	OS-3	OS-4
1A	101.8	101.7	101.7	101.7
2A	72.1	72.1	72.1	73.0
3A	80.4	80.4	80.3	73.6
4A	73.7	73.7	73.7	81.4
5A	67.8	67.8	67.9	68.1
Me-6A	18.3	18.3	18.2	19.3
1B	100.9	100.9	100.9	103.7
2B	72.9	73.1	73.3	71.8
3B	75.4	75.8	75.6	79.9
4B	73.3	73.5	73.9	73.6
5B	67.9	67.9	68.0	68.6
Me-6B	18.1	18.0	18.0	18.2
1C	103.3	100.6	104.0	100.4
2C	69.9	74.1	72.6	71.4
3C	73.8	68.0	70.3	70.3
4C	72.2	75.2	75.5	72.1
5C	68.6	68.4	68.5	67.8
Me-6C	18.1	18.0	18.1	17.9
*Me*CO	21.4	21.4	21.4	21.3
	21.1	21.3	21.3	21.0
	21.1	21.2	21.1	21.0
	21.1	21.1	21.0	20.9
	21.1	21.0	-	-
*CO*Me	171.3	171.4	171.7	171.2
	171.3	171.4	171.5	171.0
	171.1	171.3	171.4	170.9
	171.1	171.3	171.1	170.6
	170.9	171.2	-	-
OCH_2	68.6	68.6	68.7	68.4
$CH_2\beta$	30.4	30.4	30.5	30.5
$CH_2\gamma$	27.1	27.1	27.2	27.1
$(CH_2)_6$	30.6-30.2	30.6-30.2	30.6-30.3	30.5-30.2
CH_2	32.8	32.7	32.8	32.8
CH_2	23.5	23.5	23.6	23.6
Me	14.7	14.7	14.7	14.8

All data obtained in C_5D_5N-CD_3OD.

*a) Identification of **OS-1** as cleistrioside-2*

This compound was isolated from the ethyl acetate extract of *Cleistopholis patens*. FABMS (Scheme 3.1) displayed a quasi molecular ion $[M+Na]^+$ at *m/z* 857 indicating a molecular weight of 834, corresponding to the molecular formula $C_{40}H_{66}O_{18}$ (8 DBE). Peaks were observed at *m/z* 815 $[(M+Na)-COCH_2]^+$, 773 $[(M+Na)-2\times COCH_2]^+$, and 731 $[(M+Na)-3\times COCH_2]^+$.

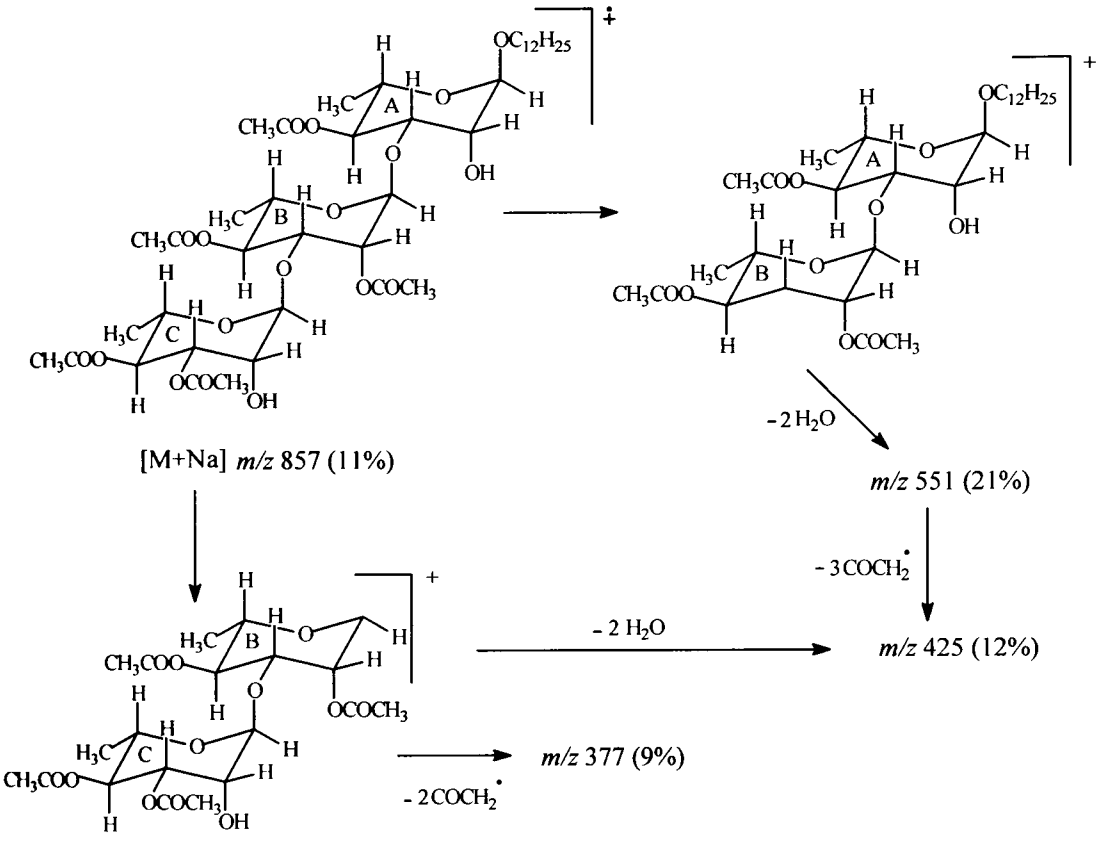
^1H and ^{13}C NMR (Spectra 3.1 & 3.2) showed a trirhamnoside with five acetoxymethyls. Acetylated positions were attributed to three H-4 at δ 5.61, 5.32 (t, J = 9.8 Hz), 5.57 (t, J = 10 Hz) and one H-3 at δ 5.33 (dd, J = 3.2, 10 Hz). A TOCSY experiment (Spectrum 3.3) established the remaining acetylated position as one H-2 at δ 5.44. Characteristic features on the HMBC (Table 3.3; Spectrum 3.4) included:

i) ^3J interactions between H-3 at δ 4.33 and C-1 at δ 103.3 and between H-3 at δ 4.22 and C-1 at δ 100.9, thus establishing the interglycosidic links as (1→3) (1→3) between the three units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.74/3.45) and the anomeric C-1 at δ 101.8 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the five deshielded protons, now identified as H-4A/H-2B/H-4B/H-3C and H-4C and the acetoxycarbonyls.

Consequently, **OS-1** was identified as the new 1-*O*-dodecanyl 3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside and given the trivial name cleistrioside-2.

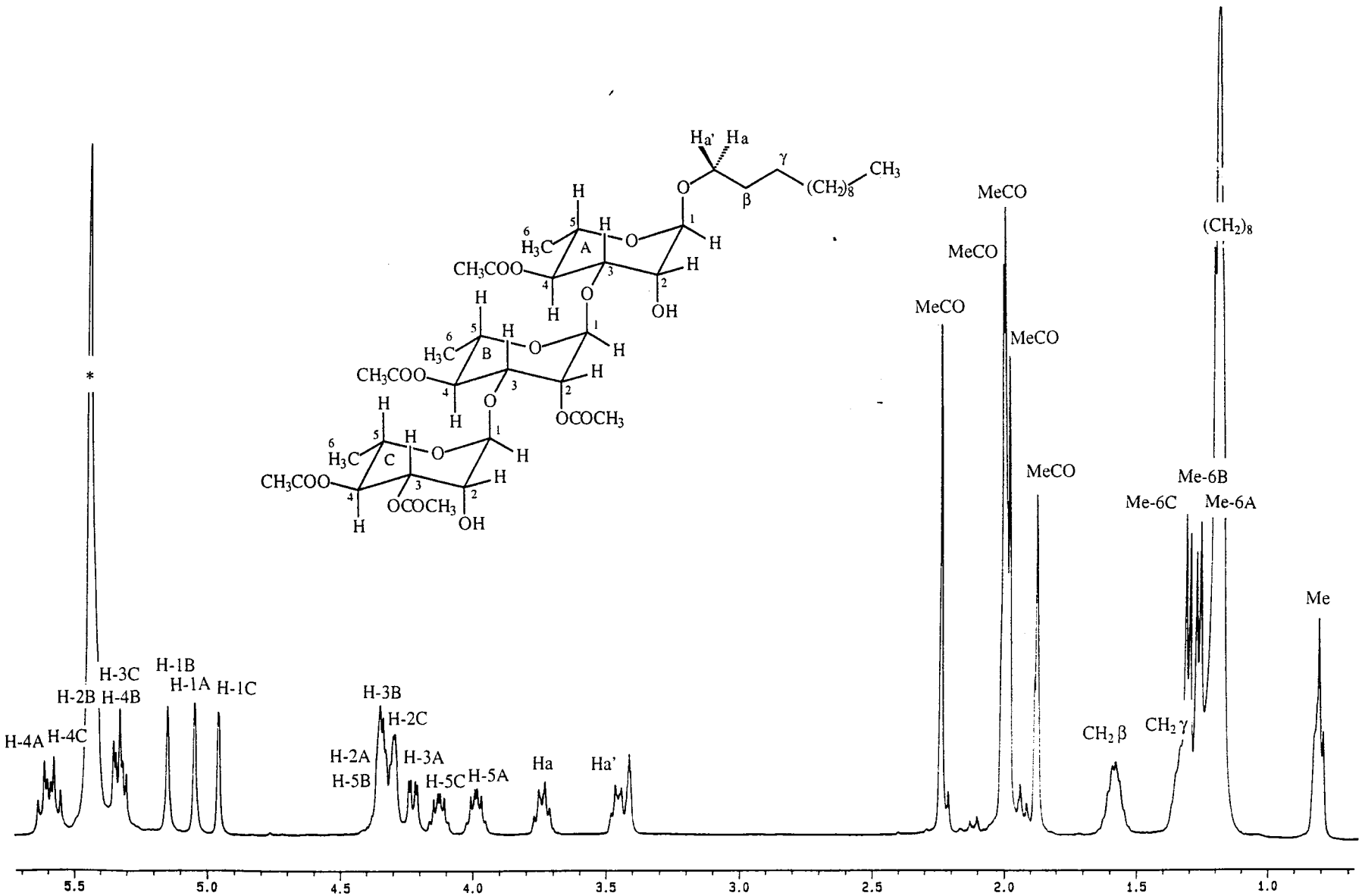


Scheme 3.1: Suggested mass fragmentation pattern of OS-1

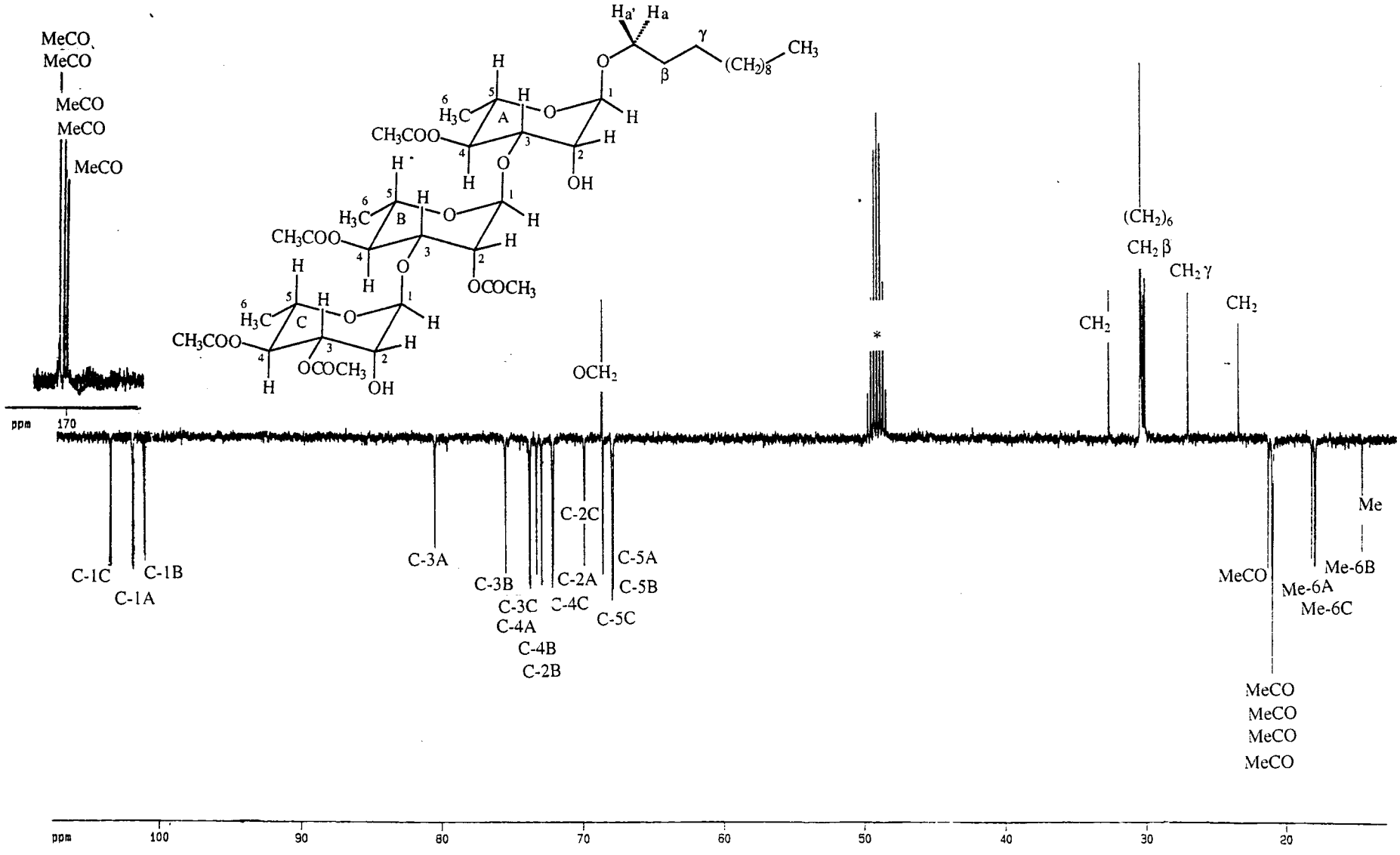
Table 3.3: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-1

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	5.05 (d, 1.2)	101.8		67.8 (C-5A), 68.6 (OCH_2), 80.4 (C-3A)
3A	4.22 (dd, 3.1, 9.8)	80.4		100.9 (C-1B)
1B	5.15 (d, 1.2)	100.9	72.9 (C-2B)	67.9 (C-5B), 75.4 (C-3B), 80.4 (C-3A)
3B	4.33 (dd)	75.4		103.3 (C-1C)
1C	4.96 (d, 1.2)	103.3	69.9 (C-2C)	68.6 (C-5C), 73.8 (C-3C), 75.4 (C-3B)
OCH_2	3.74/3.45 (m)	68.6		101.8 (C-1A)
*Me*CO	2.24 (s)	21.4	171.3 (H-4A)	
	2.01 (s)	21.1	171.3 (H-2B)	
	2.00 (s)*	21.1	171.1 (H-4B)*	
	1.98 (s)*	21.1	171.1 (H-4C)*	
	1.88 (s)	21.1	170.9 (H-3C)	
4A	5.61 (t, 9.8)	73.7	67.8 (C-5A), 80.4 (C-3A)	171.3
2B	5.44 (obsc)	72.9	75.4 (C-3B), 100.9 (C-1B)	73.3 (C-4B), 171.3
4B	5.32 (t, 9.8)	73.3	67.9 (C-5B), 75.4 (C-3B)	171.1
3C	5.33 (dd, 3.2, 10)	73.8	72.2 (C-4C)	170.9
4C	5.57 (t, 10)	72.2	68.6 (C-5C), 73.8 (C-3C)	171.1

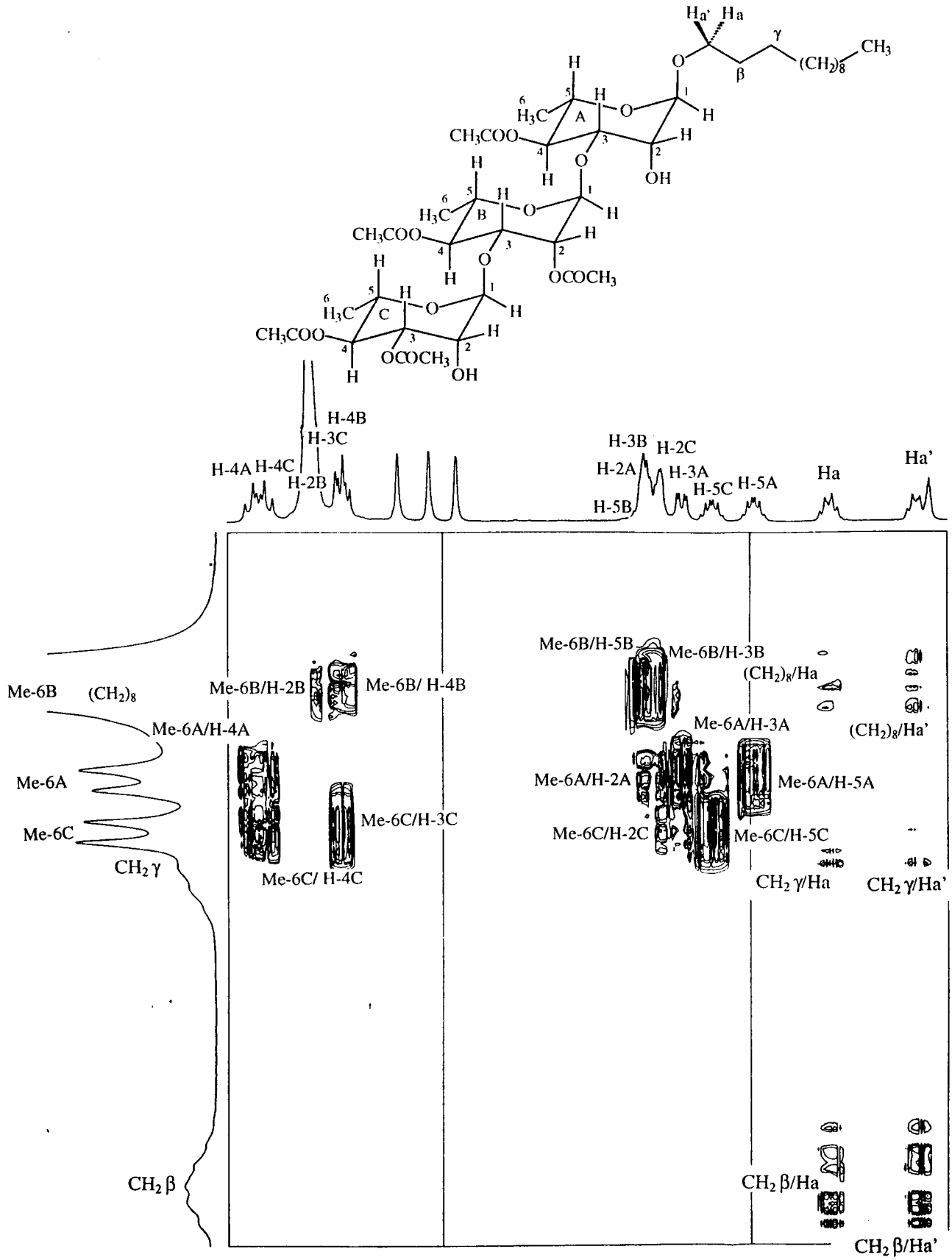
All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



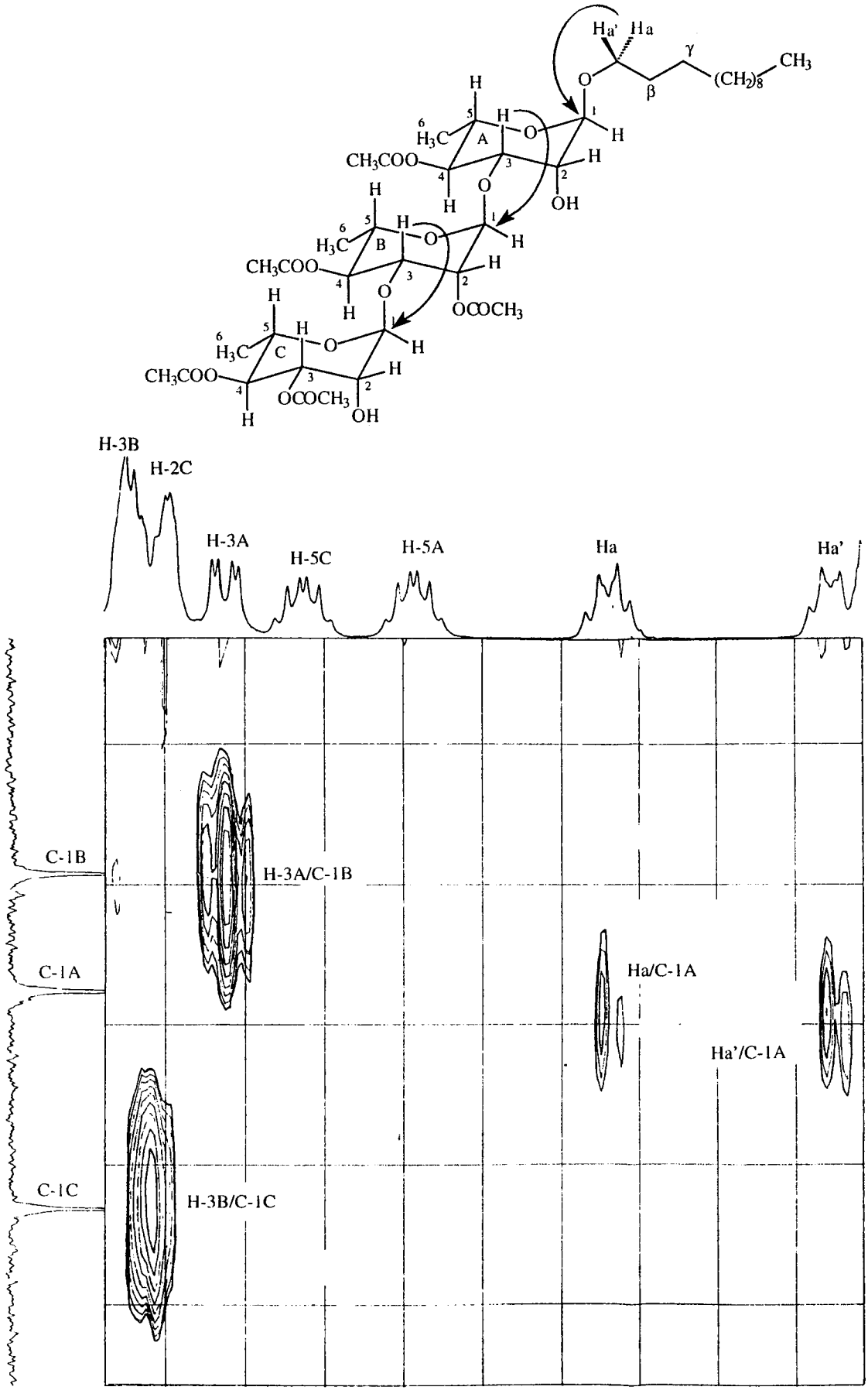
Spectrum 3.1: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-1



Spectrum 3.2: *J*-modulated ^{13}C NMR (100 MHz, C_5D_5N-CD_3OD*) of OS-1



Spectrum 3.3: Significant TOCSY correlations(400 MHz, C_5D_5N-CD_3OD) of OS-1 showing ^1H-^1H couplings for each unit



Spectrum 3.4: Significant HMBC correlations (400 MHz, C_5D_5N-CD_3OD) of OS-1 showing the location of interglycosidic links and the position of attachment of the alkyl chain

*b) Identification of **OS-2** as cleistrioside-3*

This compound was isolated from the ethyl acetate extract of *Cleistopholis patens*. FABMS (Scheme 3.2) yielded a quasi molecular ion $[M+Na]^+$ at *m/z* 857 indicating a molecular weight of 834, thus corresponding to the molecular formula $C_{40}H_{66}O_{18}$ (8 DBE). Peaks were observed at *m/z* 815 $[(M+Na)-COCH_2]^+$ and 773 $[(M+Na)-2\times COCH_2]^+$.

^1H (Spectrum 3.5) and ^{13}C NMR showed a trirhamnoside with five acetoxymethyls. Acetylated positions were attributed to three H-4 at δ 5.61 (t, J = 9.8 Hz), 5.29, 5.30 (t, J = 10 Hz) and two H-2 at δ 5.33 (dd, J = 1.2, 3.5 Hz) and 5.21 (dd, J = 1.2, obsc Hz). Characteristic features on the HMBC (Figure 3.2; Table 3.4) included:

i) ^3J interactions between H-1 at δ 4.70 and C-3 at δ 75.8 and between H-1 at δ 5.11 and C-3 at δ 80.4, thus establishing the interglycosidic links as (1→3) (1→3) between the three units.

ii) a ^3J interaction between the anomeric H-1 at δ 5.04 of one rhamnose unit and the oxymethylene at δ 68.6, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the five deshielded protons, now identified as H-4A/H-2B/H-4B/H-2C and H-4C and the acetoxycarbonyls.

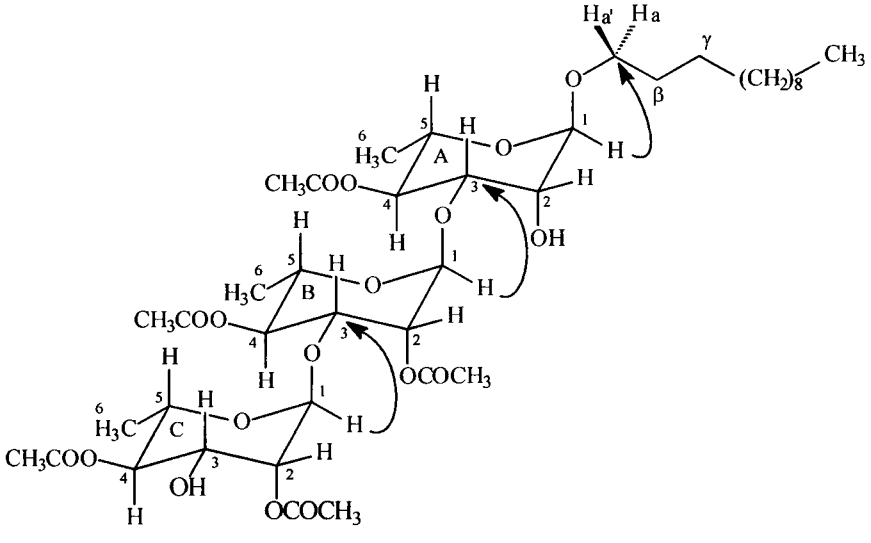
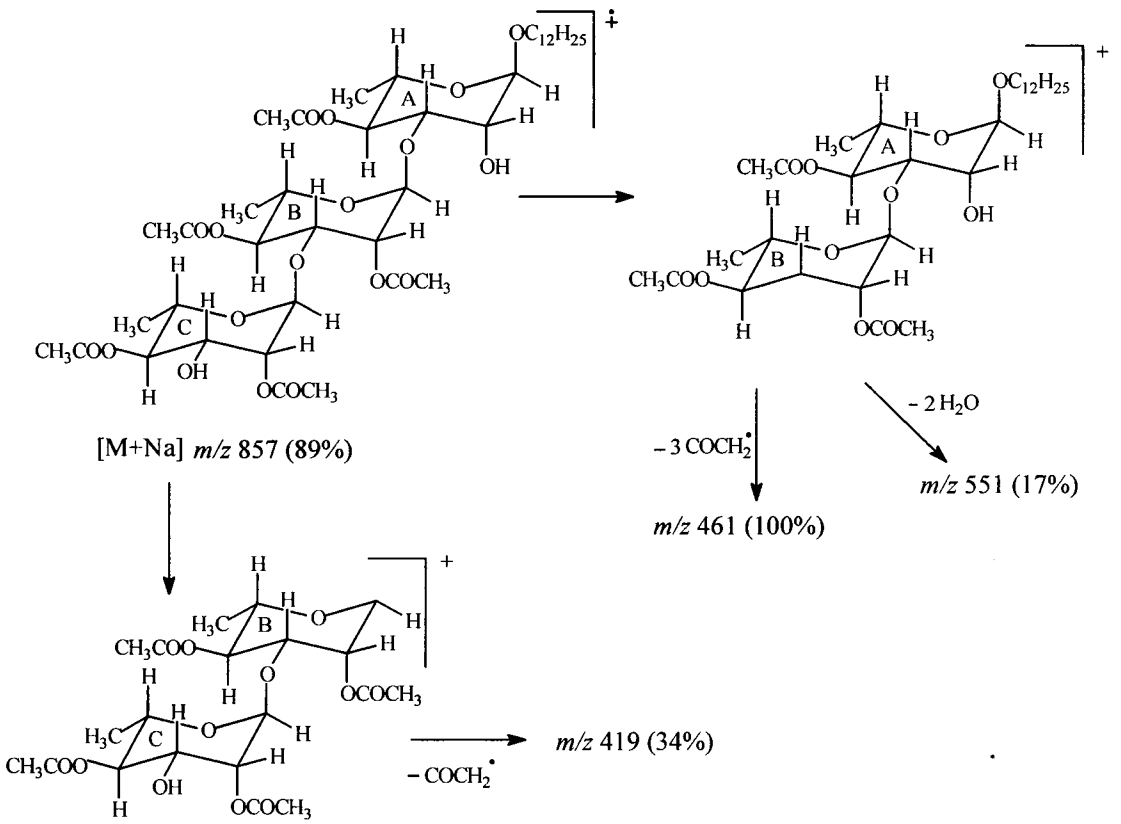


Figure 3.2: Significant HMBC correlations of OS-2

Consequently, **OS-2** was identified as the new 1-*O*-dodecanyl 2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside and given the trivial name cleistrioside-3.

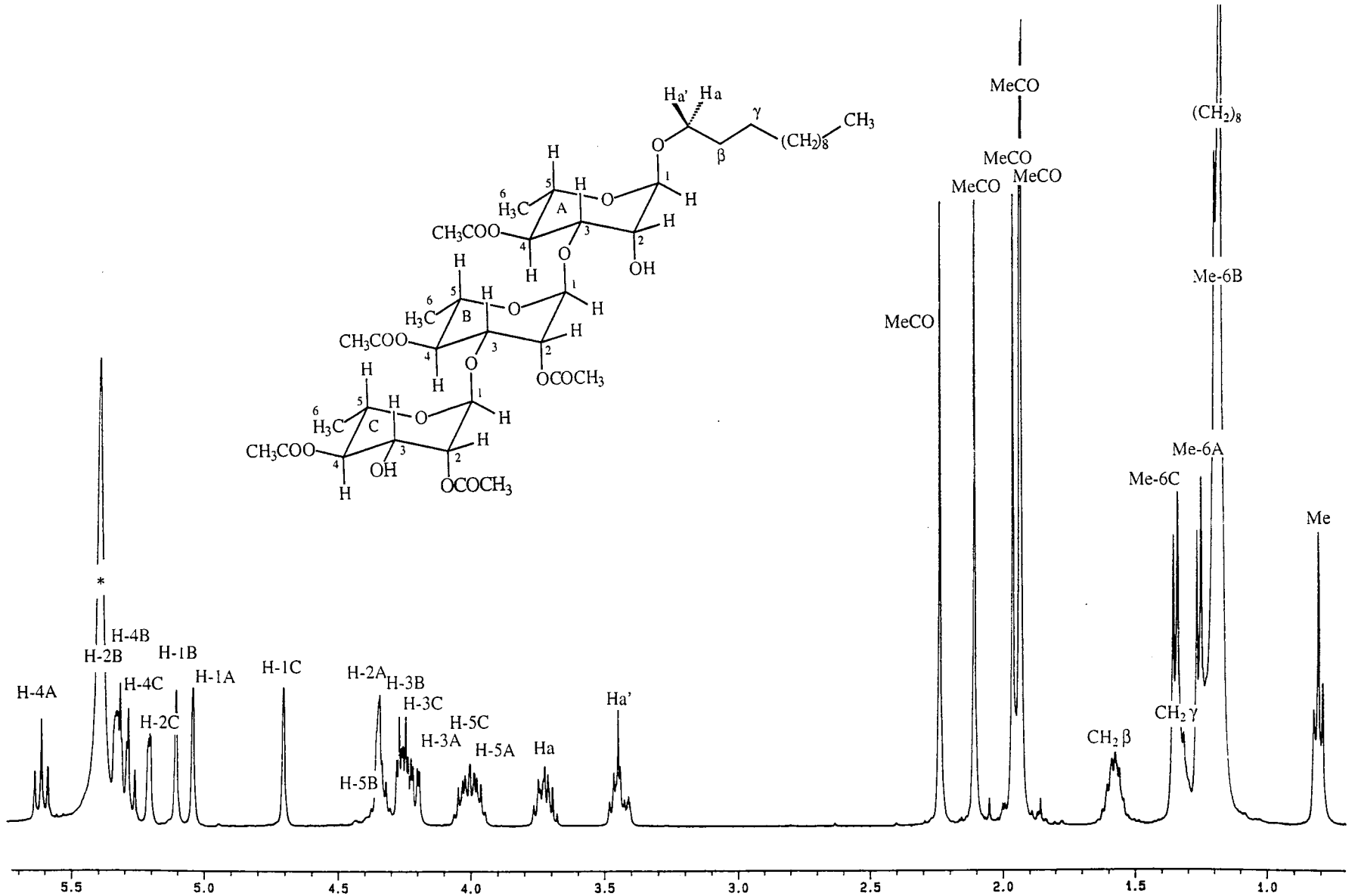


Scheme 3.2: Suggested mass fragmentation pattern of OS-2

Table 3.4: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-2

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	5.04 (d, 1.2)	101.7		67.8(C-5A), 68.6 (OCH_2), 80.4 (C-3A)
3A	4.21 (dd, 3.1, 9.8)	80.4	73.7 (C-4A)	100.9 (C-1B)
1B	5.11 (d, 1.2)	100.9	73.1 (C-2B)	67.9 (C-5B), 75.8 (C-3B), 80.4 (C-3A)
3B	4.26 (dd, 3.7, 9.9)	75.8		67.9 (C-5B),100.6 (C-1C)
1C	4.70 (d, 1.2)	100.6	74.1 (C-2C)	68.0 (C-5C), 68.4 (C-3C), 75.8 (C-3B)
OCH_2	3.73/3.45 (m)	68.6		101.7 (C-1A)
*Me*CO	2.24 (s)	21.4	171.4 (H-4A)	
	2.11 (s)*	21.3*	171.4 (H-2B/H-4B/H-2C/H-4C)*	
	1.96 (s)*	21.2*	171.3 (H-2B/H-4B/H-2C/H-4C)*	
	1.94 (s)*	21.1*	171.3 (H-2B/H-4B/H-2C/H-4C)*	
	1.93 (s)*	21.0*	171.2 (H-2B/H-4B/H-2C/H-4C)*	
4A	5.61 (t, 9.8)	73.7	67.8 (C-5A), 80.4 (C-3A)	171.4
2B	5.33 (dd, 1.2, 3.6)	73.1	75.8 (C-3B)	73.5 (C-4B), 171.4*
4B	5.29 (t, 10)	73.5	67.9 (C-5B), 75.8 (C-3B)	171.3*
2C	5.21 (dd, 1.2, obsc)	74.1	68.0 (C-3C)	75.2 (C-4C), 171.3*
4C	5.30 (t, 10)	75.2	68.4 (C-5C)	171.2*

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



Spectrum 3.5: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-2

*c) Identification of **OS-3** as cleistrioside-4*

This compound was isolated from the ethyl acetate extract of *Cleistopholis patens*. FABMS (Scheme 3.3) exhibited a quasi molecular ion $[M+Na]^+$ at *m/z* 815 indicating a molecular weight of 792, thus corresponding to the molecular formula $C_{38}H_{64}O_{17}$ (7 DBE). Peaks were observed at *m/z* 773 $[(M+Na)-COCH_2]^+$.

^1H (Spectrum 3.6) and ^{13}C NMR showed a trirhamnoside with four acetoxymethyls. Acetylated positions were attributed to two H-4 at δ 5.55 (t, J = 9.8 Hz), 5.24 (t, J = 9.9 Hz) and one H-2 at δ 5.34 (dd, J = 1.6, obsc Hz). A TOCSY experiment established the remaining acetylated position as one H-4 at δ 5.40. Characteristic features on the HMBC (Figure 3.3; Table 3.5) included:

i) ^3J interactions between H-3 at δ 4.27 and C-1 at δ 104.0 and between H-3 at δ 4.16 and C-1 at δ 100.9, thus establishing the interglycosidic links as (1→3) (1→3) between the three units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.71/3.43) and the anomeric C-1 at δ 101.7 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the four deshielded protons, now identified as H-4A/H-2B/H-4B and H-4C, and the acetoxycarbonyls.

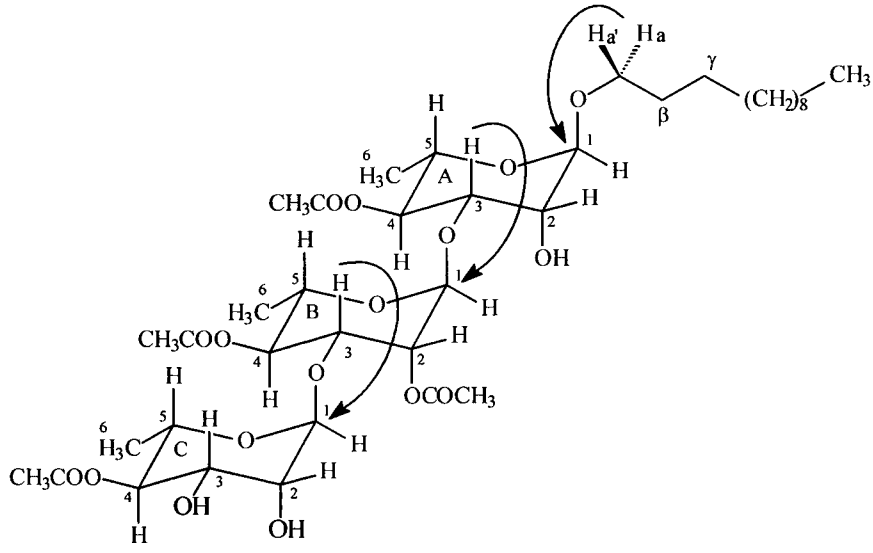
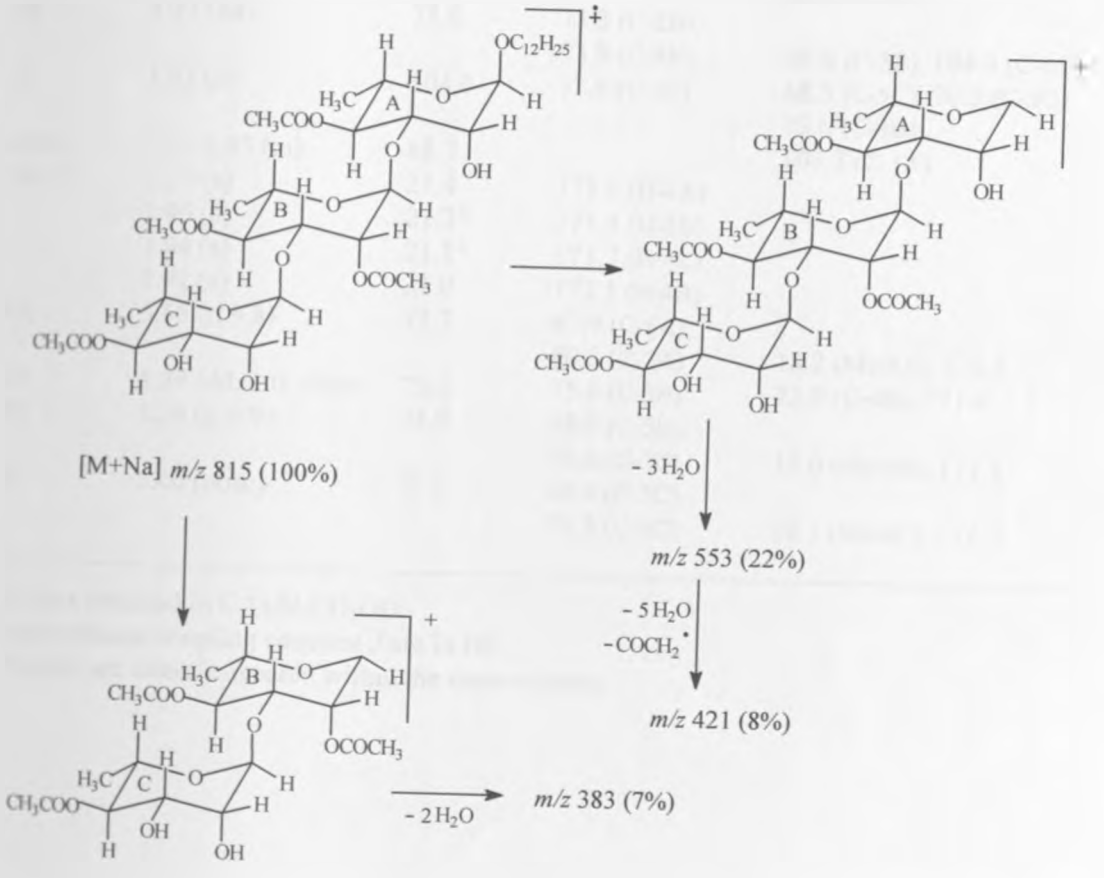


Figure 3.3: Significant HMBC correlations of OS-3

Consequently, **OS-3** was identified as the new 1-*O*-dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside and given the trivial name cleistrioside-4.

Data obtained from proton-coupled ^{13}C NMR experiments (see Section 3.1.1.1) are exemplified for this compound (Spectrum 3.7).

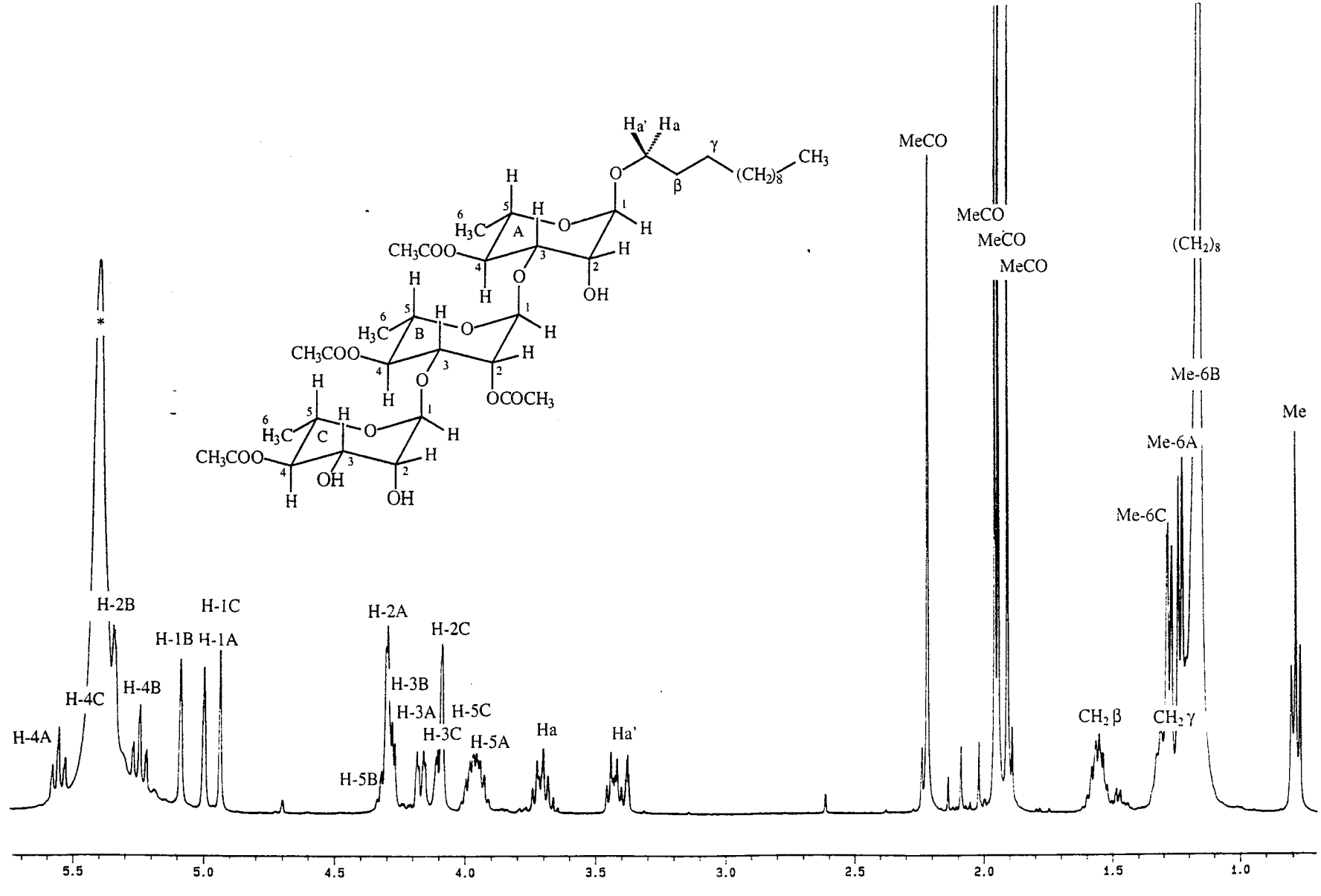


Scheme 3.3: Suggested mass fragmentation pattern of OS-3

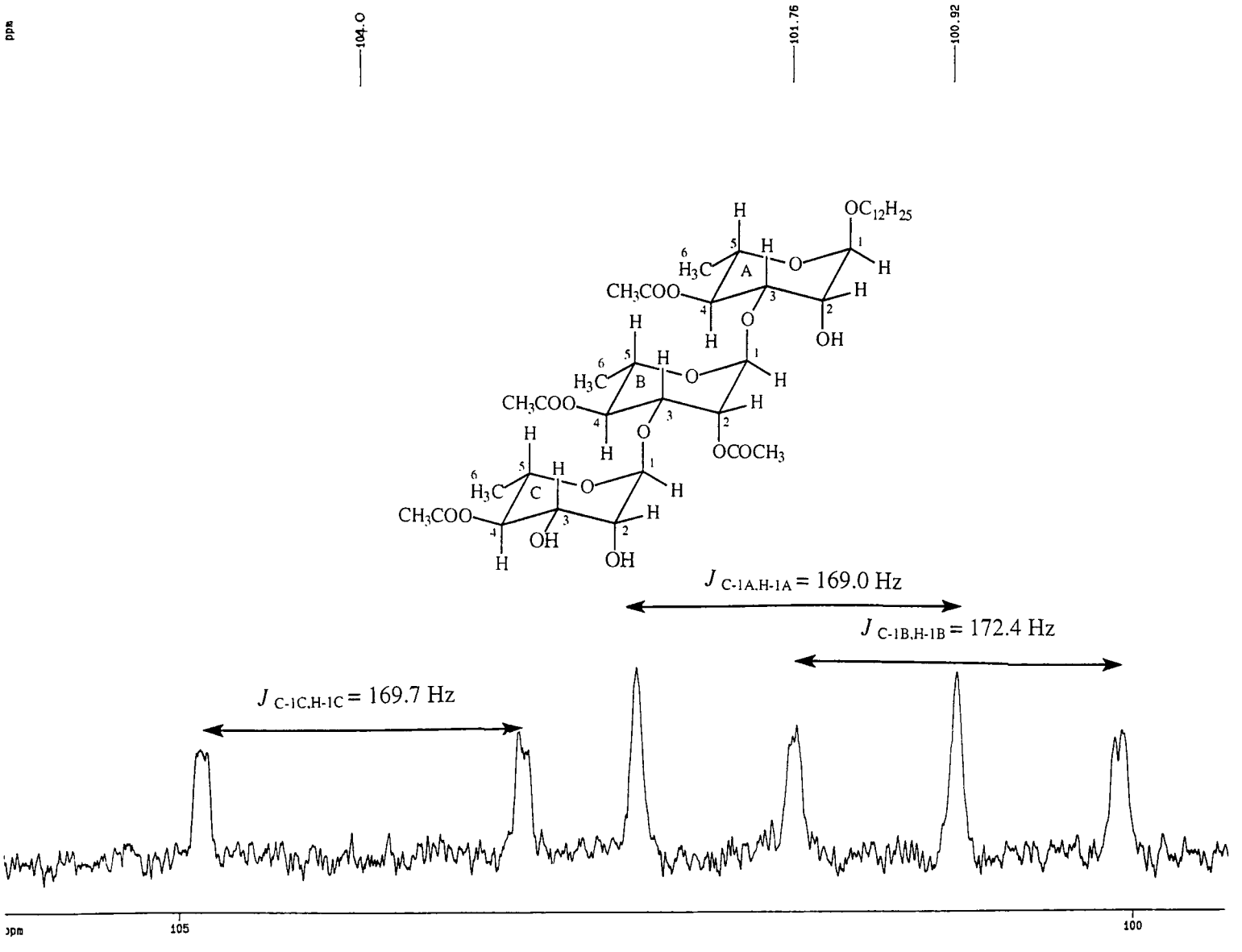
Table 3.5: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-3

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	4.99 (d, 1.5)	101.7	72.1 (C-2A)	67.9 (C-5A), 68.7 (OCH_2), 80.3 (C-3A)
3A	4.16 (dd, 2.9, 9.9)	80.3	72.1 (C-2A), 73.7 (C-4A)	67.9 (C-5A),100.9 (C-1B)
1B	5.08 (d, 1.5)	100.9	73.3 (C-2B)	68.0 (C-5B), 75.6 (C-3B), 80.3 (C-3A)
3B	4.27 (dd)	75.6	73.3 (C-2B), 73.9 (C-4B)	68.0 (C-5B), 104.0 (C-1C)
1C	4.93 (d)	104.0	72.6 (C-2C)	68.5 (C-5C), 70.3 (C-3C), 75.6 (C-3B)
OCH_2	3.71/3.43 (m)	68.7		101.7 (C-1A)
*Me*CO	2.21 (s)	21.4	171.5 (H-4A)	
	1.96 (s)	21.3*	171.4 (H-2B)	
	1.94 (s)	21.1*	171.7 (H-4C)	
	1.91 (s)	21.0	171.1 (H-4B)	
4A	5.55 (t, 9.8)	73.7	67.9 (C-5A), 80.3 (C-3A)	18.2 (Me-6A), 171.5
2B	5.34 (dd, 1.6, obsc)	73.3	75.6 (C-3B)	73.9 (C-4B), 171.4
4B	5.24 (t, 9.9)	73.9	68.0 (C-5B), 75.6 (C-3B)	18.0 (Me-6B), 171.1
4C	5.40 (obsc)	75.5	68.5 (C-5C), 70.3 (C-3C)	18.1 (Me-6C), 171.7

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



Spectrum 3.6: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-3



Spectrum 3.7: Proton-coupled ^{13}C NMR (100 MHz, C_5D_5N-CD_3OD) of OS-3 showing $^1J_{C\text{-}1,H\text{-}1}$ couplings

*d) Identification of **OS-4** as cleistrioside-1*

This compound was isolated from the petrol extract of *Cleistopholis glauca*. FABMS (Scheme 3.4) displayed a quasi molecular ion $[M+Na]^+$ at *m/z* 815 indicating a molecular weight of 792, thus corresponding to the molecular formula $C_{38}H_{64}O_{17}$ (7 DBE).

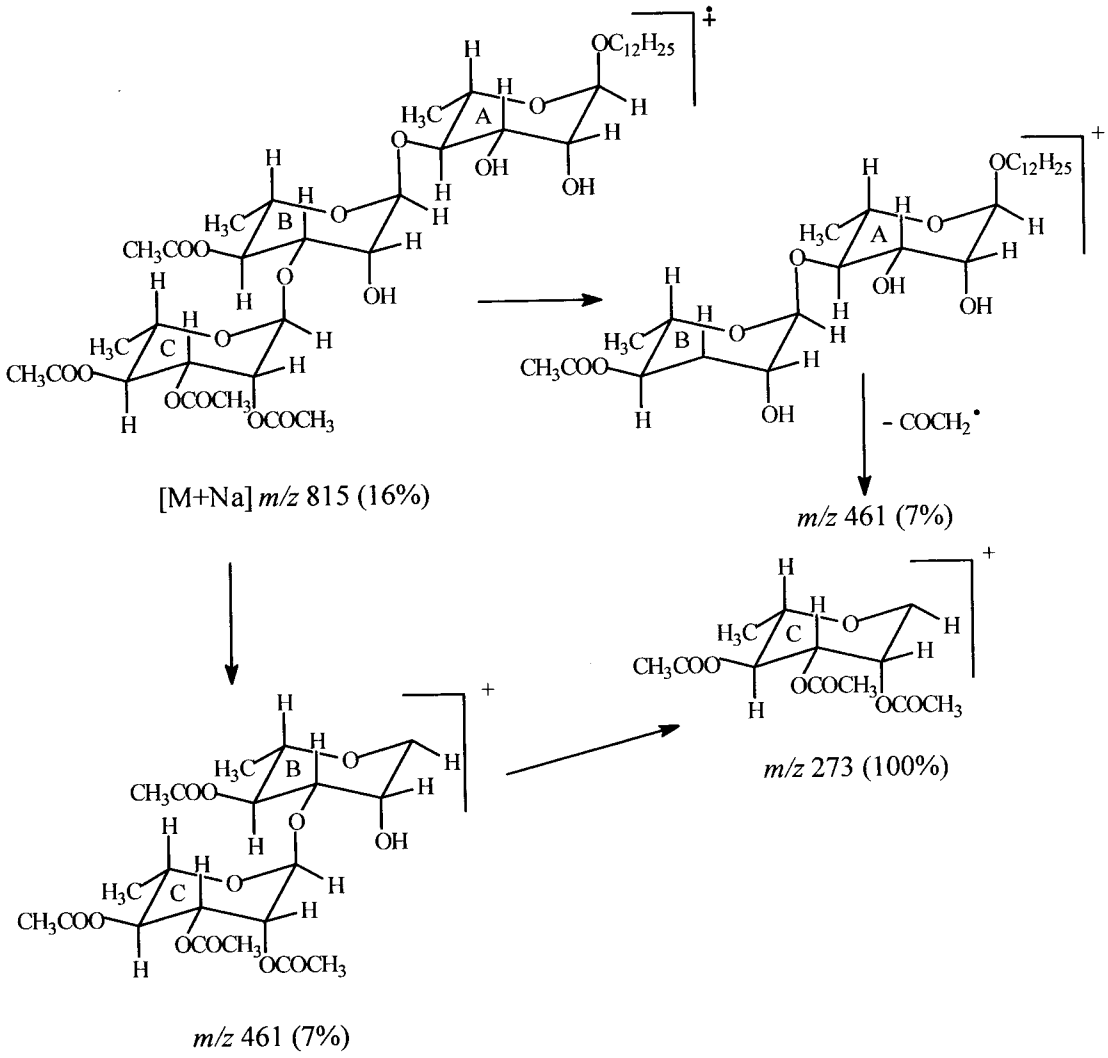
^1H (Spectrum 3.8) and ^{13}C NMR showed a trirhamnoside with four acetoxymethyls. Acetylated positions were attributed to two H-4 at δ 5.56 (t, J = 9.6 Hz), 5.22 (t, J = 10 Hz), one H-2 at δ 5.40 (brs) and one H-3 at δ 5.45 (dd, J = 3.3, 10 Hz). Characteristic features on the HMBC (Table 3.6) included:

i) ^3J interactions between H-3 at δ 4.21 and C-1 at δ 100.4 and between H-4 at δ 3.96 and C-1 at δ 103.7, thus establishing the interglycosidic links as (1→3) (1→4) between the three units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.67/3.36) and the anomeric C-1 at δ 101.7 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the four deshielded protons, now identified as H-4B/H-2C/H-3C and H-4C, and the acetoxycarbonyls

The physical and spectral data of **OS-4** showed agreement with those of the known 1-*O*-dodecanyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside, previously isolated from *Cleistopholis glauca* (Tané *et al.*, 1988b; Woods, 1989). As it was the first trirhamnoside reported in the genus, it was given the trivial name cleistrioside-1.

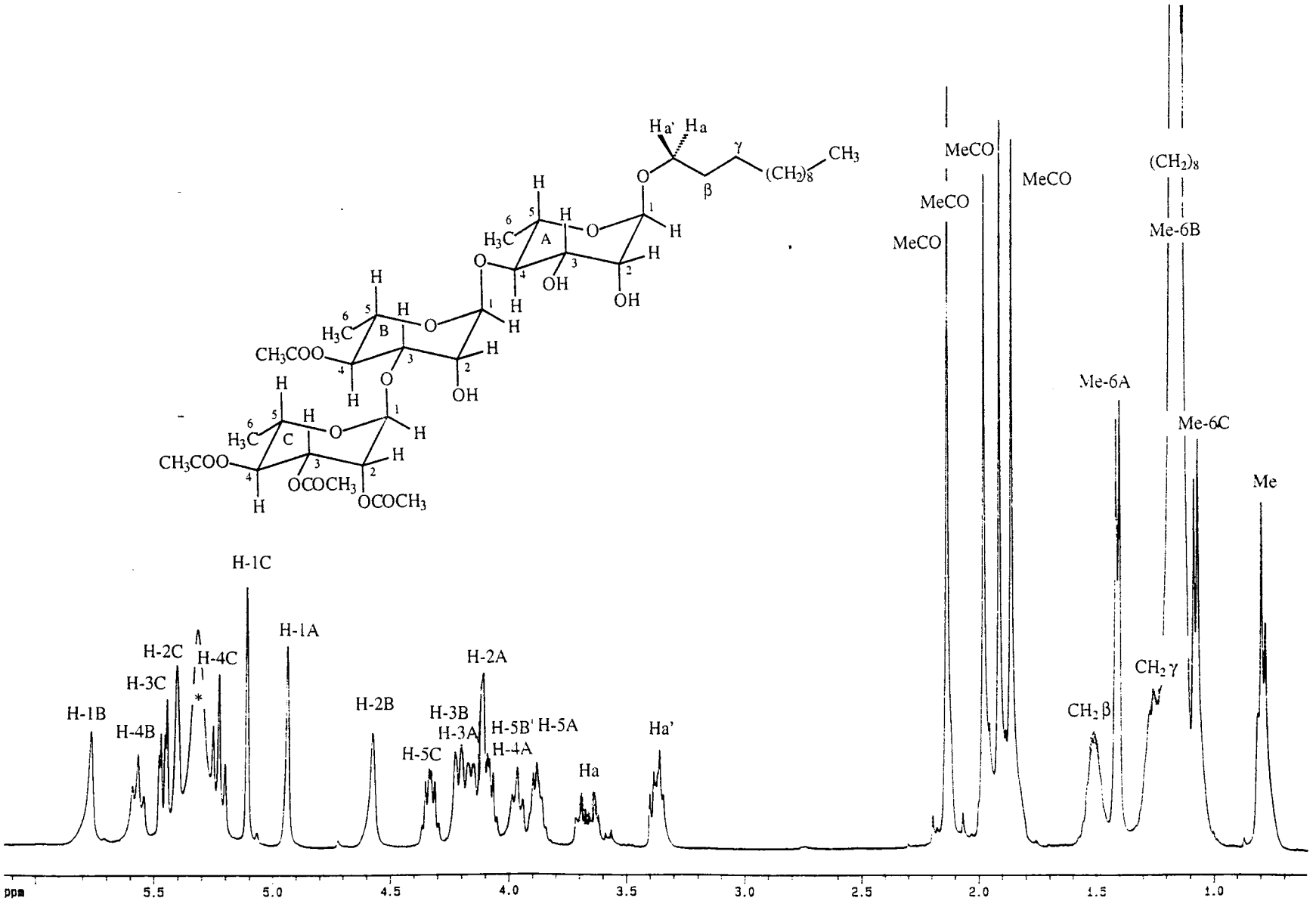


Scheme 3.4: Suggested mass fragmentation pattern of OS-4

Table 3.6: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-4

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	4.93 (brs)	101.7		68.4 (OCH_2)
4A	3.96 (t, 8.8)	81.4	68.1 (C-5A), 73.6 (C-3A)	103.7 (C-1B)
1B	5.76 (brs)	103.7		68.6 (C-5B), 79.9 (C-3B), 81.4 (C-4A)
3B	4.21 (dd, obsc, 9.8)	79.9	73.6 (C-4B)	100.4 (C-1C)
1C	5.11 (brs)	100.4		67.8 (C-5C), 70.3 (C-3C), 79.9 (C-3B)
OCH_2	3.67/3.36 (m)	68.4		101.7 (C-1A)
*Me*CO	2.14 (s)	21.3	171.2 (H-4B)	
	1.98 (s)	21.0	171.0 (H-2C)	
	1.92 (s)	21.0	170.9 (H-4C)	
	1.87 (s)	20.9	170.6 (H-3C)	
4B	5.56 (t, 9.6)	73.6	68.6 (C-5B), 79.9 (C-3B)	171.2
2C	5.40 (brs)	71.4	100.4 (C-1C)	171.0
3C	5.45 (dd, 3.3, 10)	70.3	71.4 (C-2C), 72.1 (C-4C)	100.4 (C-1C), 170.6
4C	5.22 (t, 10)	72.1	67.8 (C-5C)	170.9

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.



Spectrum 3.8: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-4

B) Partially acetylated 1-O-dodecanyl tetrarhamnosides

Six partially acetylated 1-*O*-dodecanyl tetrarhamnosides were isolated from *Cleistopholis glauca* and *Cleistopholis patens* (see Section 2.4.4). They were coded:

OS-5 = (CPE.1)

OS-6 = (CPE.9/CGE.22)

OS-7 = (CPE.4)

OS-8 = (CGP.25)

OS-9 = (CGE.23)

OS-10 = (CGE.21)

and identified respectively as :

1-*O*-dodecanyl 3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-1)

1-*O*-dodecanyl 2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-6)

1-*O*-dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-2)

1-*O*-dodecanyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-7)

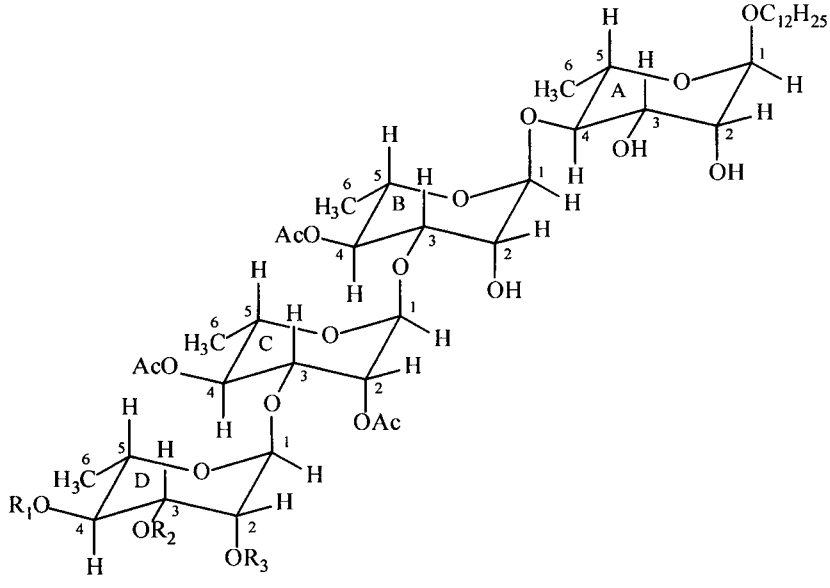
1-*O*-dodecanyl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-3)

1-*O*-dodecanyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-4) (Figure 3.4).

Characteristic features in the ^{1}H and ^{13}C NMR (Tables 3.7 to 3.10) included:

i) signals for four rhamnose units at δ 6.03-4.69 (1H each, d, J = *ca.* 1.5 Hz) and 1.54-1.06 (3H each, d, J = *ca.* 6.2 Hz) for four anomeric H-1 and four Me-6 substituents respectively.

ii) three to six acetoxymethyl resonances (δ 2.29-1.87).



Compound	R_1	R_2	R_3
OS-5	Ac	Ac	H
OS-6	Ac	H	Ac
OS-7	Ac	H	H
OS-8	Ac	Ac	Ac
OS-9	H	H	Ac
OS-10	H	H	H

Figure 3.4: Structures of OS-5 to OS-10

Table 3.7: ^{1}H NMR (400 MHz) spectral data of OS-5 to OS-7

Position	OS-5	OS-6	OS-7
1A	5.11 (d, 1.2)	4.97 (d)	4.95 (d, 1.2)
2A	4.30 (dd, 1.5, 3.4)	4.15 (dd, 1.6, 3.4)	4.12 (dd, 1.2, 3.0)
3A	4.36 (dd, 3.4, 9.3)	4.22 (dd)	4.18 (dd, 3.0, 9.4)
4A	4.18 (t, 9.4)	4.02 (t, 9.2)	3.99 (t, 9.4)
5A	4.04 (m)	3.93 (m)	3.91 (m)
Me-6A	1.54 (d, 6.2)	1.45 (d, 6.1)	1.43 (d, 6)
1B	6.03 (d, 1.5)	5.83 (d)	5.80 (d, 1.2)
2B	4.77 (dd, 1.5, 3.1)	4.60 (dd)	4.57 (dd, 1.2, 3.3)
3B	4.36 (dd)	4.22 (dd)	4.18 (dd, 3.3, 9.8)
4B	5.76 (t, 9.8)	5.61 (t, 9.8)	5.57 (t, 9.8)
5B	4.21 (m)	4.13 (m)	4.11 (m)
Me-6B	1.27 (d, 6.2)	1.20 (d, 6.3)	1.17 (obsc)
1C	5.23 (d, 1.2)	5.08 (d)	5.07 (d, 1.2)
2C	5.50 (dd, 1.6, 3.5)	5.30 (dd, obsc, 3.5)	5.31 (obsc)
3C	4.38 (dd)	4.24 (dd)	4.24 (dd, 3.4, 9.9)
4C	5.38 (t, 9.9)	5.24 (t, 9.9)	5.19 (t, 9.9)
5C	4.39 (m)	4.29 (m)	4.27 (m)
Me-6C	1.14 (d, 6.2)	1.10 (d, 6.2)	1.06 (d, 6.1)
1D	5.02 (d, 1.6)	4.69 (d)	4.90 (d, 1.1)
2D	4.37 (dd)	5.17 (dd)	4.05 (dd)
3D	5.41 (dd, 3.1, 10)	4.22 (dd)	4.08 (dd, 3.3, 9.6)
4D	5.67 (t, 9.9)	5.27 (t, 9.8)	5.36 (t, 9.6)
5D	4.19 (m)	3.99 (m)	3.94 (m)
Me-6D	1.36 (d, 6.2)	1.31 (d, 6.3)	1.25 (d, 6.2)
*Me*CO	2.29 (s)	2.23 (s)	2.21 (s)
	2.04 (s)	2.09 (s)	1.94 (s)
	2.01 (s)	1.96 (s)	1.92 (s)
	2.00 (s)	1.94 (s)	1.87 (s)
	1.87 (s)	1.93 (s)	-
OCH_2	3.78/3.45 (m)	3.71/3.39 (m)	3.69/3.37 (m)
$CH_2\beta$	1.57 (m)	1.53 (m)	1.52 (m)
$CH_2\gamma$	1.31 (m)	1.27 (m)	1.26 (m)
$(CH_2)_8$	1.21 (brs)	1.17 (brs)	1.17 (brs)
Me	0.84 (t, 6.8)	0.81 (t, 6.8)	0.80 (t, 6.8)

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
Non equivalent oxymethylene signals are listed as Ha/Ha' in each column.

Table 3.8: ^{1}H NMR (400 MHz) spectral data of OS-8 to OS-10

Position	OS-8	OS-9	OS-10
1A	4.93 (d, 1.6)	5.04 (d)	4.95 (d)
2A	4.10 (dd, 1.6, 3.4)	4.22 (dd)	4.13 (dd)
3A	4.16 (dd, 3.6, 9)	4.30 (dd)	4.17 (dd)
4A	3.96 (t, 9.1)	4.11 (t, 9.3)	3.99 (t, 9.1)
5A	3.89 (m)	3.98 (m)	3.90 (m)
Me-6A	1.43 (d, 6)	1.49 (d, 6.1)	1.43 (d, 5.6)
1B	5.76 (d, 1.6)	5.93 (d, 1.1)	5.80 (d)
2B	4.55 (dd)	4.69 (dd)	4.57 (dd)
3B	4.19 (dd, 3.3, 10)	4.28 (dd)	4.19 (dd, 3.4, 9.7)
4B	5.55 (t, 9.9)	5.70 (t, 9.8)	5.58 (t, 9.8)
5B	4.10 (m)	4.20 (m)	4.12 (m)
Me-6B	1.19 (d, 6.2)	1.24 (d, 6.2)	1.19 (obsc)
1C	5.08 (d)	5.15 (d)	5.09 (d)
2C	5.31 (dd, 1.6, 3.5)	5.38 (dd, 1.3, 3.3)	5.34 (obsc)
3C	4.27 (dd, 3.4, 9.8)	4.26 (dd)	4.26 (dd, 3.5, 9.9)
4C	5.24 (t, 10)	5.29 (t, 9.9)	5.21 (t, 9.9)
5C	4.27 (m)	4.34 (m)	4.28 (m)
Me-6C	1.12 (d, 6.2)	1.12 (d, 6.2)	1.08 (d, 6.2)
1D	4.78 (d, 1.4)	4.73 (d)	4.92 (d)
2D	5.25 (dd)	5.27 (dd)	4.06 (dd)
3D	5.35 (dd, 3.4, 10.2)	4.21 (dd)	3.99 (dd)
4D	5.24 (t, 10)	3.85 (t, 9.4)	3.84 (t)
5D	4.08 (m)	3.94 (m)	3.88 (m)
Me-6D	1.27 (d, 6.3)	1.54 (d, 6.1)	1.43 (d, 5.6)
*Me*CO	2.20 (s)	2.29 (s)	2.23 (s)
	2.08 (s)	2.11 (s)	1.89 (s)
	2.01 (s)	2.03 (s)	1.88 (s)
	2.01 (s)	1.90 (s)	-
	1.97 (s)	-	-
	1.90 (s)	-	-
OCH_2	3.69/3.38 (m)	3.73/3.42 (m)	3.68/3.38(m)
$CH_2\beta$	1.53 (m)	1.56 (m)	1.52 (m)
$CH_2\gamma$	1.27 (m)	1.31 (m)	1.27 (m)
$(CH_2)_8$	1.18 (brs)	1.18 (brs)	1.18 (brs)
Me	0.81 (t, 6.7)	0.82 (t, 6.7)	0.80 (t, 6.6)

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
Non equivalent oxymethylene signals are listed as Ha/Ha' in each column.

Table 3.9: ^{13}C NMR (100 MHz) spectral data of OS-5 to OS-7

Position	OS-5	OS-6	OS-7
1A	101.8	101.7	101.6
2A	73.1	73.0	73.0
3A	73.7	73.6	73.6
4A	81.3	81.4	81.3
5A	68.1	68.1	68.0
Me-6A	19.4	19.3	19.3
1B	103.6	103.6	103.5
2B	72.1	72.1	72.0
3B	80.5	80.3	80.3
4B	73.6	73.7	73.6
5B	68.4	68.5	68.4
Me-6B	18.2	18.1	18.1
1C	100.8	100.8	100.7
2C	72.9	73.2	73.2
3C	75.5	75.9	75.5
4C	73.7	73.5	73.7
5C	67.8	67.9	67.8
Me-6C	18.0	17.9	17.9
1D	103.3	100.6	103.9
2D	69.8	74.1	72.5
3D	73.3	67.9	70.2
4D	72.1	75.2	75.4
5D	68.5	68.3	68.4
Me-6D	18.1	18.0	18.1
*Me*CO	21.5	21.4	21.4
	21.1	21.3	21.4
	21.1	21.2	21.1
	21.1	21.1	21.0
	21.1	21.0	-
*CO*Me	171.2	171.4	171.4
	171.2	171.4	171.2
	170.9	171.3	171.2
	170.9	171.3	170.8
	170.7	171.2	-
OCH_2	68.3	68.4	68.3
$CH_2\beta$	30.4	30.4	30.4
$CH_2\gamma$	27.1	27.1	27.1
$(CH_2)_6$	30.5-30.1	30.5-30.2	30.5-30.2
CH_2	32.7	32.8	32.7
CH_2	23.5	23.5	23.5
Me	14.7	14.7	14.7

All data obtained in C_5D_5N-CD_3OD.

Table 3.10: ^{13}C NMR (100 MHz) spectral data of OS-8 to OS-10

Position	OS-8	OS-9	OS-10
1A	101.7	101.8	101.7
2A	73.0	73.1	73.0
3A	73.6	73.6	73.8
4A	81.4	81.4	81.4
5A	68.1	68.2	68.1
Me-6A	19.3	19.4	19.3
1B	103.6	103.6	103.6
2B	72.1	72.1	72.1
3B	80.1	80.4	80.2
4B	73.7	73.7	73.6
5B	68.5	68.6	68.5
Me-6B	18.1	18.2	18.2
1C	100.7	100.8	100.8
2C	72.8	73.4	73.5
3C	76.1	76.2	75.9
4C	73.4	73.5	73.8
5C	68.0	67.1	67.8
Me-6C	17.9	18.0	18.0
1D	100.0	101.2	104.4
2D	71.1	74.4	72.7
3D	70.0	70.4	72.6
4D	71.8	74.0	73.8
5D	68.3	70.9	71.0
Me-6D	17.9	18.5	18.5
*Me*CO	21.4	21.5	21.4
	21.1	21.3	21.0
	21.0	21.2	21.0
	21.0	21.0	-
	20.9	-	-
	20.9	-	-
*CO*Me	171.4	171.5	171.4
	171.4	171.4	171.3
	171.2	171.3	170.8
	171.1	171.2	-
	171.0	-	-
	170.8	-	-
OCH_2	68.4	68.4	68.4
$CH_2\beta$	30.5	30.4	30.3
$CH_2\gamma$	27.2	27.1	27.1
$(CH_2)_6$	30.6-30.2	30.6-30.2	30.6-30.2
CH_2	32.8	32.7	32.8
CH_2	23.6	23.5	23.5
Me	14.7	14.7	14.7

All data obtained in C_5D_5N-CD_3OD.

*a) Identification of **OS-5** as cleistetroside-1*

This compound was isolated from the ethyl acetate extract of *Cleistopholis patens*. FABMS (Scheme 3.5) exhibited a quasi molecular ion $[M+Na]^+$ at *m/z* 1003, indicating a molecular weight of 980, thus corresponding to the molecular formula $C_{46}H_{76}O_{22}$ (9 DBE). One peak was observed at *m/z* 961 $[(M+Na)-COCH_2]^+$.

^1H (Spectrum 3.9; Table 3.7) and ^{13}C (Table 3.9) NMR data showed a tetra-rhamnoside with five acetoxymethyls. Acetylated positions were attributed to three H-4 at δ 5.76 (t, J = 9.8 Hz), 5.38, 5.5.67 (t, J = 9.9 Hz), one H-3 at δ 5.41 (dd, J = 3.1, 10 Hz) and one H-2 at δ 5.50 (dd, J = 1.6, 3.5 Hz). Characteristic features on the HMBC (Table 3.11) included:

i) ^3J interactions between H-3 at δ 4.38 and C-1 at δ 103.3, between H-3 at δ 4.36 and C-1 at δ 100.8 and between H-4 at δ 4.18 and C-1 at δ 103.6, thus establishing the interglycosidic links as (1→3) (1→3) (1→4) between the four units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.78/3.45) and the anomeric C-1 at δ 101.8 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

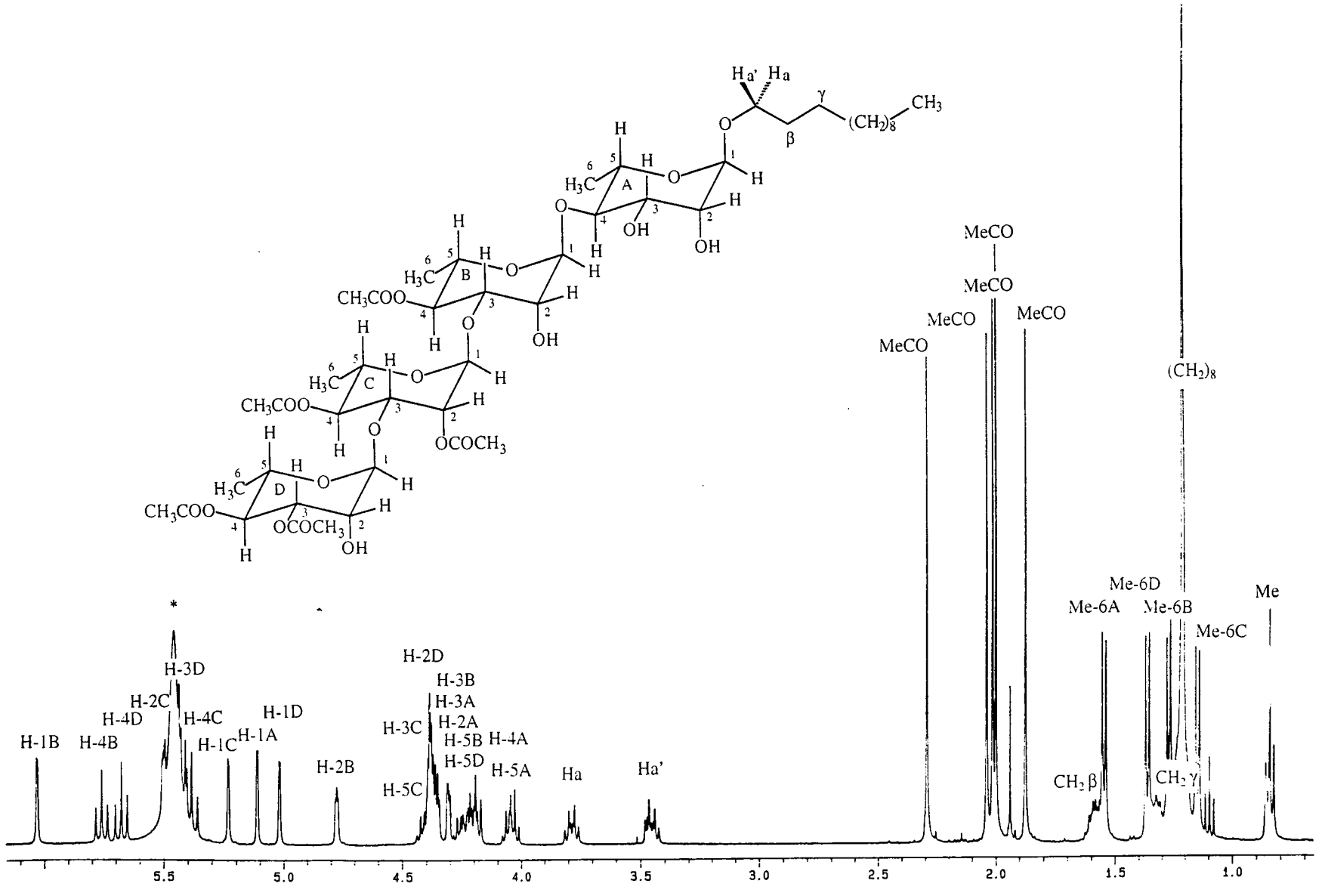
iii) ^3J-interactions between the five deshielded protons, now identified as H-4B/ H-2C/H-4C/H-3D and H-4D and the acetoxycarbonyls.

The physical and spectral data of **OS-5** showed agreement with those of the known 1-*O*-dodecanyl 3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamno-pyranoside, previously isolated from *Cleistopholis glauca* (Tané *et al.*, 1988b; Woods, 1989).

Table 3.11: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-5

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	5.11 (d, 1.2)	101.8		68.1(C-5A), 68.3 (OCH_2), 73.7 (C-3A)
4A	4.18 (t, 9.4)	81.3		19.4 (Me-6A), 103.6 (C-1B)
1B	6.03 (d, 1.5)	103.6	72.1 (C-2B)	68.4 (C-5B), 80.5 (C-3B), 81.3 (C-4A)
3B	4.36 (dd)	80.5		103.6 (C-1B)
1C	5.23 (d, 1.2)	100.8	72.9 (C-2C)	67.8 (C-5C), 75.5 (C-3C), 80.5 (C-3B)
3C	4.38 (dd)	75.5		103.3 (C-1D)
1D	5.02 (d, 1.6)	103.3	69.8 (C-2D)	68.5 (C-5D), 73.3 (C-3D), 75.5 (C-3C)
OCH_2	3.78/3.45 (m)	68.3		101.8
*Me*CO	2.29 (s)	21.5	171.2 (H-4B)	
	2.04 (s)	21.1	171.2 (H-2C)	
	2.01 (s)*	21.1	170.7* (H-4C)*	
	2.00 (s)*	21.1	170.9 (H-4D)*	
	1.87 (s)	21.1	170.9* (H-3D)*	
4B	5.76 (t, 9.8)	73.6	68.4 (C-5B), 80.5 (C-3B)	18.2 (Me-6B), 171.2
2C	5.50 (dd, 1.6, 3.5)	72.9	75.5 (C-3C)	171.2
4C	5.38 (t, 9.9)	73.7	67.8 (C-5C), 75.5 (C-3C)	18.0 (Me-6C), 170.7*
3D	5.41 (dd, 3.1, 10)	73.3	72.1 (C-4D)	170.9*
4D	5.67 (t, 9.9)	72.1	68.5 (C-5D), 73.3 (C-3D)	18.1 (Me-6D), 170.9

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



Spectrum 3.9: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-5

*b) Identification of **OS-6** as cleistetroside-6*

This compound was isolated from the ethyl acetate extract of *Cleistopholis patens*. FABMS (Scheme 3.5) yielded a quasi molecular ion $[M+Na]^+$ at *m/z* 1003, indicating a molecular weight of 980, thus corresponding to the molecular formula $C_{46}H_{76}O_{22}$ (9 DBE). Peaks were observed at *m/z* 961 $[(M+Na)-COCH_2]^+$, 919 $[(M+Na)-2\times COCH_2]^+$ and 877 $[(M+Na)-3\times COCH_2]^+$. ^1H (Spectrum 3.10; Table 3.7) and ^{13}C (Table 3.9) NMR data showed a tetrarhamnoside with five acetoxymethyls. Acetylated positions were attributed to three H-4 at δ 5.24 (t, J = 9.9 Hz), 5.61, 5.27 (t, J = 9.8 Hz) and two H-2 at δ 5.30 (dd, J = obsc, 3.5 Hz) and 5.17 (dd). Characteristic features on the HMBC (Figure 3.5; Table 3.12) included:

i) ^3J interactions between H-3 at δ 4.24 and C-1 at δ 100.6, between H-3 at δ 4.22 and C-1 at δ 100.8 and between H-4 at δ 4.02 and C-1 at δ 103.6, thus establishing the interglycosidic links as (1→3) (1→3) (1→4) between the four units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.71/3.39) and the anomeric C-1 at δ 101.7 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the five deshielded protons, now identified as H-4B/ H-2C/H-4C/H-2D and H-4D, and the acetoxycarbonyls.

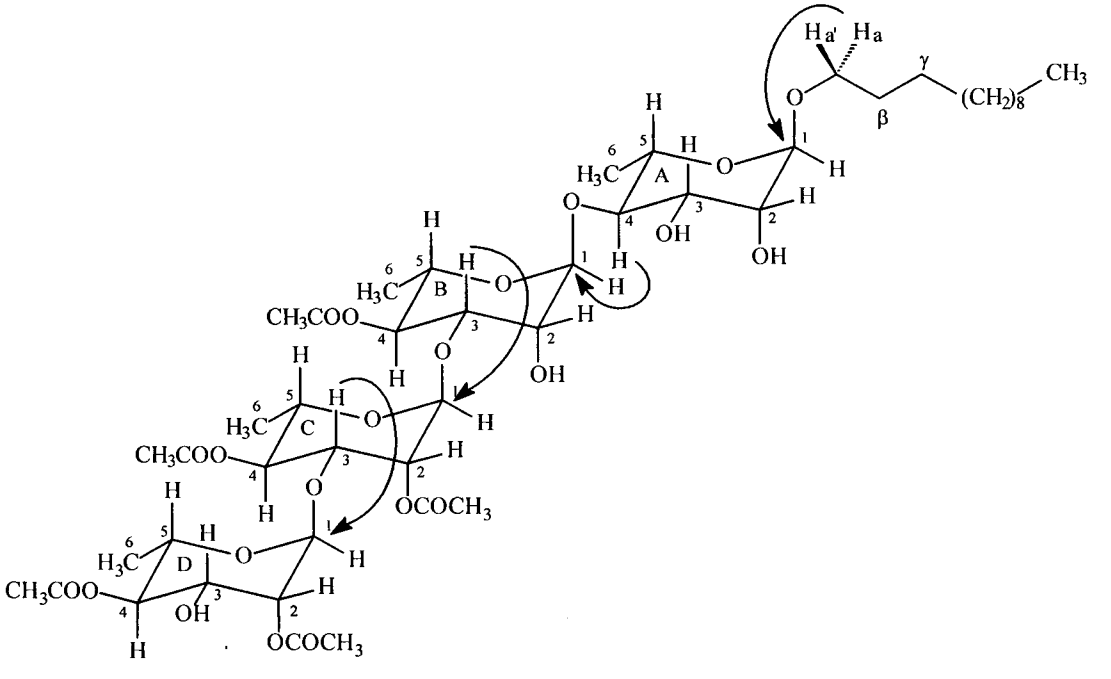
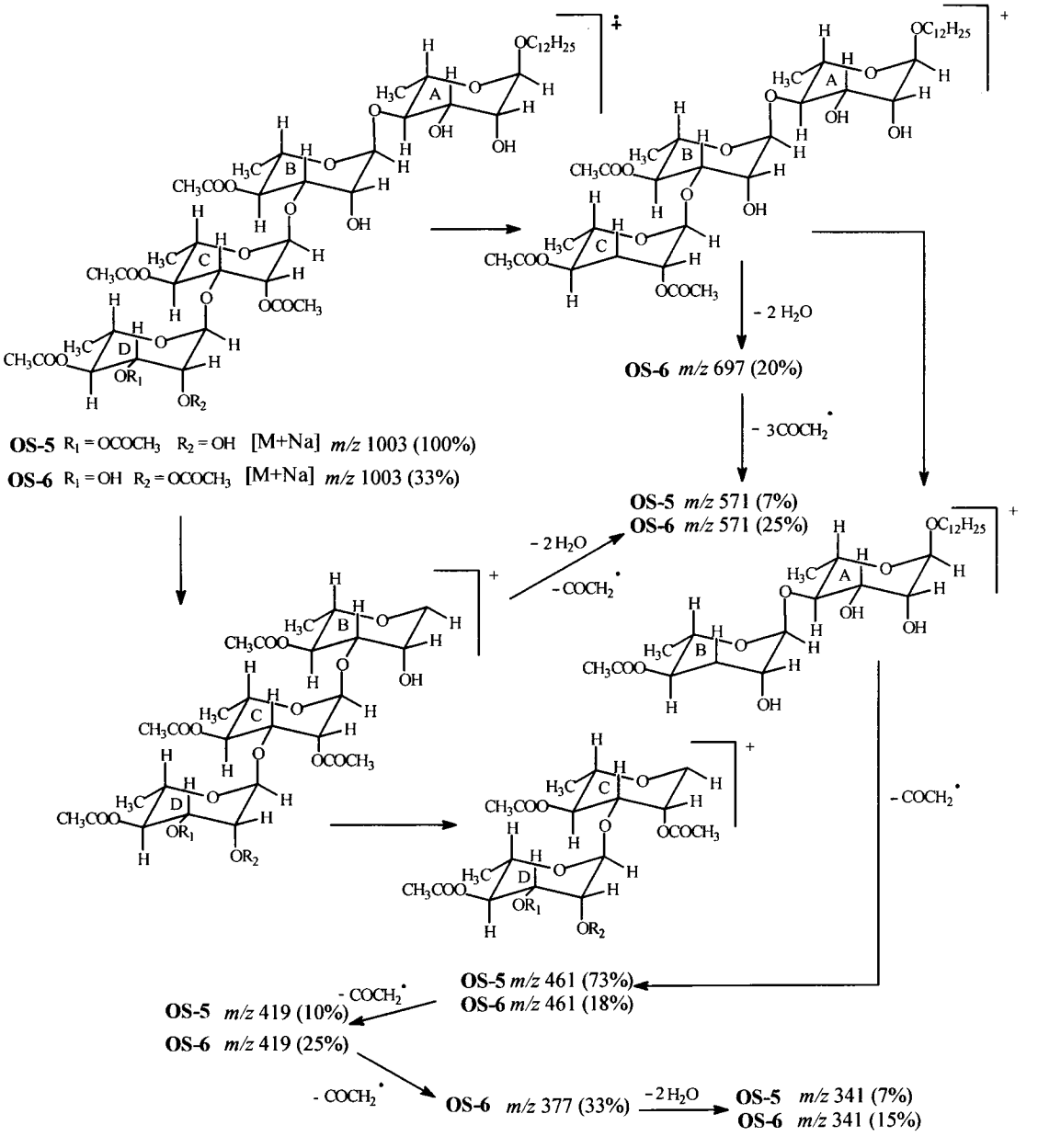


Figure 3.5: Significant HMBC correlations of OS-6

On this basis, **OS-6** was identified as the new 1-*O*-dodecanyl 2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside and given the trivial name cleistetroside-6.

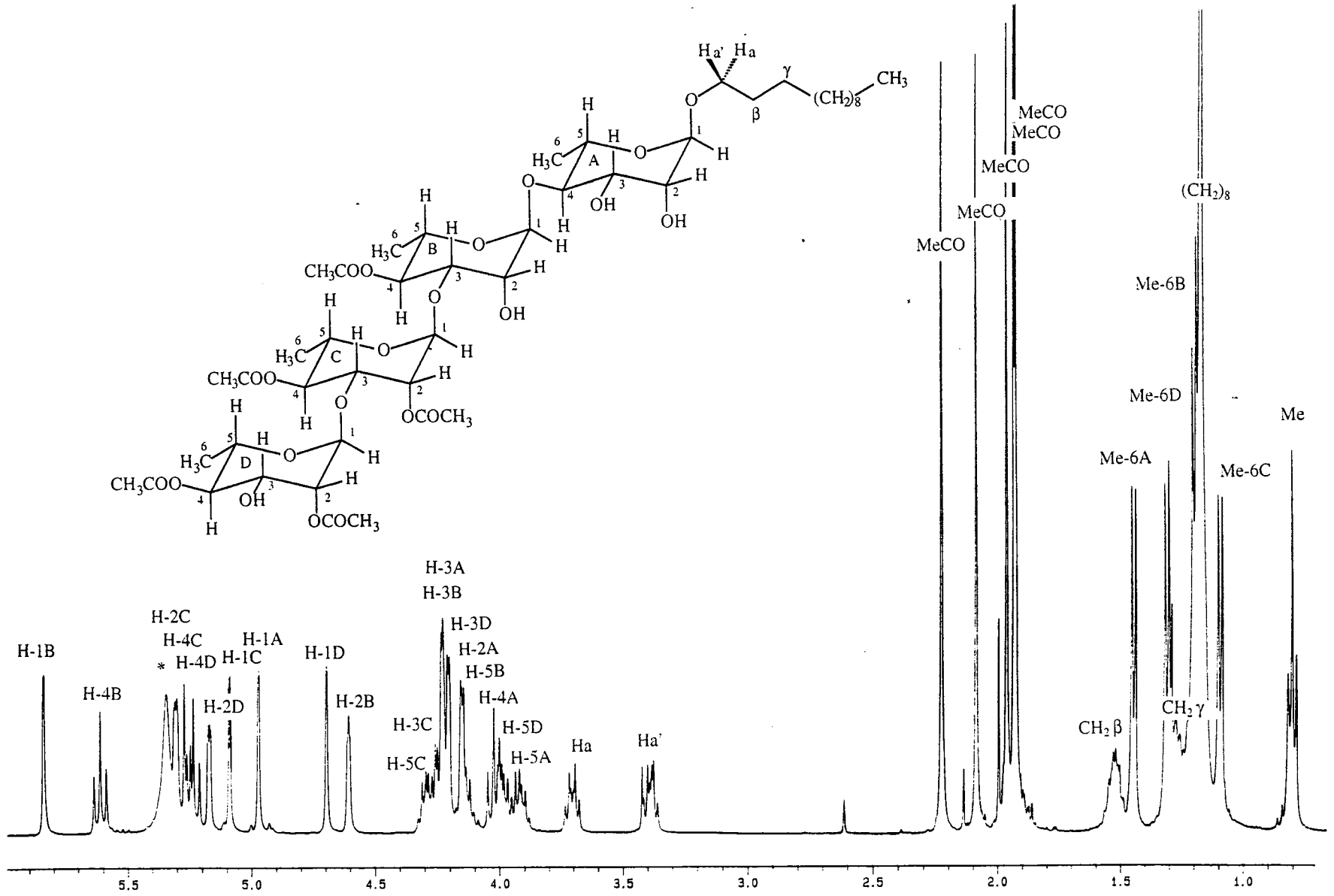


Scheme 3.5: Suggested mass fragmentation pattern of OS-5 and OS-6

Table 3.12: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-6

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	4.97 (d)	101.7		68.1(C-5A), 68.4 (OCH_2), 73.6 (C-3A)
4A	4.02 (t, 9.2)	81.4		103.6 (C-1B)
1B	5.83 (d)	103.6		68.5 (C-5B), 80.3 (C-3B), 81.4 (C-4A)
3B	4.22 (dd)	80.3		100.8 (C-1C),
1C	5.08 (d)	100.8		67.9 (C-5C), 75.9 (C-3C), 80.3 (C-3B)
3C	4.24 (dd)	75.9		100.6 (C-1D)
1D	4.69 (d)	100.6		67.9 (C-3D), 68.3 (C-5D), 75.9 (C-3C)
OCH_2	3.71/3.39 (m)	68.4		101.7
*Me*CO	2.23 (s)	21.4	171.3 (H-4B)	
	2.09 (s)	21.3*	171.2 (H-4C)	
	1.96 (s)	21.2*	171.3 (H-4D)	
	1.94 (s)*	21.1*	171.4 (H-2C)	
	1.93 (s)*	21.0*	171.4 (H-2D)	
4B	5.61 (t, 9.8)	73.7	68.5 (C-5B), 80.3 (C-3B)	171.3
2C	5.30 (dd, obsc, 3.5)	73.2	75.9 (C-3C)	171.4
4C	5.24 (t, 9.9)	73.5	67.9 (C-5C), 75.9 (C-3C)	171.2
2D	5.17 (dd)	74.1	67.9 (C-3D)	171.4, 75.2 (C-4D)
4D	5.27 (t, 9.8)	75.2	68.3 (C-5D), 67.9 (C-3D)	171.3

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column



Spectrum 3.10: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-6

c) Identification of ***OS-7*** *as cleistetroside-2*

This compound was isolated from the ethyl acetate extract of *Cleistopholis patens*. FABMS (Scheme 3.7) yielded a quasi molecular ion $[M+Na]^+$ at *m/z* 961, indicating a molecular weight of 938, thus corresponding to the molecular formula $C_{44}H_{74}O_{21}$ (8 DBE). Peaks were observed at *m/z* 919 $[(M+Na)-COCH_2]^+$ and 901 $[(M+Na)-COCH_2-H_2O]^+$.

^1H (Spectrum 3.11; Table 3.7) and ^{13}C (Table 3.9) NMR data showed a tetrarhamnoside with four acetoxymethyls. Acetylated positions were attributed to three H-4 at δ 5.57 (t, J = 9.8 Hz), 5.19 (t, J = 9.9 Hz), 5.36 (t, J = 9.6 Hz). A TOCSY experiment established the remaining acetylated position as one H-2 at δ 5.31. Characteristic features on the HMBC (Table 3.13) included:

i) ^3J interactions between H-3 at δ 4.24 and C-1 at δ 103.9, between H-3 at δ 4.18 and C-1 at δ 100.7 and between H-4 at δ 3.99 and C-1 at δ 103.5, thus establishing the interglycosidic links as (1→3) (1→3) (1→4) between the four units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.69/3.37) and the anomeric C-1 at δ 101.6 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the four deshielded protons, now identified as H-4B/H-2C/H-4C and H-4D and the acetoxycarbonyls.

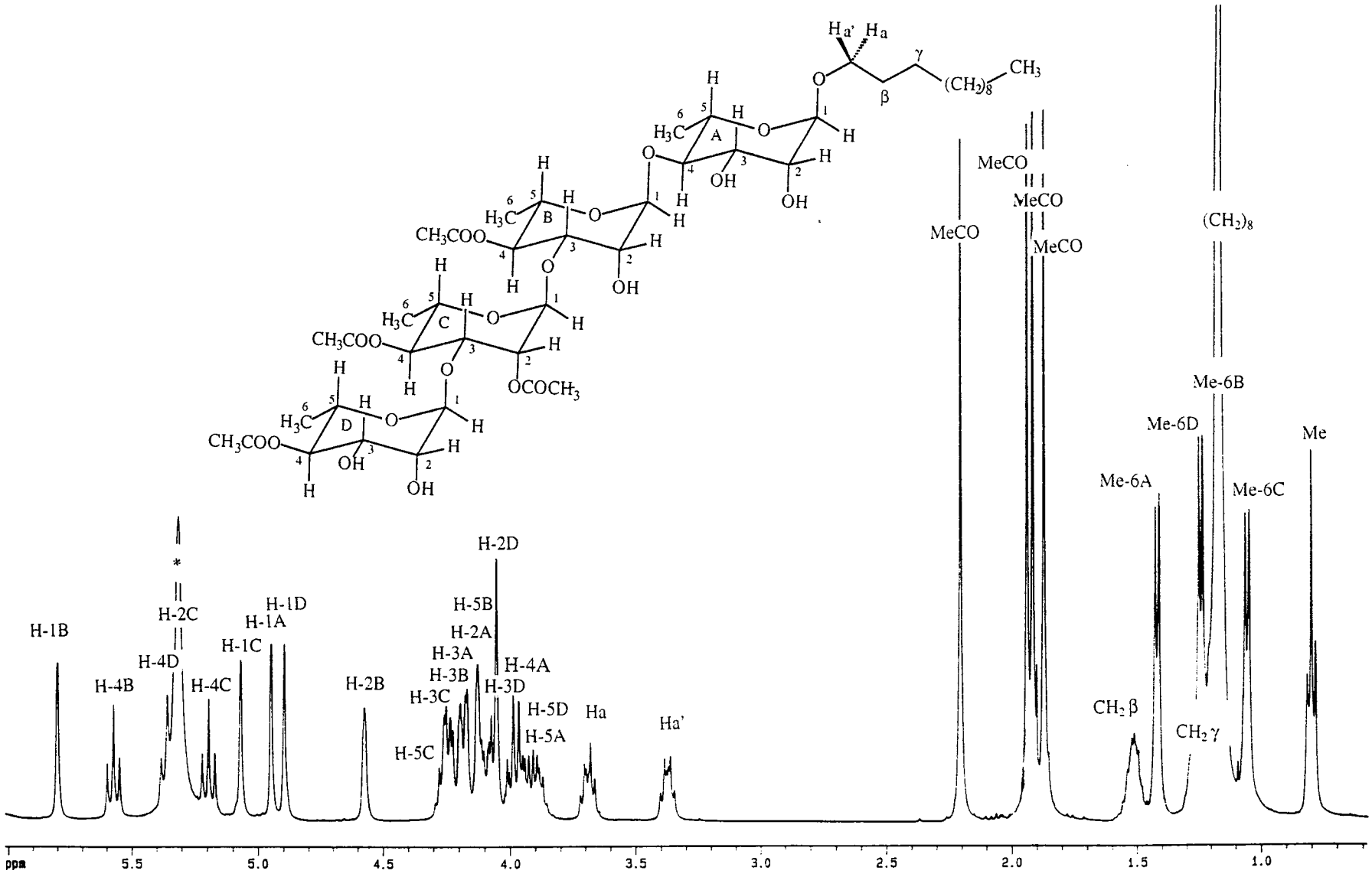
Comparison of the physical and spectral data of **OS-7** showed good agreement with those reported for the known 1-*O*-dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside, previously isolated from *Cleistopholis glauca* (Tané *et al.*, 1988b; Woods, 1989).

Data obtained in LR-COSY experiments (see Section 3.1.1.1) are exemplified for this compound (Spectrum 3.12).

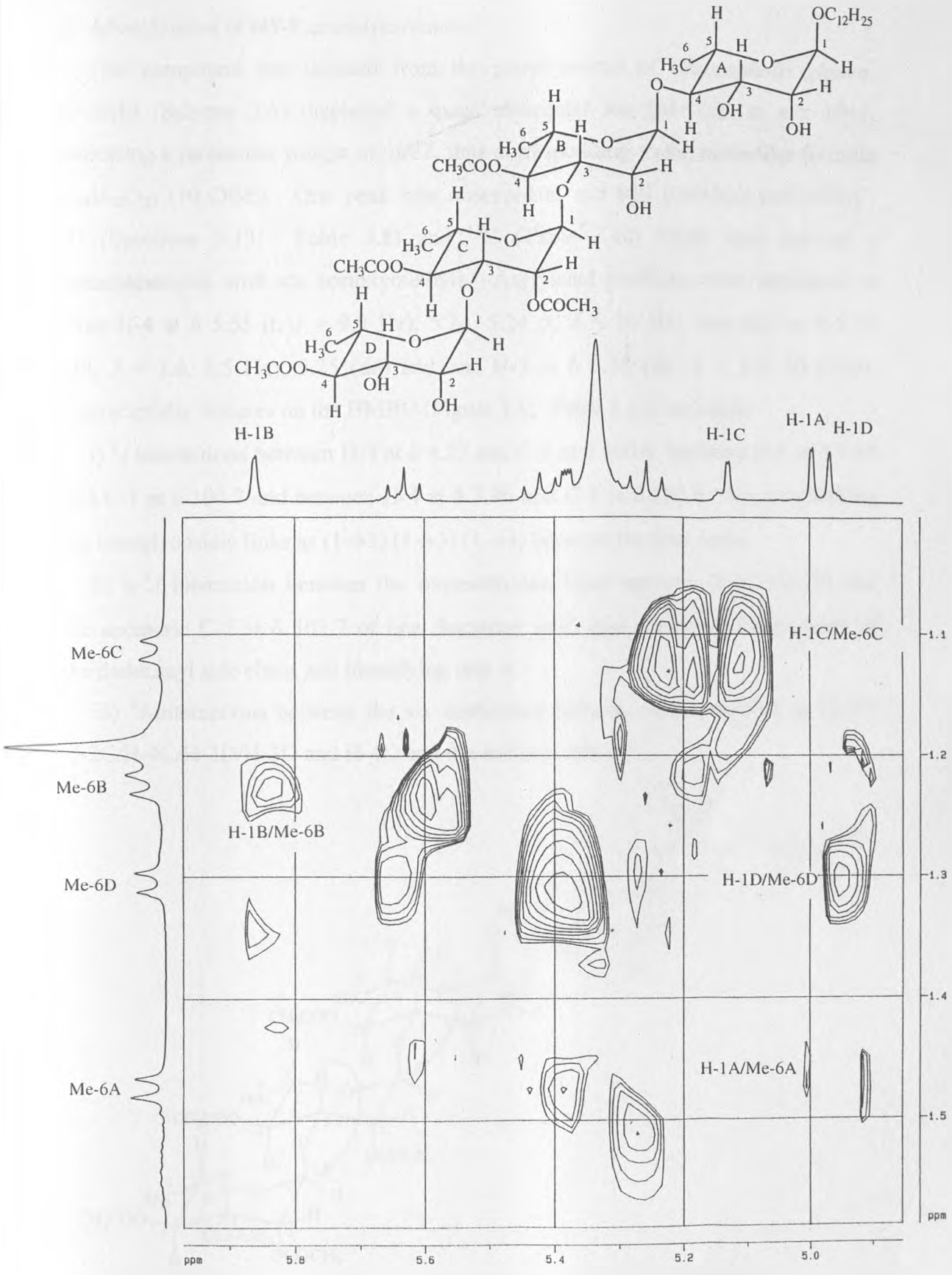
Table 3.13: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-7

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	4.95 (d, 1.2)	101.6		68.0 (C-5A), 68.3 (OCH_2) 73.6 (C-3A)
4A	3.99 (t, 9.4)	81.3	68.0 (C-5A)	103.5 (C-1B)
1B	5.80 (d, 1.2)	103.5	72.0 (C-2B)	68.4 (C-5B), 80.3 (C-3B), 81.3 (C-4A)
3B	4.18 (dd, 3.3, 9.8)	80.3		100.7 (C-1C)
1C	5.07 (d, 1.2)	100.7		67.8 (C-5C), 75.5 (C-3C), 80.3 (C-3B)
3C	4.24 (dd, 3.4, 9.9)	75.5	73.7 (C-4C)	103.9 (C-1D)
1D	4.90 (d, 1.2)	103.9	72.5 (C-2D)	68.4 (C-5D), 70.2 (C-3D), 75.5 (C-3C)
OCH_2	3.69/3.37 (m)	68.3		101.6
*Me*CO	2.21 (s)	21.4	171.2 (H-4B)	
	1.94 (s)	21.4	171.2 (H-2C)	
	1.92 (s)	21.1	171.4 (H-4D)	
	1.87 (s)	21.0	170.8 (H-4C)	
4B	5.57 (t, 9.8)	73.6	68.4 (C-5B), 80.3 (C-3B)	171.2
2C	5.31 (obsc)	73.2	75.5 (C-3C)	73.7 (C-4C), 171.2
4C	5.19 (t, 9.9)	73.7	67.8 (C-5C), 75.5 (C-3C)	170.8
4D	5.36 (t, 9.6)	75.4	68.4 (C-5D), 70.2 (C-3D)	171.4

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.



Spectrum 3.11: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-7



Spectrum 3.12: Significant LR-COSY correlations (400 MHz, C_5D_5N-CD_3OD) of OS-7 showing ^5J-bond couplings between H-1 and Me-6 protons

*d) Identification of **OS-8** as cleistetroside-7*

This compound was isolated from the petrol extract of *Cleistopholis glauca*. FABMS (Scheme 3.6) displayed a quasi molecular ion $[M+Na]^+$ at *m/z* 1045, indicating a molecular weight of 1022, thus corresponding to the molecular formula $C_{48}H_{78}O_{23}$ (10 DBE). One peak was observed at *m/z* 919 $[(M+Na)-3\times COCH_2]^+$. ^1H (Spectrum 3.13; Table 3.8) and ^{13}C (Table 3.10) NMR data showed a tetrarhamnoside with six acetoxymethyls. Acetylated positions were attributed to three H-4 at δ 5.55 (t, *J* = 9.9 Hz), 5.24, 5.24 (t, *J* = 10 Hz), two H-2 at δ 5.31 (dd, *J* = 1.6, 3.5 Hz), 5.25 (dd) and one H-3 at δ 5.35 (dd, *J* = 3.4, 10.2 Hz). Characteristic features on the HMBC (Figure 3.6; Table 3.14) included:

i) ^3J interactions between H-3 at δ 4.27 and C-1 at δ 100.0, between H-3 at δ 4.19 and C-1 at δ 100.7 and between H-4 at δ 3.96 and C-1 at δ 103.6, thus establishing the interglycosidic links as (1→3) (1→3) (1→4) between the four units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.69/3.38) and the anomeric C-1 at δ 101.7 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the six deshielded protons, now identified as H-4B/ H-2C/H-4C/H-2D/H-3D and H-4D and the acetoxycarbonyls.

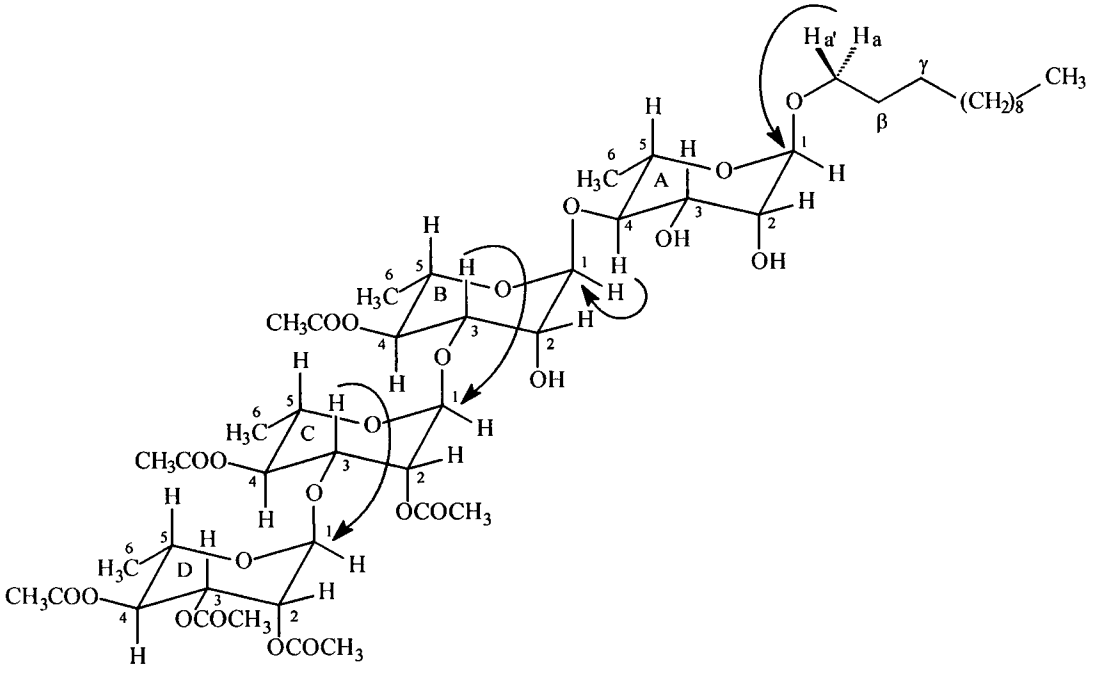
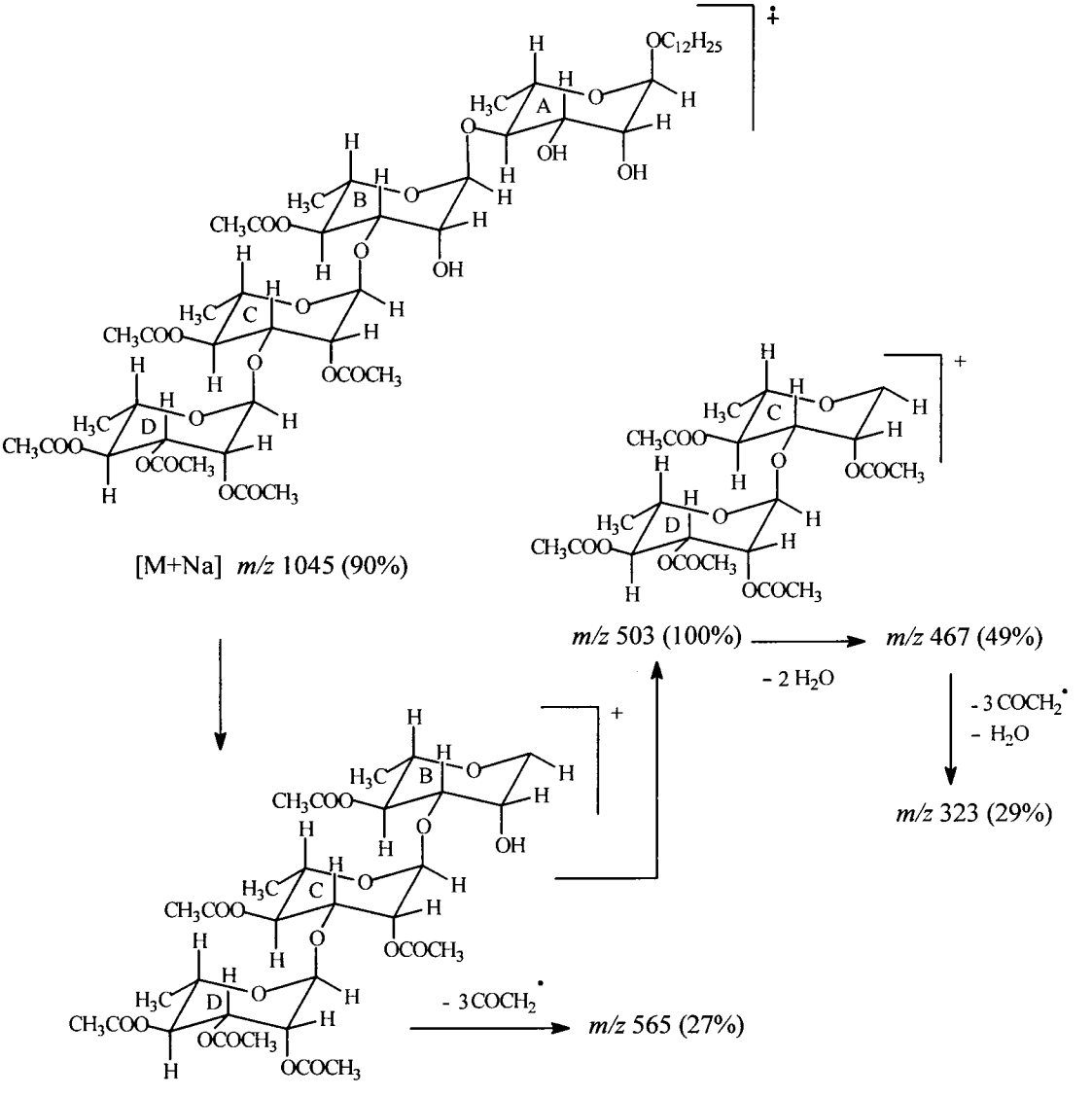


Figure 3.6: Significant HMBC correlations of OS-8

On this basis, **OS-8** was identified as the new 1-*O*-dodecanyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside and given the trivial name cleistetroside-7.

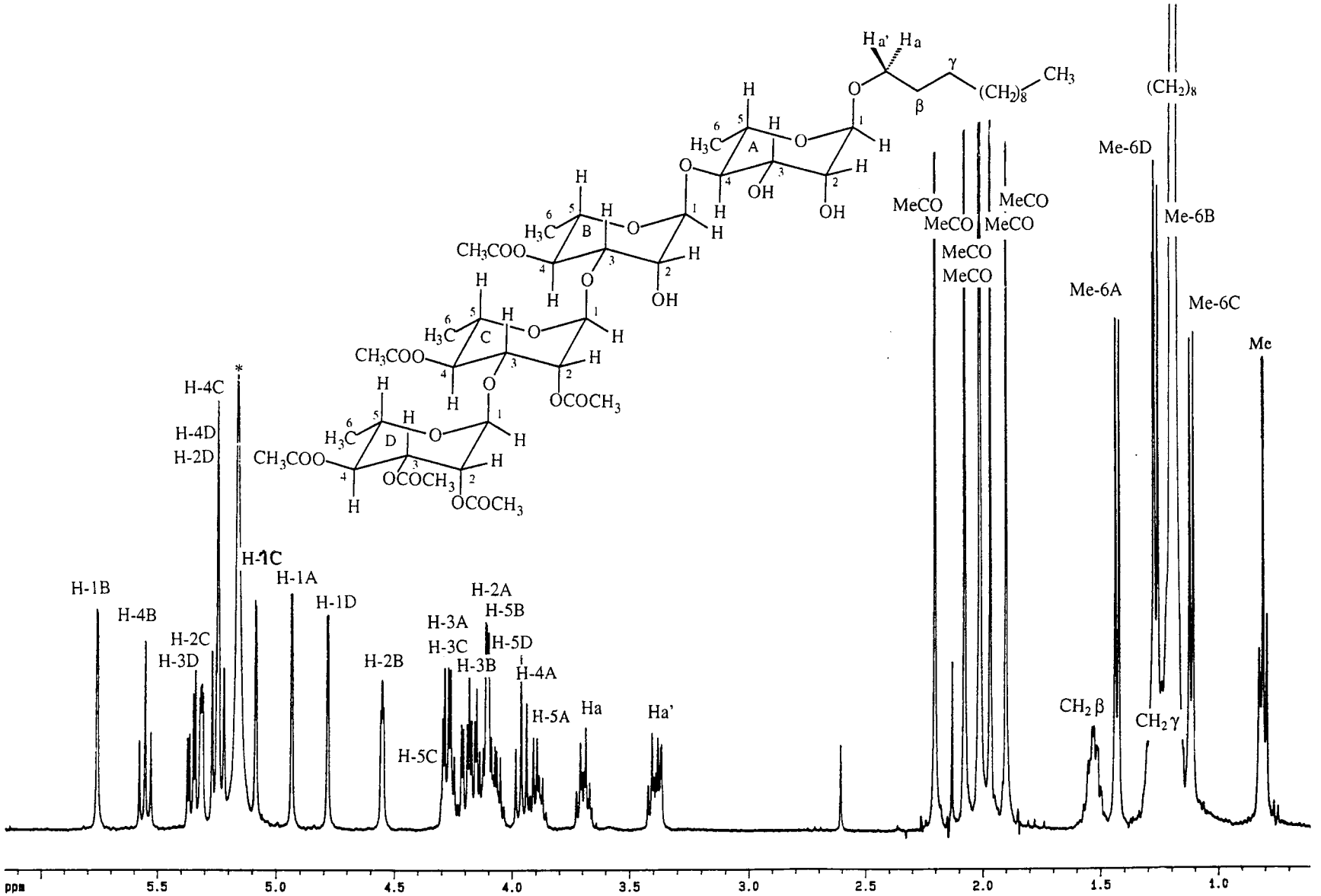


Scheme 3.6: Suggested mass fragmentation pattern of OS-8

Table 3.14: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-8

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	4.93 (d, 1.6)	101.7		68.1(C-5A), 68.4 (OCH_2), 73.6 (C-3A)
4A	3.96 (t, 9.1)	81.4	68.1 (C-5A)	19.3 (Me-6A), 103.6 (C-1B)
1B	5.76 (d, 1.6)	103.6	72.1 (C-2B)	68.5 (C-5B), 80.1 (C-3B), 81.4 (C-4A)
3B	4.19 (dd, 3.3, 10)	80.1		100.7 (C-1C)
1C	5.08 (d)	100.7	72.8 (C-2C)	68.0 (C-5C), 76.1 (C-3C), 80.1 (C-3B)
3C	4.27 (dd, 3.4, 9.8)	76.1		100.0 (C-1D)
1D	4.78 (d, 1.4)	100.0		68.3 (C-5D), 70.0 (C-3D), 76.1 (C-3C)
OCH_2	3.69/3.38 (m)	68.4		101.7
*Me*CO	2.20 (s)	21.4	171.4 (H-4B)	
	2.08 (s)*	21.1*	171.4 (H-2C)	
	2.01 (s)*	21.0*	171.2 (H-2D)	
	2.01 (s)*	21.0*	171.1* (H-4C)*	
	1.97 (s)*	20.9*	171.0* (H-4D)*	
	1.90 (s)	20.9*	170.8 (H-3D)	
4B	5.55 (t, 9.9)	73.7	68.5 (C-5B), 80.1 (C-3B)	18.1 (Me-6B), 171.4
2C	5.31 (dd, 1.6, 3.5)	72.8	76.1 (C-3C)	73.4 (C-4C), 171.4
4C	5.24 (t, 10)	73.4	68.0 (C-5C), 76.1 (C-3C)	17.9 (Me-6C), 171.0*
2D	5.25 (dd)	71.1	70.0 (C-3D)	171.2
3D	5.35 (dd, 3.4, 10.2)	70.0	71.8 (C-4D)	170.8
4D	5.24 (t, 10)	71.8	68.3 (C-5D), 70.0 (C-3D)	17.9 (Me-6D), 171.1*

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



Spectrum 3.13: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-8

*e) Identification of **OS-9** as cleistetroside-3*

This compound was isolated from the petrol extract of *Cleistopholis glauca.* FABMS (Scheme 3.7) displayed a quasi molecular ion $[M+Na]^+$ at *m/z* 961, indicating a molecular weight of 938, thus corresponding to the molecular formula $C_{44}H_{74}O_{21}$ (8 DBE). One peak was observed at *m/z* 919 $[(M+Na)-COCH_2]^+$.

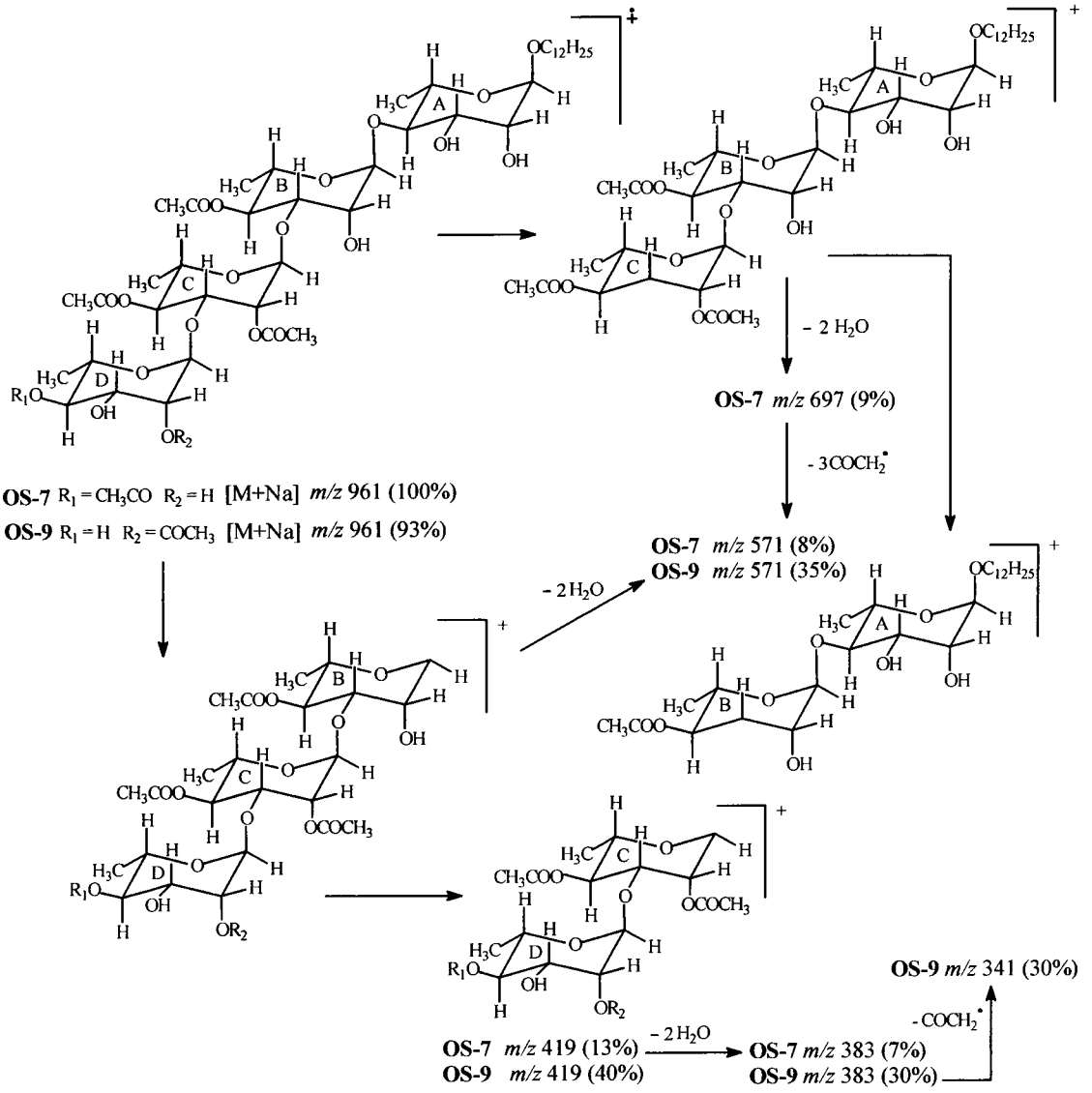
^1H (Spectrum 3.14; Table 3.8) and ^{13}C (Table 3.10) NMR data showed a tetrarhamnoside with four acetoxymethyls. Acetylated positions were attributed to two H-4 at δ 5.70 (t, *J* = 9.8 Hz), 5.29 (t, *J* = 9.9 Hz) and two H-2 at δ 5.38 (dd, *J* = 1.3, 3.3 Hz), 5.27 (dd). Characteristic features on the HMBC (Table 3.15) included:

i) ^3J interactions between H-3 at δ 4.26 and C-1 at δ 101.2, between H-3 at δ 4.28 and C-1 at δ 100.8 and between H-4 at δ 4.11 and C-1 at δ 103.6, thus establishing the interglycosidic links as (1→3) (1→3) (1→4) between the four units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.73/3.42) and the anomeric C-1 at δ 101.8 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the four deshielded protons, now identified as H-4B/ H-2C/H-4C and H-2D and the acetoxycarbonyls.

The physical and spectral results of **OS-9** complied with those reported for the known 1-*O*-dodecanyl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside, previously isolated from *Cleistopholis glauca* (Tané *et al.*, 1988b; Woods, 1989).

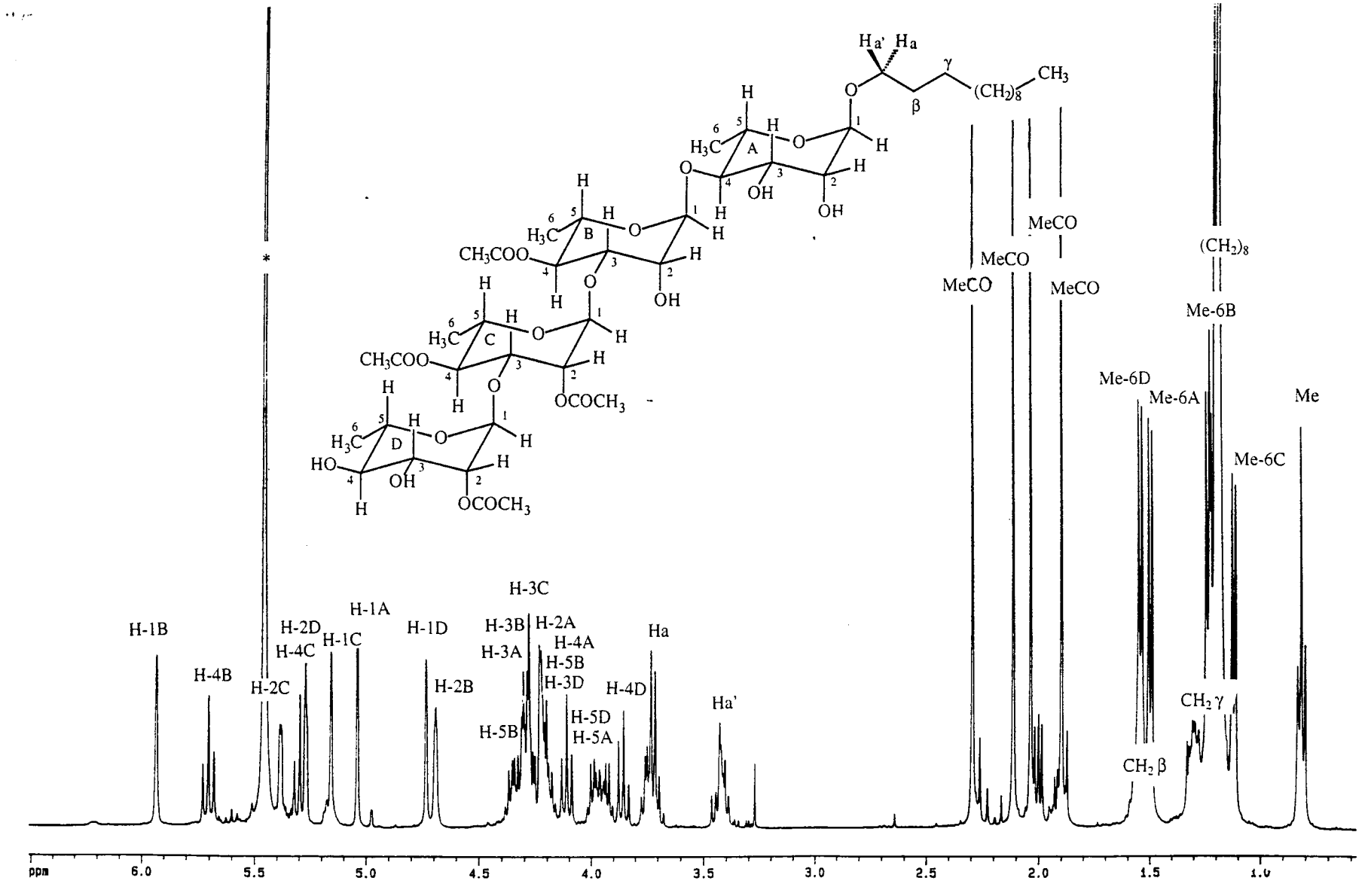


Scheme 3.7: Suggested mass fragmentation pattern of OS-7 and OS-9

Table 3.15: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-9

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	5.04 (d)	101.8		68.2 (C-5A),68.4 (OCH_2), 73.6 (C-3A)
4A	4.11 (t, 9.3)	81.4	68.2 (C-5A), 73.6 (C-3A)	103.6 (C-1B)
1B	5.93 (d, 1.1)	103.6	72.2 (C-2B)	68.6 (C-5B), 80.4 (C-3B), 81.4 (C-4A)
3B	4.28 (dd)	80.4		68.6 (C-5B), 100.8 (C-1C)
1C	5.15 (d)	100.8	73.4 (C-2C)	67.1 (C-5C), 76.2 (C-3C), 80.4 (C-3B)
3C	4.26 (dd)	76.2		101.2 (C-1C)
1D	4.73 (d)	101.2		70.9 (C-5D), 76.2 (C-3C)
OCH_2	3.73/3.42 (m)	68.4		101.8 (C-1A)
*Me*CO	2.29 (s)	21.5	171.5 (H-4B)	
	2.11 (s)*	21.3*	171.4* (H-2C)*	
	2.03 (s)*	21.2*	171.3* (H-2D)*	
	1.90 (s)*	21.0*	171.2* (H-4C)*	
4B	5.70 (t, 9.8)	73.7	68.6 (C-5B), 80.4 (C-3B)	72.1 (C-2B), 171.5
2C	5.38 (dd, 1.3, 3.3)	73.4		171.4*
4C	5.29 (t, 9.9)	73.5	67.1 (C-5C), 76.2 (C-3C)	171.2*
2D	5.27 (dd)	74.4	70.4 (C-3D)	74.0 (C-4D), 171.3*

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



Spectrum 3.14: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-9

f) Identification of ***OS-10*** *as cleistetroside-4*

This compound was isolated from the petrol extract of *Cleistopholis glauca*. FABMS (Scheme 3.8) displayed a quasi molecular ion $[M+Na]^+$ at *m/z* 919, indicating a molecular weight of 896, thus corresponding to the molecular formula $C_{42}H_{72}O_{20}$ (7 DBE). Peaks were observed at *m/z* 877 $[(M+Na)-COCH_2]^+$ and 835 $[(M+Na)-2\times COCH_2]^+$.

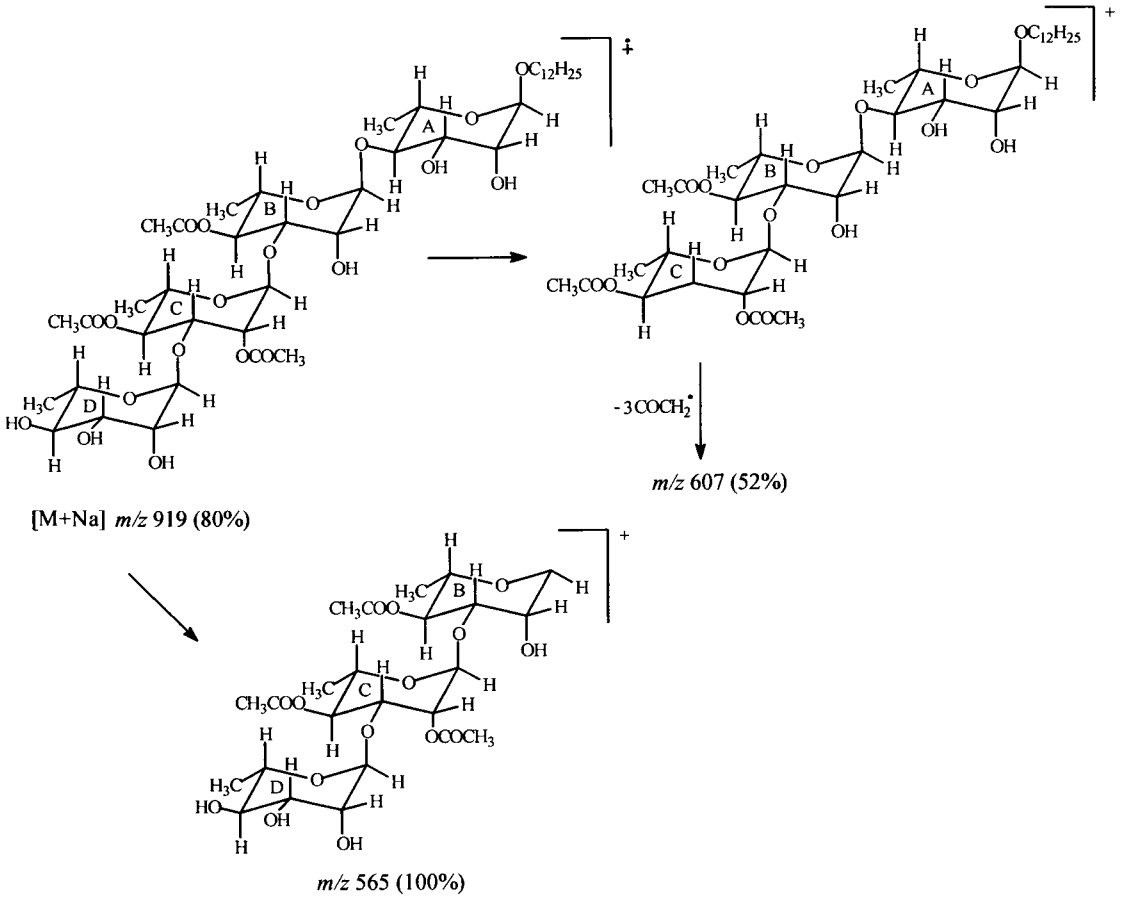
^1H (Spectrum 3.15; Table 3.8) and ^{13}C (Table 3.10) NMR data showed a tetrarhamnoside with three acetoxymethyls. Acetylated positions were attributed to two H-4 at δ 5.58 (t, J = 9.8 Hz), 5.21 (t, J = 9.9 Hz). A TOCSY experiment established the remaining acetylated position as one H-2 at δ 5.34. Characteristic features on the HMBC (Table 3.16) included:

i) ^3J interactions between H-3 at δ 4.26 and C-1 at δ 104.4, between H-3 at δ 4.19 and C-1 at δ 100.8 and between H-4 at δ 3.99 and C-1 at δ 103.6, thus establishing the interglycosidic links as (1→3) (1→3) (1→4) between the four units.

ii) a ^3J interaction between the oxymethylene Ha/a' protons (δ 3.68/3.38) and the anomeric C-1 at δ 101.7 of one rhamnose unit, thus assigning the position of the dodecanyl side chain and identifying unit A.

iii) ^3J-interactions between the three deshielded protons, now identified as H-4B/H-2C and H-4C and the acetoxycarbonyls.

The physical and spectral data of **OS-10** complied with those reported for the known 1-*O*-dodecanyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside, previously isolated from *Cleistopholis glauca* (Tané *et al.*, 1988b; Woods, 1989).

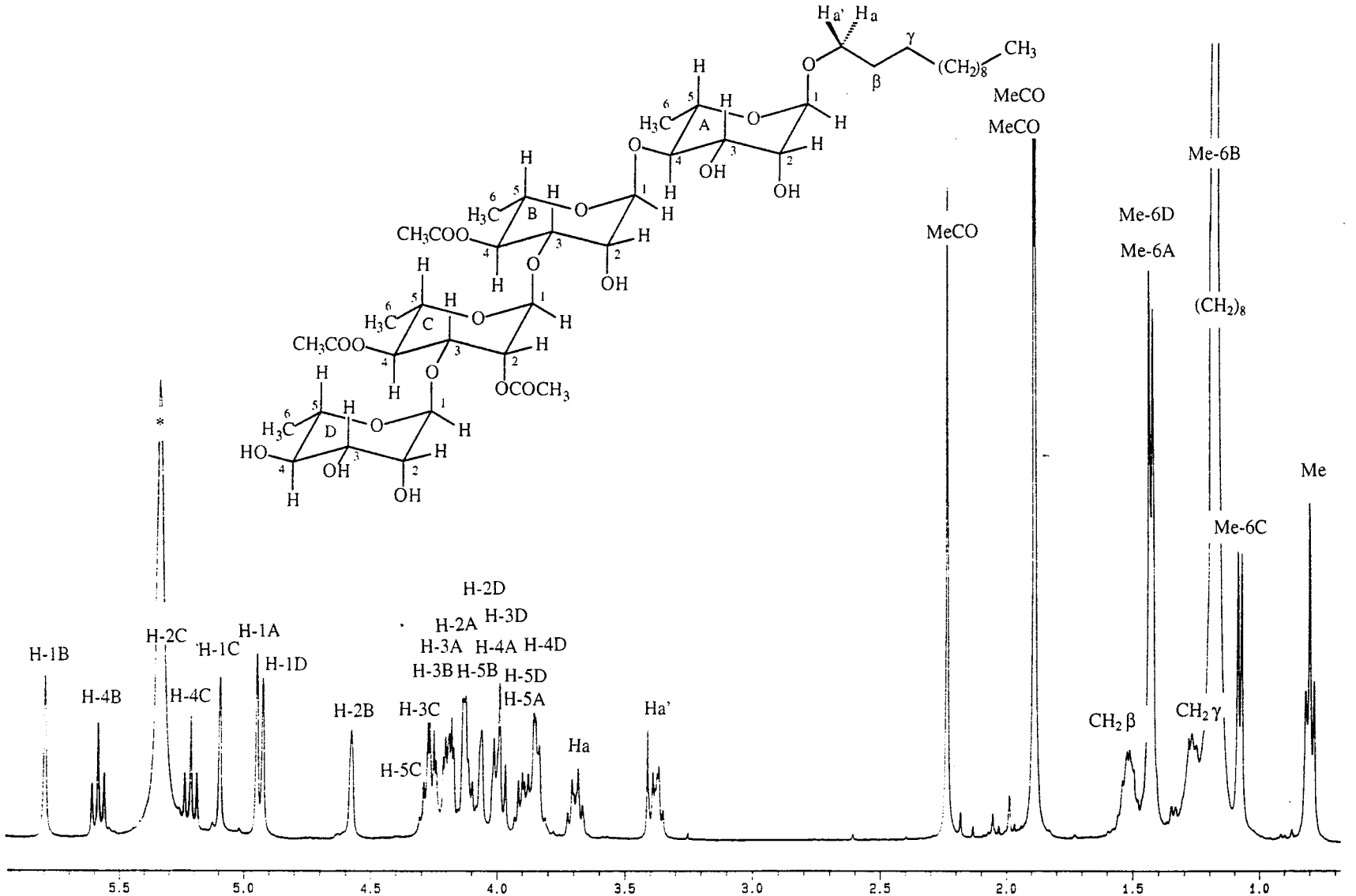


Scheme 3.8: Suggested mass fragmentation pattern of OS-10

Table 3.16: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of OS-10

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1A	4.95 (d)	101.7	73.0 (C-2A)	68.1 (C-5A), 68.4 (OCH_2) 73.8 (C-3A)
4A	3.99 (t, 9.1)	81.4		103.6 (C-1B)
1B	5.80 (d)	103.6	72.1 (C-2B)	68.5 (C-5B), 80.2 (C-3B), 81.4 (C-4A)
3B	4.19 (dd, 3.4, 9.7)	80.2		100.8 (C-1C),103.6(C-1B)
1C	5.09 (d)	100.8		67.8 (C-5C), 75.9 (C-3C), 80.2 (C-3B)
3C	4.26 (dd, 3.5, 9.9)	75.9		104.4 (C-1D)
1D	4.92 (d)	104.4	72.7 (C-2D)	71.0 (C-5D), 72.6 (C-3D), 75.9 (C-3C)
OCH_2	3.68/3.38 (m)	68.4		101.7 (C-1A)
*Me*CO	2.23 (s)	21.4	171.4 (H-4B)	
	1.89 (s)*	21.0	171.3 (H-2C)	
	1.88 (s)*	21.0	170.8 (H-4C)	
4B	5.58 (t, 9.8)	73.6	68.5 (C-5B), 80.5 (C-3B)	171.4
2C	5.34 (obsc)	73.5		171.3
4C	5.21 (t, 9.9)	73.8	75.9 (C-3D)	170.8

All data obtained in C_5D_5N-CD_3OD.
In parentheses coupling constant *J* are in Hz.
*Signals are interchangeable within the same column.



Spectrum 3.15: ^{1}H NMR (400 MHz, C_5D_5N-CD_3OD*) of OS-10

3.1.1.2 Properties and spectral data of isolated partially acetylated 1-O-dodecanyl oligorhamnosides

OS-1 (Cleistrioside-2)

Gummy solid. $[\alpha]_D^{23}$ -39.4° (MeOH, *c* 0.66). IR ν_{max} (film) cm^{-1}: 3472 (OH), 2980, 2925, 2855, 1745 (C=O), 1375, 1227, 1135, 1075, 1046. Found $[M+Na]^+$ 857.4073 ($C_{40}H_{66}O_{18}Na$ requires 857.4147) FABMS *m/z* (rel. int. %): 857 (11), 815 (23), 773 (100), 731 (34), 655 (18), 627 (12), 551 (21), 425 (12), 377 (9). ^1H & ^{13}C NMR: Tables 3.1 & 3.2.

OS-2 (Cleistrioside-3)

Gummy solid. $[\alpha]_D^{23.1}$ -52.9° (MeOH, *c* 0.17). IR ν_{max} (film) cm^{-1}: 3483 (OH), 2980, 2926, 2854, 1745 (C=O), 1375, 1230, 1137, 1076, 1046. Found $[M+Na]^+$ 857.4203 ($C_{40}H_{66}O_{18}Na$ requires 857.4147) FABMS *m/z* (rel. int. %): 857 (89), 815 (57), 773 (18), 655 (17), 627 (13), 551 (17), 461 (100), 419 (34). ^1H & ^{13}C NMR: Tables 3.1 & 3.2.

OS-3 (Cleistrioside-4)

Gummy solid. $[\alpha]_D^{23.5}$ -48.8° (MeOH, *c* 0.164). IR ν_{max} (film) cm^{-1}: 3464 (OH), 2979, 2925, 2855, 1744 (C=O), 1375, 1230, 1136, 1076, 1046. Found $[M+Na]^+$ 815.4020 ($C_{38}H_{64}O_{17}Na$ requires 815.4041) FABMS *m/z* (rel. int. %): 815 (100), 773 (14), 657 (25), 553 (22), 421 (8), 383 (7). ^1H & ^{13}C NMR: Tables 3.1 & 3.2.

OS-4 (Cleistrioside-1)

Gummy solid. $[\alpha]_D^{23.3}$ -59.1° (MeOH, *c* 0.4735) [Lit. $[\alpha]_D$ -35.7° (MeOH, *c* 0.82), Woods, 1989]. IR ν_{max} (film) cm^{-1}: 3478 (OH), 2977, 2925, 2854, 1748 (C=O), 1371, 1227, 1134, 1076, 1047. Found $[M+Na]^+$ 815.3997 ($C_{38}H_{64}O_{17}Na$ requires 815.4041) FABMS *m/z* (rel. int. %): 815 (16), 461 (7), 273 (100). ^1H & ^{13}C NMR: Tables 3.1 & 3.2.

OS-5 (Cleistetroside-1)

Gummy solid. $[\alpha]_D^{23.6}$ -65.3° (MeOH, *c* 0.5665) [Lit. $[\alpha]_D$ -54.4° (MeOH, *c* 0.75), Woods, 1989]. IR ν_{max} (film) cm^{-1}: 3461 (OH), 2979, 2925, 2855, 1745 (C=O), 1375, 1231, 1135, 1075, 1045. Found $[M+Na]^+$ 1003.4785 ($C_{46}H_{76}O_{22}Na$ requires 1003.4726) FABMS *m/z* (rel. int. %): 1003 (100), 961 (19), 801 (8), 729 (7), 687 (8), 571 (7), 461 (73), 419 (10), 341 (7). ^1H & ^{13}C NMR: Tables 3.7 & 3.9.

OS-6 (Cleistetroside-6)

Gummy solid. $[\alpha]_D^{23.8}$ -54.8° (MeOH, *c* 0.474). IR ν_{max} (film) cm^{-1}: 3474 (OH), 2979, 2926, 2855, 1745 (C=O), 1375, 1230, 1136, 1076, 1045. Found $[M+Na]^+$ 1003.4729 ($C_{46}H_{76}O_{22}Na$ requires 1003.4726) FABMS *m/z* (rel. int. %): 1003 (33), 961 (77), 919 (100), 877 (28), 801 (25), 697 (20), 571 (25), 461 (18), 419 (25), 377 (33), 341 (15). ^1H & ^{13}C NMR: Tables 3.7 & 3.9.

OS-7 (Cleistetroside-2)

Gummy solid. $[\alpha]_D^{23.9}$ -63.1° (MeOH, *c* 0.301) [Lit. $[\alpha]_D$ -41.5° (MeOH, *c* 0.6), Woods, 1989]. IR ν_{max} (film) cm^{-1}: 3466 (OH), 2979, 2925, 2855, 1745 (C=O), 1375, 1233, 1136, 1076, 1044. Found $[M+Na]^+$ 961.4739 ($C_{44}H_{74}O_{21}Na$ requires 961.4620) FABMS *m/z* (rel. int. %): 961 (100), 919 (12), 801 (10), 901 (7), 697 (10), 571 (8), 419 (13), 383 (7). ^1H & ^{13}C NMR: Tables 3.7 & 3.9.

OS-8 (Cleistetroside-7)

Gummy solid. $[\alpha]_D^{23}$ -58.8° (MeOH, *c* 0.238). IR ν_{max} (film) cm^{-1}: 3490 (OH), 2977, 2925, 2856, 1748 (C=O), 1372, 1224, 1137, 1076, 1044. Found $[M+Na]^+$ 1045.4768 ($C_{48}H_{78}O_{23}Na$ requires 1045.4831) FABMS *m/z* (rel. int. %): 1045 (90), 919 (32), 801 (26), 771 (28), 729 (24), 565 (27), 503 (100), 467 (49), 323 (29). ^1H & ^{13}C NMR: Tables 3.8 & 3.10.

OS-9 (Cleistetroside-3)

Gummy solid. $[\alpha]_D^{23.2}$ -54.7° (MeOH, *c* 0.256) [Lit. $[\alpha]_D$ -44.5° (MeOH, *c* 0.51), Woods, 1989]. IR ν_{max} (film) cm^{-1}: 3461 (OH), 2977, 2925, 2855, 1742 (C=O), 1375, 1233, 1137, 1076, 1047. Found $[M+Na]^+$ 961.4722 ($C_{44}H_{74}O_{21}Na$ requires 961.4620) FABMS *m/z* (rel. int. %): 961 (93), 919 (83), 801 (40), 697 (30), 645 (30), 571 (35), 419 (40), 383 (30), 341 (30). ^1H & ^{13}C NMR: Tables 3.8 & 3.10.

OS-10 (Cleistetroside-4)

Gummy solid. $[\alpha]_D^{21.8}$ -79.5° (MeOH, *c* 0.176) [Lit. $[\alpha]_D$ -67.8° (MeOH, *c* 1.82), Woods, 1989]. IR ν_{max} (film) cm^{-1}: 3424 (OH), 2976, 2925, 2854, 1742 (C=O), 1377, 1233, 1136, 1075, 1047. Found $[M+Na]^+$ 919.4449 ($C_{42}H_{72}O_{20}Na$ requires 919.4515) FABMS *m/z* (rel. int. %): 919 (80), 877 (32), 835 (30), 731 (30), 607 (52), 565 (100). ^1H & ^{13}C NMR: Tables 3.8 & 3.10.

3.2 Terpenes

Eight terpenes were isolated from *Cleistopholis glauca*, *Piptostigma fasciculata* and *Goniothalamus thwaitesii*. They included four sesquiterpenes, one diterpene and three triterpenes.

3.2.1 Sesquiterpenes

Four sesquiterpenes were obtained from *Cleistopholis glauca* and *Piptostigma fasciculata* (see Section 2.4.4). They included three farnesane derivatives (Figure 3.7), coded **S-1** (CGP.2), **S-2** (CGP.3), **S-3** (CGP.7), and identified respectively as:

methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate

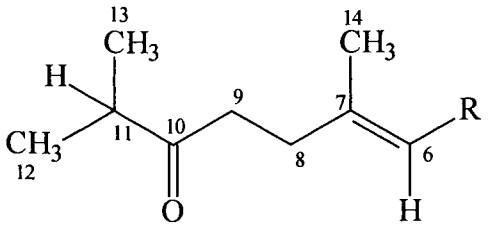
methyl-(2*E*,6*E*,10ξ)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate

methyl-(2*E*,6*E*,10ξ)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate

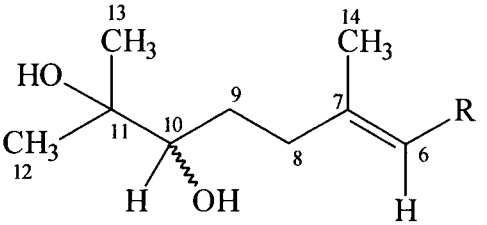
and one caryophyllane sesquiterpene, coded **S-4** (PFH.1), identified as β-caryophyllene-4,5-oxide (Figure 3.8).

3.2.1.1 General characterisation of isolated farnesane sesquiterpenes

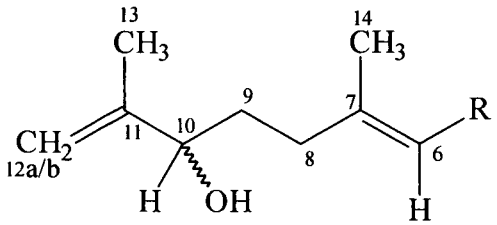
The three farnesane sesquiterpenes were isolated from the petrol extract of *Cleistopholis glauca*. On TLC analysis, they showed quenching spots (λ = 254 nm) and a purple colour on spraying with anisaldehyde-H_2SO_4 reagent, turning yellow after heating. Their IR spectra indicated the presence of an α,β-unsaturated ester carbonyl function (*ca.* 1720 cm^{-1}) which was further suggested in the UV spectra with absorption maxima at 218 nm.



S-1



S-2



S-3

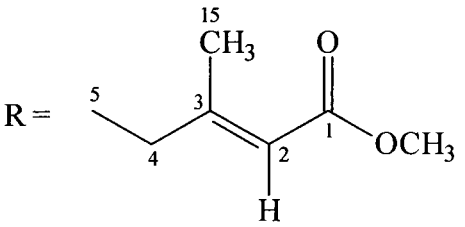


Figure 3.7: Structures of S-1 to S-3

Common features on the ^{1}H and ^{13}C NMR (Tables 3.17 & 3.18) included:

i) two singlets for olefinic methines at *ca.* δ 5.65 (*ca.* δ_C 115.5) and *ca.* δ 5.10 (*ca.* δ_C 123.5).

ii) a methoxyl group resonating at *ca.* δ 3.65 (*ca.* δ_C 51.0).

iii) two methylene resonances at *ca.* δ 2.20-2.15 (*ca.* δ_C 41.0 and 26.0).

iv) two vinylic methyl resonances at *ca.* δ 2.15 (*ca.* δ_C 19.0) and 1.60 (*ca.* δ_C 16.2) attached to two olefinic quaternary carbons *ca.* δ 160.0 and 135.8.

v) 16 carbons, thus establishing a methyl ester substitution on the sesquiterpene skeleton for a farnesoate derivative with the ester carbonyl resonance at *ca.* δ 167.5.

Table 3.17: ^{1}H NMR (400 MHz) spectral data of S-1 to S-3

Position	S-1	S-2	S-3
2	5.66 (s)	5.62 (s)	5.67 (s)
4	2.20-2.14 (m)	2.22-2.13 (m)	2.20-2.16 (m)
5	2.20-2.14 (m)	2.22-2.13 (m)	2.20-2.16 (m)
6	5.09 (brs)	5.09 (brs)	5.13 (brs)
8	2.24 (t, 7.7)	2.01 (m)	2.03 (m)
9	2.52 (t)	1.52/1.35 (m)	1.63 (m)
10	-	3.27 (dd, 1.9, 10.4)	4.03 (t, 6.4)
11	2.58 (m, 6.9)	-	-
12	1.09 (d, 6.9)	1.10 (s)*	4.93/4.84 (d, 1.6)
Me-13	1.09 (d, 6.9)	1.14 (s)*	1.73 (s)
Me-14	1.61 (d)	1.57 (d)	1.58 (d)
Me-15	2.16 (d, 1.3)	2.11 (d, 1.3)	2.16 (d, 1.2)
OMe	3.69 (s)	3.63 (s)	3.69 (s)
OH		2.62 (brs)	

All data obtained in $CDCl_3$. In parentheses coupling constant J are in Hz. *Signals within columns are interchangeable. Methylene signals are listed as Ha/Hb in each column

Table 3.18: ^{13}C NMR (100 MHz) spectral data of S-1 to S-3

Position	S-1	S-2	S-3
1	167.4	167.5	167.4
2	115.5	115.7	115.6
3	160.0	159.9	160.1
4	41.0	41.0	41.0
5	26.1	26.0	26.1
6	123.5	123.8	123.5
7	135.3	136.2	136.1
8	33.6	36.8	35.8
9	39.2	29.9	33.3
10	214.5	78.2	75.7
11	41.1	73.2	147.8
Me-12	18.4	23.4*	111.1
Me-13	18.4	26.6*	17.8
Me-14	16.3	16.1	16.2
Me-15	19.0	18.9	19.0
OMe	51.0	51.0	50.9

All data obtained in $CDCl_3$. *Signals within columns are interchangeable

*a) Identification of **S-1** as methyl-(2E,6E)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate*

In addition to the common features already mentioned, HREIMS exhibited a molecular ion at *m/z* 266, analysing for the molecular formula $C_{16}H_{26}O_3$ (4 DBE).

Characteristic features in the ^{1}H NMR (Spectrum 3.16; Table 3.17) showed:

i) an isopropyl unit with a methine at δ 2.58 (m, J = 6.9 Hz) coupling to two equivalent methyls at δ 1.09 (d, J = 6.9 Hz).

ii) two methylenes at δ 2.52 (adjacent to a carbonyl) and δ 2.24 (adjacent to a double bond).

Characteristic features on the J-modulated ^{13}C NMR (Spectrum 3.17; Table 3.18), assigned by ^1J ^{1}H-^{13}C HC-COBI, included one extra carbonyl resonance for a saturated ketone (δ 214.5); two methylenes at δ 39.2 and 33.6, respectively for carbons adjacent to a carbonyl and a double bond; the methine (δ 41.1) and the two equivalent methyls (δ 18.4) of the isopropyl group.

Unambiguous identification of **S-1** as methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyl-dodeca-2,6-dienoate was established by HMBC (Spectrum 3.18; Table 3.19) which showed ^3J couplings between:

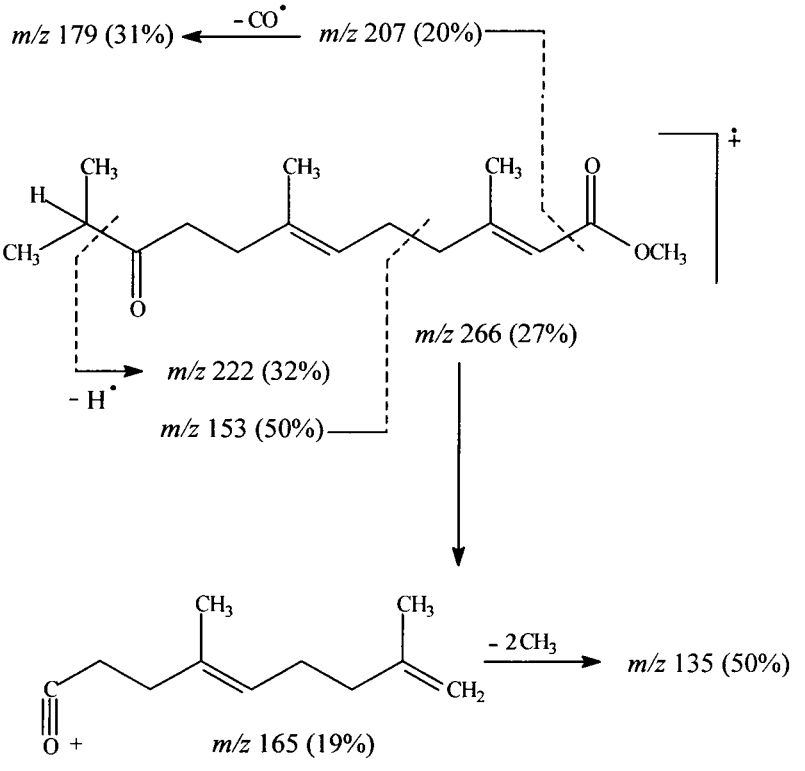
i) the two methyls at δ 1.09 and the carbonyl at δ 214.5 (C-10), thus establishing the substitution of the isopropyl unit next to the ketone group.

ii) the methylene at δ 2.24 with the ketone at δ 214.5 (C-10) and the methine at δ 123.5 (C-6), thus establishing the assignment of the C-8 position at δ 2.24.

iii) one methylene at the region δ 2.14-2.20 with the C-6 (123.5) and C-2 methines (δ 115.5).

iv) the methoxyl group at δ 3.69 and the carbonyl at δ 167.4 (C-1), thus confirming the methyl ester substitution at C-1.

The presence of the isopropyl, OMe ester and ketone carbonyl groups were rationalised from the HREIMS respectively with fragment ions at *m/z* 222 $[M\text{-}C_3H_7\text{-}H]^+$, 207 $[M\text{-}COOMe]^+$ and 179 $[M\text{-}COOMe\text{-}CO]^+$ (Scheme 3.9).



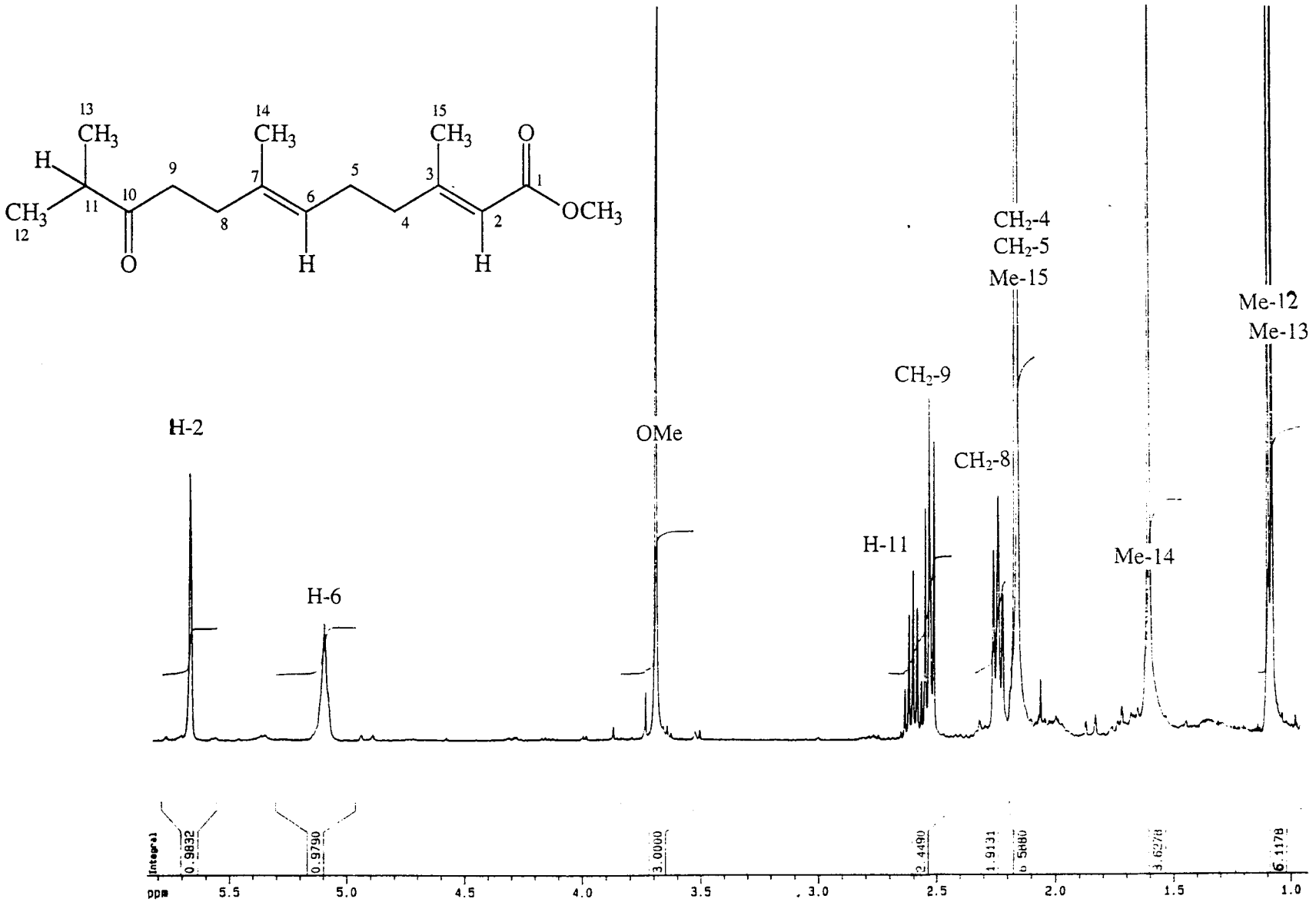
Scheme 3.9: Suggested mass fragmentation pattern of S-1

On the basis of these spectral data, **S-1** was identified as the new farnesane sesquiterpene, methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate.

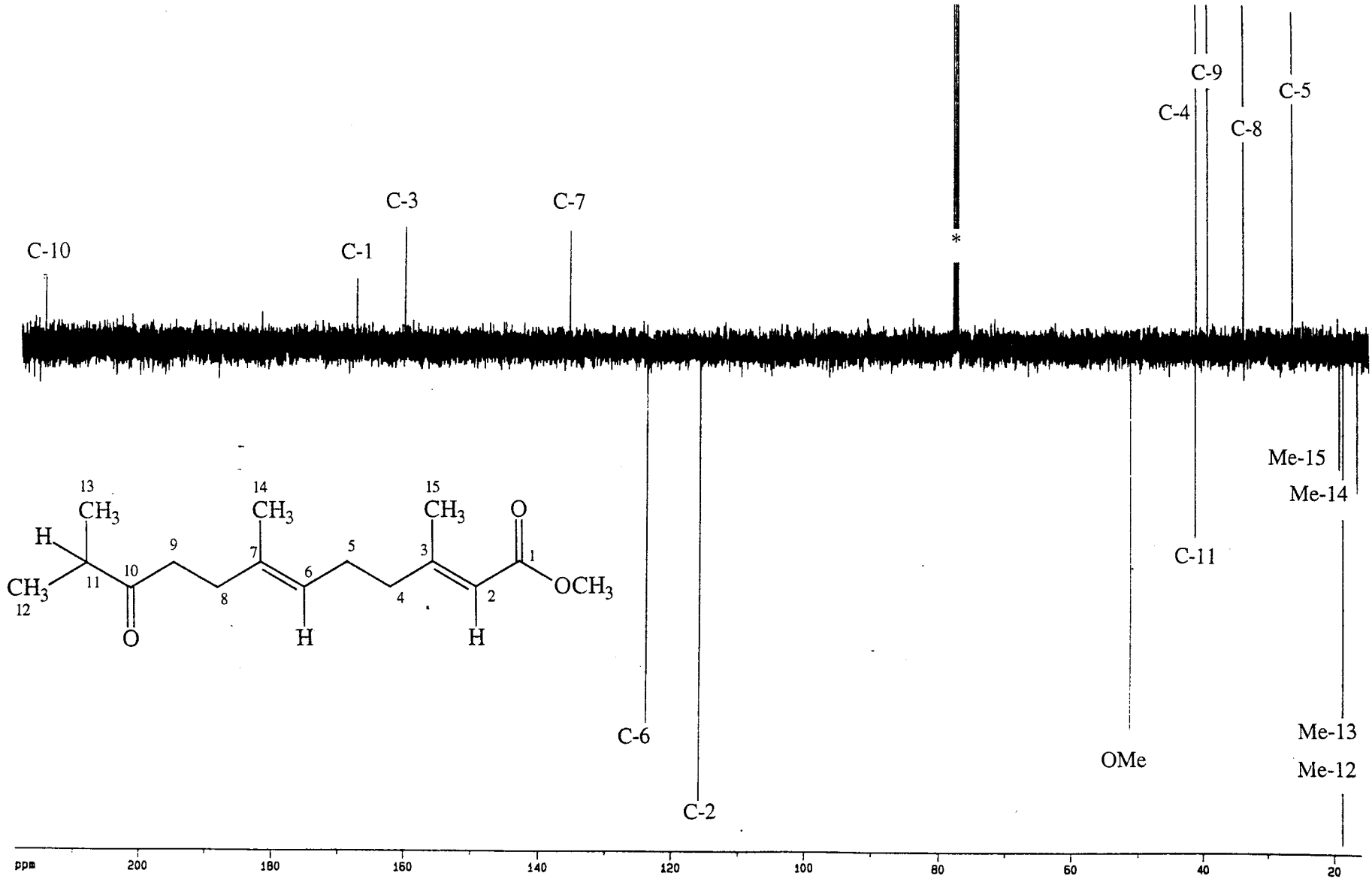
Table 3.19: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of S-1

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2	5.66 (s)	115.5	167.4 (C-1)	19.0 (C-15), 41.0 (C-4)
4	2.20-2.14 (m)	41.0	26.1 (C-5), 160.0 (C-3)	19.0 (C-15), 115.5 (C-2), 123.5 (C-6)
5	2.20-2.14 (m)	26.1	41.0 (C-4), 123.5 (C-6)	135.3 (C-7), 160.0 (C-3)
6	5.09 (brs)	123.5	26.1 (C-5)	16.3 (C-14), 33.6 (C-8), 41.0 (C-4)
8	2.24 (t, 7.7)	33.6	39.2 (C-9), 135.3 (C-7)	16.3 (C-14), 123.5 (C-6), 214.5 (C-10)
9	2.52 (t)	39.2	33.6 (C-8), 214.5 (C-10)	135.3 (C-7)
11	2.58 (m, 6.9)	41.1	18.4 (C-12/13), 214.5 (C-10)	
Me-12	1.09 (d, 6.9)	18.4	18.4 (C-13), 41.1 (C-11)	214.5 (C-10)
Me-13	1.09 (d, 6.9)	18.4	18.4 (C-12), 41.1(C-11)	214.5 (C-10)
Me-14	1.61 (d)	16.3	135.3 (C-7)	33.6 (C-8), 123.5 (C-6)
Me-15	2.16 (d, 1.3)	19.0	160.0 (C-3)	41.0 (C-4), 115.5 (C-2)
OMe	3.69 (s)	51.0		167.4 (C-1)

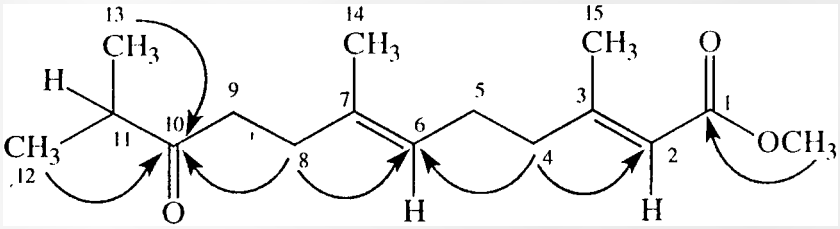
All data obtained in $CDCl_3$. In parentheses coupling constant J are in Hz.

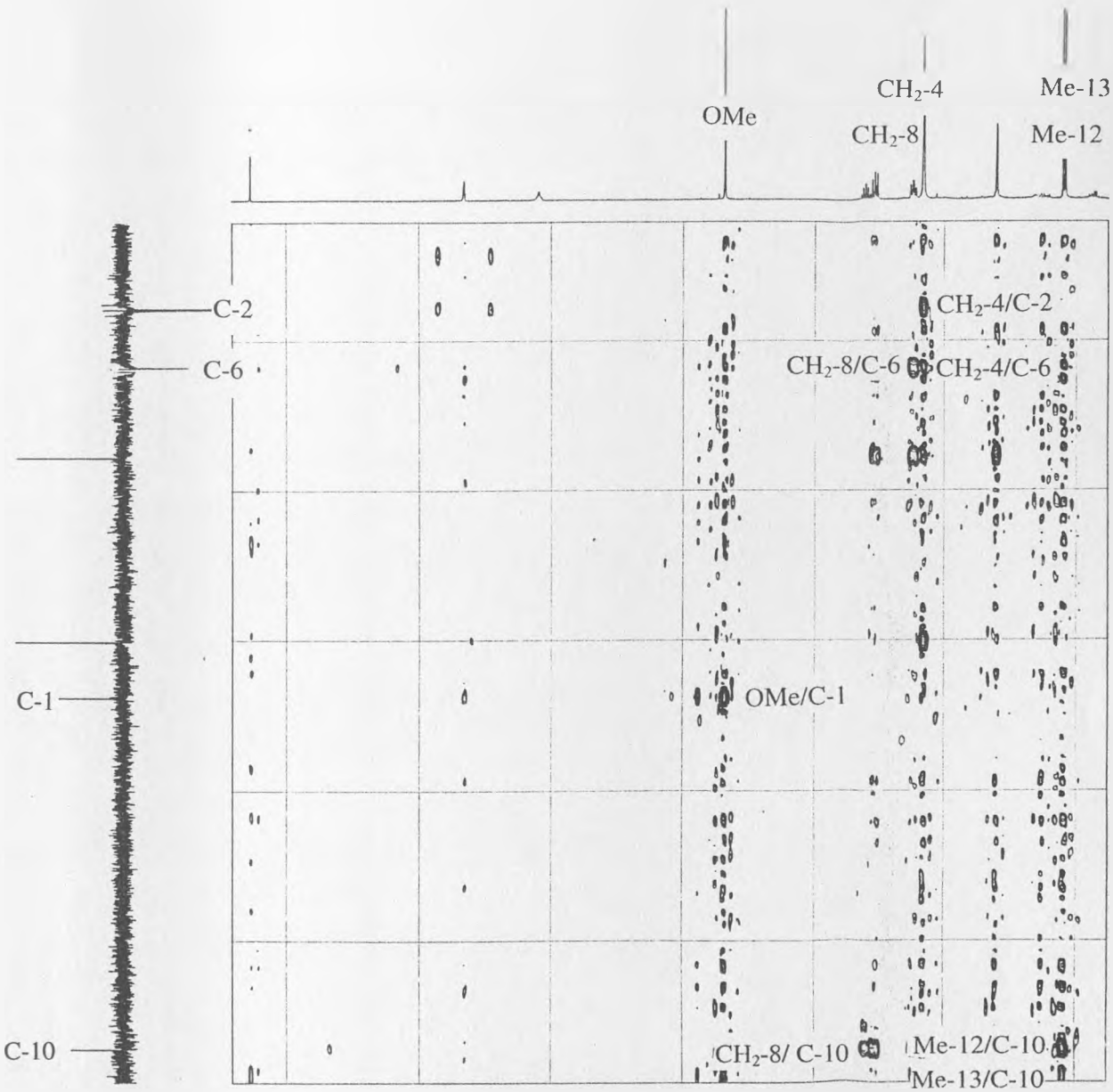


Spectrum 3.16: ^{1}H NMR (400 MHz, $CDCl_3$) of S-1



Spectrum 3.17: *J*-modulated ^{13}C NMR (100 MHz, $CDCl_3$*) of S-1





Spectrum 3.18: Significant HMBC (400 MHz, $CDCl_3$) correlations of S-1

*b) Identification of **S-2** as methyl-(2E,6E,10ξ)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate*

In addition to the ester carbonyl (1720 cm^{-1}), IR spectral data indicated the presence of hydroxyl group(s) (3443 cm^{-1}). The HREIMS displayed a weak molecular ion at *m/z* 284 analysing for the molecular formula of $C_{16}H_{28}O_4$ (3 DBE). Fragment ions were observed at *m/z* 266 $[M\text{-}H_2O]^+$ and 235 $[M\text{-}H_2O\text{-}OCH_3]^+$.

^1H and ^{13}C NMR displayed the common features of farnesoate sesquiterpenes. In addition the ^1H NMR (Table 3.17) revealed the presence of an oxymethine at δ 3.27 (dd, J = 1.9, 10.4 Hz); a methylene adjacent to a double bond at δ 2.01 (m), and one at 1.52/1.35 (m); two OH groups at δ 2.62 (brs); a *gem*-dimethyl group at δ 1.14 and 1.10. Characteristic features on the *J*-modulated ^{13}C NMR (Table 3.18) included the presence of two oxygenated carbon at δ 78.2 (oxymethine) and 73.2 (quaternary); two methylenes at δ 36.8 (adjacent to a double bond) and 29.9; two methyls at δ 26.6 and 23.4.

Comparison of IR, UV, MS, ^1H and ^{13}C NMR data of **S-2** showed good agreement with those of the known farnesane sesquiterpene methyl-(2*E*,6*E*,10ξ)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate, previously isolated from *Cleistopholis patens* (Waterman and Muhammad, 1985) and *C. glauca* (Etse *et al.*, 1988). $[\alpha]_D$ measurements of **S-2** agreed with that recorded by Etse *et al.* (1988).

*c) Identification of **S-3** as methyl-(2E,6E,10ξ)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate*

In addition to the ester carbonyl (1717 cm^{-1}), the IR spectrum indicated the presence of hydroxyl group(s) (3457 cm^{-1}). The HREIMS exhibited a molecular ion peak at *m/z* 266 analysing for $C_{16}H_{26}O_3$ (4 DBE). Fragment ions were observed at *m/z* 248 $[M\text{-}H_2O]^+$ and 233 $[M\text{-}H_2O\text{-}CH_3]^+$.

The ^{1}H NMR (Table 3.17) revealed the presence of an exomethylene moiety with two non equivalent protons at δ 4.93 and 4.84 (d, J = 1.6 Hz); a vinylic methyl at δ 1.73; an oxymethine at δ 4.03 (t, J = 6.4 Hz) and methylenes at δ 2.03 (adjacent to a double bond) and δ 1.63. The J-modulated ^{13}C NMR (Table 3.18) confirmed the presence of the exomethylene (δ 111.1), the vinylic methyl (δ 17.8), the oxymethine (δ 75.7) and the two methylenes at δ 35.8 (adjacent to a double bond) and δ 33.3. It further showed one quaternary olefinic carbon at δ 147.8.

Comparison of the spectral and physical data of **S-3** showed good agreement with those of the known farnesane sesquiterpene methyl-(2*E*,6*E*,10ξ)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate, previously isolated from *Cleistopholis glauca* (Etse *et al.*, 1988).

3.2.1.2 Characterisation of the isolated caryophyllane sesquiterpene S-4 as β-caryophyllene-4,5-oxide

This sesquiterpene was obtained from the *n*-hexane extract of *Piptostigma fasciculata.*

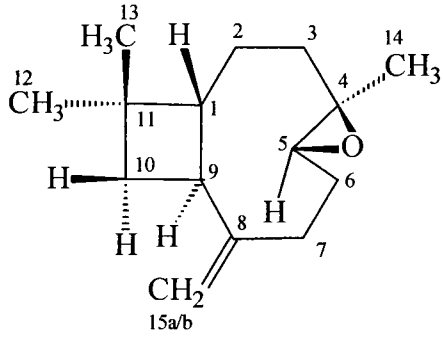


Figure 3.8: Structure of S-4

On TLC analysis, **S-4** showed a very light quenching spot (λ = 254 nm) and a pink colour on spraying with anisaldehyde-H_2SO_4 reagent, followed by heating. IR spectral data suggested the presence of an exomethylene group (3066, 1631, 890 cm^{-1}) (Williams and Fleming, 1995). The HREIMS showed a molecular ion at *m/z* 220 corresponding to the molecular formula $C_{15}H_{24}O$ (4 DBE).

Characteristic signals on the ^{1}H NMR included two deshielded doublets for coupling protons at δ 4.98 and 4.87 (J = 1.3 Hz) of the exomethylene group; an oxymethine at δ 2.88 (dd, J = 4.2, 10.6 Hz); a tertiary methyl at δ 1.12 adjacent to a carbon bearing oxygen and a *gem*-dimethyl group with singlets at δ 1.01 and 0.99. The presence of the latter was confirmed with an IR absorption band at 1384 cm^{-1}.

The J-modulated ^{13}C NMR revealed a sesquiterpene skeleton with 15 carbons. They included three quaternary carbons including one oxygenated position (δ 60.0), three methines including one oxygenated position (δ 64.0), six methylenes including the exomethylene (δ 113.0), three methyls including the tertiary methyl (δ 17.2) and the *gem*-dimethyl group (δ 30.1, 21.8). The two oxygenated carbons at δ 64.0 and 60.0 suggested the presence of an epoxide since **S-4** had only one oxygen. This was further confirmed with IR absorption bands for C-O-C stretch (1122, 1076 cm^{-1}) and the epoxide ring (1261, 910 and 821 cm^{-1}).

Comparison of the physical and spectral data of **S-4** agreed with those reported for the known tricyclic mono-oxygenated sesquiterpene β-caryophyllene-4,5-oxide (Heymann *et al.*, 1994; Fleischer, 1997).

3.2.2 Diterpene

One diterpene, coded **D-1** (PFE-1), was isolated from the ethyl acetate extract of *Piptostigma fasciculata* (see Section 2.4.4) and identified as (2*E*,7ξ,11ξ)-phytol (Figure 3.9).

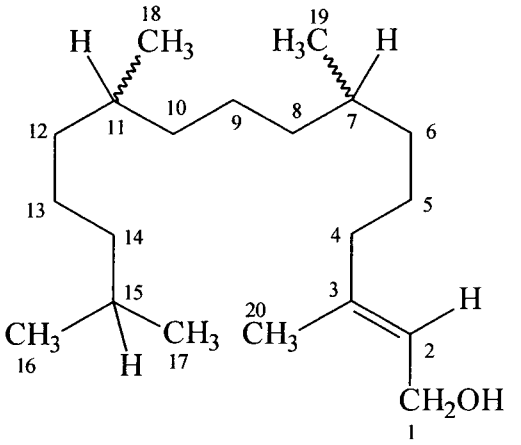
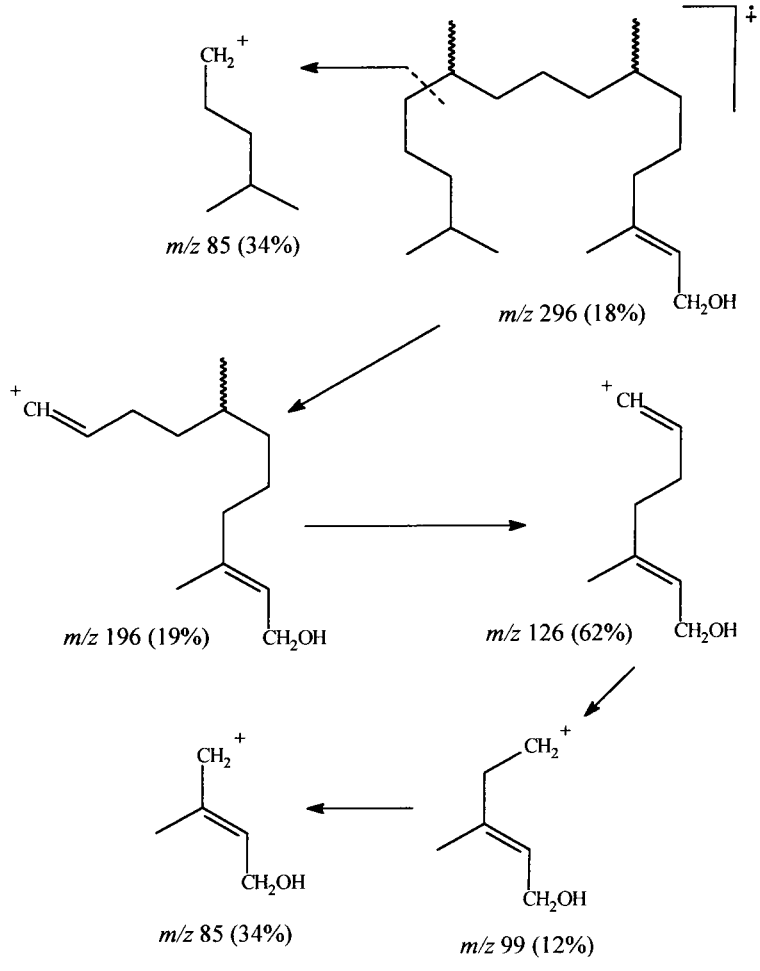


Figure 3.9: Structure of D-1

3.2.2.1 Characterisation of the isolated diterpene D-1 as (2E,7ξ,11ξ)-phytol

The terpenic structure of **D-1** was suggested on TLC analysis as it revealed a purple spot on spraying with anisaldehyde-H_2SO_4 reagent, followed by heating. Its IR spectrum indicated the presence of hydroxyl (3370 cm^{-1}), non conjugated double bond (1698 cm^{-1}) and olefinic methine (3023, 838 cm^{-1}) groups (Williams and Fleming, 1995).

HREIMS indicated a molecular ion at *m/z* 296, which analysed for the molecular formula $C_{20}H_{40}O$ (1 DBE). Two fragments at *m/z* 278 $[M\text{-}H_2O]^+$ and 263 $[M\text{-}H_2O\text{-}CH_3]^+$ as well as other characteristic fragment ions were observed on the mass fragmentation pattern (Scheme 3.10).



Scheme 3.10: Suggested mass fragmentation pattern of D-1

The ^{1}H NMR (Spectrum 3.19; Table 3.20) displayed an olefinic proton at δ 5.42 (t, J = 7 Hz) coupling to an oxymethylene at δ 4.16 (d, J = 6.9 Hz); a methylene attached to a centre of unsaturation at δ 2.00 (t, J = 7.2 Hz); a tertiary methyl attached to an olefinic carbon at δ 1.67 (s); a series of resonances at δ 1.43-1.08 (m) integrating for 18 protons; two equivalent methyls at δ 0.87 (d, J = 6.4 Hz) and one methine at δ 1.53 (m, J = 6.6 Hz) typical of an isopropyl unit; two methyls each adjacent to only one proton at δ 0.85 (d, J = 6.6 Hz) and 0.86 (d, J = 6.1 Hz).

The diterpenic structure of **D-1** was established with the presence of 20 carbons in the J-modulated ^{13}C NMR (Spectrum 3.20; Table 3.20). Assignments established by ^{1}J ^{1}H-^{13}C HC-COBI revealed one quaternary olefinic carbon (δ 140.5), ten methylenes including the oxymethylene (δ 59.6) and the methylene attached to

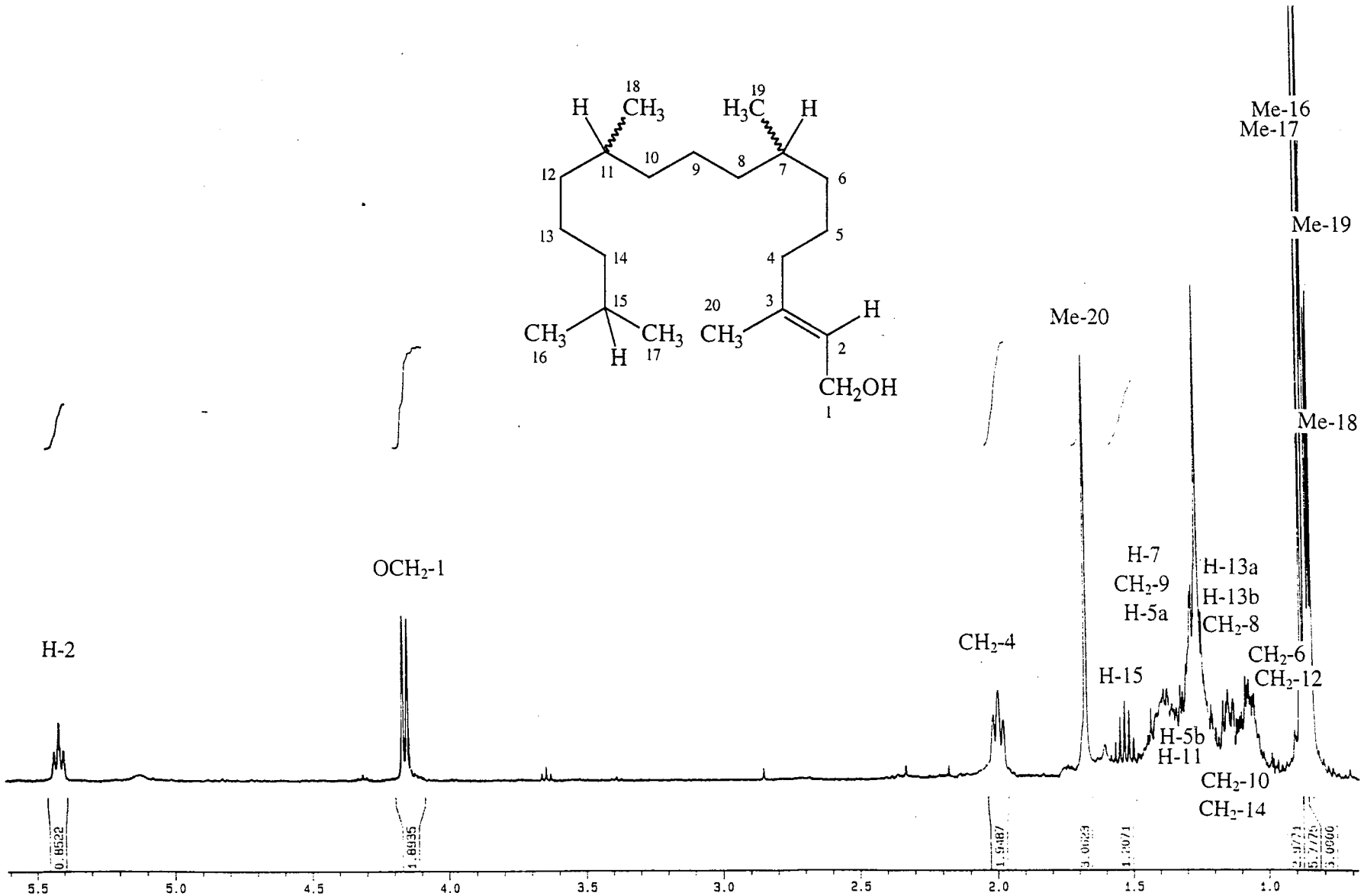
the centre of unsaturation (δ 40.1); four methines including the olefinic (δ 123.3), the isopropyl (δ 28.2) and two resonances at δ 33.0 and 32.9; five methyls including the isopropyl (δ 22.8 and 22.9), the two resonances each adjacent to one proton (δ 19.9 and 20.0) and the tertiary position (δ 16.4).

Comparison of the physical and spectral data of **D-1** complied with those reported for the known acyclic diterpene alcohol (2*E*,7ξ,11ξ)-phytol, previously isolated from species of marine algae (Iwata and Sakurai, 1963; De Souza and Nes, 1969; Sims and Pettus, 1976), *Fatsia japonica* (Araliaceae) (Suga and Aoki, 1974), *Tetragonia tetragonoides* (Aizoaceae) (Aoki *et al.*, 1982) and *Artemisia annua* (Compositae) (Brown, 1994). Complete ^{1}H NMR assignments, established by direct and long-range ^{1}H-^{13}C couplings, are reported unambiguously for the first time.

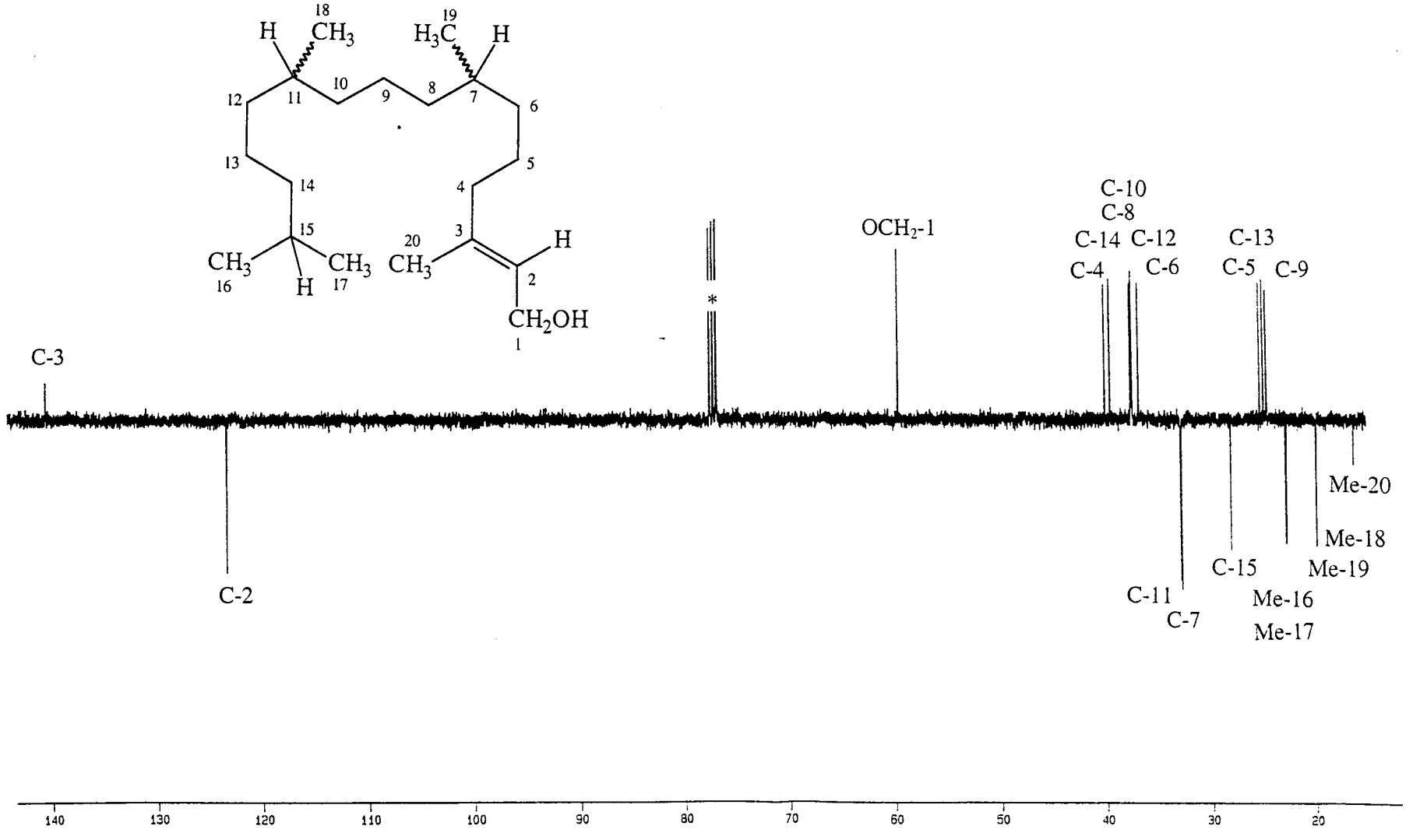
Table 3.20: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of D-1

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1	4.16 (d, 6.9)	59.6	123.3 (C-2)	140.5 (C-3)
2	5.42 (t, 7)	123.3		16.4 (C-20), 40.1 (C-4)
3	-	140.5		
4	2.00 (t, 7.2)	40.1	25.3 (C-5), 140.5 (C-3)	16.4 (Me-20),36.9 (C-6), 123.3 (C-2)
5	1.43/1.38 (m)	25.3		
6	1.18 (m)	36.9		
7	1.41 (m)	33.0		24.7 (C-9)
8	1.24 (m)	37.6	24.7 (C-9)	
9	1.41 (m)	24.7		
10	1.17 (m)	37.6		37.5 (C-12)
11	1.36 (m)	32.9		
12	1.08 (m)	37.5		37.6 (C-10)
13	1.24/1.18 (m)	25.0		
14	1.15 (m)	39.6		22.9 (Me-17), 22.8 (Me-16)
15	1.53 (m, 6.6)	28.2	22.8 (Me-16), 22.9 (Me-17), 39.6 (C-14)	
16	0.87 (d, 6.4)	22.8	28.2 (C-15)	39.6 (C-14)
17	0.87 (d, 6.4)	22.9	28.2 (C-15)	39.6 (C-14)
18	0.85 (d, 6.6)*	20.0	32.9 (C-11)	37.5 (C-12), 37.6 (C-10)
19	0.86 (d, 6.1)*	20.0	33.0 (C-7)	36.9 (C-6), 37.6 (C-8)
20	1.67 (s)	16.4	140.5 (C-3)	40.1 (C-4), 123.3 (C-2)

All data obtained in $CDCl_3$
In parentheses coupling constant J are in Hz.
*Signals within columns are interchangeable



Spectrum 3.19: ^{1}H NMR (400 MHz, $CDCl_3$) of D-1



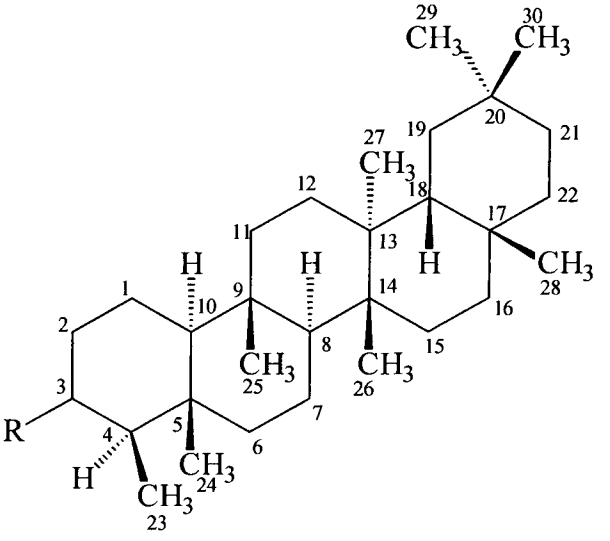
Spectrum 3.20: *J*-modulated ^{13}C NMR (100 MHz, $CDCl_3$*) of D-1

3.2.3 Triterpenes

Three triterpenes were isolated from *Goniothalamus thwaitesii* (see Section 2.4.4). They included two friedelane compounds, coded **T-1** (GTH.1) and **T-2** (GTH.2), identified respectively as friedelan-3α-ol (friedelinol) and friedelan-3-one (friedelin) (Figure 3.10), and one lupane derivative, coded **T-3** (GTE.1) and identified as betulinic acid (Figure 3.12).

3.2.3.1 General characterisation of isolated friedelane triterpenes

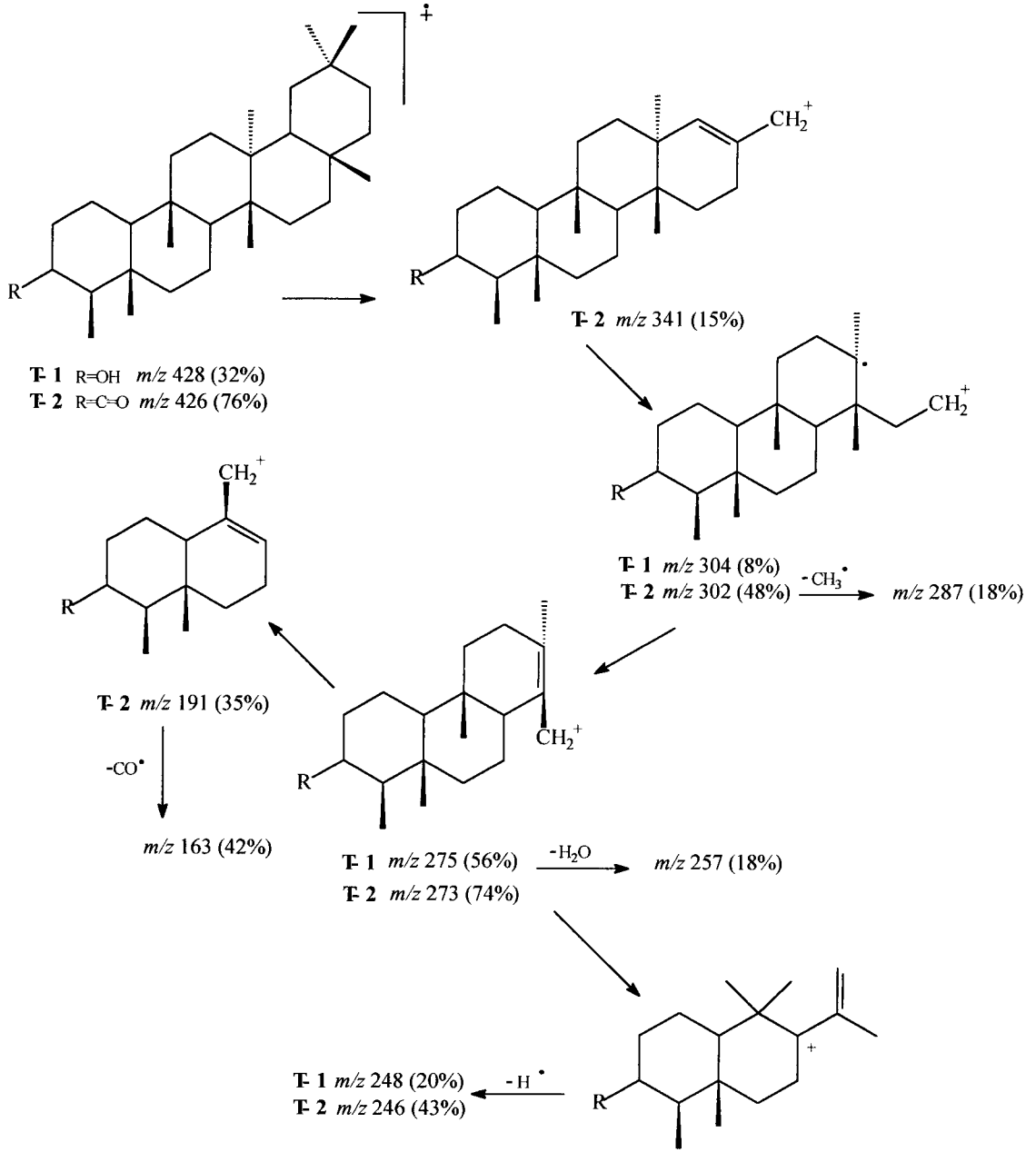
The two friedelane triterpenes were obtained from the *n*-hexane extract of *Goniothalamus thwaitesii*.



Compound	R
T-1	·····OH
T-2	=O

Figure 3.10: Structures of T-1 and T-2

TLC analysis showed pink spots on spraying with anisaldehyde-H_2SO_4 reagent, followed by heating, suggesting terpene structures. The IR spectra indicated the presence of *gem*-dimethyl groups (*ca.* 1385 cm^{-1}) (Williams and Fleming, 1995). HREIMS and *J*-modulated ^{13}C NMR revealed triterpenic skeletons with 30 carbon atoms. Common features readily identified on the ^{1}H and ^{13}C NMR (Tables 3.21 & 3.22) included eight methyls, occurring as seven singlets and one doublet, six quaternary carbons, four methines and eleven methylenes, which suggested the structures as pentacyclic triterpenes of the friedelane group. This was further confirmed with typical fragments in the HREIMS (Scheme 3.11) (Budzikiewicz *et al.*, 1963; Shiojima *et al.*, 1992). Assignments were established by means of COSY, HC-COBI, HMBC, NOESY experiments and comparison with published data.



Scheme 3.11: Suggested mass fragmentation pattern of T-1 and T-2

Table 3.21: ^{1}H NMR (400 MHz) spectral data of T-1 and T-2

Position	T-1[a]	T-2
1	1.50/1.68 (m)	1.96/1.69 (m)
2	1.91/2.18 (m)	2.40/2.31 (m)
3	3.99 (td, 2.5, obsc)	-
4	1.32 (m)	2.27 (m)
6	obsc/1.85 (m)	1.28/1.76 (m)
7	1.40 (m)	1.38/1.49 (m)
8	1.37 (m)	1.39 (m)
10	1.05 (m)	1.54 (m)
11		1.26/1.46 (m)
12	1.31 (m)	1.36 (m)
15		1.51/1.32 (m)
16		1.39/1.59 (m)
18	1.57 (m)	1.57 (m)
19		1.36/1.21 (m)
21		1.28/1.43 (m)
22		0.94/1.52 (m)
Me-23	1.18 (d, 6.9)	0.89 (d, 6.4)
Me-24	1.30 (s)	0.73 (s)
Me-25	0.95 (s)	0.88 (s)
Me-26	1.02 (s)	1.02 (s)
Me-27	1.06 (s)	1.06 (s)
Me-28	1.21 (s)	1.19 (s)
Me-29	1.01 (s)	0.96 (s)
Me-30	1.08 (s)	1.01 (s)

All data obtained in $CDCl_3$, a = Spectrum run in C_5D_5N
In parentheses coupling constant *J* are in Hz.
Methylene signals are listed as Ha/Hb in each column

Table 3.22: ^{13}C NMR (100 MHz) spectral data of T-1 and T-2

Position	T-1[a]	T-2
1	17.1	22.5
2	37.0	41.7
3	71.9	213.3
4	50.5	58.5
5	39.0	42.4
6	42.8	41.5
7	18.5	18.4
8	54.0	53.3
9	37.9	37.7
10	62.4	59.7
11	36.4	35.8
12	31.4	30.7
13	38.9	38.5
14	40.4	39.9
15	33.0	32.6
16	36.9	36.2
17	30.7	30.2
18	43.6	43.0
19	36.0	35.6
20	28.8	28.4
21	33.6	33.0
22	39.9	39.5
Me-23	13.0	7.0
Me-24	17.5	14.8
Me-25	19.1	18.1
Me-26	20.8	20.4
Me-27	19.3	18.8
Me-28	32.7	32.3
Me-29	35.5	35.2
Me-30	32.5	32.0

All data obtained in $CDCl_3$, a = Spectrum run in C_5D_5N

*a) Identification of **T-1** as friedelinol*

The presence of a broad absorption band at 3471 cm^{-1} in the IR spectrum indicated an hydroxyl group. HREIMS gave a molecular ion peak at *m/z* 428, accounting for the molecular formula $C_{30}H_{52}O$ (5 DBE).

Characteristic features in the ^{1}H (Spectrum 3.21; Table 3.21) and ^{13}C NMR (Spectrum 3.22; Table 3.22) included one oxymethine at δ 3.99 (δ_C 71.8). The structure of a C-3 hydroxylated friedelane triterpene was rationalised by comparison of IR, MS, ^{1}H and ^{13}C NMR chemical shifts of **T-1** with published data for friedelan-3β-ol or epifriedelinol (Duwiejua, 1992). A combination of COSY, HC-COBI and HMBC (Table 3.23) experiments established some ^{1}H and corrected ^{13}C NMR assignments. Distinction of signals for C-19 from C-21 and C-16 from C-22, previously misreported by Duwiejua (1992), were corrected on the basis of the observation of a ^{2}J coupling in the HMBC between H-18 and the methylene at δ 36.0, thus assigned as C-19. Consequently C-21 was assigned at δ 33.6. Positions C-16 and C-22 were respectively assigned at δ 36.9 and 39.9, in agreement with values obtained for friedelanes (Patra and Chaudhuri, 1987). The relative stereochemistry at C-3 (OH group in axial or 3α position) was established on the basis of the $[\alpha]_D$ measurement which was in agreement with published data for friedelan-3α-ol or friedelinol (Shoppee *et al.*, 1962) and observation of NOE interactions between H-3 and Me-23, Me-24 (Figure 3.11).

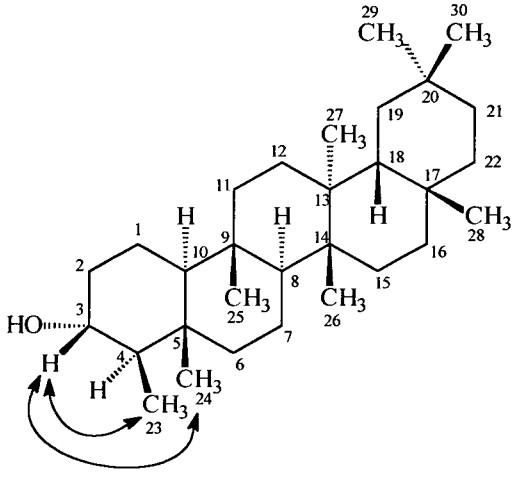
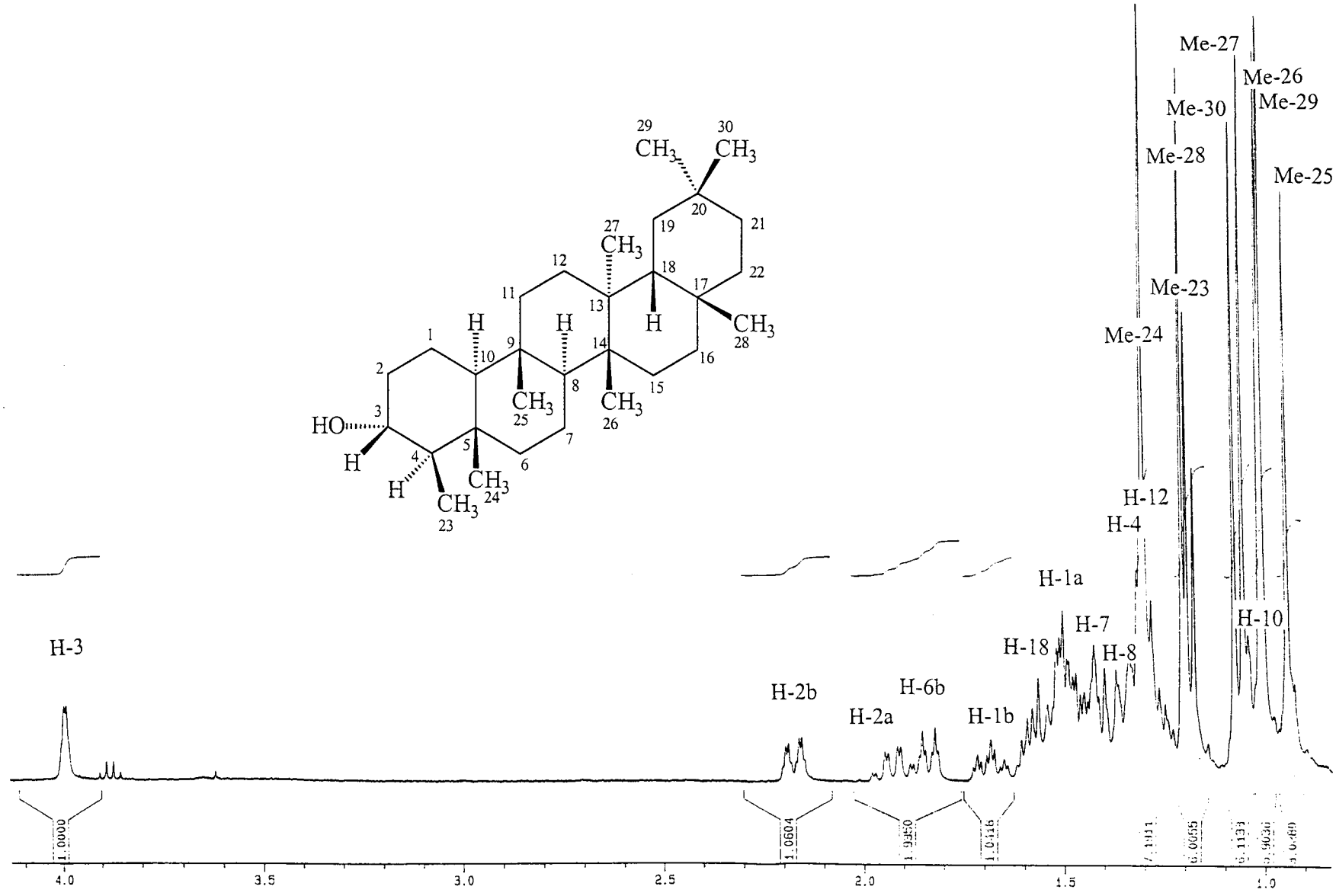


Figure 3.11: Significant NOE interactions of T-1

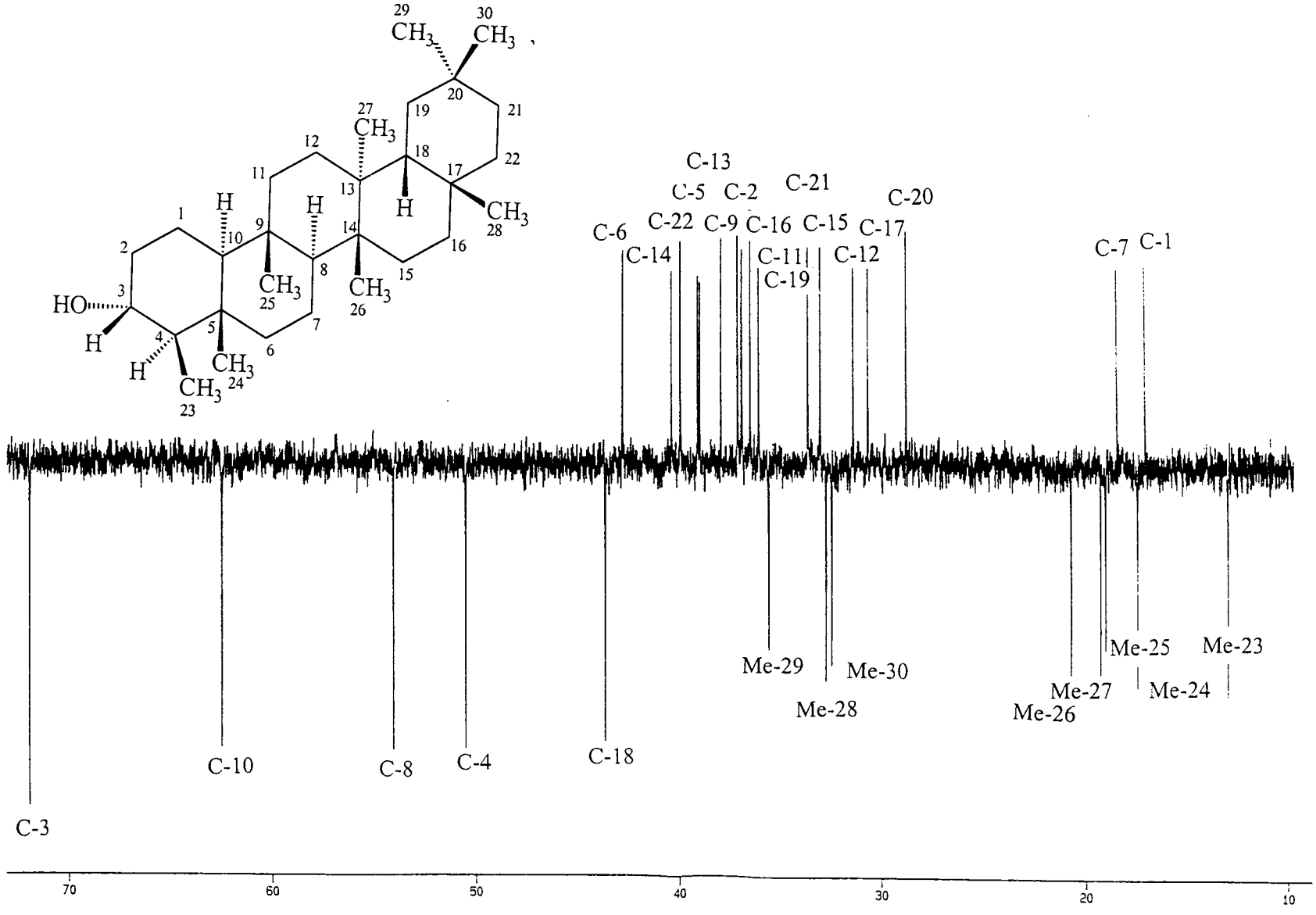
Table 3.23: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of T-1

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
3	3.99 (td, 2.5, obsc)	71.9		17.1 (C-1)
4	1.32 (m)	50.5	39.0 (C-5)	17.5 (C-24), 42.8 (C-6), 62.4 (C-10)
8	1.37 (m)	54.0	18.5 (C-7)	20.8 (C-26), 33.0 (C-15), 38.9 (C-13), 42.8 (C-6)
10	1.05 (m)	62.4	39.0 (C-5)	42.8 (C-6)
18	1.57 (m)	43.6	30.7 (C-17), 36.0 (C-19)	19.3 (C-27), 31.4 (C-12), 32.7 (C-28),40.4 (C-14)
Me-23	1.18 (d, 6.9)	13.0	50.5 (C-4)	39.0 (C-5), 71.9 (C-3)
Me-24	1.30 (s)	17.5	39.0 (C-5)	42.8 (C-6), 50.5 (C-24), 62.4 (C-10)
Me-25	0.95 (s)	19.1	37.9 (C-9)	36.4(C-11), 54.0 (C-8), 62.4 (C-10)
Me-26	1.02 (s)	20.8	40.4 (C-14)	33.0 (C-15), 38.9 (C-13), 54.0 (C-8)
Me-27	1.06 (s)	19.3	38.9 (C-13)	31.4 (C-12), 40.4 (C-14), 43.6 (C-18)
Me-28	1.21 (s)	32.7	30.7 (C-17)	36.9 (C-16), 43.6 (C-18), 39.9 (C-22)
Me-29	1.01 (s)	35.5	28.8 (C-20)	32.5 (C-30), 33.6 (C-21), 36.0 (C-19)
Me-30	1.08 (s)	32.5	28.8 (C-20)	33.6 (C-21), 35.5 (C-29), 36.0 (C-19)

All data obtained in C_5D_5N.
In parentheses coupling constant *J* are in Hz.
Methylene signals are listed as Ha/Hb in each column



Spectrum 3.21: ^{1}H NMR (400 MHz, C_5D_5N) of T-1



Spectrum 3.22: *J*-modulated ^{13}C NMR (100 MHz, C_5D_5N) of T-1

*b) Identification of **T-2** as friedelin*

The presence of a sharp absorption band at 1716 cm^{-1} in the IR spectrum indicated a saturated ketone group. HREIMS gave a molecular ion at *m/z* 426, consistent with the molecular formula $C_{30}H_{50}O$ (6 DBE). Characteristic features in the ^{1}H (Table 3.21) and ^{13}C NMR (Table 3.22) included non equivalent methylene protons at δ 2.40/2.31 (δ_C 41.7) and a methine at δ 2.27 (δ_C 58.5) both adjacent to a carbonyl group (δ 213.3).

Comparison of the mp, $[\alpha]_D$, IR, MS, ^{1}H and ^{13}C NMR of **T-2** showed good agreement with data published for the known pentacyclic triterpene friedelan-3-one or friedelin (Sainsbury 1970; Klass *et al.*, 1992; Ageta *et al.*, 1995).

3.2.3.2 Characterisation of the isolated lupane triterpene T-3 as betulinic acid

This compound was isolated from the ethyl acetate extract of *Goniothalamus thwaitesii.*

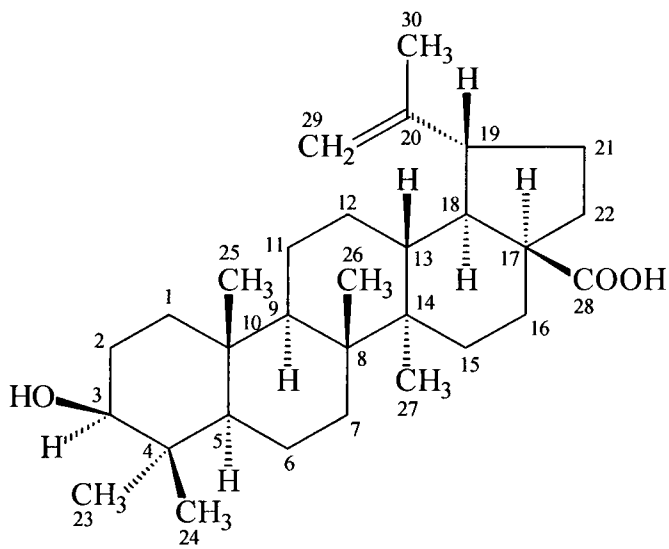
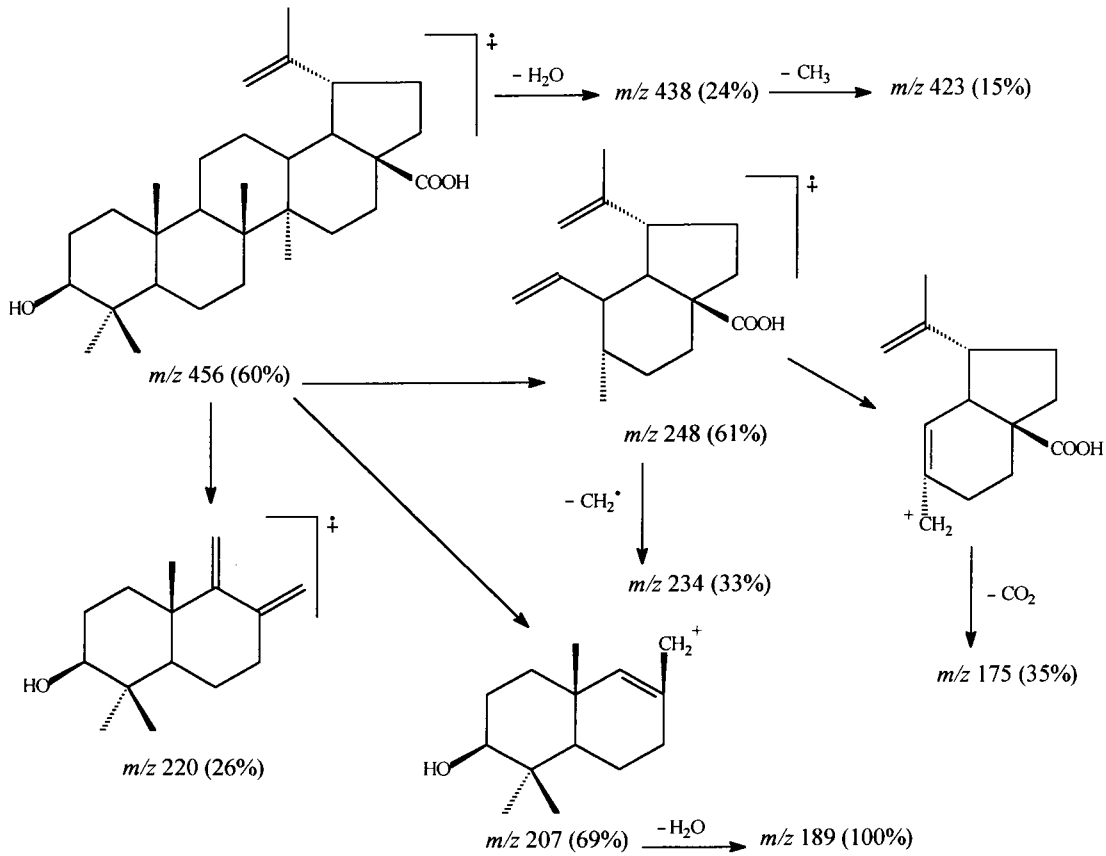


Figure 3.12: Structure of T-3

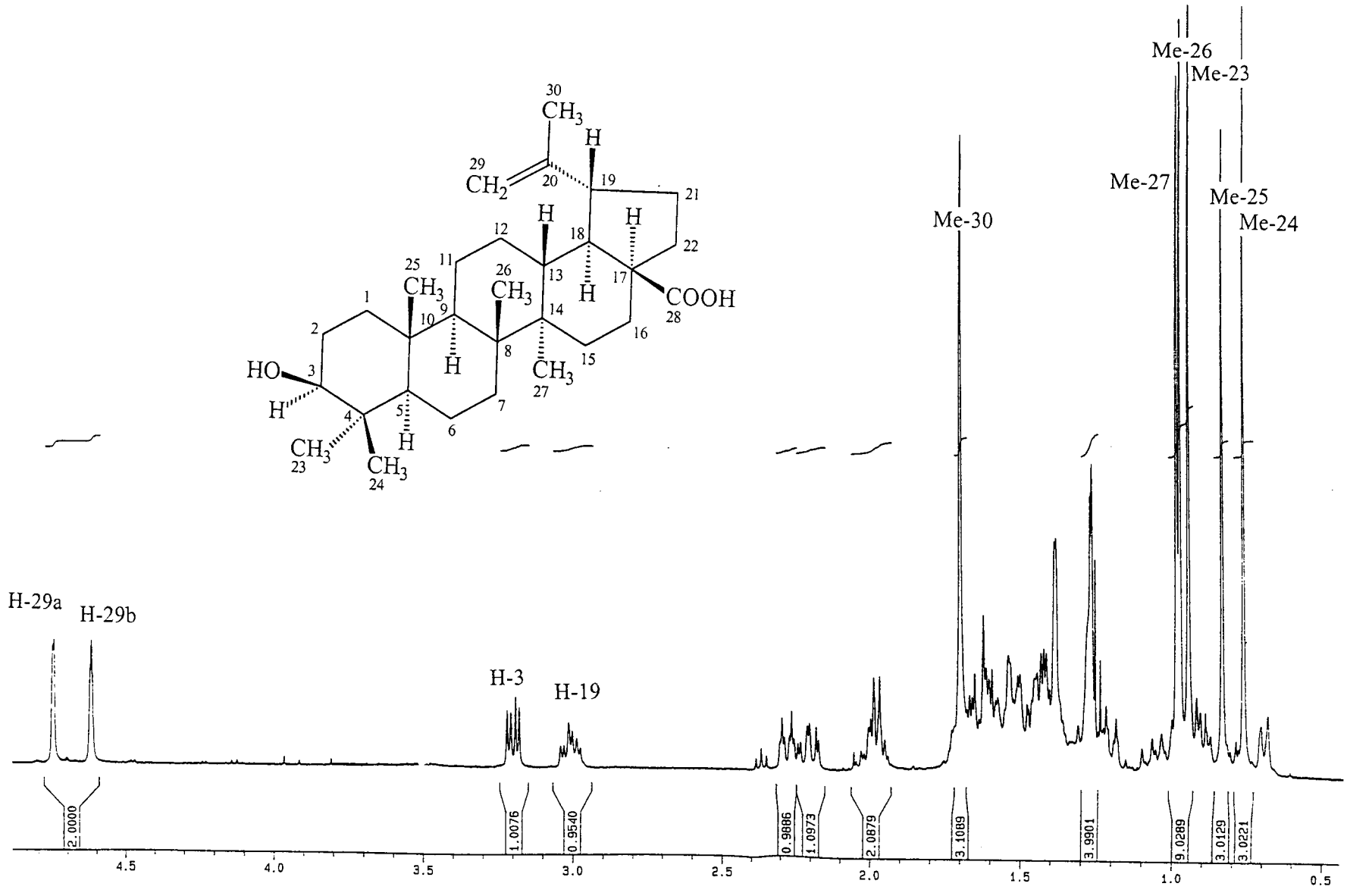
TLC analysis displayed a pink spot on spraying with anisaldehyde-H_2SO_4 reagent, followed by heating, suggesting the presence of a terpene. The IR spectrum showed hydroxyl (3446 cm^{-1}), carbonyl (1685 cm^{-1}), exomethylene (3068, 1637, 881 cm^{-1}) and *gem*-dimethyl groups (1384 cm^{-1}) (Williams and Fleming, 1995).

HREIMS yielded a molecular ion at *m/z* 456, consistent with the molecular formula $C_{30}H_{48}O_3$ (7 DBE) indicating a triterpenic structure, which was confirmed in the *J*-modulated ^{13}C NMR. ^1H (Spectrum 3.23) and ^{13}C NMR suggested the presence of an isopropenyl unit with a pair of deshielded doublets for exomethylene protons at δ 4.75 and 4.61 (J = 1.6 Hz) (δ_C 109.9) and a methyl singlet at δ 1.70 (δ_C 19.6) adjacent to an olefinic quaternary sp^2 carbon (δ 150.6). Other readily identified features were the presence of an oxymethine at δ 3.20 (dd, J = 5, 11.2 Hz) (δ_C 79.2) and five remaining methyls, occurring as singlets in the ^1H NMR spectrum. Characteristic features in the *J*-modulated ^{13}C NMR included a carboxylic acid carbonyl resonance (δ 180.5), five quaternary carbons, ten methylenes and five methines.

The structure of 3β-OH-20(19)-lupaen-28-oic acid or betulinic acid was rationalised by comparison of the ^{13}C NMR chemical shifts of **T-3** with those published in the recent review on pentacyclic triterpenoids by Mahato and Kundu (1994). Mp, $[\alpha]_D$, IR, mass fragmentation pattern (Scheme 3.12), ^1H NMR and the relative stereochemistry at C-3 (OH group in equatorial or 3β position) also complied with published data (Budzikiewicz *et al.*, 1963; Robinson and Martel, 1970; Siddiqui *et al.*, 1988; Shiojima *et al.*, 1992).



Scheme 3.12: Suggested mass fragmentation pattern of T-3



Spectrum 3.23: ^{1}H NMR (400 MHz, $CDCl_3$) of T-3

3.2.4 Properties and spectral data of isolated terpenes

S-1 [Methyl-(2E,6E)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate]

Yellow oil. IR ν_{max} (film) cm^{-1}: 2966, 2948, 2934, 2875, 1717 (C=O), 1648, 1436, 1384, 1358, 1224, 1148, 1075. UV λ_{max} (EtOH) nm: 218, 262. Found $[M]^+$ 266.1911 ($C_{16}H_{26}O_3$ requires 266.1882) HREIMS *m/z* (rel. int. %): 266 (27), 234 (25), 222 (32), 204 (93), 207 (20), 189 (37), 179 (31), 161 (100), 153 (50), 135 (54), 121 (82), 109 (98), 105 (72), 95 (100), 81 (100). ^1H & ^{13}C NMR: Tables 3.17 & 3.18.

S-2 [Methyl-(2E,6E,10ξ)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate]

Yellow oil. $[\alpha]_D^{23.9}$ +8.7° (MeOH, *c* 0.92) [Lit. $[\alpha]_D$ +13° (MeOH, *c* 0.6), Etse *et al.*, 1988]. IR ν_{max} (film) cm^{-1}: 3443 (OH), 2971, 2948, 2929, 2856, 1720 (C=O), 1648, 1436, 1384, 1358, 1226, 1148, 1078. UV λ_{max} (EtOH) nm: 218. Found $[M]^+$ 284.1996 ($C_{16}H_{28}O_4$ requires 284.1987) HREIMS *m/z* (rel. int. %): 284 (2), 266 (6), 235 (48), 224 (84), 207 (9), 193 (99), 153 (52), 151 (38). ^1H & ^{13}C NMR: Tables 3.17 & 3.18.

S-3 [Methyl-(2E,6E,10ξ)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate]

Yellow oil. $[\alpha]_D^{23.5}$ +8 ° ($CHCl_3$, *c* 0.25) [Lit. $[\alpha]_D$ +5° ($CHCl_3$, *c* 0.3), Etse *et al.*, 1988]. IR ν_{max} (film) cm^{-1}: 3457 (OH), 2935, 2870, 1717 (C=O), 1645, 1436, 1384, 1358, 1226, 1150, 1024, 920, 893, 864. UV λ_{max} (EtOH) nm: 219. Found $[M]^+$ 266.1884 ($C_{16}H_{26}O_3$ requires 266.1882) HREIMS *m/z* (rel. int. %): 266 (7), 264 (9), 248 (22), 233 (24), 189 (30), 175 (38), 135 (100), 122 (64), 107 (77). ^1H & ^{13}C NMR: Tables 3.17 & 3.18.

S-4 (β-Caryophyllene-4,5-oxide)

Colourless oil. $[\alpha]_D^{24}$ -54.5 ° ($CHCl_3$, *c* 0.11) [Lit. $[\alpha]_D$ -57.7° ($CHCl_3$, *c* 0.6), Heymann *et al.*, 1994]. IR ν_{max} (film) cm^{-1}: 3066, 2958, 2927, 2861, 1631, 1454, 1384, 1365, 1261, 1122, 1076, 1014, 964, 910, 890, 821. Found $[M]^+$ 220.1453 ($C_{15}H_{24}O$ requires 220.1827) HREIMS *m/z* (rel. int. %): 220 (5), 205 (50), 167 (70), 149 (100), 136 (20), 109 (30), 93 (35), 79 (22), 69 (62). ^1H NMR (400 MHz, $CDCl_3$) (*J* in Hz): δ 1.75 (t, 10.1, H-1), 1.33/1.72-1.55 (m, H-2), 0.96/2.12 (m, H-3), 2.88 (dd, 4.2, 10.6, H-5), 1.42/2.24 (m, H-6), 2.12/2.33 (m, H-7), 2.61 (dt, H-9), 1.72-1.55/1.72-1.55 (m, H-10), 1.01 (s, Me-12), 0.99 (s, Me-13), 1.12 (s, Me-14) 4.98/4.87 (d, 1.3, Me-15). ^{13}C (100 MHz, $CDCl_3$): δ 51.0 (C-1), 27.4 (C-2), 39.5 (C-3), 60.0 (C-4), 64.0 (C-5), 30.4 (C-6), 30.0 (C-7), 152.0 (C-8), 49.0 (C-9), 40.1 (C-10), 34.2 (C-11), 21.8 (Me-12), 30.1 (Me-13), 17.2 (Me-14), 113.0 (Me-15).

D-1 [(2E,7ξ,11ξ)-Phytol]

Oil. IR ν_{max} (film) cm^{-1}: 3370 (OH), 3023, 2952, 2925, 2869, 1698, 1538, 1519, 1463, 1365, 1126, 1058, 894, 838. Found $[M]^+$ 296.3068 ($C_{20}H_{40}O$ requires 296.3079) HREIMS *m/z* (rel. int. %): 296 (18), 278 (19), 263 (7), 196 (19), 138 (36), 126 (62), 123 (100), 99 (12), 85 (34). ^1H & ^{13}C NMR: Table 3.20.

T-1 [Friedelan-3α-ol (friedelinol)]

Colourless needles (*n*-hexane/$CHCl_3$). Mp 268-270° (Lit. mp 292-301°, Shoppee *et al.*, 1962). $[\alpha]_D^{21.9}$ +22.7° ($CHCl_3$, *c* 0.176) [Lit. $[\alpha]_D^{20}$ +18° ($CHCl_3$, *c* 1.0), Shoppee *et al.*, 1962]. IR ν_{max} (KBr disc) cm^{-1}: 3471 (OH), 2979, 2933, 2871, 1446, 1384. Found $[M]^+$ 428.4021 ($C_{30}H_{52}O$ requires 428.4018) HREIMS *m/z* (rel. int. %): 428 (32), 413 (37), 395 (8), 304 (8), 275 (56), 257 (18), 248 (20), 220 (41), 205 (33), 177 (46), 165 (94), 150 (21), 137 (42), 125 (75), 123 (74), 109 (88). ^1H & ^{13}C: Tables 3.21 & 3.22.

T-2 [Friedelan-3-one (friedelin)]

Colourless needles (*n*-hexane/$CHCl_3$). Mp 261-262° (Lit. mp 261-262°, Sainsbury 1970). $[\alpha]_D^{19.1}$ -28.4° ($CHCl_3$, *c* 0.176) (Lit. $[\alpha]_D$ -22.5° ($CHCl_3$, *c* 1.0), Klass *et al.*, 1992). IR ν_{max} (KBr disc) cm^{-1}: 2971, 2948, 2927, 2869, 1716 (C=O), 1456, 1390. Found $[M]^+$ 426.3844 ($C_{30}H_{50}O$ requires 426.3861) HREIMS *m/z* (rel. int. %): 426 (76), 411 (27), 341 (15), 302 (48), 287 (18), 273 (74), 257 (18), 246 (43), 231 (43), 218 (59), 207 (12), 191 (35), 179 (50), 163 (42), 149 (36), 137 (51), 125 (100). ^1H &^{13}C: Tables 3.21 & 3.22.

T-3 (Betulinic acid)

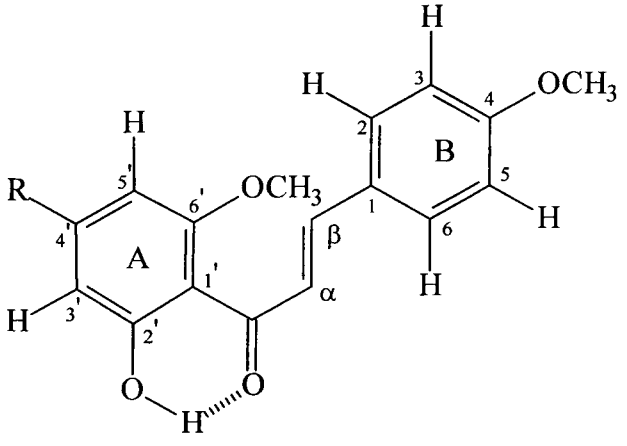
Colourless prisms (EtOH). Mp 279-284° (Lit. mp 275-278°, Robinson and Martel, 1970). $[\alpha]_D^{23.3}$ +8.4° (Pyr, *c* 0.83) (Lit. $[\alpha]_D^{23}$ +7.9° (Pyr, *c* 0.57), Robinson and Martel, 1970). IR ν_{max} (KBr disc) cm^{-1}: 3446 (OH), 3068, 2942, 2869, 1685 (C=O), 1637, 1456, 1384, 1031, 881. Found $[M]^+$ 456.3582 ($C_{30}H_{48}O_3$ requires 456.3603) HREIMS *m/z* (rel. int. %): 456 (60), 438 (24), 423 (15), 395 (14), 316 (14), 281 (16), 248 (61), 234 (33), 220 (26), 207 (69), 189 (100), 175 (35), 135 (59). ^1H (400 MHz, $CDCl_3$) (*J* in Hz): δ 3.20 (dd, 5, 11.2, H-3), 3.00 (td, 4.6, 10.6, H-19), 0.95 (s, Me-23), 0.76 (s, Me-24), 0.83 (s, Me-25), 0.97 (s, Me-26), 0.98 (s, Me-27), 4.61/4.75 (d, 1.6, H-29a/b), 1.70 (s, Me-30). ^{13}C (100 MHz, $CDCl_3$): δ 38.9 (C-1), 27.6 (C-2), 79.2 (C-3), 39.1 (C-4), 55.6 (C-5), 18.5 (C-6), 34.6 (C-7), 40.9 (C-8), 50.8 (C-9), 37.4 (C-10), 21.1 (C-11), 25.7 (C-12), 38.6 (C-13), 42.7 (C-14), 30.8 (C-15), 32.4 (C-16), 56.5 (C-17), 47.1 (C-18), 49.5 (C-19), 150.6 (C-20), 29.9 (C-21), 37.3 (C-22), 28.2 (Me-23), 15.5 (Me-24), 16.2 (Me-25), 16.2 (Me-26), 14.9 (Me-27), 180.5 (Me-28), 109.9 (H-29a/b), 19.6 (Me-30).

3.3 Flavonoids

Thirteen flavonoids were obtained from *Cleistopholis glauca*, *Piptostigma fasciculata*, *Goniothalamus thwaitesii* and *Goniothalamus gardneri*. They included two chalcones, three dihydrochalcones, one chalcone dimer, two flavanones, one dihydroflavonol, three flavonols including two glycosides, one flavonol-3-*O*-methyl ether.

3.3.1 Chalcones

Two chalcones were isolated from *Goniothalamus gardneri* (see Section 2.4.4). They were coded **F-1** (GGP.3) and **F-2** (GGE.2) and identified respectively as 2'-hydroxy-4,4',6'-trimethoxychalcone (flavokawain A) and 2',4'-dihydroxy-4,6'-dimethoxychalcone (Figure 3.13).



Compound	R
F-1	OCH_3
F-2	OH

Figure 3.13: Structures of F-1 and F-2

3.3.1.1 General characterisation of isolated chalcones

IR spectral data indicated hydroxyl (*ca.* 3435-3200 cm^{-1}) and aryl ether (*ca.* 2840 cm^{-1}) groups, aromatic rings (*ca.* 3000, 1604, 1580, 1510 cm^{-1}) with one *para*-disubstitution (*ca.* 830 cm^{-1}), hydrogen bonded α,β-unsaturated carbonyl functions conjugated with aromatic rings (*ca.* 1620 cm^{-1}) and *trans*-olefinic protons (*ca.* 970 cm^{-1}) (Williams and Fleming, 1995).

Both compounds were yellow in colour. UV spectral behaviour (Table 3.24) was typical of chalcones with a major absorption (band I/340-390 nm) associated with the B-ring cinnamoyl system and a relatively minor absorption (band II/220-270 nm) associated with the A-ring benzoyl moiety (Jurd, 1962; Mabry *et al.*, 1970). Spectra were recorded in MeOH before and after addition of shift reagents, specifically interacting with the different hydroxyl sites, thus establishing the substitution pattern of the chalcones. Bathochromic shifts (band I/*ca.* 35 nm) on addition of $AlCl_3$ revealed free 2'-OH groups (Mabry *et al.*, 1970).

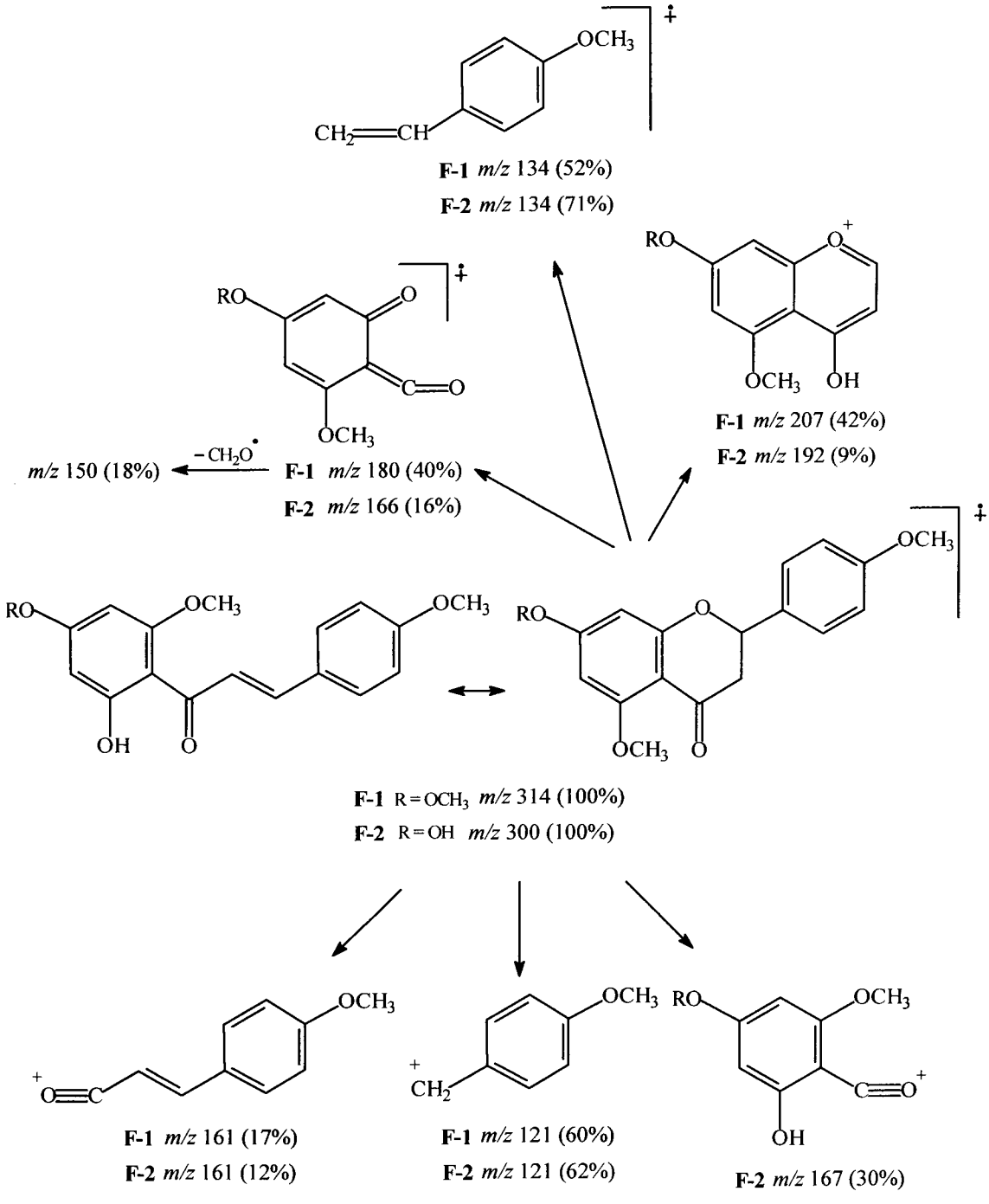
Table 3.24: UV (λ_{max}, nm) spectral data of isolated chalcones

		F-1	F-2
MeOH	Band II	233 (sh)	237 (sh)
	Band I	363	363
$AlCl_3$	Band II	241	239
	Band I	400, 336 (sh)	397, 334 (sh)
NaOAc	Band II	233 (sh)	237 (sh)
	Band I	364	372
NaOMe	Band II	233 (sh)	246 (sh)
	Band I	363 (sh), 324	390, 332 (sh)

HREIMS (Scheme 3.13) enabled preliminary identification of the substitution patterns on each ring. Both compounds yielded intense molecular ions and peaks at $[M-H]^+$, $[M-OH]^+$, $[M-CO]^+$ and $[M-CO-CH_3]^+$. They showed typical chalcone fragmentation (Mabry and Markham, 1975). Fragments from the corresponding flavanones were also observed (Audier, 1966).

The ^1H NMR (Table 3.25) displayed a low-field singlet (δ *ca.* 14.3) assignable to the strongly chelated 2'-OH. The chalcone skeletons were confirmed with the presence of a pair of olefinic protons showing a *trans* coupling constant at δ *ca.* 7.80 and 7.75 (1H each, J = 15.6 Hz) assigned respectively to H-α and H-β. Two *ortho* coupled doublets at δ 7.57 and 6.94 (2H each, J = 8.8 Hz) were attributable respectively to H-2/6 and H-3/5, establishing the *para*-disubstitution on the B-ring. Two *meta* coupled doublets at δ *ca.* 6.10 and 5.95 (1H each, J = 2.4 Hz) indicated the presence of a 2',4',6'-trisubstituted A-ring (Mabry *et al.*, 1970; Markham and Mabry, 1975). Furthermore, the ^1H NMR spectra showed singlets (3H) in the range δ 3.95-3.85 attributable to aromatic methoxyl groups.

The *J*-modulated ^{13}C NMR (Table 3.26), assigned by ^1H-^{13}C HC-COBI, were typical for the flavonoid skeletons (15 carbons) bearing methoxyl substituents. Carbonyls were within the range of chalcone carbonyls (δ *ca.* 193) and two olefinic carbons at δ *ca.* 142 (C-β) and 125 (C-α) further supported the chalcone structures (Agrawal and Bansal, 1989). A pair of two equivalent aromatic methines, at δ *ca.* 130 and δ *ca.* 114, were assigned to C-2/6 and C-3/5 on the B-ring while signals at δ *ca.* 95 and 91 were attributed to the A-ring methines. The ^{13}C NMR also displayed four oxygenated quaternary carbons and relatively shielded methoxyl resonances (δ *ca.* 56.0-55.6), requiring them to be adjacent to at least one free *ortho* position (Panichpol and Waterman, 1978). A combination of HMBC and NOESY experiments established unambiguous ^1H and ^{13}C NMR assignments and the placement of oxygenated positions on each ring.



Scheme 3.13: Suggested mass fragmentation pattern of F-1 and F-2

Table 3.25: ^{1}H NMR (400 MHz) spectral data of F-1 and F-2

Position	F-1	F-2
2/6	7.57 (d, 8.8)	7.57 (d, 8.8)
3/5	6.94 (d, 8.8)	6.94 (d, 8.8)
3'	6.12 (d, 2.4)	6.04 (d, 2.4)
5'	5.97 (d, 2.4)	5.96 (d, 2.4)
4-OMe	3.86 (s)	3.87 (s)
2'-OH	14.40 (s)	14.26 (s)
4'-OMe	3.84 (s)	-
4'-OH	-	5.43 (brs)
6'-OMe	3.92 (s)	3.94 (s)
α	7.82 (d, 15.6)	7.81 (d, 15.6)
β	7.78 (d, 15.6)	7.77 (d, 15.6)

All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.

Table 3.26: ^{13}C NMR (100 MHz) spectral data of F-1 and F-2

Position	F-1	F-2
1	128.6	128.5
2/6	130.3	130.4
3/5	114.6	114.6
4	161.6	161.6
1'	106.6	106.8
2'	168.6	168.1
3'	94.0	97.0
4'	166.2	162.5
5'	91.5	91.2
6'	162.7	163.4
4-OMe	55.6	55.6
4'-OMe	55.8	-
6'-OMe	56.0	56.1
α	125.3	125.3
β	142.7	142.9
C=O	192.8	192.9

All data obtained in $CDCl_3$

*a) Identification of **F-1** as flavokawain A*

This compound was isolated from the petrol extract of *Goniothalamus gardneri*. UV spectral data (Table 3.24) indicated a chalcone with a free 2'-OH. Addition of alkali did not cause a bathochromic shift of band I, therefore requiring positions 4 and/or 4' to be substituted (Jurd and Horowitz, 1961; Jurd, 1962; Mabry *et al.*, 1970).

The HREIMS (Scheme 3.13) yielded a molecular ion at *m/z* 314, indicating the molecular formula $C_{18}H_{18}O_5$ (10 DBE) and showed fragments at *m/z* 313 $[M-H]^+$, 297 $[M-OH]^+$, 286 $[M-CO]^+$ and 271 $[M-CO-CH_3]^+$. Ions at *m/z* 207 and 180 were consistent with an A-ring substituted by two methoxyls and one hydroxyl while those at *m/z* 161, 134 and 121 indicated the presence of one methoxyl on the B-ring.

The ^1H NMR (Spectrum 3.24; Table 3.25) displayed signals typical for a chalcone with a 4-mono-substituted B-ring and a 2',4',6'-tri-substituted A-ring. Three methoxyl groups were present at δ 3.92, 3.86 and 3.84. These combined data required the placement of oxygenated positions to be 2'-OH, 4'-OMe, 6'-OMe on the A-ring and 4-OMe on the B-ring.

Confirmation of the placements of the oxygenated positions and unambiguous ^1H and ^{13}C NMR assignments were established with HMBC (Figure 3.14; Table 3.27) and NOESY experiments (Figure 3.15). The latter revealed cross-peaks between H-3' and the methoxyl at δ 3.84 and between H-5' and the methoxyl at δ 3.92. On the B-ring, the presence of a methoxyl group in C-4 was confirmed by a NOE interaction between H-3/5 and the signal at δ 3.86.

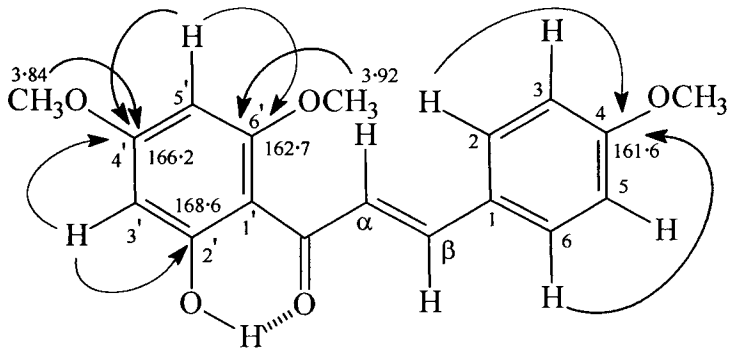


Figure 3.14: Significant HMBC correlations of F-1

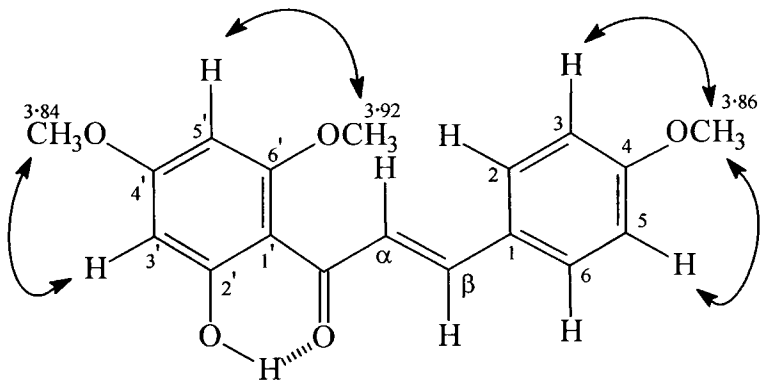


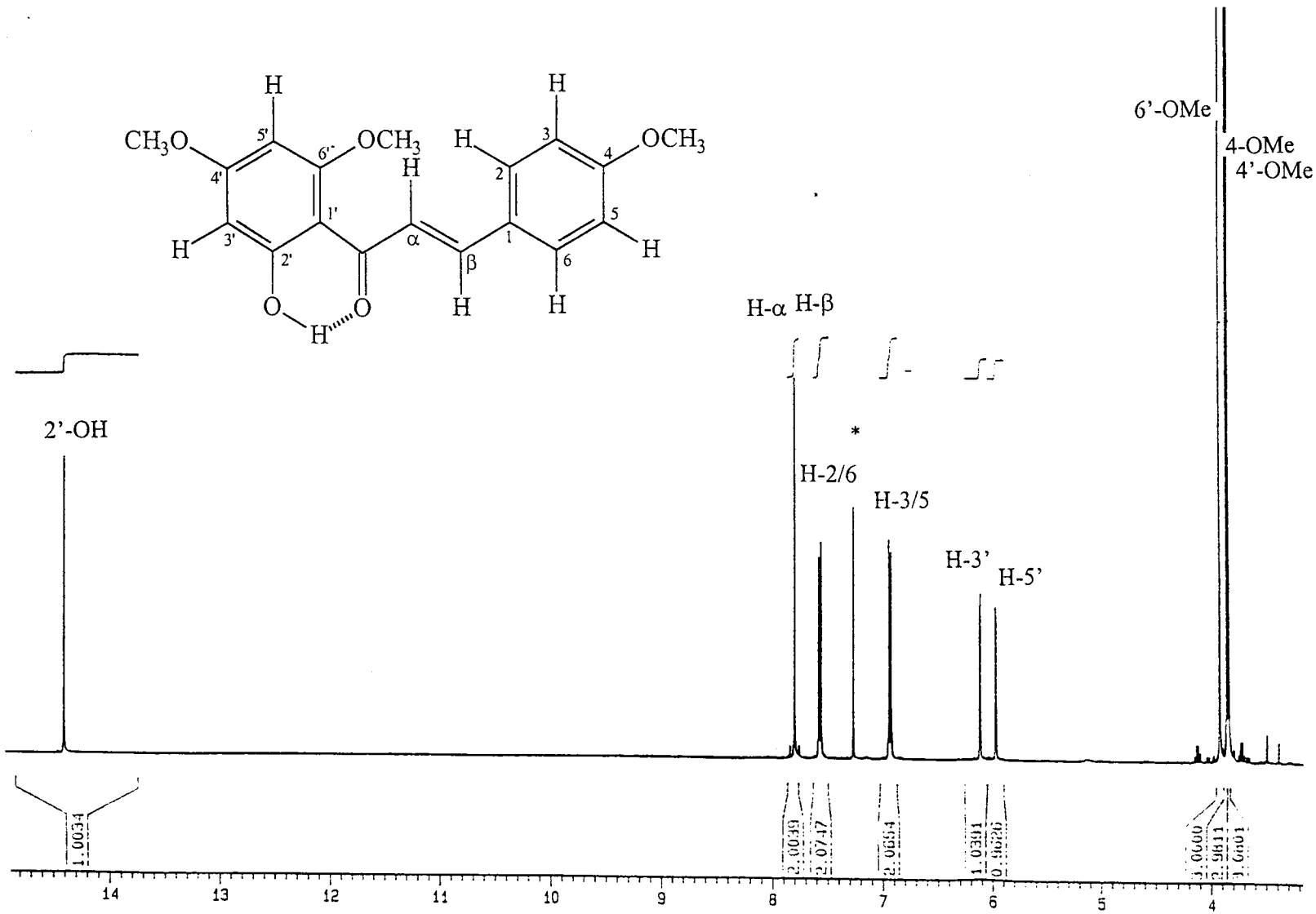
Figure 3.15: Significant NOE interactions of F-1

The mp, IR, UV and MS data of **F-1** complied with those reported for the known 2'-hydroxy-4,4',6'-trimethoxychalcone or flavokawain A, previously reported from *Xanthorrhoea preissii* (Xanthorrhoeaceae) (Birch and Ryan, unpublished results-see Birch and Hextall, 1955), *Piper methysticum* (Piperaceae) (Hansel *et al.*, 1963) and *Dahlia tenuicaulis* (Compositae) (Lam and Wrang, 1975). ^{1}H NMR assignments are reported for the first time. ^{13}C NMR assignments agreed with published data (Duddeck *et al.*, 1978) except for the C-2', C-4' and C-6' resonances respectively corrected at δ 168.6, 166.2 and 162.7.

Table 3.27: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-1

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2/6	7.57 (d, 8.8)	130.3		142.7 (C-β), 161.6 (C-4)
3/5	6.94 (d, 8.8)	114.6	161.6 (C-4)	128.6 (C-1)
3'	6.12 (d, 2.4)	94.0	166.2 (C-4'), 168.6 (C-2')	91.5 (C-5'), 106.6 (C-1')
5'	5.97 (d, 2.4)	91.5	162.7 (C-6'), 166.2 (C-4')	94.0 (C-3'), 106.6 (C-1')
4-OMe	3.86 (s)	55.6		161.6 (C-4)
4'-OMe	3.84 (s)	55.8		166.2 (C-4')
6'-OMe	3.92 (s)	56.0		162.7 (C-6')
α	7.82 (d, 15.6)	125.3	192.8 (C=O)	128.6 (C-1)
β	7.78 (d, 15.6)	142.7	125.3 (C-α), 128.6 (C-1)	130.3 (C-2/6), 192.8 (C=O)

All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.



Spectrum 3.24: ^{1}H NMR (400 MHz, $CDCl_3$*) of F-1

*b) Identification of **F-2** as 2',4'-dihydroxy-4,6'-dimethoxychalcone*

This compound was isolated from the ethyl acetate extract of *Goniothalamus gardneri*. UV data again revealed a chalcone with a free 2'-OH (Table 3.24). A bathochromic shift (band I/9 nm) on addition of NaOAc indicated a free 4 and/or 4'-OH (Mabry *et al.*, 1970), while a bathochromic shift (band I/27 nm) with no increase in peak intensity on addition of NaOMe indicated no free 4-OH but a free 2- or 4'-OH (Jurd and Horowitz, 1961; Jurd, 1962).

The HREIMS (Scheme 3.13) revealed a molecular ion at *m/z* 300 consistent with the molecular formula $C_{17}H_{16}O_5$ (10 DBE) with fragments at *m/z* 299 $[M-H]^+$, 283 $[M-OH]^+$, 272 $[M-CO]^+$ and 257 $[M-CO-CH_3]^+$. The mass fragmentation displayed further ions at *m/z* 192, 167 and 166 for an A-ring with two hydroxyls and one methoxyl while fragments at *m/z* 161, 134 and 121 indicated the presence of one methoxyl on the B-ring.

The ^1H NMR (Spectrum 3.25; Table 3.25) showed a chalcone with a 4-mono-substituted B-ring and a 2',4',6'-tri-substituted A-ring. Two methoxyl groups were present at δ 3.94 and 3.87 and one free OH group at δ 5.43. These combined data required the placement of oxygenated positions to be 2'-OH, 4'-OH, 6'-OMe on the A-ring and 4-OMe on the B-ring.

A combination of HMBC and NOESY experiments allowed the unambiguous assignments of these oxygenated positions. The HMBC (Spectrum 3.26; Table 3.28) displayed:

i) ^3J couplings between both the methoxyl at δ 3.87 and H-2/6 and the oxygenated carbon at δ 161.6, thus assigning the methoxylated position C-4 on the B-ring.

ii) a ^3J interaction between the remaining methoxyl at δ 3.94 and the oxygenated carbon at δ 163.4, thus establishing the assignment of the second methoxylated position C-6' on the A-ring.

iii) common ^2J interactions between the A-ring methines and the carbon at δ 162.5, thus establishing the assignment of the C-4' position.

iv) a ^{2}J correlation between the proton at δ 6.04 and the carbon at δ 168.1, which could only be assigned to the remaining oxygenated C-2' resonance. Therefore, oxygenated positions on the A-ring were established as hydroxylated C-2' (δ 168.1), C-4' (δ 162.5) and methoxylated C-6' (δ 163.4). This was in good agreement with the ^{1}H and ^{13}C NMR data which had revealed two distinctive chemical shifts for positions 3' and 5' now respectively assigned at δ 6.04 and 5.96. If the only methoxyl on the A-ring had been located in position 4', then signals for 3' and 5' would have been equivalent because of a symmetric A-ring. The presence of free phenolic 2'- and 4'-OH were also in good agreement with the UV spectral data.

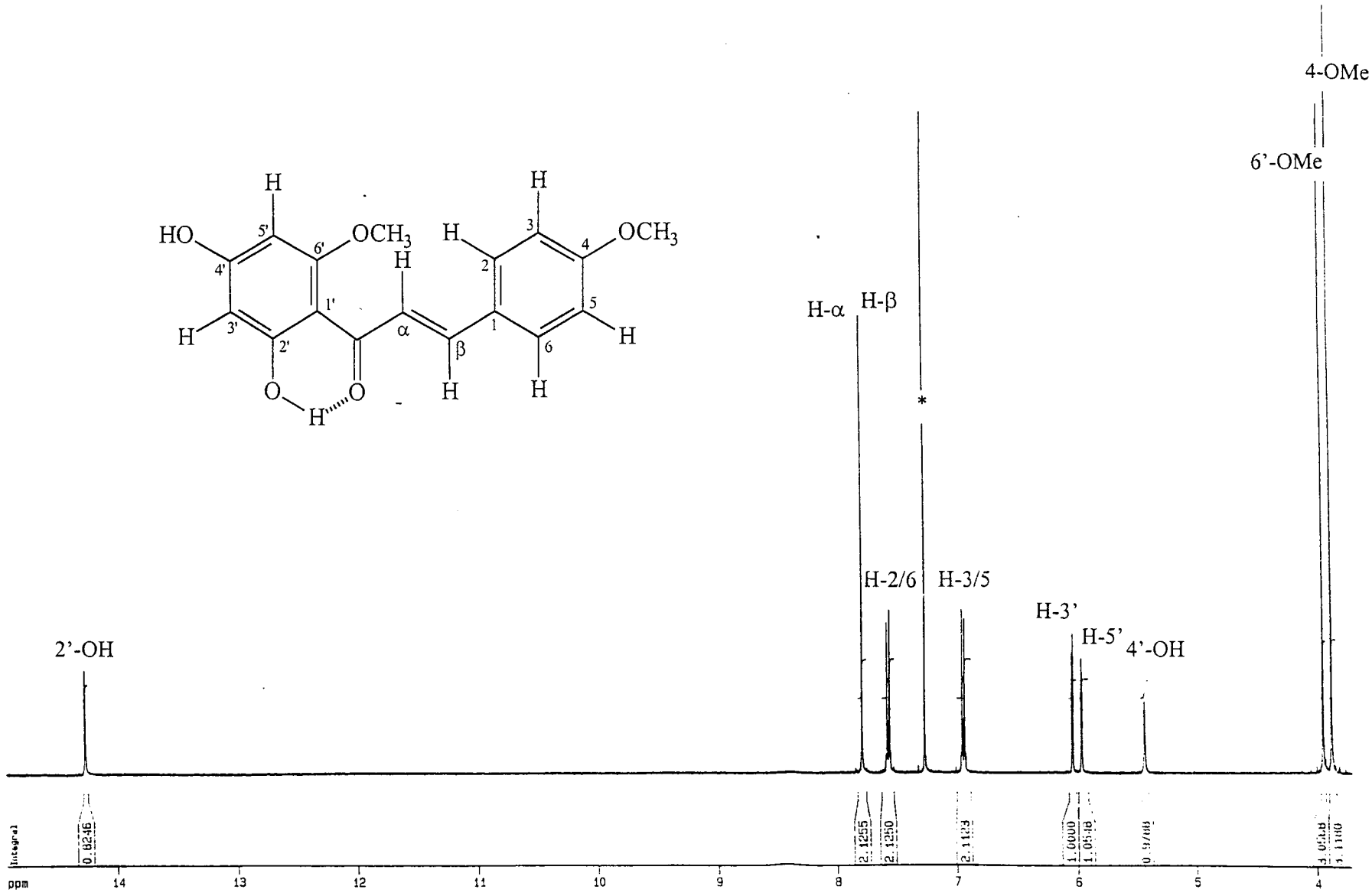
The NOESY experiment (Spectrum 3.33) revealed cross-peaks between H-5' and the methoxyl at δ 3.94, while both H-3' and H-5' protons showed correlations to the 4'-OH. On the B-ring, the presence of a methoxyl group in C-4 was confirmed by a NOE interaction between H-3/5 and the signal at δ 3.87.

On this basis, **F-2** was identified as 2',4'-dihydroxy-4,6'-dimethoxychalcone, which has previously been synthesised but has not been recorded as a natural product. The melting point showed good agreement with published data (Bhardwaj *et al.*, 1982) and complete ^{1}H and ^{13}C NMR assignments are reported for the first time.

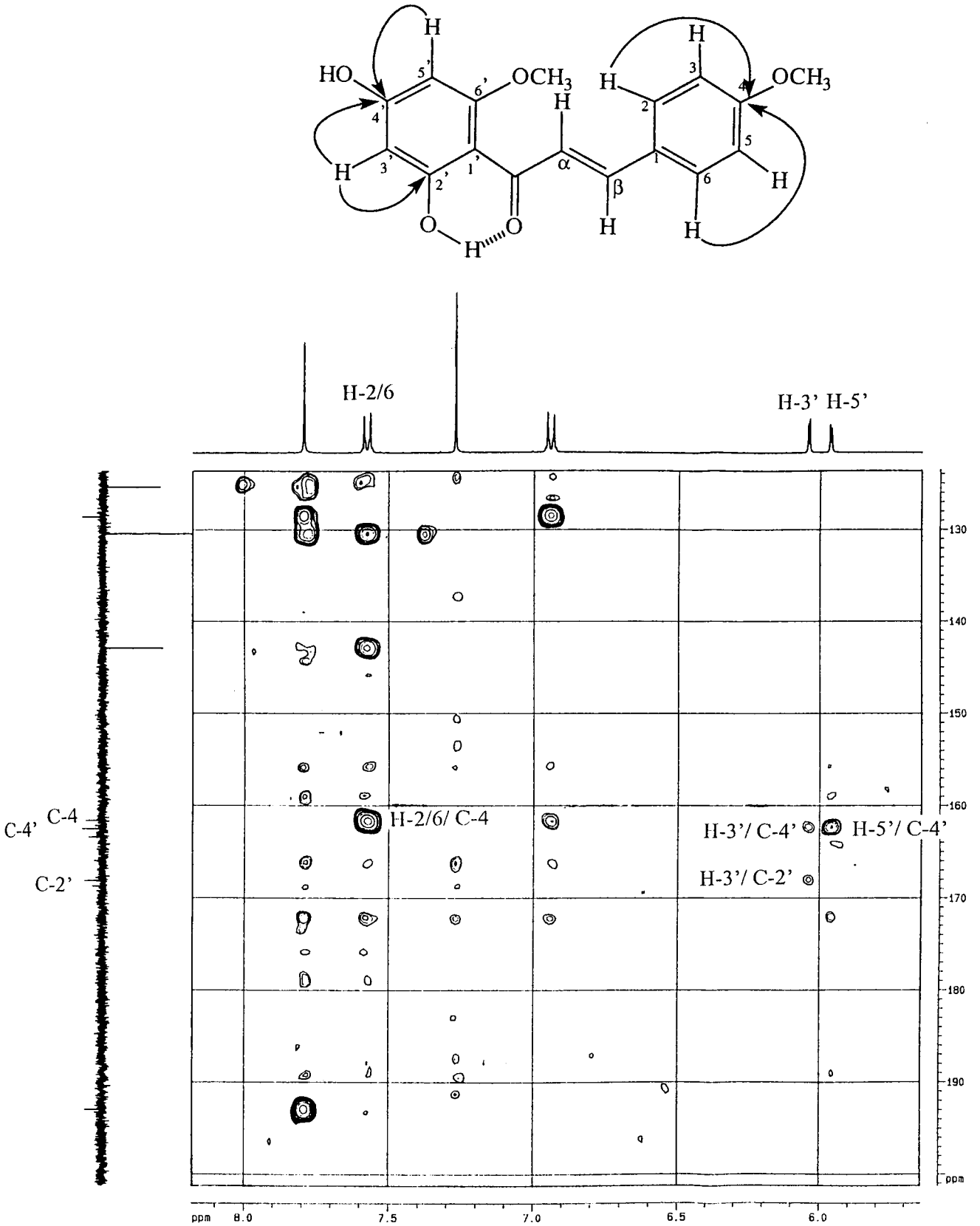
Table 3.28: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-2

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2/6	7.57 (d, 8.8)	130.4		142.9 (C-β), 161.6 (C-4)
3/5	6.94 (d, 8.8)	114.6	161.6 (C-4)	128.5 (C-1)
3'	6.04 (d, 2.4)	97.0	162.5 (C-4'), 168.1 (C-2')	91.2 (C-5'), 106.8 (C-1')
5'	5.96 (d, 2.4)	91.2	162.5 (C-4')	97.0 (C-3'), 106.8 (C-1')
4-OMe	3.87 (s)	55.6		161.6 (C-4)
6'-OMe	3.94 (s)	56.1		163.4 (C-6')
α	7.81 (d, 15.6)	125.3	192.9 (C=O)	128.5 (C-1)
β	7.77 (d, 15.6)	142.9	125.3 (C-α), 128.5 (C-1)	130.4 (C-2/6), 192.9 (C=O)

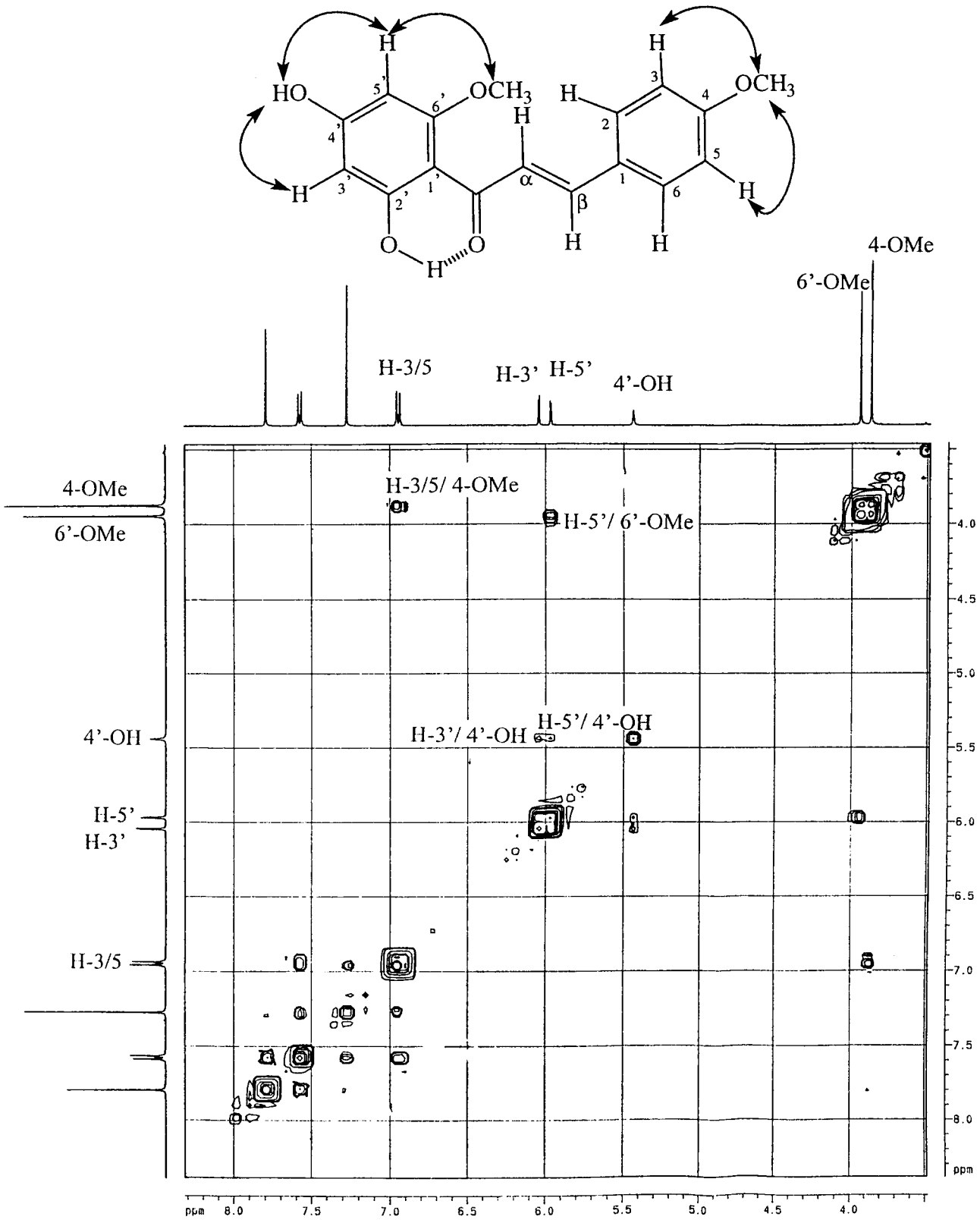
All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.



Spectrum 3.25: ^{1}H NMR (400 MHz, $CDCl_3$*) of F-2



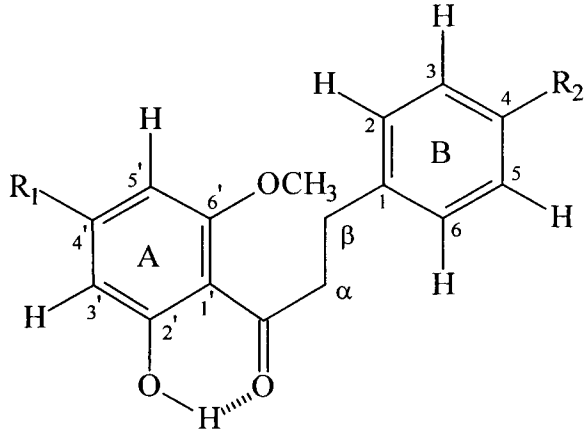
Spectrum 3.26: Significant HMBC correlations (400 MHz, $CDCl_3$) of F-2



Spectrum 3.27: Significant NOE interactions (400 MHz, $CDCl_3$) of F-2

3.3.2 Dihydrochalcones

Three dihydrochalcones were isolated from *Goniothalamus gardneri* (see Section 2.4.4). They were coded **F-3** (GGP.9), **F-4** (GGP.1), **F-5** (GGE.5) and identified respectively as 2'-hydroxy-4,4',6'-trimethoxydihydrochalcone (dihydroflavokawain A), 2',4'-dihydroxy-4,6'-dimethoxydihydrochalcone and 4,2',4'-trihydroxy-6'-methoxydihydrochalcone (Figure 3.16).



Compound	R_1	R_2
F-3	OCH_3	OCH_3
F-4	OH	OCH_3
F-5	OH	OH

Figure 3.16: Structures of F-3 to F-5

3.3.2.1 General characterisation of isolated dihydrochalcones

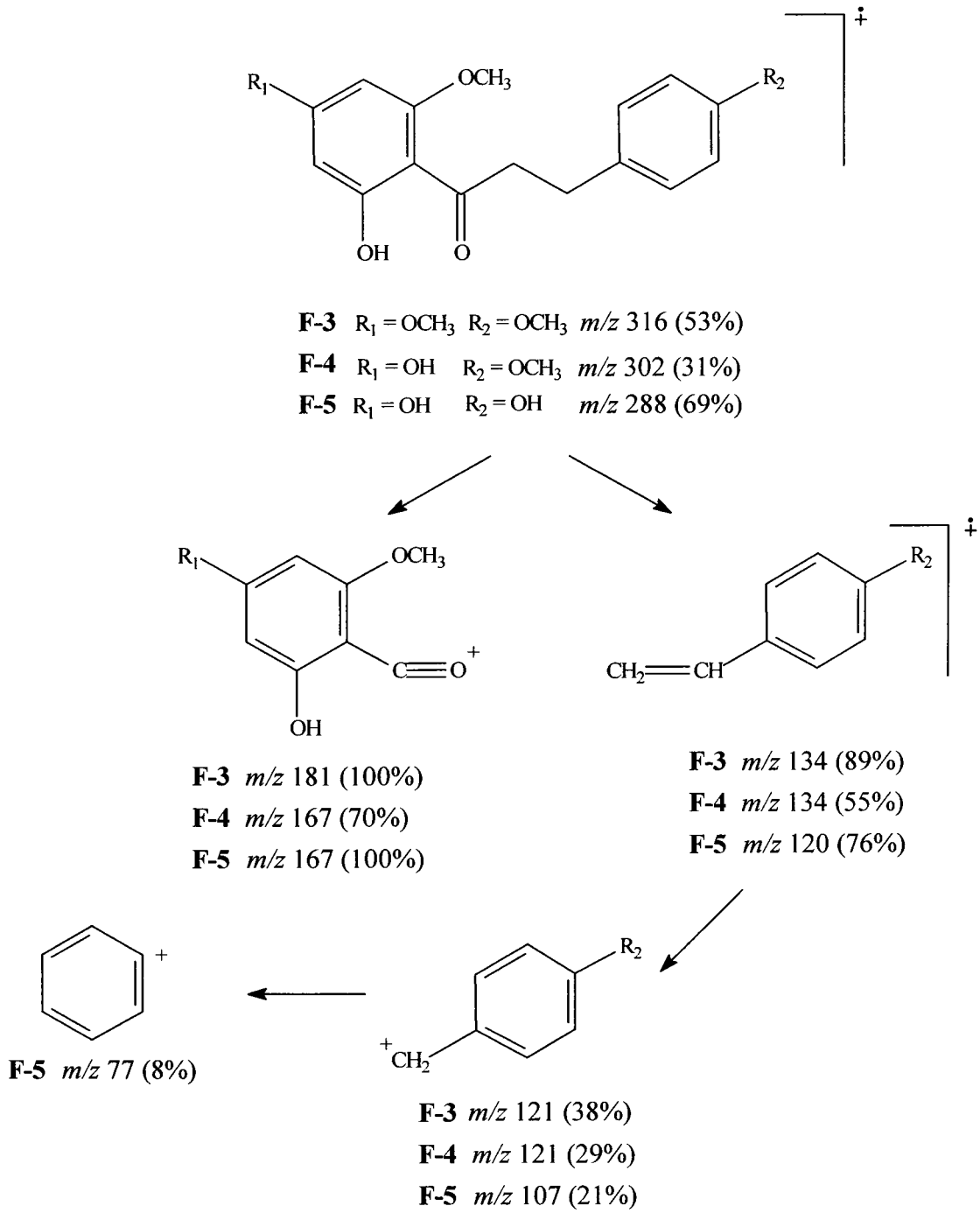
The presence of hydroxyl (*ca.* 3430-3250 cm^{-1}), aryl ether (*ca.* 2855-2835 cm^{-1}) groups, aromatic rings (*ca.* 3015, 1610, 1580, 1510 cm^{-1}) with *para*-disubstitution (*ca.* 820 cm^{-1}) and chelated carbonyl functions conjugated with aromatic rings (*ca.* 1635 cm^{-1}) were inferred from IR spectral data (Williams and Fleming, 1995).

The dihydrochalcones were colourless in visible light. UV spectral behaviour (Table 3.29) was similar to flavanones and dihydroflavonols as a result of a lack of conjugation between the A and B-rings. Thus, the dihydrochalcones exhibited a low intensity band I (300-340 nm), often appearing as a shoulder to the major band II absorption (270-295 nm) (Jurd, 1962; Mabry *et al.*, 1970). The UV spectra of **F-3** to **F-5** showed bathochromic shifts (band II/*ca.* 20 nm) on addition of $AlCl_3$, thus indicating the presence of free 2'-OH groups (Mabry *et al.*, 1970).

Table 3.29: UV (λ_{max}, nm) spectral data of isolated dihydrochalcones

		F-3	F-4	F-5
MeOH	Band II	286	287	286
	Band I	331 (sh)	335 (sh)	314 (sh)
$AlCl_3$	Band II	305	308	304
	Band I	378 (sh)	381 (sh)	345
NaOAc	Band II	286	323	324
	Band I	334 (sh)		
NaOMe	Band II	286	325	325
	Band I	334 (sh)		

The HREIMS (Scheme 3.14) gave characteristic fragment ions as for chalcones.



Scheme 3.14: Suggested mass fragmentation pattern of F-3 to F-5

Similarly to the chalcones, common features on the ^{1}H NMR spectra (Table 3.30) of dihydrochalcones included the sharp deshielded singlet for the chelated 2'-OH, the *para*-disubstituted B-ring with *ortho* coupled H-2/6 and H-3/5, the 2',4',6'-trisubstituted A-ring with *meta* coupled H-3' and H-5' and a number of methoxyl groups. A characteristic feature for dihydrochalcones was the presence of a pair of aliphatic methylenes (t, $J = 7.7$ Hz) in the range δ *ca.* 3.45-3.30 and 3.15-2.95, which were respectively attributable to H-α and H-β.

Characteristic features on the *J*-modulated ^{13}C NMR spectra (Table 3.31), assigned by ^{1}H-^{13}C HC-COBI, included deshielded carbonyls within the range of dihydrochalcone carbonyls (δ *ca.* 205), more deshielded C-1 (δ *ca.* 134), more shielded C-4 (δ *ca.* 158) and the pair of aliphatic methylenes at δ *ca.* 46.5 (C-α) and 30.5 (C-β) establishing the presence of a -C=O-CH_2-CH_2 moiety (Agrawal and Bansal, 1989). The spectra also included relatively shielded methoxyl resonances (δ *ca.* 56.1-55.4), requiring their placements adjacent to at least one free *ortho* position (Panichpol and Waterman, 1978). As for chalcones, a combination of HMBC and NOESY experiments established unambiguous assignments of oxygenated positions on each ring.

Table 3.30: ^{1}H NMR (400 MHz) spectral data of F-3 to F-5

Position	F-3[a]	F-4	F-5
2/6	7.17 (d, 8.5)	7.31 (d, 8.6)	7.32 (d, 8.3)
3/5	6.85 (d, 8.6)	6.97 (d, 8.6)	7.17 (d, 8.3)
3'	6.08 (d, 2.4)	6.51 (d, 2.2)	6.50 (d, 2.4)
5'	5.93 (d, 2.4)	6.28 (d, 2.2)	6.27 (d, 2.4)
4-OMe	3.80 (s)	3.67 (s)	-
2'-OH	14.03 (s)	14.64 (s)	14.67 (s)
4'-OMe	3.83 (s)	-	-
4'-OH	-	13.00 (brs)	-
6'-OMe	3.84 (s)	3.69 (s)	3.68 (s)
α	3.29 (t, 7.7)	3.41 (t, 7.7)	3.43 (t, 7.7)
β	2.95 (t, 7.7)	3.08 (t, 7.7)	3.12 (t, 7.7)

All data obtained in C_5D_5N
a = Spectrum run in $CDCl_3$
In parentheses coupling constant *J* are in Hz.

Table 3.31: ^{13}C NMR (100 MHz) spectral data of F-3 to F-5

Position	F-3[a]	F-4	F-5
1	133.9	134.8	133.2
2/6	129.5	130.3	130.6
3/5	114.0	114.9	116.9
4	158.1	159.0	157.7
1'	106.0	106.0	106.0
2'	167.9	168.7	168.8
3'	93.9	97.6	97.6
4'	166.1	167.2	167.2
5'	91.0	92.7	92.8
6'	162.9	164.5	164.6
4-OMe	55.4	55.6	-
4'-OMe	55.7	-	-
6'-OMe	55.8	56.0	56.1
α	46.2	46.8	47.2
β	30.0	30.8	31.0
C=O	204.8	205.0	205.3

All data obtained in C_5D_5N
a = Spectrum run in $CDCl_3$

*a) Identification of **F-3** as dihydroflavokawain A*

This compound was isolated from the petrol extract of *Goniothalamus gardneri*. UV spectral data was typical for a dihydrochalcone with a free 2'-OH (Table 3.29). Addition of alkali gave no bathochromic shift of band II, indicating the absence of a 2',4'-dihydroxyl system (Mabry *et al.*, 1970).

The HREIMS (Scheme 3.14) afforded a molecular ion at *m/z* 316, which analysed for $C_{18}H_{20}O_5$ (9 DBE), with fragments at *m/z* 285 $[M\text{-}OCH_3]^+$ and 267 $[M\text{-}H_2\text{-}CH_2O\text{-}OH]^+$. A further fragment ion at *m/z* 181 was consistent with an A-ring bearing two methoxyls and one hydroxyl group, while fragments at *m/z* 134 and 121 indicated the presence of one methoxyl group on the B-ring.

The ^1H NMR (Spectrum 3.28; Table 3.30) was typical of a dihydrochalcone substituted by three methoxyls at δ 3.84, 3.83 and 3.80. The HMBC (Figure 3.17; Table 3.32) displayed:

i) ^3J coupling between both the methoxyl signal at δ 3.80 and H-2/6 and the oxygenated carbon at δ 158.1, thus establishing the assignment of the methoxylated position C-4 on the B-ring.

ii) ^3J interactions between the methoxyls at δ 3.83 and 3.84 and the oxygenated carbons respectively at δ 166.1 and 162.9.

iii) a ^2J interaction between H-3' and δ 167.9, as well as a ^2J interaction between H-5' and δ 166.1. The carbon at δ 167.9, showing no interaction with methoxyls, was assigned to the hydroxylated C-2'.

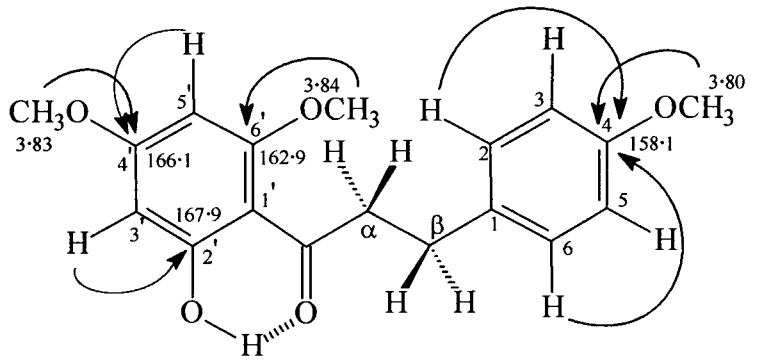


Figure 3.17: Significant HMBC correlations of F-3

A NOESY experiment (Figure 3.18) established the unambiguous assignments of all methoxyl groups. The presence of a correlation between H-3' and the methoxyl at δ 3.83 established the methoxylated C-4' position at δ 166.1. Cross-peaks between H-5' and both the methoxyls at δ 3.83 and 3.84 established the remaining methoxylated C-6' position at δ 162.9. On the B-ring, the presence of a methoxyl group in C-4 was confirmed by a NOE interaction between H-3/5 and the signal at δ 3.80.

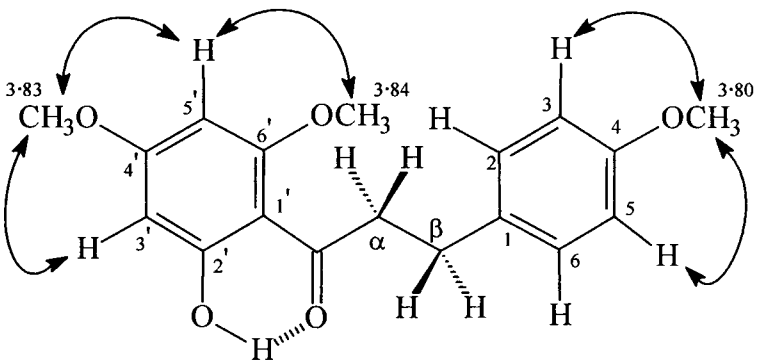


Figure 3.18: Significant NOE interactions of F-3

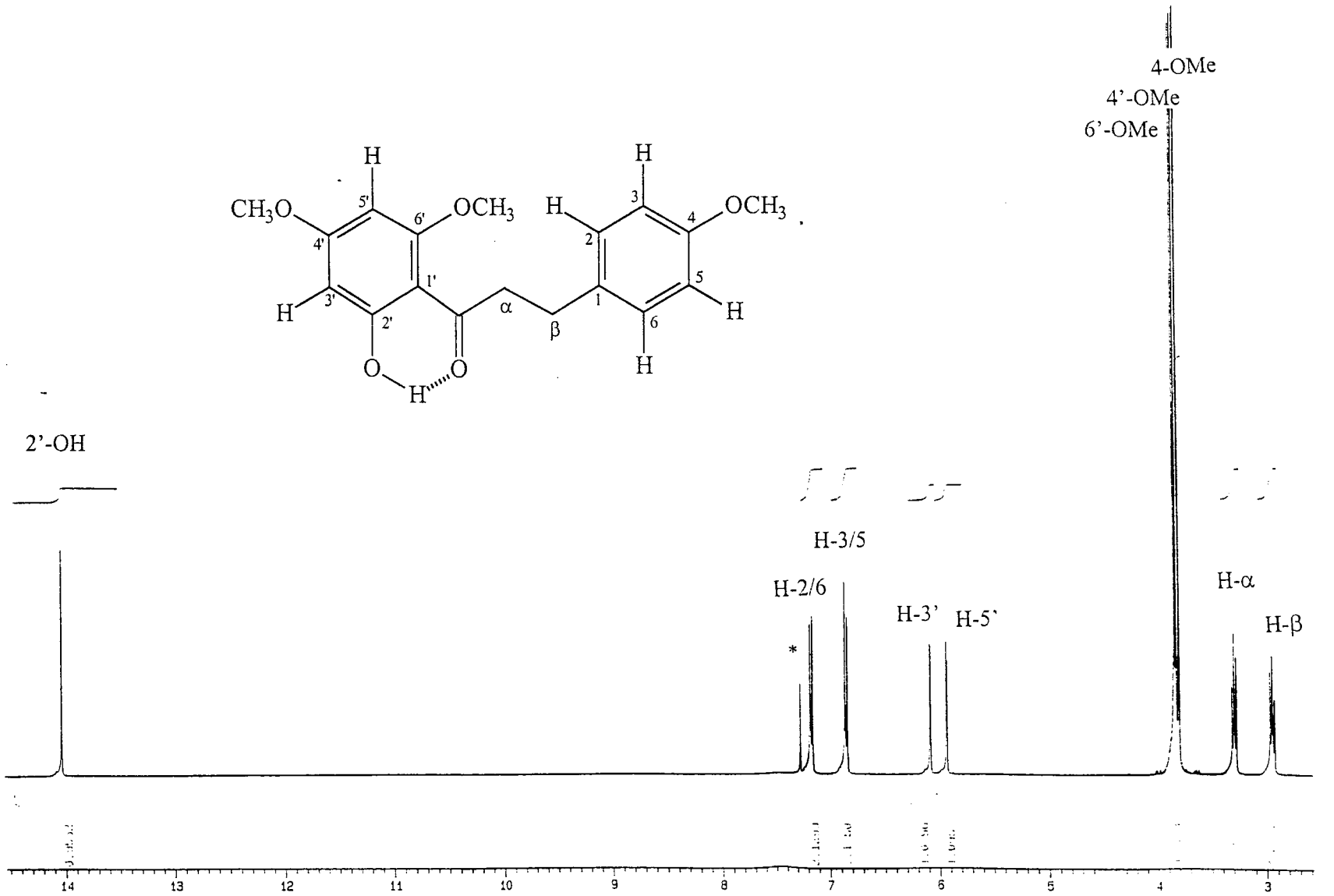
On this basis, **F-3** was identified as 2'-hydroxy-4,4',6'-trimethoxydihydro-chalcone, which has previously been synthesised but has not been recorded as a natural product. Mp, IR, UV and MS data complied with the literature (Braz Filho *et al.*, 1980; Bhardwaj *et al.*, 1982). ^{1}H NMR assignments showed good agreement with results obtained by Braz Filho *et al.* (1980). Unambiguous ^{1}H NMR

reported for the first time. Taking into account the presence in the same plant of the corresponding chalcone, flavokawain A, **F-3** was given the trivial name dihydroflavokawain A.

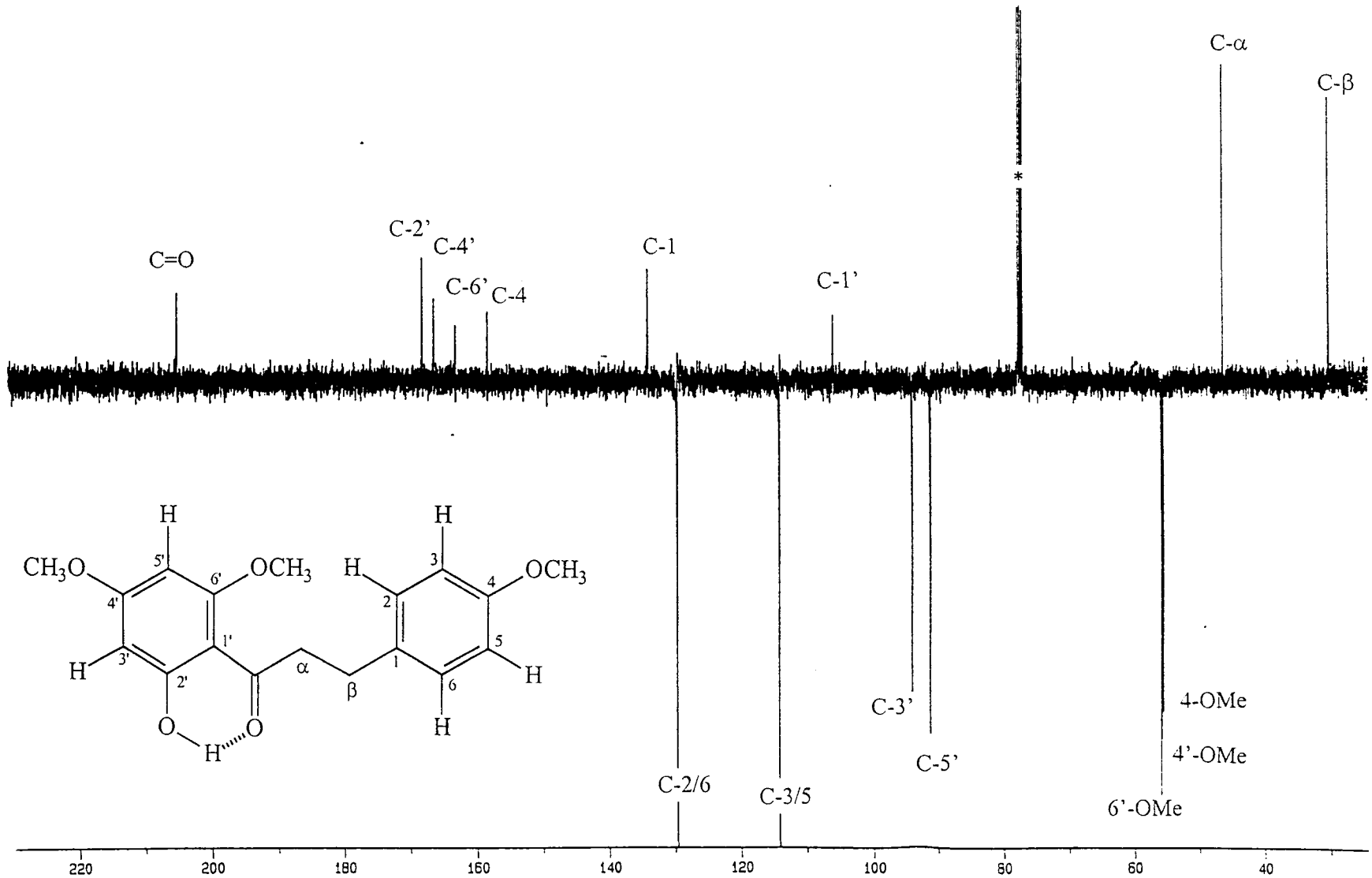
Table 3.32: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-3

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2/6	7.17 (d, 8.5)	129.5		30.0 (C-β), 158.1 (C-4)
3/5	6.85 (d, 8.6)	114.0	158.1 (C-4)	133.9 (C-1)
3'	6.08 (d, 2.4)	93.9	167.9 (C-2')	91.0 (C-5'), 106.0 (C-1')
5'	5.93 (d, 2.4)	91.0	166.1 (C-4')	93.9 (C-3'), 106.0 (C-1')
4'-OMe	3.83 (s)	55.7		166.1 (C-4')
6'-OMe	3.84 (s)	55.8		162.9 (C-6')
4-OMe	3.80 (s)	55.4		158.1 (C-4)
α	3.29 (t, 7.7)	46.2	30.0 (C-β), 204.8 (C=O)	133.9 (C-1)
β	2.95 (t, 7.7)	30.0	46.2 (C-α), 133.9 (C-1)	129.5 (C-2/6), 204.8 (C=O)

All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.



Spectrum 3.28: ^{1}H NMR (400 MHz, $CDCl_3$*) of F-3



Spectrum 3.29: *J*-modulated ^{13}C NMR (100 MHz, $CDCl_3$*) of F-3

*b) Identification of **F-4** as 2',4'-dihydroxy-4,6'-dimethoxydihydrochalcone*

This compound was isolated from the petrol extract of *Goniothalamus gardneri*. The UV spectral data (Table 3.29) was typical for a dihydrochalcone with a free 2'-OH and in this case a bathochromic shift on addition of alkali (band II/*ca.* 37 nm), accompanied by an increase in peak intensity in the presence of NaOMe, revealed a 2',4'-dihydroxyl system (Mabry *et al.*, 1970).

The HREIMS (Scheme 3.14) afforded a molecular ion at *m/z* 302, which analysed for $C_{17}H_{18}O_5$ (9 DBE) with common ions at *m/z* 271 $[M\text{-}OCH_3]^+$ and 253 $[M\text{-}H_2\text{-}CH_2O\text{-}OH]^+$ and 228 $[M\text{-}OCH_3\text{-}CO\text{-}CH_3]^+$. A fragment ion at *m/z* 167 showed two hydroxyls and one methoxyl group on the A-ring, while fragments at *m/z* 134 and 121 indicated the presence of one methoxyl group on the B-ring.

The ^1H NMR (Spectrum 3.30; Table 3.30) was typical of a dihydrochalcone substituted by two methoxyls at δ 3.69 and 3.67. A broad singlet at δ 13.0 confirmed the presence of the free 4'-OH.

The mp, IR, UV and MS of **F-4** complied with those recorded for 2',4'-dihydroxy-4,6'-dimethoxydihydrochalcone, previously synthesised (Bhardwaj *et al.*, 1982) and isolated from *Iryanthera laevis* (Braz Filho *et al.*, 1980; Garzon *et al.*, 1987), *Iryanthera ulei* (Conserva *et al.*, 1990a/b), *Iryanthera sagotiana* (Kawanishi *et al.*, 1990) and *Iryanthera paraensis* (Myristicaceae) (Conserva *et al.*, 1990b). ^1H and ^{13}C NMR assignments were not directly comparable as the original authors recorded them in different solvents. However, comparison of the ^1H NMR values in relative order of shielding/deshielding showed good agreement with those reported by Conserva *et al.* (1990b), except for the resonances H-3' and H-5' which were reversed. Similarly, comparison of the ^{13}C NMR chemical shifts showed good agreement with those reported by Conserva *et al.* (1990b). All assignments were established on the basis of detailed direct, long-range correlations (Table 3.33) and NOE interactions (Figure 3.19).

Table 3.33: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-4

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2/6	7.31 (d, 8.6)	130.3		30.8 (C-β), 159.0 (C-4)
3/5	6.97 (d, 8.6)	114.9	159.0 (C-4)	134.8 (C-1)
3'	6.51 (d, 2.2)	97.6	167.2 (C-4'), 168.7 (C-2')	92.7 (C-5'), 106.0 (C-1')
5'	6.28 (d, 2.2)	92.7	164.5 (C-6'), 167.2 (C-4')	97.6 (C-3'), 106.0 (C-1')
4-OMe	3.67 (s)	55.6		159.0 (C-4)
6'-OMe	3.69 (s)	56.0		164.5 (C-6')
α	3.41 (t, 7.7)	46.8	30.8 (C-β), 205.0 (C=O)	134.8 (C-1)
β	3.08 (t, 7.7)	30.8	46.8 (C-α), 134.8 (C-1)	130.3 (C-2/6), 205.0 (C=O)

All data obtained in C_5D_5N.
In parentheses coupling constant J are in Hz.

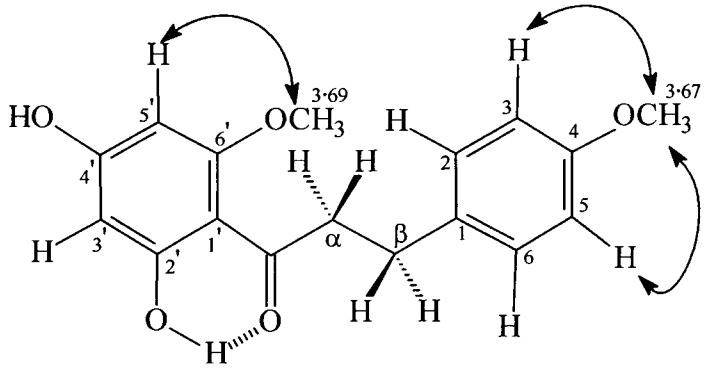
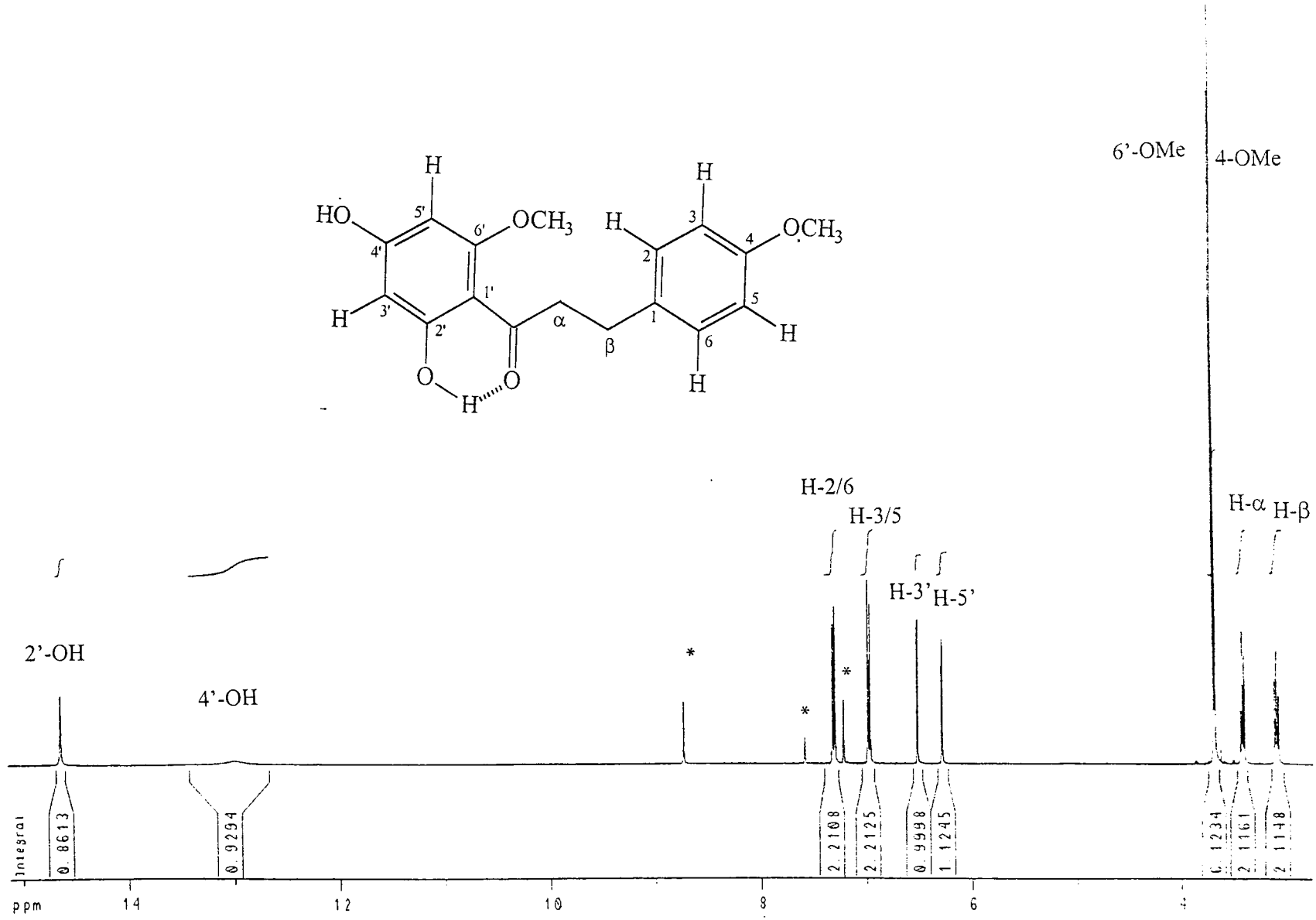


Figure 3.19: Significant NOE interactions of F-4



Spectrum 3.30: ^{1}H NMR (400 MHz, C_5D_5N*) of F-4

c) Identification of ***F-5*** *as 4,2',4'-trihydroxy-6'-methoxydihydrochalcone*

This compound was isolated from the ethyl acetate extract of *Goniothalamus gardneri*. The UV spectral data (Table 3.29) was typical for a dihydrochalcone with a free 2'-OH. A bathochromic shift on addition of alkali (band II/*ca.* 38 nm), accompanied by an increase in peak intensity in the presence of NaOMe, again revealed a 2',4'-dihydroxyl system (Mabry *et al.*, 1970).

The molecular formula of $C_{16}H_{16}O_5$ (9 DBE) was established from the HREIMS with a molecular ion at *m/z* 288 (Scheme 3.14). A fragment at *m/z* 167 revealed two hydroxyls and one methoxyl on the A-ring. The presence of one hydroxyl on the B-ring, instead of the usual methoxyl, was evidenced by fragments at *m/z* 120 and 107.

The ^{1}H NMR (Spectrum 3.31; Table 3.30) confirmed the presence of only one methoxyl at δ 3.68. The HMBC (Figure 3.20; Table 3.34) displayed:

i) a ^3J coupling between H-2/6 and the oxygenated carbon at δ 157.7, which must therefore be the hydroxyl bearing C-4 on the B-ring.

ii) a ^3J interaction between the methoxyl at δ 3.68 and the oxygenated carbon at δ 164.6.

iii) common ^2J interactions between the A-ring methines and δ 167.2, thus establishing the assignment of the C-4' position.

iv) a ^2J correlation between the proton at δ 6.50 and the carbon at δ 168.8, which could only be assigned to the remaining oxygenated C-2' resonance and a ^2J interaction between the proton at δ 6.27 and the methoxylated carbon at δ 164.6 assigned to C-6'. Therefore, oxygenated positions on the A-ring were established as hydroxylated C-2' (δ 168.8), C-4' (δ 167.2) and methoxylated C-6' (δ 164.6).

This was in good agreement with the ^{1}H and ^{13}C NMR data which had revealed two distinctive chemical shifts for positions 3' and 5' now respectively assigned at δ 6.50 and 6.27. If the only methoxyl on the A-ring had been located in position 4', then signals for 3' and 5' would have been equivalent because of a symmetric A-ring.

The presence of free phenolic 2'- and 4'-OH were also in good agreement with the UV spectral data.

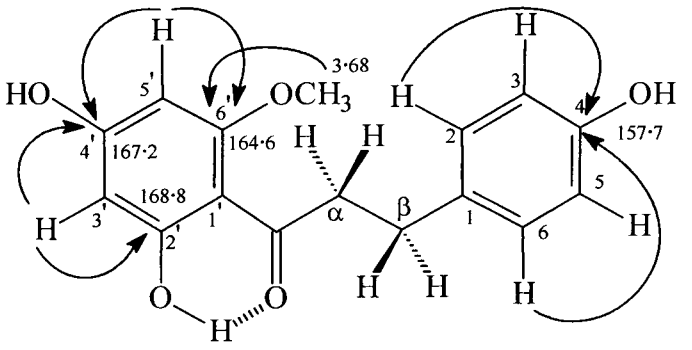


Figure 3.20: Significant HMBC correlations of F-5

A NOE interaction between H-5' and the methoxyl at δ 3.68 in the NOESY experiment assigned the 6'-OMe (Figure 3.21).

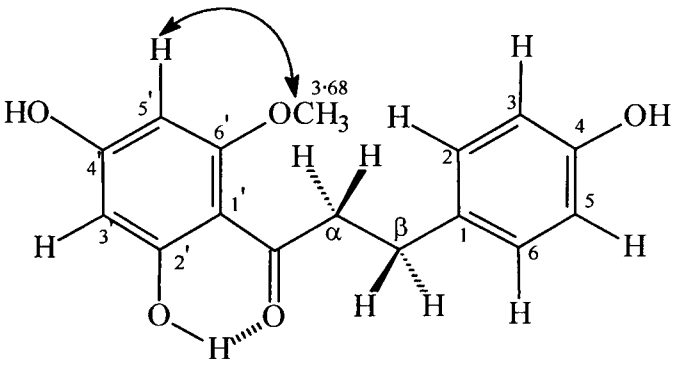


Figure 3.21: Significant NOE interactions of F-5

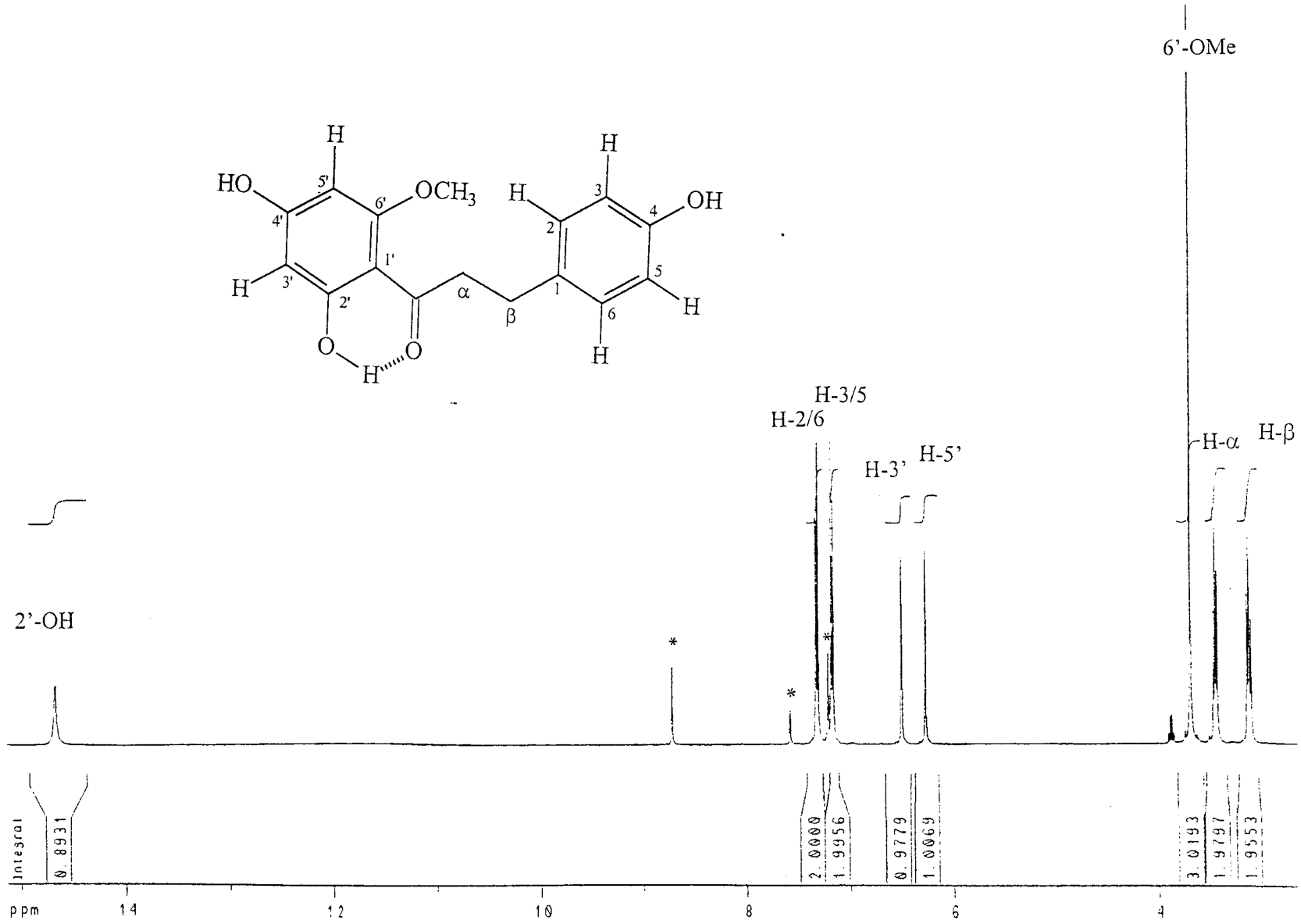
On this basis, **F-5** was identified as the known 4,2',4'-trihydroxy-6'-methoxydihydrochalcone, previously isolated from *Coptis japonica* var. *dissecta* (Ranunculaceae) (Mizuno *et al.*, 1987). MS data was in agreement with the literature. ^{1}H NMR assignments were not directly comparable as the original authors recorded them in a different solvent. However, comparison of the chemical shifts in relative order of shielding/deshielding showed good agreement, except for the

resonances H-3' and H-5' which were reversed. This is the first report of ^{13}C NMR assignments.

Table 3.34: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-5

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2/6	7.32 (d, 8.3)	130.6		31.0 (C-β), 157.7 (C-4)
3/5	7.17 (d, 8.3)	116.9	157.7 (C-4)	133.2 (C-1)
3'	6.50 (d, 2.4)	97.6	168.8 (C-2'), 167.2 (C-4')	92.8 (C-5'), 106.0 (C-1')
5'	6.27 (d, 2.4)	92.8	167.2 (C-4'), 164.6 (C-6')	97.6 (C-3'), 106.0 (C-1')
6'-OMe	3.68 (s)	56.1		164.6 (C-6')
α	3.43 (t, 7.7)	47.2	31.0 (C-β), 205.3 (C=O)	133.2 (C-1)
β	3.12 (t, 7.7)	31.0	47.2 (C-α), 133.2 (C-1)	130.6 (C-2/6), 205.3 (C=O)

All data obtained in C_5D_5N.
In parentheses coupling constant *J* are in Hz.



Spectrum 3.31: ^{1}H NMR (400 MHz, C_5D_5N*) of F-5

3.3.3 Chalcone dimer

One chalcone dimer, coded **F-6** (GGE.4), was isolated from the ethyl acetate extract of *Goniothalamus gardneri* (see Section 2.4.4) and identified as *rel*-(1β,2α)-di-(2,4-dihydroxy-6-methoxy)benzoyl-*rel*-(3β,4α)-di-(4-methoxy)phenyl-cyclobutane or 2',4'-dihydroxy-4,6'-dimethoxychalcone dimer (Figure 3.22).

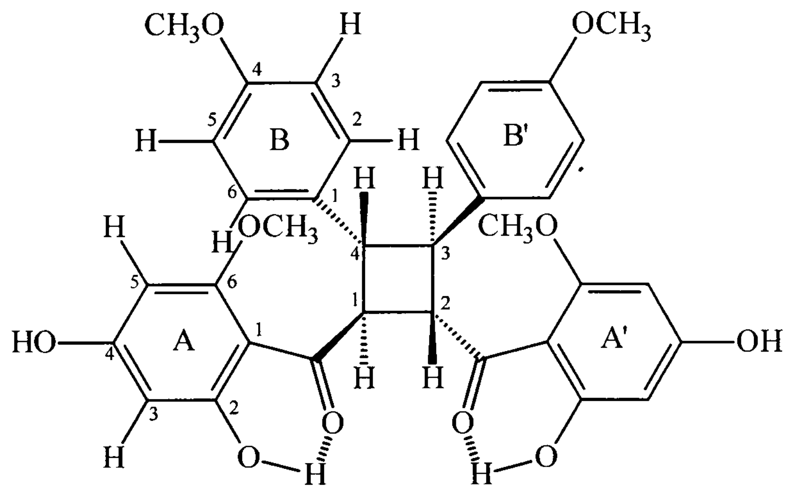


Figure 3.22: Structure of F-6

3.3.3.1 Characterisation of the isolated chalcone dimer F-6 as 2',4'-dihydroxy-4,6'-dimethoxychalcone dimer

The presence of hydroxyl (3409 cm^{-1}), aryl ether groups (2836 cm^{-1}), aromatic rings (1596, 1513 cm^{-1}) with *para*-disubstitution (829 cm^{-1}) and chelated carbonyl functions conjugated with aromatic rings (1623 cm^{-1}) were inferred from IR spectral data (Williams and Fleming, 1995).

The UV spectrum (Table 3.35) was similar to dihydrochalcones [*i.e* low intensity band I (300-340 nm), appearing as a shoulder to the major band II absorption (270-295 nm)]. **F-6** showed a bathochromic shift (band II/20 nm) on addition of $AlCl_3$, accounting for a free 2-OH on the A-ring. A bathochromic shift (band II/*ca.* 35 nm) on addition of alkali, accompanied by an increase in peak intensity in the presence of NaOMe, further characterised a 2,4-dihydroxyl system on this ring (Mabry *et al.*, 1970).

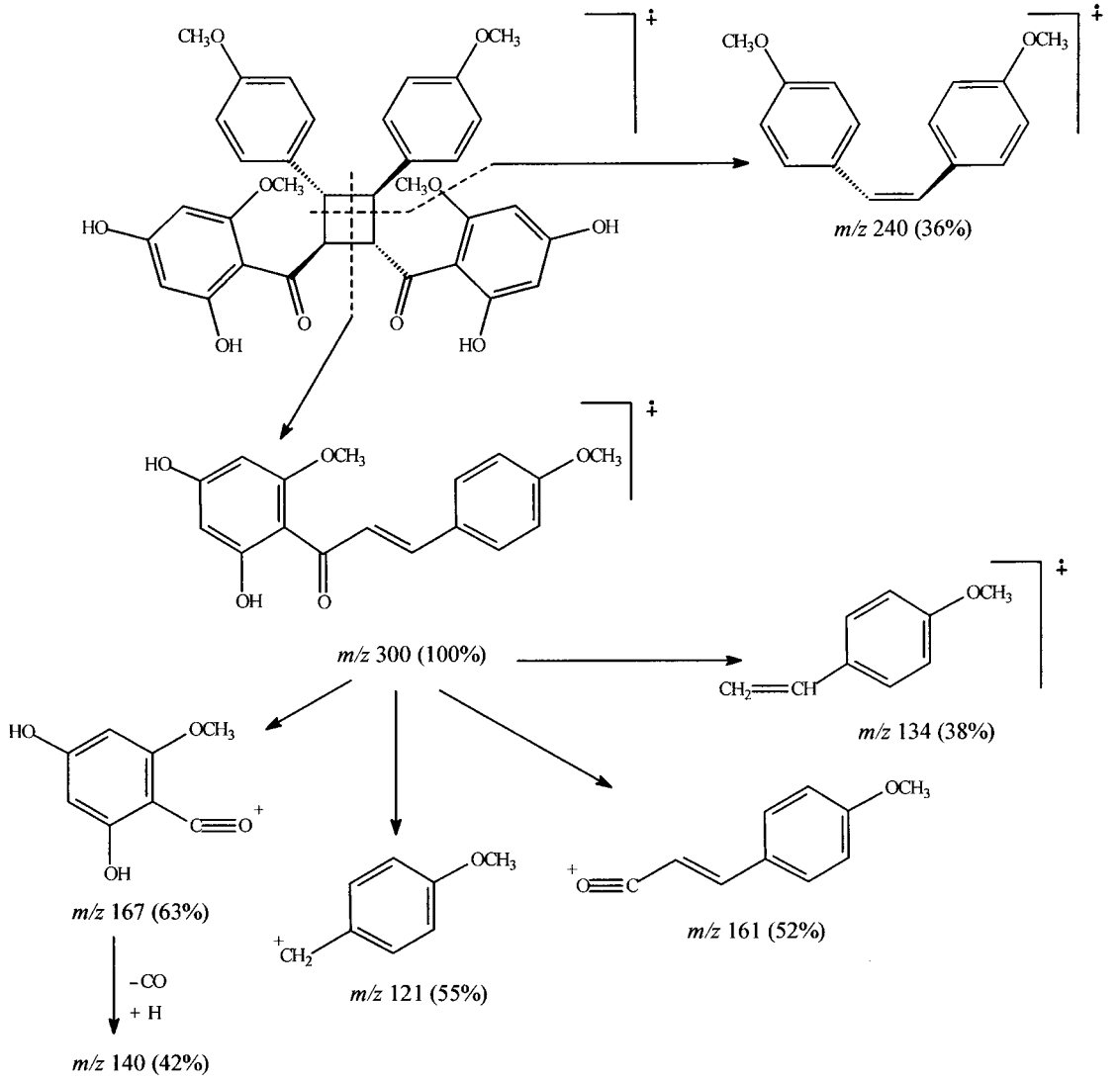
Table 3.35: UV (λ_{max}, nm) spectral data of F-6

MeOH	Band II	288
	Band I	335 (sh)
$AlCl_3$	Band II	308
	Band I	365 (sh)
NaOAc	Band II	324
	Band I	
NaOMe	Band II	323
	Band I	

The FABMS revealed a quasi-molecular ion at *m/z* 601, which was consistent with the molecular formula $C_{34}H_{32}O_{10}$ (19 DBE) and suggested a dimeric structure. In the HREIMS **F-6** showed a typical chalcone fragmentation (Audier, 1966; Mabry and Markham, 1975). The dimeric structure was confirmed by the presence of a major fragment ion at *m/z* 300, which was attributed to chalcone monomers of molecular formula $C_{17}H_{16}O_5$. Further fragmentation revealed two hydroxyls and one methoxyl on the A and A'-rings (*m/z* 167 and 140), and one methoxyl group on the B and B'-rings (*m/z* 161, 134 and 121) of each monomer (Scheme 3.15).

The ^{1}H NMR (Spectrum 3.32; Table 3.36) revealed signals comparable with the chalcone **F-2** and the dihydrochalcone **F-4**, both isolated from this species. These were a low-field singlet at δ 14.52 for the chelated 2-OH on the A ring, a pair of *ortho* coupled doublets at δ 7.29 and 6.89 (2H each, J = 8.4 Hz) for a *para*-disubstituted B-ring, a pair of *meta* coupled doublets at δ 6.49 and 6.23 (1H each, J = 2.4 Hz) for a 2,4,6-trisubstituted A-ring, and two methoxyl resonances at δ 3.53 and 3.47. A characteristic feature of **F-6** were two coupled aliphatic methine doublets at δ 5.14 and 4.52 (J = 6.4 Hz). These combined data required the

placement of oxygenated positions to be 2-OH, 4-OH, 6-OMe on the A ring and 4-OMe on the B ring.



Scheme 3.15: Suggested mass fragmentation pattern of F-6

The *J*-modulated ^{13}C NMR (Spectrum 3.33; Table 3.36), assigned by ^{1}H-^{13}C HC-COBI, confirmed the presence of the *para*-disubstituted B ring (two equivalent aromatic methines at δ 130.1 and δ 114.3) and the 2,4,6-trisubstituted A-ring (pair of methines at δ 97.7 and 92.5). Furthermore it revealed six quaternary carbons including four oxygenated positions and two relatively shielded methoxyl resonances at δ 55.9 and 55.4, requiring their placement adjacent to at least one free *ortho* position (Panichpol and Waterman, 1978). Other characteristic features were a carbonyl, within the range of dihydrochalcone carbonyls (δ 204.1) (*i.e* showing the presence of a saturated α,β bond) (Agrawal and Bansal, 1989), and the pair of aliphatic methines at δ 55.4 and 45.3.

The presence of identical chemical shifts and splitting patterns for all NMR assignments required a plane of symmetry in the molecule. This was satisfied with the presence of a cyclobutane ring symmetrically substituted by two identical A/A'-rings on one side, and two identical B/B'-rings on the opposite side (*i.e* head-to-head substitution). This was further confirmed in the HREIMS with the fragment ion at *m/z* 240, attributable to the loss of the B/B' system (Scheme 3.15).

The structure of *rel*-(1β,2α)-di-(2,4-dihydroxy-6-methoxy)benzoyl-*rel*-(3β,4α)-di-(4-methoxy)phenyl-cyclobutane was elucidated by a series of HMBC and NOESY experiments. The HMBC (Figure 3.23; Table 3.36) displayed:

i) ^{3}J couplings between both the methoxyls at δ 3.53 and H-2/6B/B' and the oxygenated carbons at δ 158.7, thus establishing the assignments of the methoxylated C-4B/B' positions.

ii) ^{3}J interactions between the remaining methoxyls at δ 3.47 and the oxygenated carbons at δ 164.1.

iii) ^{2}J interactions between H-3A/A' and δ 167.1 and 168.6, and ^{2}J interactions between H-5A/A' and δ 167.1 and 164.1, thus allowing the assignments of the hydroxylated C-4A/A' positions at δ 167.1. Consequently, the methoxylated C-6A/A' positions were established at δ 164.1, and the remaining hydroxylated C-2A/A' positions at δ 168.6.

iv) a ^3J coupling between the methines at δ 5.14 and C-1B/B', a ^3J coupling between the methines at δ 4.52 and both C-2/6B/B' and the carbonyl.

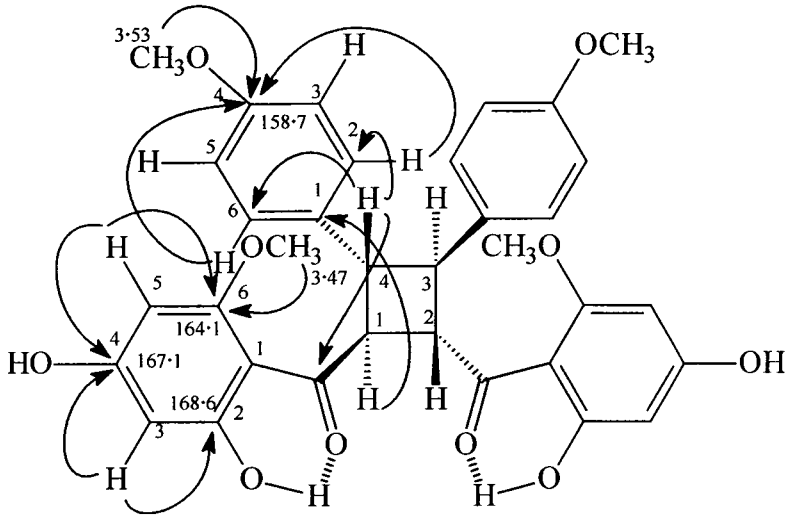


Figure 3.23: Significant HMBC correlations of F-6

The NOESY experiment (Figure 3.24) revealed cross-peaks between H-5A/A' and methoxyls at δ 3.47, thus confirming the assignment of the 6A/A'-OMe. The presence of methoxyl groups in C-4B/B' was confirmed by a NOE interaction between H-3/5B/B' and the resonances at δ 3.53. Furthermore, a strong NOE interaction between H-1/2 and H-2/6B/B' required the placement of the B and B' rings *trans* respectively to the A and A' rings. This was satisfied by the only two possible structures **(a)** and **(b)**. In both cases, it established the relative stereochemistry of **F-6** as *rel*-(1β,4α). Another NOE interaction between H-1/2 and H-3/4 indicated the relative stereochemistry at C-2/C-3 to be *rel*-(2α,3β) as in structure **(b)** (Figure 3.24).

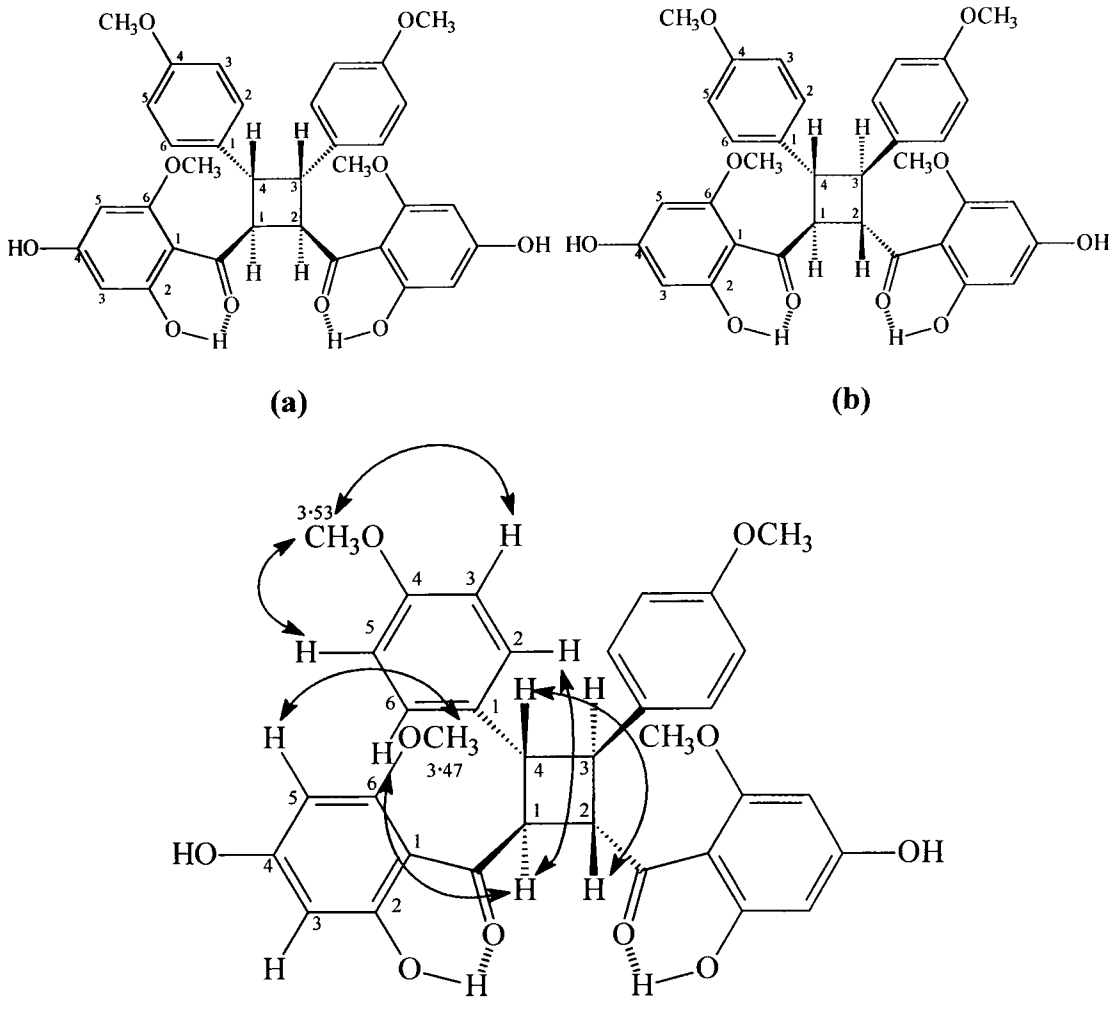


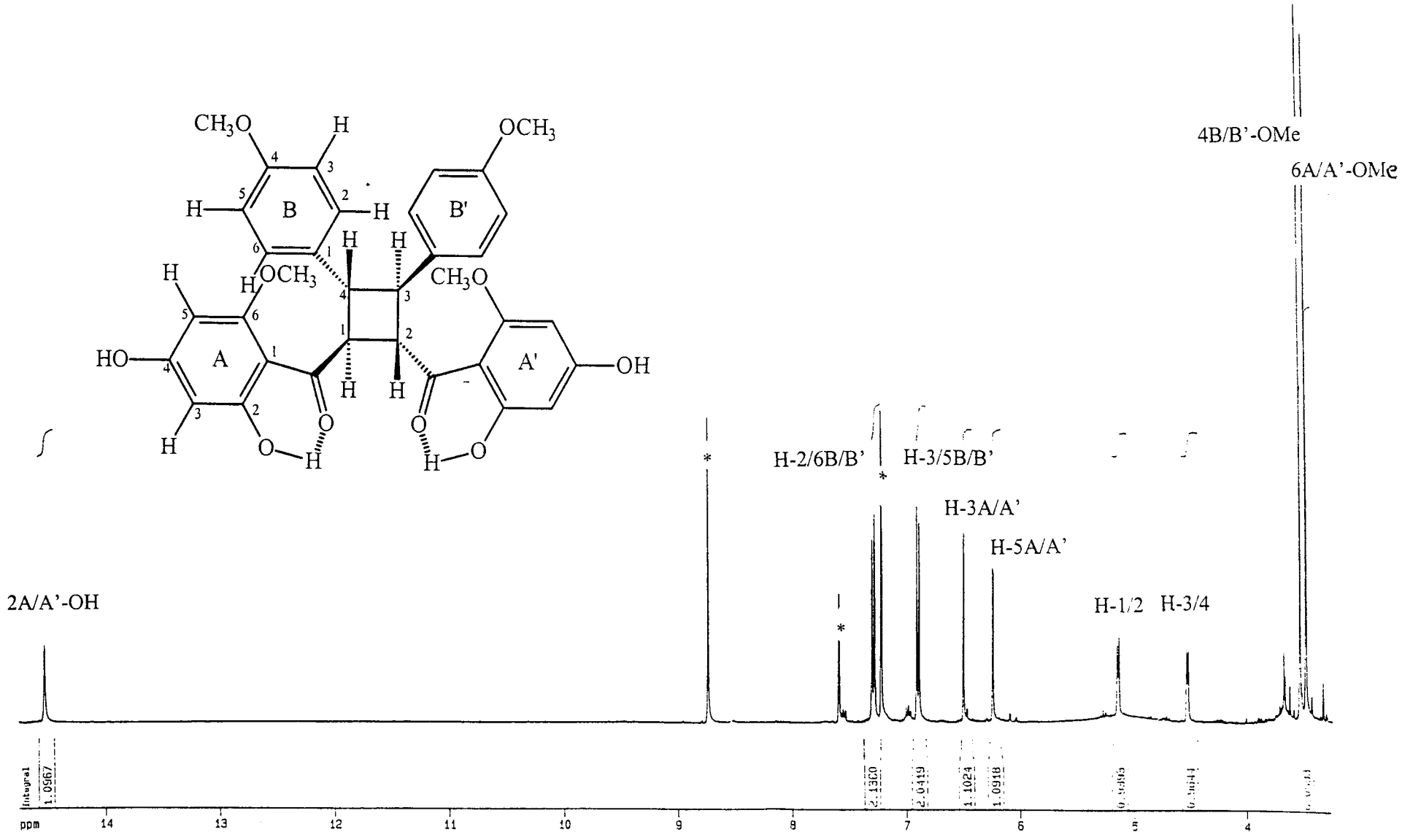
Figure 3.24: Possible structures and significant NOE interactions of F-6

This led to the identification of **F-6** as the new chalcone dimer *rel*-(1β,2α)-di-(2,4-dihydroxy-6-methoxy)benzoyl-*rel*-(3β,4α)-di-(4-methoxy)phenyl-cyclobutane or 2',4'-dihydroxy-4,6'-dimethoxychalcone dimer.

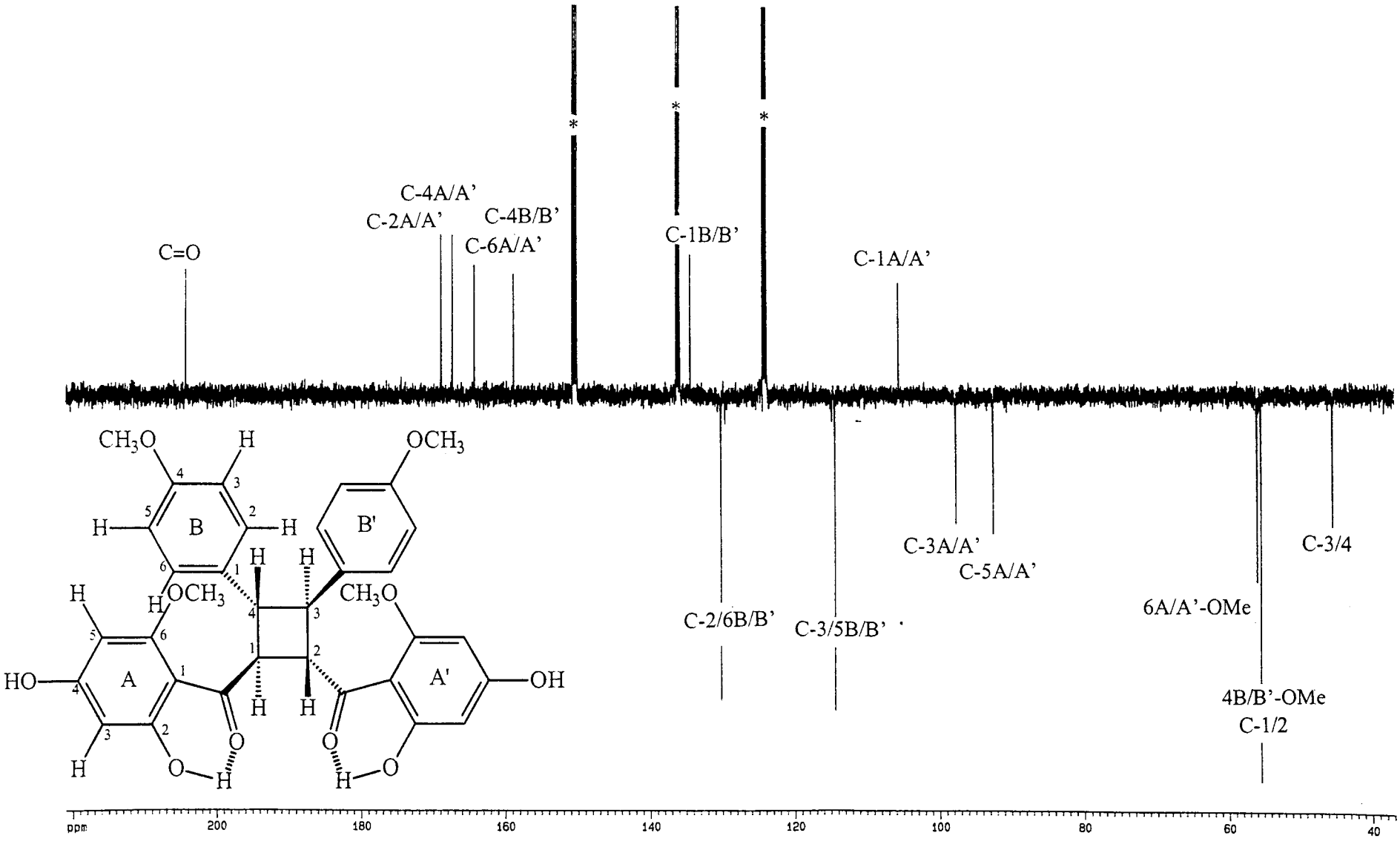
Table 3.36: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of F-6

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
1B/B'	-	134.3		
2/6B/B'	7.29 (d, 8.4)	130.1		45.3 (C-3/4), 158.7 (C-4B/B')
3/5B/B'	6.89 (d, 8.4)	114.3	158.7 (C-4B/B')	134.3 (C-1B/B')
4B/B'	-	158.7		
1A/A'	-	105.6		
2A/A'	-	168.6		
3A/A'	6.49 (d, 2.4)	97.7	167.1 (C-4A/A'), 168.6 (C-2A/A')	92.5 (C-5A/A'), 105.6 (C-1A/A')
4A/A'	-	167.1		
5A/A'	6.23 (d, 2.4)	92.5	164.1 (C-6A/A'), 167.1 (C-4A/A')	97.7 (C-3A/A'), 105.6 (C-1A/A')
6A/A'	-	164.1		
6A/A'-OMe	3.47 (s)	55.9		164.1 (C-6A/A')
4B/B'-OMe	3.53 (s)	55.4		158.7 (C-4B/B')
1/2	5.14 (d, 6.4)	55.4		134.3 (C-1B/B')
3/4	4.52 (d, 6.4)	45.3	204.1 (C=O)	130.1 (C-2/6B/B')
C=O	-	204.1		
2A/A'-OH	14.52 (s)			

All data obtained in C_5D_5N.
In parentheses coupling constant *J* are in Hz.



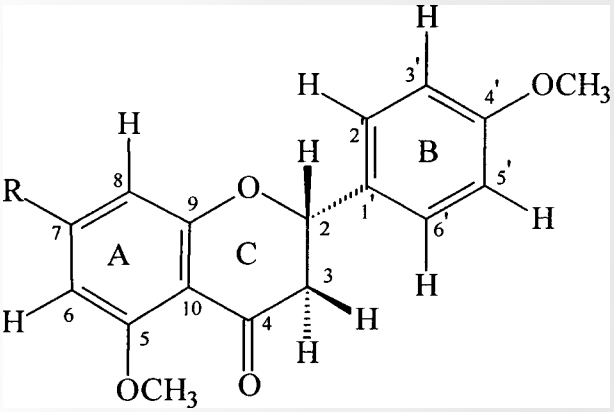
Spectrum 3.32: ^{1}H NMR (400 MHz, C_5D_5N*) of F-6



Spectrum 3.33: *J*-modulated ^{13}C NMR (100 MHz, C_5D_5N*) of F-6

3.3.4 Flavanones

Two flavanones were isolated from *Goniothalamus gardneri* (see Section 2.4.4). They were coded **F-7** (GGP.6) and **F-8** (GGE.3) and identified respectively as 5,7,4'-trimethoxyflavanone (naringenin trimethyl ether) and 7-hydroxy-5,4'-dimethoxyflavanone (tsugafolin) (Figure 3.25).



Compound	R
F-7	OCH_3
F-8	OH

Figure 3.25: Structures of F-7 and F-8

3.3.4.1 General characterisation of isolated flavanones

IR spectral data suggested the presence of aryl ether groups (*ca.* 2840 cm^{-1}), aromatic rings (*ca.* 3015, 1605, 1575, 1520 cm^{-1}) with *para*-disubstitution (*ca.* 820 cm^{-1}) and carbonyl functions conjugated with aromatic rings (*ca.* 1665 cm^{-1}) (Williams and Fleming, 1995).

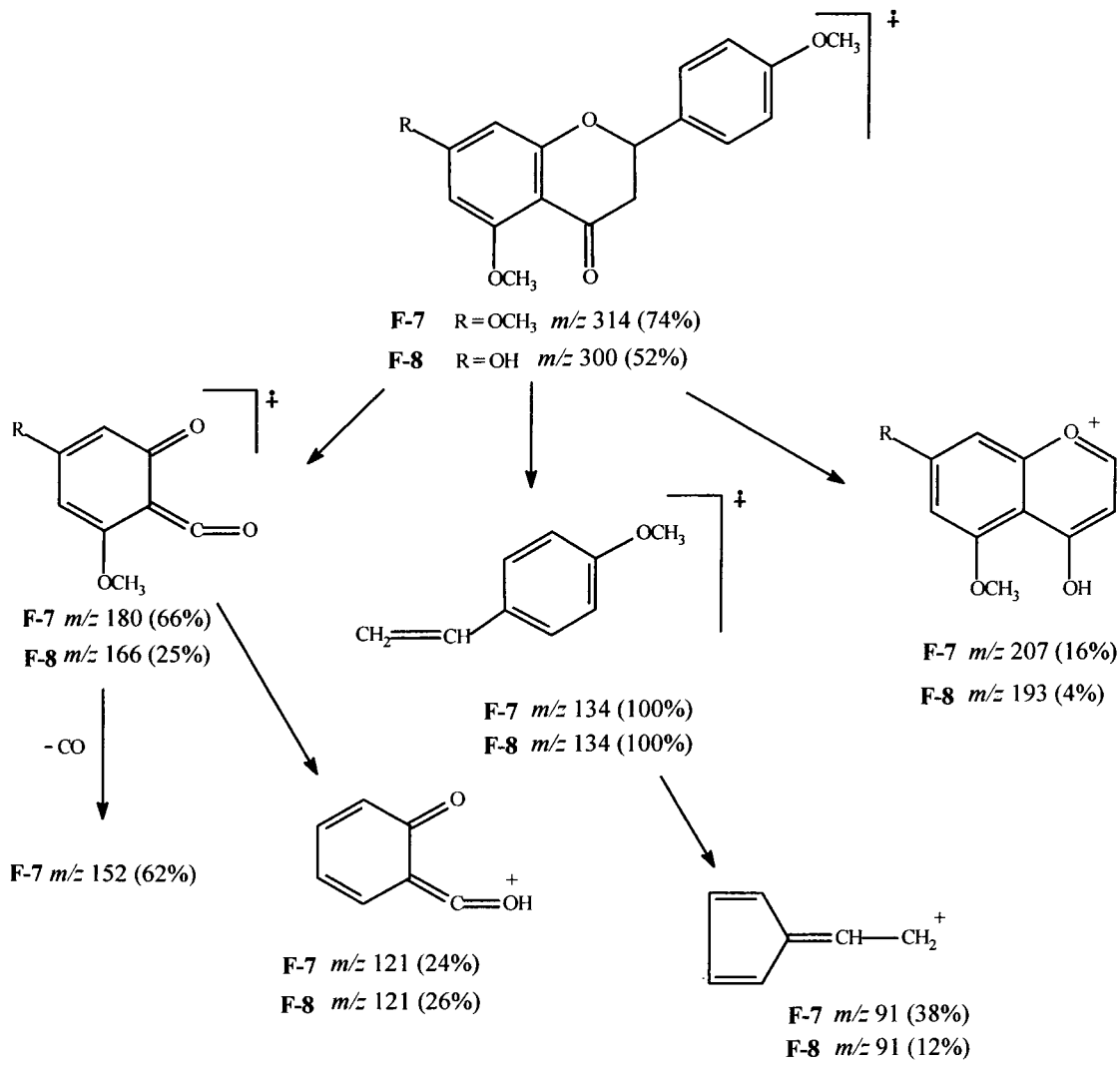
The isolated flavanones were colourless in visible light. UV spectra exhibited a prominent band II maximum (270-295 nm) accompanied by a low intensity band I absorption (Jurd, 1962; Mabry *et al.*, 1970) (Table 3.37). **F-7** and **F-8** showed no significant shifts on addition of $AlCl_3$, thus suggesting the absence of

a 5-OH group. This was in agreement with the light blue fluorescences that they exhibited under long-wave UV light (Mabry *et al.*, 1970).

Table 3.37: UV (λ_{max}, nm) spectral data of isolated flavanones

		F-7	F-8
MeOH	Band II	282	283
	Band I	323 (sh)	324 (sh)
$AlCl_3$	Band II	282	283
	Band I	323 (sh)	324 (sh)
NaOAc	Band II	282	321
	Band I	324 (sh)	
NaOMe	Band II	283	322
	Band I	324 (sh)	

HREIMS (Scheme 3.16) revealed a fragmentation pattern typical of flavanones (Audier, 1966; Mabry and Markham, 1975). Other fragments observed included $[M-H]^+$ and $[M-CO]^+$.



Scheme 3.16: Suggested mass fragmentation pattern of F-7 and F-8

Common features on the ^{1}H NMR (Table 3.38) of flavanones included a pair of *ortho* coupled doublets H-2'/6' and H-3'/5' characterising the *para*-disubstituted B-ring, a pair of *meta*-coupled doublets H-6 and H-8 for the A-ring methines and a number of methoxyl groups. Characteristic features for **F-7** and **F-8** were the absence of a low-field sharp singlet for a chelated 5-OH and the presence of an ABX system at δ *ca.* 5.4 (dd, *ca.* J = 3, 13 Hz, H-2), δ *ca.* 3.1 (dd, *ca.* J = 13, 16.5 Hz, H-3_{ax}) and δ *ca.* 2.8 (dd, *ca.* J = 3, 16.5 Hz, H-3_{eq}).

Characteristic features on the J-modulated ^{13}C NMR (Table 3.39), assigned by ^{1}H-^{13}C HC-COBI, included carbonyls within the range of flavanone carbonyls (δ *ca.* 189), an oxymethine at δ *ca.* 79 (C-2), a methylene at δ *ca.* 46 (C-3) (Agrawal *et al.*, 1989), six quaternary carbons including four oxygenated positions, and relatively shielded methoxyl resonances (δ *ca.* 56.3-55.3), requiring them to be adjacent to at least one free *ortho* position (Panichpol and Waterman, 1978). As for the chalcones and dihydrochalcones, a combination of HMBC and NOESY experiments established unambiguous assignments of oxygenated positions on each ring.

Table 3.38: ^{1}H NMR (400 MHz) spectral data of F-7 and F-8

Position	F-7	F-8[a]
2	5.34 (dd, 2.8, 13.1)	5.52 (dd, 2.8, 12.8)
3_{ax}	3.02 (dd, 13.1, 16.5)	3.22 (dd, 12.8, 16.2)
3_{eq}	2.75 (dd, 2.9, 16.5)	2.92 (dd, 2.8, 16.2)
6	6.08 (d, 2.3)	6.47 (d, 2)
8	6.13 (d, 2.3)	6.52 (d, 2)
2'/6'	7.37 (d, 8.7)	7.52 (d, 8.6)
3'/5'	6.93 (d, 8.7)	7.01 (d, 8.6)
5-OMe	3.88 (s)	3.81 (s)
7-OMe	3.80 (s)	-
7'-OH	-	5.02 (brs)
4'-OMe	3.81 (s)	3.67 (s)

All data obtained in $CDCl_3$
a = Spectrum run in C_5D_5N.
In parentheses coupling constant *J* are in Hz.

Table 3.39: ^{13}C NMR (100 MHz) spectral data of F-7 and F-8

Position	F-7	F-8[a]
2	78.8	79.7
3	45.3	46.6
4	189.3	188.6
5	162.2	163.9
6	93.0	95.0
7	165.9	166.6
8	93.5	97.3
9	165.0	165.9
10	105.9	106.2
1'	130.8	132.4
2'/6'	127.6	128.8
3'/5'	114.1	114.9
4'	159.8	160.7
5-OMe	56.0	56.3
7-OMe	55.3	-
4'-OMe	55.5	55.7

All data obtained in $CDCl_3$
a = Spectrum run in C_5D_5N.

*a) Identification of **F-7** as naringenin trimethyl ether*

This compound was isolated from the petrol extract of *Goniothalamus gardneri*. The UV spectrum revealed a flavanone which exhibited no significant shifts in the presence of $AlCl_3$ or alkali, therefore indicating the absence of A-ring hydroxylation in positions 5 and 7 (Horowitz and Jurd, 1961; Mabry *et al.*, 1970).

HREIMS (Scheme 3.16) analysed for $C_{18}H_{18}O_5$ (10 DBE) based on a molecular ion at *m/z* 314. The presence of two methoxyl groups on the A-ring was evidenced by fragments at *m/z* 207, 180 and 152, while fragments at *m/z* 134 and 91 indicated the presence of one methoxyl group on the B-ring.

The ^{1}H NMR (Spectrum 3.34; Table 3.38) displayed signals typical of a flavanone with a 4'-substituted B-ring, an A-ring with two *meta*-coupled protons at δ 6.13 and 6.08 (d, J = 2.3 Hz) and three methoxyl groups (δ 3.88, 3.81 and 3.80). These combined data required positions 5, 7 and 4' to be methoxylated. Unambiguous assignments of the methoxylated positions were established by a combination of HMBC and NOESY experiments. The HMBC (Figure 3.26; Table 3.40) displayed:

i) a ^3J coupling between H-2'/6' and the oxygenated carbon at δ 159.8, which must therefore be the methoxylated C-4' on the B-ring.

ii) ^3J interactions between the methoxyl at δ 3.81 and the carbon at δ 159. 8, the methoxyl at δ 3.80 and the carbon at δ 165.9, the methoxyl at δ 3.88 and the carbon at δ 162.2.

iii) ^2J interactions between H-6 and δ 162.2 and 165.9, as well as ^2J interactions between H-8 and δ 165.0 and 165.9, thus allowing the assignment of the methoxylated C-7 position at δ 165.9.

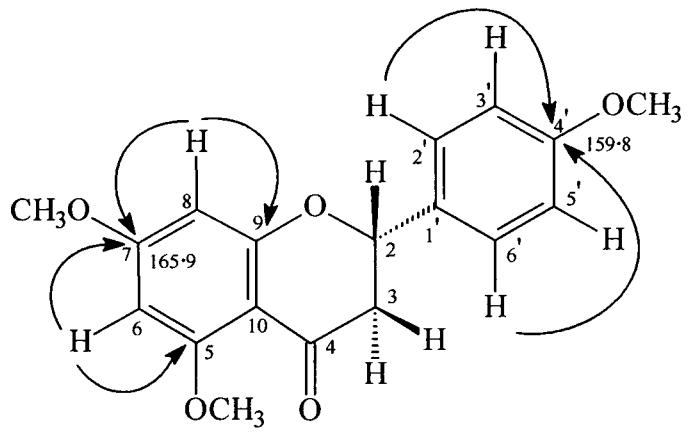


Figure 3.26: Significant HMBC correlations of F-7

The NOESY experiment (Figure 3.27) revealed interactions between both H-8 and H-6 and the methoxyl at δ 3.80, and between H-3'/5' and the methoxyl at δ 3.81, thus confirming the C-7 and C-4' assignments respectively at δ 165.9 and 159.8. Further interactions between H-6 and the methoxyl at δ 3.88 established the assignment of C-5 at δ 162.2. The aromatic B-ring was assigned to the equatorial position in view of the ^{3}J coupling values between H-2 and H-3, and this was further confirmed with a NOE interaction between H-3_{ax} and H-2'/6'.

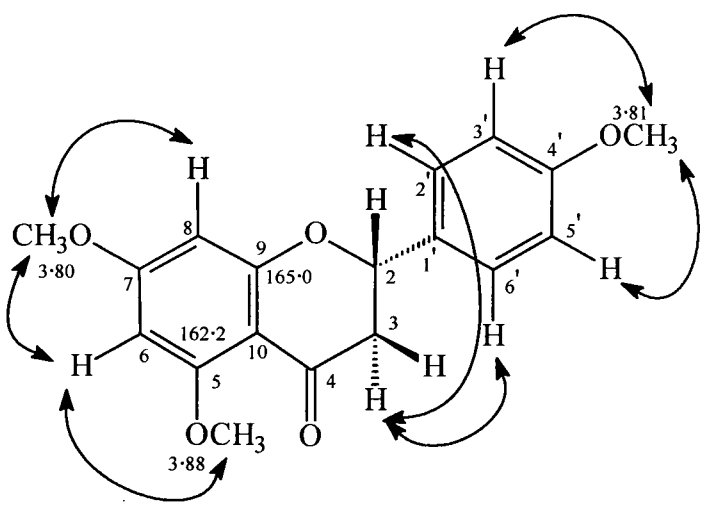


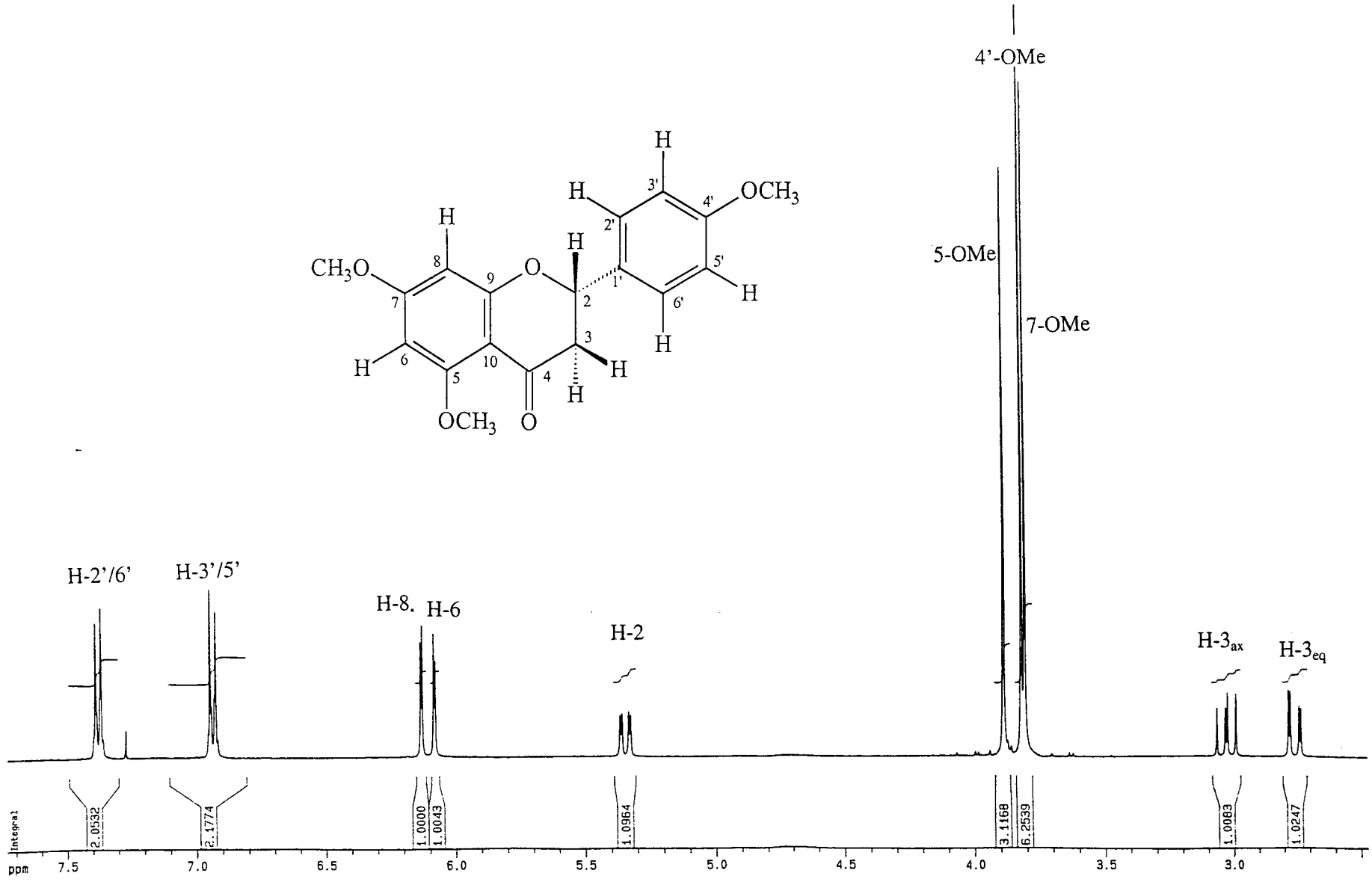
Figure 3.27: Significant NOE interactions of F-7

The mp, IR, UV and MS data of **F-7** complied with those reported for the known 5,7,4'-trimethoxyflavanone or naringenin trimethyl ether, previously isolated from *Dahlia tenuicaulis* and *Dahlia lehmanni* (Compositae) (Kaufmann and Lam, 1967; Lam and Wrang, 1975). ^{1}H NMR assignments showed agreement with values reported by Kaufmann and Lam (1967), with the methoxyl resonances and the methines H-6 and H-8 assignments established unambiguously for the first time. ^{13}C NMR assignments agreed with published data (Duddeck *et al.*, 1978) except for the C-5, C-6, C-8 and C-9 resonances respectively corrected at δ 162.2, 93.0, 93.5 and 165.0.

Table 3.40: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-7

Position	$^{\delta}$H	$^{\delta}$C	^{2}J	^{3}J
2	5.34 (dd, 2.8, 13.1)	78.8	130.8 (C-1')	127.6 (C-2'/6')
3_{ax}	3.02 (dd, 13.1, 16.5)	45.3	78.8 (C-2), 189.3 (C=O)	130.8 (C-1')
3_{eq}	2.75 (dd, 2.9, 16.5)	45.3	189.3 (C=O)	105.9 (C-10)
6	6.08 (d, 2.3)	93.0	162.2 (C-5), 165.9 (C-7)	93.5 (C-8), 105.9 (C-10)
8	6.13 (d, 2.3)	93.5	165.0 (C-9), 165.9 (C-7)	93.0 (C-6), 105.9 (C-10)
2'/6'	7.37 (d, 8.7)	127.6		78.8 (C-2), 159.8 (C-4')
3'/5'	6.93 (d, 8.7)	114.1	159.8 (C-4')	130.8 (C-1')
5-OMe	3.88 (s)	56.0		162.2 (C-5)
7-OMe	3.80 (s)	55.3		165.9 (C-7)
4'-OMe	3.81 (s)	55.5		159.8 (C-4')

All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.



Spectrum 3.34: ^{1}H NMR (400 MHz, $CDCl_3$*) of F-7

*b) Identification of **F-8** as tsugafolin*

This compound was isolated from the ethyl acetate extract of *Goniothalamus gardneri*. IR spectral data showed a broad absorption band (3207 cm^{-1}) for an hydroxyl group.

The UV spectrum (Table 3.37) indicated a flavanone substituted in position 5. A bathochromic shift (band II) on addition of alkali suggested a free 7-OH group (Horowitz and Jurd, 1961).

HREIMS (Scheme 3.16) gave a molecular ion at *m/z* 300, which analysed for $C_{17}H_{16}O_5$ (10 DBE). Fragments at *m/z* 193 and 166 confirmed the presence of one methoxyl and one hydroxyl group on the A-ring while fragments at *m/z* 134 and 91 were consistent with a B-ring substituted by one methoxyl group.

The ^1H NMR (Spectrum 3.35; Table 3.38) displayed signals typical of a flavanone with a 4'-mono-substituted B-ring and an A-ring with two *meta*-coupled protons at δ 6.52 and 6.47 (d, J = 2 Hz). Furthermore, it showed a broad singlet (free 7-OH) and two methoxyl groups at δ 3.81 and 3.67. These combined data required the placement of oxygenated positions to be 7-OH, 5-OMe on the A-ring and 4'-OMe on the B-ring. A combination of HMBC and NOESY experiments allowed the unambiguous assignments of these oxygenated positions. The HMBC (Figure 3.28; Table 3.41) displayed:

i) a ^3J coupling between H-2'/6' and the oxygenated carbon at δ 160.7, which must therefore be the methoxylated C-4' on the B-ring.

ii) ^3J interactions between the methoxyl at δ 3.67 and the carbon at δ 160. 7, the methoxyl at δ 3.81 and the carbon at δ 163.9.

iii) a ^2J interaction between H-6 and δ 166.6, as well as ^2J interactions between H-8 and δ 165.9 and 166.6, thus allowing the assignment of the methoxylated C-7 position at δ 166.6.

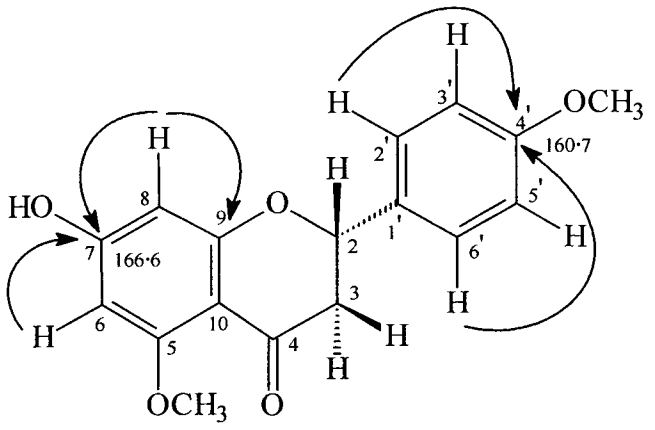


Figure 3.28: Significant HMBC correlations of F-8

The NOESY experiment (Figure 3.29) revealed interactions between H-6 and the methoxyl at δ 3.81, thus establishing the C-5 assignment at δ 163.9 and between H-3'/5' and the methoxyl at δ 3.67, thus confirming the C-4' assignment at δ 160.7. The aromatic B-ring was assigned to the equatorial position in view of the ^{3}J coupling values between H-2 and H-3, and this was further confirmed with a NOE interaction between H-3_{ax} and H-2'/6'.

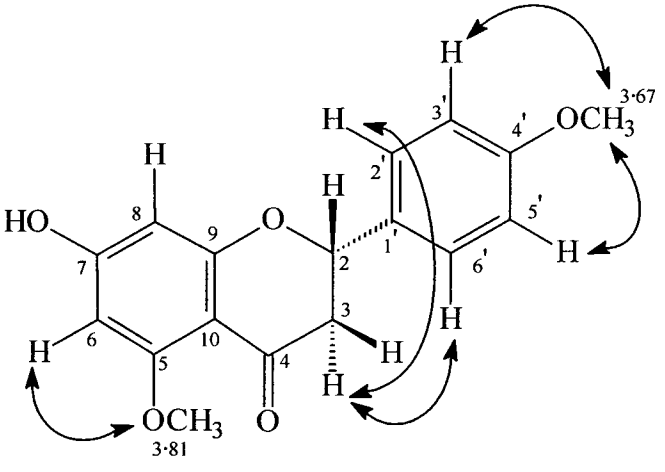


Figure 3.29: Significant NOE interactions of F-8

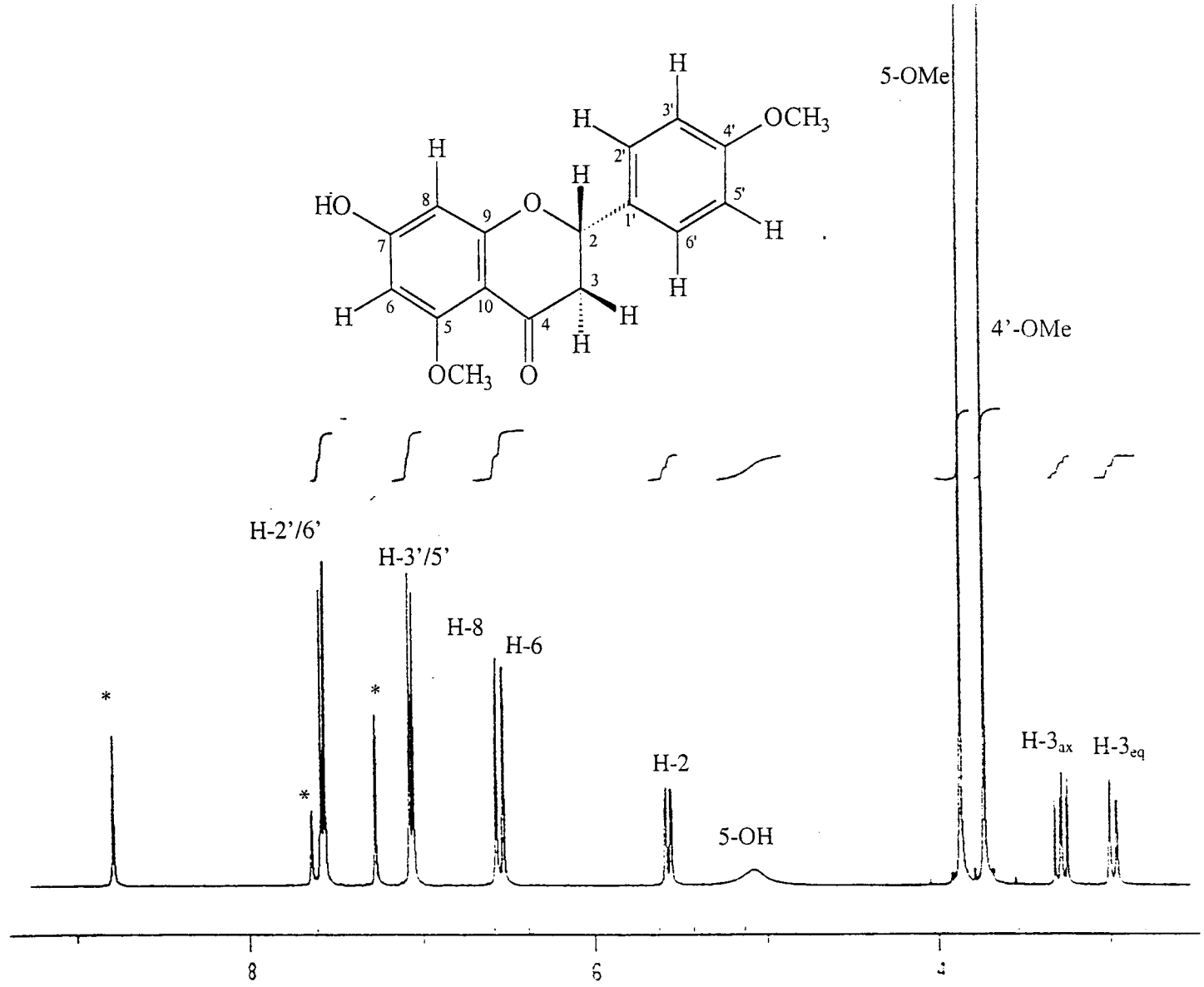
This led to the identification of **F-8** as the known 7-hydroxy-5,4'-dimethoxyflavanone or tsugafolin, previously isolated from *Tsuga diversifolia* (Pinaceae) (Tanaka *et al.*, 1989). IR, UV and MS results showed good agreement with published data. ^{1}H and ^{13}C NMR assignments were not directly comparable as

the ^{1}H NMR values in relative order of shielding/deshielding showed good agreement, except for the resonances H-3' and H-5' which were reversed. Similarly, comparison of the ^{13}C NMR chemical shifts showed good agreement, except for the two methoxyl resonances which were reversed.

Table 3.41: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-8

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
3_{ax}	3.22 (dd, 12.8, 16.2)	46.6	79.7 (C-2), 188.6 (C=O)	132.4 (C-1')
3_{eq}	2.92 (dd, 2.8, 16.2)		188.6 (C=O)	
6	6.47 (d, 2)	95.0	166.6 (C-7)	97.3 (C-8), 106.2 (C-10)
8	6.52 (d, 2)	97.3	165.9 (C-9), 166.6 (C-7)	95.0 (C-6), 106.2 (C-10)
2'/6'	7.52 (d, 8.6)	128.8		79.7 (C-2), 160.7 (C-4')
3'/5'	7.01 (d, 8.6)	114.9	160.7 (C-4')	132.4 (C-1')
5-OMe	3.81 (s)	56.3		163.9 (C-5)
4'-OMe	3.67 (s)	55.7		160.7 (C-4')
7'-OH	5.02 (brs)			

All data obtained in C_5D_5N.
In parentheses coupling constant J are in Hz.



Spectrum 3.35: ^{1}H NMR (400 MHz, C_5D_5N*) of F-8

3.3.5 Dihydroflavonol

One dihydroflavonol, coded **F-9** (CGM.1), was isolated from the methanol extract of *Cleistopholis glauca* (see Section 2.4.4) and identified as 3',4',5,7-tetrahydroxydihydroflavonol or dihydroquercetin (Figure 3.30).

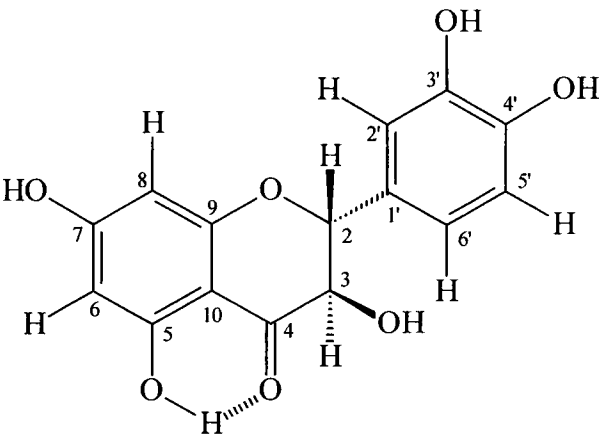


Figure 3.30: Structure of F-9

3.3.5.1 Characterisation of the isolated dihydroflavonol F-9 as dihydroquercetin

The presence of hydroxyls (3432 cm^{-1}) and hydrogen-bonded carbonyl group (1629 cm^{-1}) conjugated with an aromatic ring (3087, 1531 cm^{-1}) were established from the IR spectrum.

UV spectral behaviour was typical of a dihydroflavonol with a major band II (270-295 nm) and low intensity band I absorptions (Jurd, 1962; Mabry *et al.*, 1970). Bathochromic shifts (band II/36 nm) on addition of alkali, accompanied by an increase in peak intensity in the presence of NaOMe, indicated a 5,7-dihydroxyl system (Mabry *et al.*, 1970).

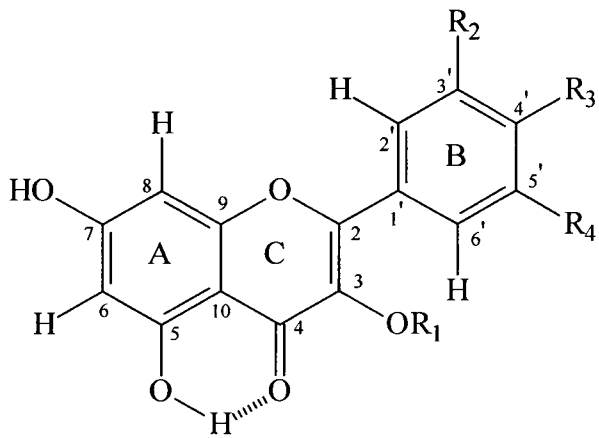
HREIMS afforded a molecular ion at *m/z* 304, which analysed for $C_{15}H_{12}O_7$ (10 DBE). The fragmentation pattern was consistent with a dihydroflavonol nucleus with two hydroxyl groups on the A-ring (*m/z* 165 and 153) and two hydroxyl groups on the B-ring (*m/z* 152 and 123) (Audier, 1966; Mabry and Markham, 1975).

The ^{1}H NMR revealed a chelated 5-OH (δ 11.62), an ABD system for B-ring protons with signals at δ 7.05 (d, J = 1.8 Hz), 6.89 (dd, J = 1.8, 8.1 Hz) and 6.83 (d, J = 8.1 Hz), *meta*-coupled protons on the A-ring at δ 5.96 and 5.91 (d, J = 2 Hz), two oxygenated C-ring protons at δ 4.98 and 4.59 showing a *trans*-diaxially relative configuration (d, J = 11.5 Hz) and four free OH groups. Fifteen carbons on the J-modulated ^{13}C NMR spectrum included the carbonyl at δ 198.2, the two A-ring methines at δ 97.0 and 96.1, the three B-ring methines at δ 120.9, 115.9, 115.8, the two C-ring oxymethines at δ 84.6 and 73.2 and seven quaternary carbons including five oxygenated positions.

Comparison of the physical and spectral data of **F-9** showed good agreement with those reported for the known 3',4',5,7-tetrahydroxydihydroflavonol or dihydroquercetin (Aft, 1961; Grisebach and Kellner, 1965; Audier, 1966; Shen *et al.*, 1993). The identification of **F-9** was also confirmed by co-TLC and comparison of its spectral data with those of an authentic sample.

3.3.6 Flavonol and flavonol glycosides

One flavonol, coded **F-10** (CGM.2), and two flavonol glycosides, coded **F-11** (PFM-1) and **F-12** (GTM-2), were isolated from *Cleistopholis glauca, Piptostigma fasciculata and Goniothalamus thwaitesii* (see Section 2.4.4). They were identified respectively as 3',4',5,7-tetrahydroxyflavonol (quercetin), kaempferol-3-*O*-rutinoside (nicotiflorin) and myricetin-4'-*O*-methyl ether-3-α-L-rhamnoside (mearnsitrin) (Figure 3.31).



Compound	R_1	R_2	R_3	R_4
F-10	H	OH	OH	H
F-11	Rha-glc**	H	OH	H
F-12	Rha*	OH	OCH_3	OH

Figure 3.31: Structures of F-10 to F-12

3.3.6.1 General characterisation of isolated flavonols and flavonols glycosides

IR spectral data suggested the presence of hydroxyl groups (3427-3208 cm^{-1}) and hydrogen bonded carbonyl functions (*ca.* 1655 cm^{-1}) conjugated with aromatic rings (1610-1504 cm^{-1}) (Williams and Fleming, 1995).

* Rha = rhamnose
**Rha-glc = rhamnoglucosyl

UV spectral behaviour (Table 3.42) was typical of flavonols with two major absorption peaks, band II (240-280 nm) and band I. The position of the latter helped to distinguish between free 3-hydroxyflavones (352-385 nm) and 3-substituted (methylated or glycosylated) flavonols (328-357 nm). Bathochromic shifts (band II/*ca.* 10 nm) on addition of NaOAc, indicated the presence of free 7-OH groups (Mabry *et al.*, 1970).

Table 3.42: UV (λ_{max}, nm) spectral data of isolated flavonols and flavonol glycosides

		F-10	F-11	F-12
MeOH	Band II	256, 261 (sh)	267	265
	Band I	372	347	337
$AlCl_3$	Band II	270, 301 (sh)	275	270,301 (sh)
	Band I	442	394	388
$AlCl_3$/HCl	Band II	267, 300 (sh)	275	275, 300 (sh)
	Band I	362 (sh), 431	395	393
NaOAc	Band II	257, 276 (sh)	274	273
	Band I	321 (sh), 380 (dec.)	373	352
NaOAc/H_3BO_3				
	Band II	260, 294 (sh)	267	265
	Band I	386	351	339
NaOMe	Band II	277 (sh)	275	271
	Band I	418 (dec.)	400	372

Common features on the ^{1}H and ^{13}C NMR (Tables 3.43 & 3.44) included a chelated 5-OH (δ 12.5), *meta*-coupled protons on the A-ring, carbonyls within the range of flavonol carbonyls (δ *ca.* 178) (Agrawal *et al.*, 1989) and six oxygenated quaternary carbons.

Table 3.43: ^{1}H NMR (400 MHz) spectral data of F-10 to F-12

Position	F-10[a]	F-11[b]	F-12[c]
6	6.26 (d, 1.8)	6.23 (d, 2.1)	6.69 (d, 2)
8	6.52 (d, 1.8)	6.41 (d, 2.1)	6.63 (d, 2)
2'	7.82 (d, 2)	8.08 (d, 8.7)	7.58 (s)
3'	-	6.92 (d, 8.7)	-
5'	6.99 (d, 8.5)	6.92 (d, 8.7)	-
6'	7.69 (dd, 2, 8.5)	8.08 (d, 8.7)	7.58 (s)
3-OH	2.94 (brs)		
5-OH	12.15 (s)		13.25 (s)
OH	8.60 (brs)		11.84 (brs)
1''		5.14 (d, 7.1)	6.22 (d, 1)
2''		3.47 (dd)	5.07 (dd, 1.5, 3.2)
3''		3.46 (m)	4.61 (dd, 3.4, 8.6)
4''		3.29 (t)	4.30 (t)
5''		3.38 (m)	4.28 (m)
6''		3.84/3.42 (m)	1.52 (d, 5.2)
1'''		4.55 (d, 1.2)	
2'''		3.68 (dd)	
3'''		3.56 (dd, 3.3, 9.4)	
4'''		3.32 (t, 9.7)	
5'''		3.47 (m)	
Me-6'''		1.16 (d, 6.1)	
4'-OMe			4.08 (s)

a = Spectrum run in acetone-d_6
b = Spectrum run in CD_3OD
c = Spectrum run in C_5D_5N
In parentheses coupling constant *J* are in Hz.

Table 3.44: ^{13}C NMR (100 MHz) spectral data of F-10 to F-12

Position	F-10[a]	F-11[b]	F-12[c]
2	147.4	159.5	158.6
3	137.2	135.7	137.1
4	176.9	179.4	179.6
5	162.6	163.0	163.5
6	99.5	100.2	100.3
7	165.4	166.2	166.5
8	94.9	95.1	95.1
9	158.3	158.6	158.3
10	104.3	105.7	106.1
1'	124.3	122.8	127.3
2'	116.2	132.5	110.2
3'	146.2	116.2	153.0
4'	148.7	161.5	140.1
5'	116.7	116.2	153.0
6'	121.9	132.5	110.2
1''		104.9	104.7
2''		75.8	72.5
3''		78.2	73.0
4''		71.5	73.8
5''		77.2	72.6
6''		68.7	18.9
1'''		102.5	
2'''		72.1	
3'''		72.4	
4'''		74.0	
5'''		69.8	
Me-6'''		18.0	
4'-OMe			60.8

a = Spectrum run in acetone-d_6
b = Spectrum run in CD_3OD
c = Spectrum run in C_5D_5N.

*a) Identification of **F-10** as quercetin*

This compound was isolated from the methanol extract of *Cleistopholis glauca*. Its UV spectrum (Table 3.42) was typical of a 3-hydroxyflavone with a free 7-OH group. A bathochromic shift (band I/59 nm) on addition of $AlCl_3$/HCl revealed free 3-OH and 5-OH groups (Mabry *et al.*, 1970). Bathochromic shifts both on addition of $AlCl_3$ (band I/11 nm above the one observed in $AlCl_3$/HCl) and NaOAc/H_3BO_3 (band I/14 nm) revealed a 3',4' *ortho*-dihydroxyl group (Mabry *et al.*, 1970). Furthermore, degeneration of the spectra recorded in alkali with time was characteristic of a flavonol containing free 3,4'- or 3,3',4'-hydroxyl groups (Mabry *et al.*, 1970).

HREIMS gave a molecular ion at *m/z* 302 corresponding to the molecular formula $C_{15}H_{10}O_7$ (11 DBE). The fragmentation pattern was consistent with a flavonol bearing two hydroxyl groups on the A-ring (*m/z* 153) and two hydroxyl groups on the B-ring (*m/z* 137) (Audier, 1966; Mabry and Markham, 1975).

Characteristic features on the ^1H and ^{13}C NMR (Tables 3.43 & 3.44) showed an ABD system for B-ring protons with signals at δ 7.82 (d, J = 2 Hz), 7.69 (dd, J = 2, 8.5 Hz) and 6.99 (d, J = 8.5 Hz), four free OH and seven oxygenated quaternary carbons, thus confirming the presence of 5,7 dihydroxyl and 3',4'-*ortho*-dihydroxyl groups.

Comparison of the physical and spectral data of **F-10** showed good agreement with those reported for the known 3',4',5,7-tetrahydroxyflavonol or quercetin (Audier, 1966; Shen, *et al.*, 1993). The identification of **F-10** was also confirmed by co-TLC and comparison of its spectral data with those of an authentic sample.

*b) Identification of **F-11** as nicotiflorin*

This compound was isolated from the methanol extract of *Piptostigma fasciculata*. An absorption band at 821 cm^{-1} in the IR spectrum suggested the presence of a *para*-disubstituted aromatic ring (Williams and Fleming, 1995).

UV spectral data (Table 3.42) showed a 3-substituted flavonol with a free 7-OH. A bathochromic shift (band I/*ca.* 48 nm) on addition of $AlCl_3$/HCl confirmed the 3-OH substitution and indicated a free 5-OH (Jurd, 1962; Mabry *et al.*, 1970). No significant shift of band I, both on addition of $AlCl_3$ (compared to the one observed in $AlCl_3$/HCl) and NaOAc/H_3BO_3, confirmed the absence of an *o*-dihydroxyl group on the B-ring (Mabry *et al.*, 1970). The presence of a free 4'-OH was indicated by a bathochromic shift (band I/53 nm) on addition of NaOMe without any decrease in intensity (Mabry *et al.*, 1970).

Characteristic features on the ^1H NMR (Table 3.43; Spectrum 3.36) included a pair of *ortho*-coupled methines at δ 8.08 and 6.92 (2H each, d, 8.7), thus confirming the 4'-substitution on the B-ring and identifying the flavonol as kaempferol. This was confirmed in the FABMS with a fragment ion at *m/z* 286. The ^1H NMR further displayed signals accounting for the presence of two sugar units with anomeric protons at δ 5.14 (d, J = 7.1 Hz) and 4.55 (d, J = 1.2 Hz) and a doublet at δ 1.16 (3H, J = 6.1 Hz), suggesting that one of the sugars was a rhamnose unit. The presence of a (1→6)-rhamnoglucosyl moiety, substituted in C-3 of kaemperol was evidenced by the HMBC experiment (Table 3.45) with ^3J couplings between:

i) the anomeric proton of the rhamnose unit H-1'" at δ 4.55 and the methylene carbon in C-6" of the glucose unit at δ 68.7.

ii) the anomeric proton of the glucose unit H-1" at δ 5.14 and the C-3 position of the flavonol nucleus at δ 135.7.

On the basis of these data and by co-TLC and comparison of physical and spectral data with those of an authentic sample*, **F-11** was identified as kaempferol-3-*O*-rutinoside or nicotiflorin, previously isolated in the Annonaceae from *Cananga*

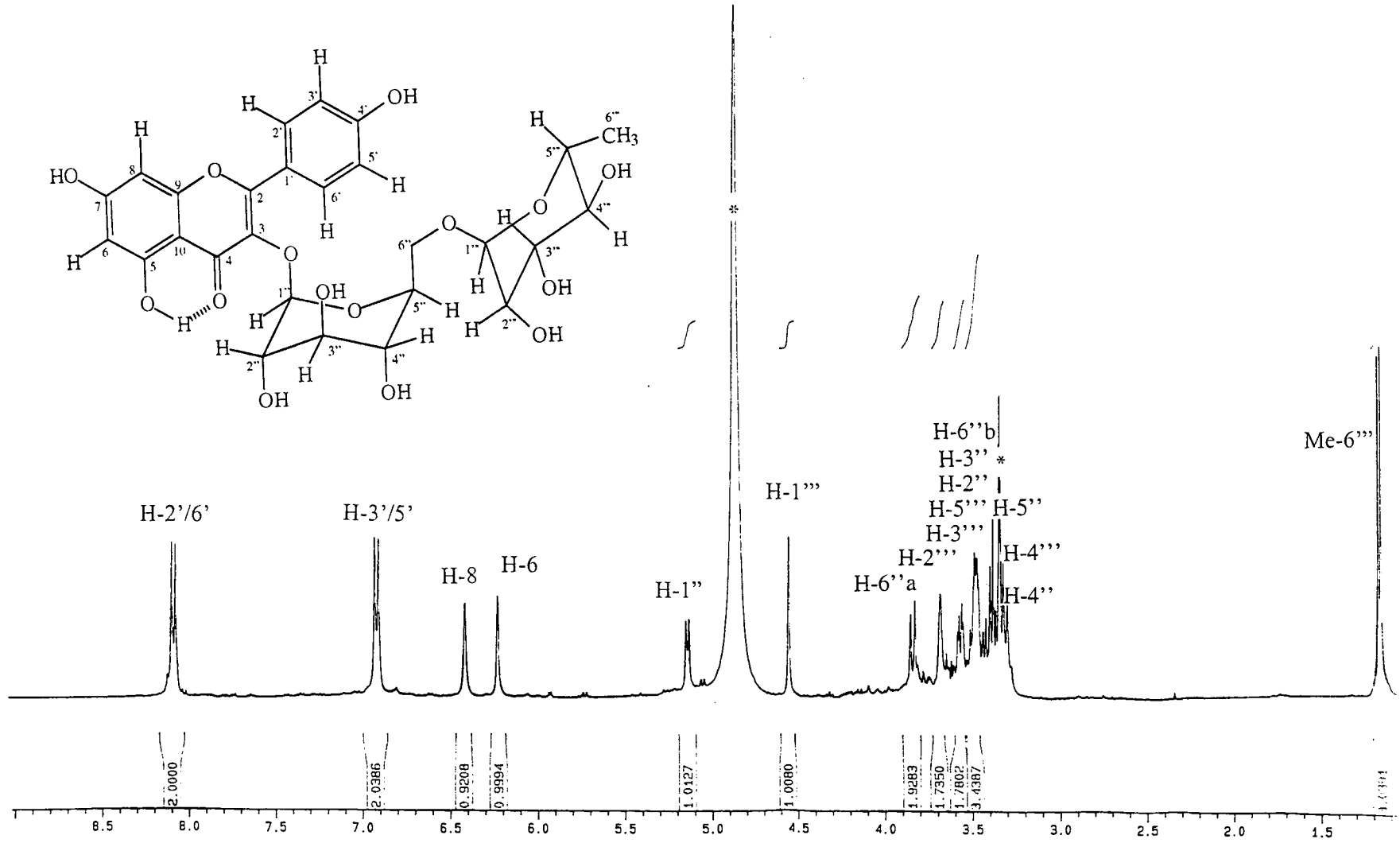
latifolia (Siv and Paris, 1972). IR, UV and MS data showed good agreement with published data (Siv and Paris, 1972; Satake *et al.*, 1984). ^{1}H and ^{13}C NMR assignments were not directly comparable as Satake *et al.* (1984) recorded them in a different solvent. However, comparison of the ^{13}C NMR chemical shifts in relative order of shielding/deshielding showed good agreement, with unambiguous assignments of C-2 (δ 159.5) and C-9 (δ 158.6) positions. ^{1}H NMR chemical shifts are assigned unambiguously for the first time.

Table 3.45: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-11

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
6	6.23 (d, 2.1)	100.2	163.0 (C-5), 166.2 (C-7)	95.1 (C-8), 105.7 (C-10)
8	6.41 (d, 2.1)	95.1	158.6 (C-9), 166.2 (C-7)	100.2 (C-6), 105.7 (C-10)
2'/6'	8.08 (d, 8.7)	132.5		159.5 (C-2), 161.5 (C-4')
3'/5'	6.92 (d, 8.7)	116.2	161.5 (C-4')	122.8 (C-1')
1"	5.14 (d, 7.1)	104.9		135.7 (C-3)
2"	3.47 (dd)	75.8	78.2 (C-3")	71.5 (C-4")
3"	3.46 (m)	78.2	71.5 (C-4"), 75.8 (C-2")	
4"	3.29 (t)	71.5	77.2 (C-5")	
5"	3.38 (m)	77.2	68.7 (C-6"), 71.5 (C-4")	
6"	3.84/3.42 (m)	68.7	71.5 (C-4"), 77.2 (C-5")	
1"'	4.55 (d, 1.2)	102.5		68.7 (C-6"), 69.8 (C-5"'), 72.4(C-3"')
2"'	3.68 (dd)	72.1		74.0 (C-4")
4"'	3.32 (t, 9.7)	74.0	69.8 (C-5"'), 72.4 (C-3"')	
5"'	3.47 (m)	69.8	74.0 (C-4"')	
Me-6"'	1.16 (d, 6.1)	18.0	69.8 (C-5"')	74.0 (C-4"')

All data obtained in CD_3OD.
In parentheses coupling constant *J* are in Hz.

* Authentic sample kindly provided by Professor F. Bailleul, Pharmacognosy Laboratories, Faculty of Pharmacy, Lille, France.



Spectrum 3.36: ^{1}H NMR (400 MHz, CD_3OD*) of F-11

c) Identification of ***F-12*** *as mearnsitrin*

This compound was isolated from the methanol extract of *Goniothalamus thwaitesii*. Characteristic feature in the IR data revealed an aryl ether group (2850 cm^{-1}).

UV data (Table 3.42) showed a 3-substituted flavonol with a free 7-OH. A bathochromic shift (band I/*ca.* 56 nm) on addition of $AlCl_3$/HCl confirmed the 3-OH substitution and indicated a free 5-OH (Jurd, 1962; Mabry *et al.*, 1970). No significant shift of band I both on addition of $AlCl_3$ (compared to the one observed in $AlCl_3$/HCl) and NaOAc/H_3BO_3, confirmed the absence of an *o*-dihydroxyl group on the B-ring (Mabry *et al.*, 1970). Furthermore, addition of NaOMe caused no decomposition of the spectrum, showing the absence of alkali sensitive 3,4'- or 3,3',4'-hydroxyl groups (Mabry *et al.*, 1970).

HREIMS revealed a molecular ion at *m/z* 478, consistent with the molecular formula $C_{22}H_{22}O_{12}$ (12 DBE).

Characteristic features on the ^1H NMR (Spectrum 3.37; Table 3.43) included a singlet at δ 7.58 (2H each) for two equivalent H-2'/6', thus indicating a symmetrical substitution pattern on the B-ring. It further revealed a series of resonances accounting for the presence of a rhamnose unit with an anomeric proton H-1'' at δ 6.22 (d, J = 1 Hz), oxymethines H-2'', H-3'', H-4'', H-5" respectively at δ 5.07 (dd, J = 1.5, 3.2 Hz), 4.61 (dd, J = 3.4, 8.6 Hz), 4.30 (t), 4.28 (m) and a characteristic Me-6" at δ 1.52 (d, J = 5.2 Hz). The presence of a rhamnose moiety was confirmed in the HREIMS with fragment ions at *m/z* 332 $[M\text{-rhamnosyl}]^+$, 317 $[M\text{-rhamnosyl-}CH_3]^+$and 297 $[M\text{-rhamnosyl-H-2}\times OH]^+$. The ^1H NMR further showed some free hydroxyl groups at δ 11.84 (brs) and one aromatic methoxyl at δ 4.08.

The *J*-modulated ^{13}C NMR (Table 3.44), assigned by ^{1}H-^{13}C HC-COBI, confirmed the presence of the two equivalent methines H-2'/6' (δ 110.2), the rhamnose unit with the anomeric methine (δ 104.7) and the Me-6" (δ 18.9) and the methoxyl resonance (δ 60.8). The relatively deshielded position of the latter required its placement adjacent to two *ortho* substituents (Panichpol and Waterman, 1978). The *J*-modulated ^{13}C NMR further showed eight oxygenated quaternary carbons.

The nature of the aglycone and the location of the rhamnosyl and methoxyl substituents were evidenced by a combination of HMBC and NOESY experiments. The HMBC (Spectrum 3.38; Table 3.46) displayed:

i) a ^{3}J coupling between H-2'/6' and the oxygenated carbon C-4' at δ 140.1, as well as a ^{3}J interaction between the methoxyl at δ 4.08 and this same oxygenated C-4', thus requiring the methoxyl group at the C-4'-position on the B-ring and identifying the aglycone as myricetin 4'-*O*-methyl ether or mearnsetin.

ii) ^{2}J interactions between H-6 and δ 166.5 and 163.5, as well as ^{2}J interactions between H-8 and δ 166.5 and 158.3, thus allowing the assignment of the C-7 position at δ 166.5.

iii) a ^{3}J coupling between the anomeric H-1" and the oxygenated carbon C-3 at δ 137.1, thus establishing the glycosylation site in position C-3.

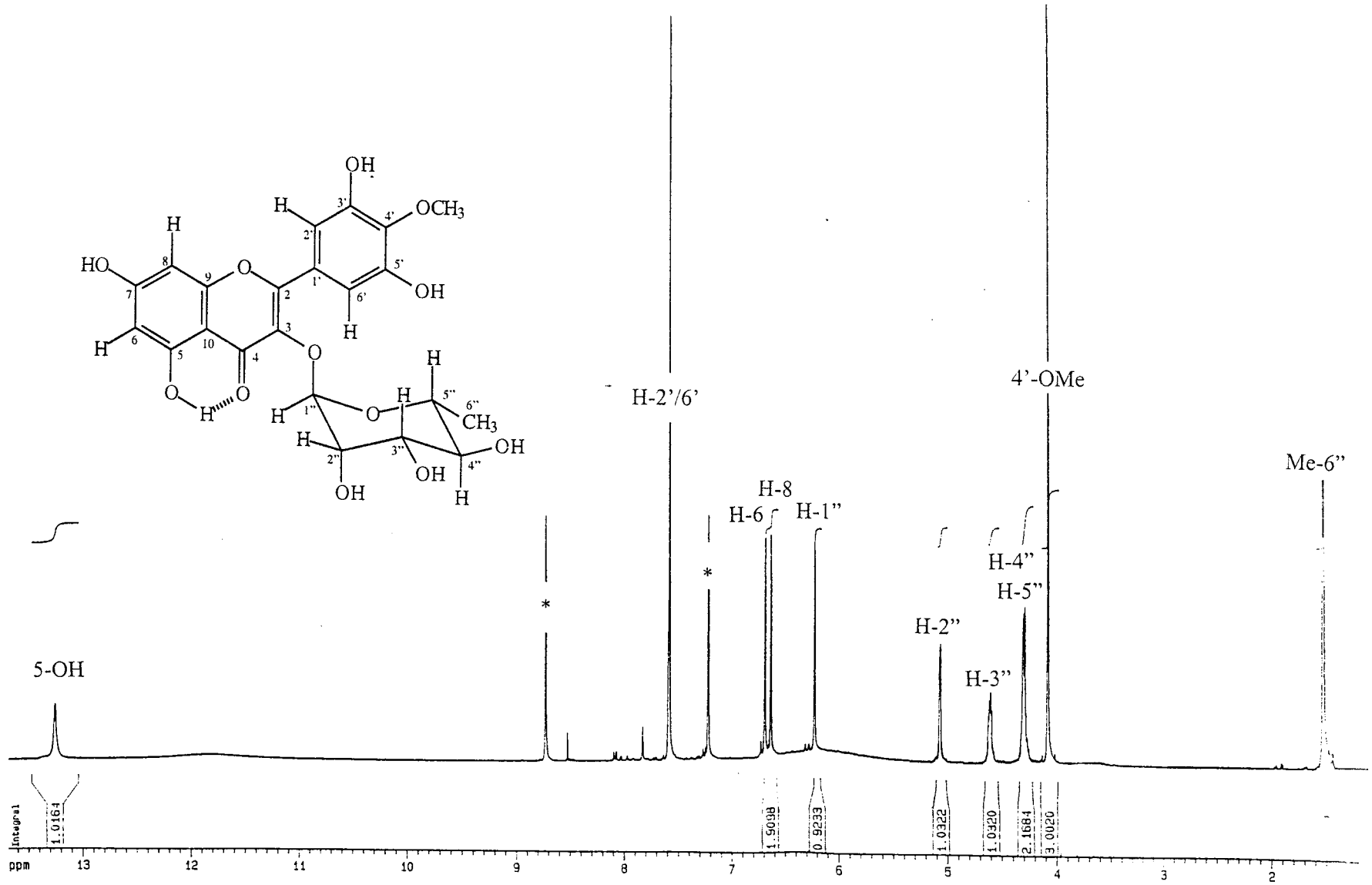
In the NOESY experiment, the methoxyl resonance did not show any correlation, thus confirming its position at C-4', flanked by two hydroxyl groups.

On this basis, **F-12** was identified as myricetin 4'-*O*-methyl ether-3-*O*-α-L-rhamnopyranoside or mearnsitrin previously reported from *Acacia mearnsii* (Leguminosae) (Mackenzie, 1969). ^{1}H and ^{13}C NMR assignments, established on the basis of extensive 2D NMR experiments as well as comparison with assignments reported for the known myricetin-3-*O*-α-L-rhamnopyranoside or myricitrin (Nicollier and Thompson, 1983), are reported unambiguously for the first time.

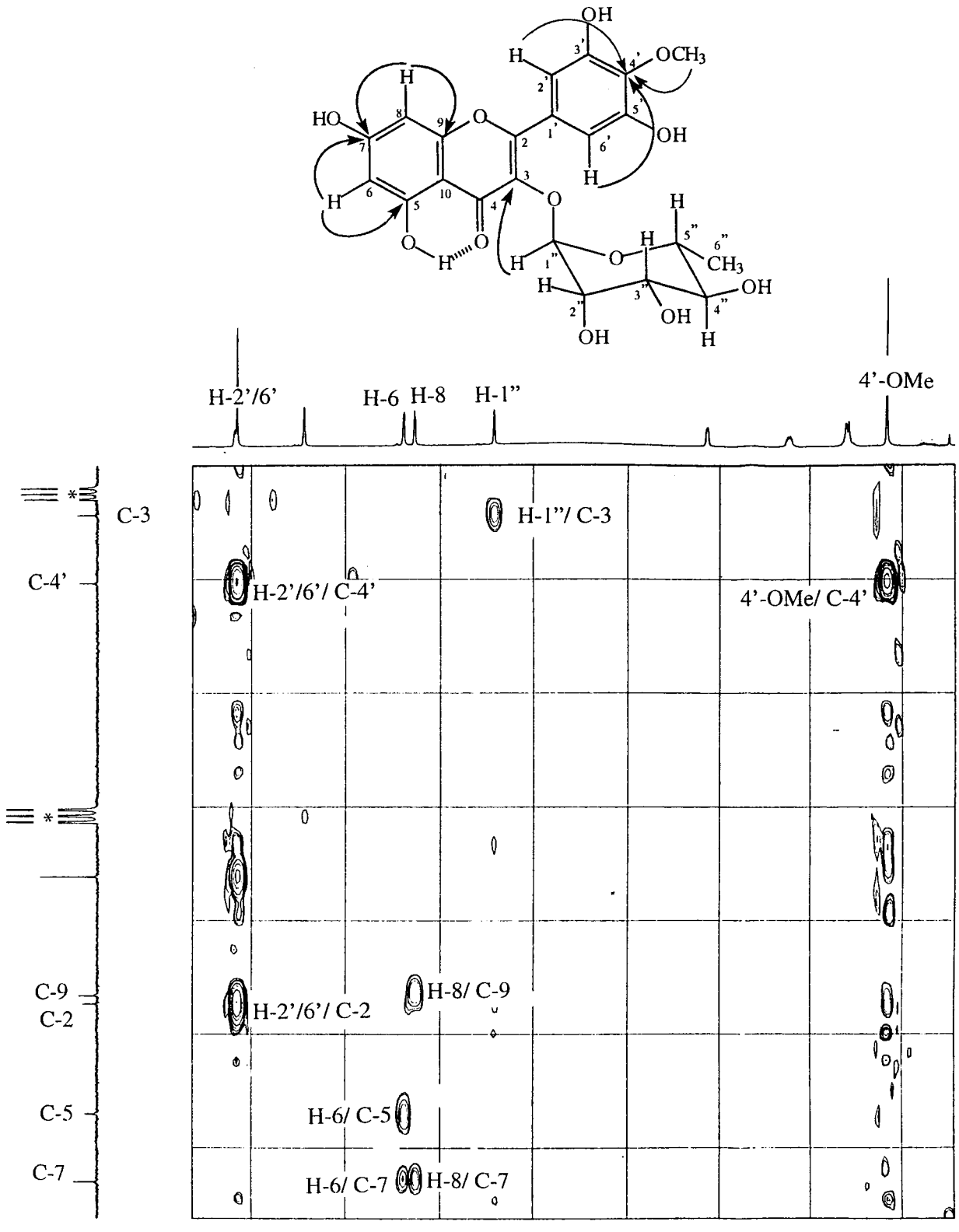
Table 3.46: Significant ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations of F-12

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
6	6.69 (d, 2)	100.3	163.5 (C-5), 166.5 (C-7)	95.1 (C-8), 106.1 (C-10)
8	6.63 (d, 2)	95.1	158.3 (C-9), 166.5 (C-7)	100.3 (C-6), 106.1 (C-10)
2'/6'	7.58 (s)	110.2	153.0 (C-3'/5')	140.1 (C-4'), 158.6 (C-2)
4'-OMe	4.08 (s)	60.8		140.1 (C-4')
1"	6.22 (d, 1)	104.7	72.5 (C-2")	72.6 (C-5"), 73.0 (C-3"), 137.1 (C-3)
2"	5.07 (dd, 1.5, 3.2)	72.5		73.8 (C-4")
3"	4.61 (dd, 3.4, 8.6)	73.0	73.8 (C-4")	
4"	4.30 (t)	73.8	72.6 (C-5"), 73.0 (C-3")	
Me-6"	1.52 (d, 5.2)	18.9	72.6 (C-5")	73.8 (C-4")

All data obtained in C_5D_5N.
In parentheses coupling constant J are in Hz.



Spectrum 3.37: ^{1}H NMR (400 MHz, C_5D_5N*) of F-12



Spectrum 3.38: Significant HMBC correlations (400 MHz, C_5D_5N) of F-12

3.3.7 Flavonol-3-*O*-methyl ether

One flavonol-3-*O*-methyl ether, coded **F-13** (GTM-1), was isolated from the methanol extract of *Goniothalamus thwaitesii* (see Section 2.4.4) and identified as myricetin-3-*O*-methyl ether or annulatin (Figure 3.32).

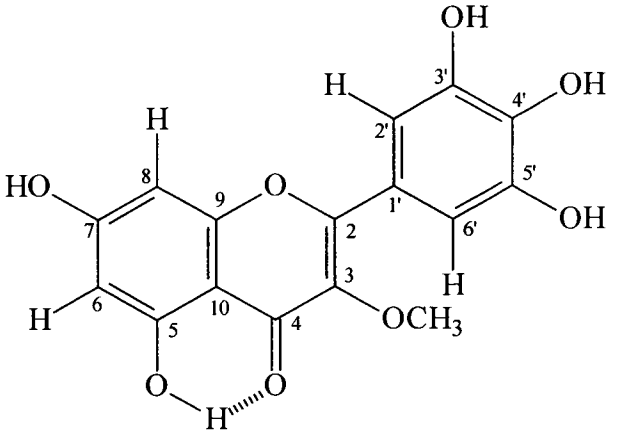


Figure 3.32: Structure of F-13

3.3.7.1 Characterisation of the isolated flavonol-3-O-methyl ether F-13 as annulatin

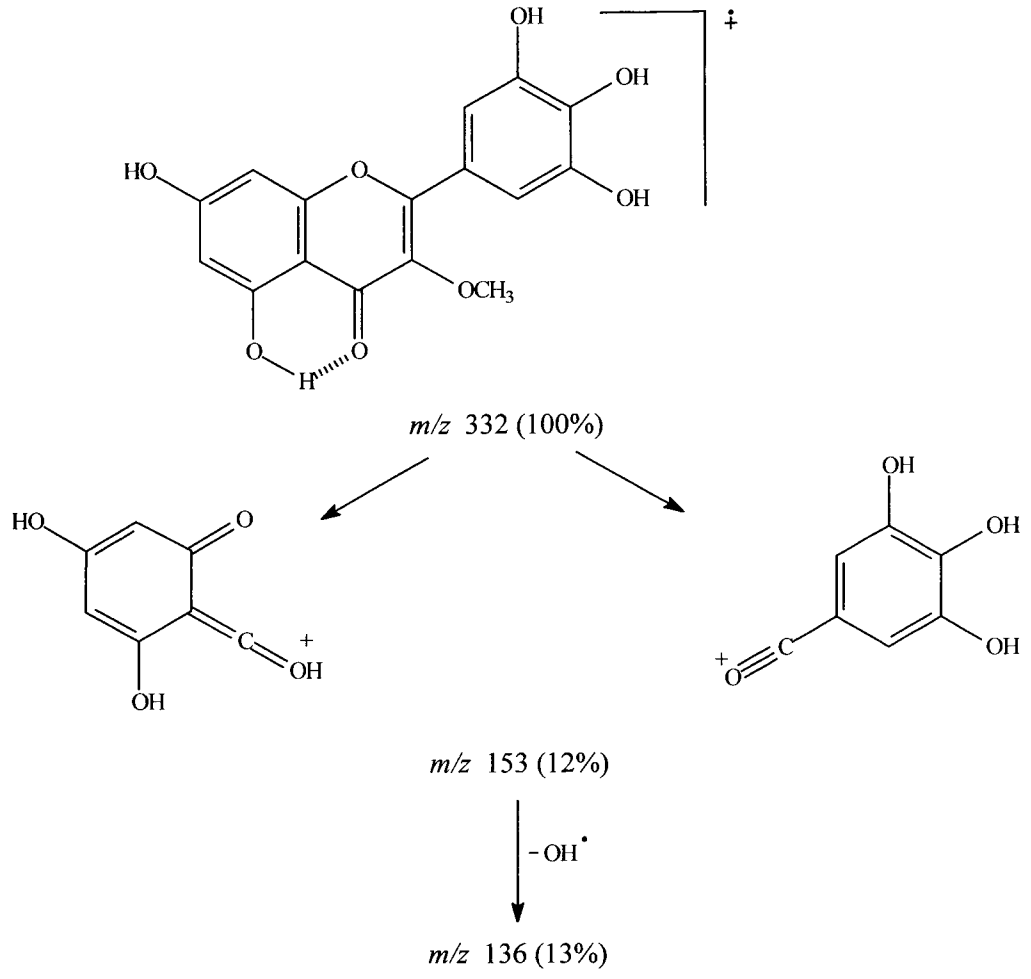
The presence of hydroxyl (3313 cm^{-1}), aryl ether groups (2848 cm^{-1}) and α,β-unsaturated, hydrogen-bonded, carbonyl function (1654 cm^{-1}) conjugated with aromatic rings (3068, 1602, 1511 cm^{-1}) were established from the IR spectrum (Williams and Fleming, 1995).

UV spectral behaviour (Table 3.47) was typical of a 3-substituted flavonol. A bathochromic shift (band II/12 nm) on addition of NaOAc, indicated a free 7-OH group (Mabry *et al.*, 1970). A bathochromic shift (band I/39 nm) on addition of $AlCl_3$/HCl indicated a 3-substituted flavonol with a free 5-OH (Jurd, 1962; Mabry *et al.*, 1970). The structure of a 3-methoxyflavone with a phoroglucinol type A-ring was confirmed by comparison with published data (Voirin, 1983). The presence of a free 4'-OH was indicated by a bathochromic shift (band I/44 nm) on addition of NaOMe without any decrease in intensity (Mabry *et al.*, 1970). Degeneration with time of the spectra recorded in alkali, was in accord with a flavonol containing an alkali sensitive 3',4',5'-trihydroxyl system (Mabry *et al.*, 1970).

Table 3.47: UV (λ_{max}, nm) spectral data of F-13

MeOH	Band II	264
	Band I	304, 366
$AlCl_3$	Band II	271
	Band I	347, 424
$AlCl_3$/HCl	Band II	271
	Band I	347, 405
NaOAc	Band II	276 (dec)
	Band I	377 (dec)
NaOAc/H_3BO_3		
	Band II	264
	Band I	367
NaOMe	Band II	277 (dec)
	Band I	410 (dec)

HREIMS afforded a molecular ion at *m/z* 332, which analysed for $C_{16}H_{12}O_8$ (11 DBE). Peaks were observed at *m/z* 317 $[M\text{-}CH_3]^+$ and 286 $[M\text{-}CO\text{-}OH\text{-}H]^+$. A fragment ion at *m/z* 153 revealed two hydroxyl groups on the A-ring and/or three hydroxyl groups on the B-ring (Scheme 3.17).



Scheme 3.17: Suggested mass fragmentation pattern of F-13

The ^{1}H NMR (Spectrum 3.39; Table 3.48) showed a low-field singlet (δ 13.22) for the chelated 5-OH, a singlet (2H) at δ 8.14 attributed to H-2'/6', showing a symmetrical substitution on the B-ring, two *meta* coupled doublets at δ 6.72 and 6.70 (1H each, J = 2 Hz), confirming the phloroglucinol like pattern on the A-ring, one aromatic methoxyl (δ 4.11) and some free hydroxyl groups.

The J-modulated ^{13}C NMR (Spectrum 3.40; Table 3.48), assigned by ^{1}H-^{13}C HC-COBI, confirmed the presence of the two equivalent H-2'/6' (δ 109.3), the *meta*-coupled methines H-6, H-8 (δ 99.8 and 94.8) and the methoxyl (δ 60.8) whose relatively deshielded resonance required its placement adjacent to two *ortho* substituents (Panichpol and Waterman, 1978). The J-modulated ^{13}C NMR further

showed a carbonyl (δ 178.0) and ten quaternary carbons including eight oxygenated positions.

The structure of myricetin-3-*O*-methyl ether was evidenced by a combination of HMBC and NOESY experiments. The HMBC (Table 3.48; Figure 3.33) displayed:

i) a ^{3}J coupling between H-2'/6' and the oxygenated carbon at δ 139.3, which must therefore be the hydroxyl bearing C-4' on the B-ring.

ii) a ^{3}J interaction between the methoxyl at δ 4.11 and the oxygenated carbon at δ 139.4, thus identifying the flavonol as myricetin 3-*O*-methyl ether.

iii) ^{2}J interactions between H-6 and both δ 166.3 and 162.9, as well as ^{2}J interactions between H-8 and both δ 166.3 and 158.0, thus allowing the assignment of the C-7 position at δ 166.3.

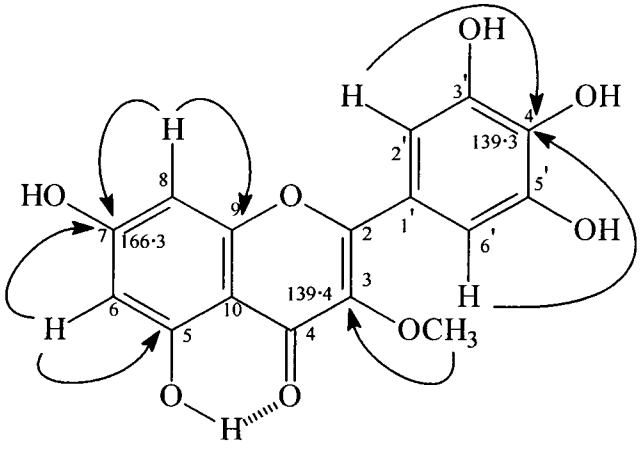


Figure 3.33: Significant HMBC correlations of F-13

A NOE interaction between H-2'/6' and the methoxyl group at δ 4.11 confirmed the presence of the 3-*O*-methylated position (Figure 3.34).

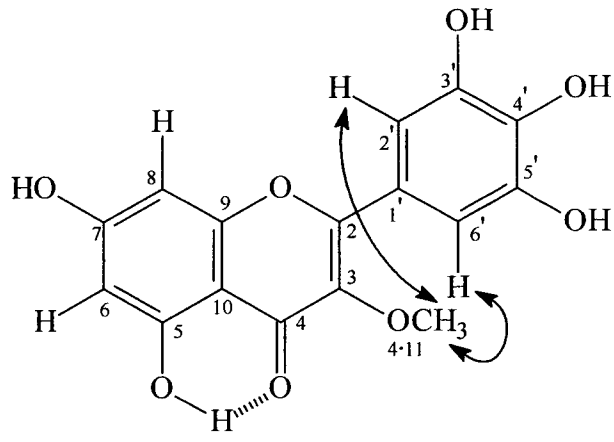


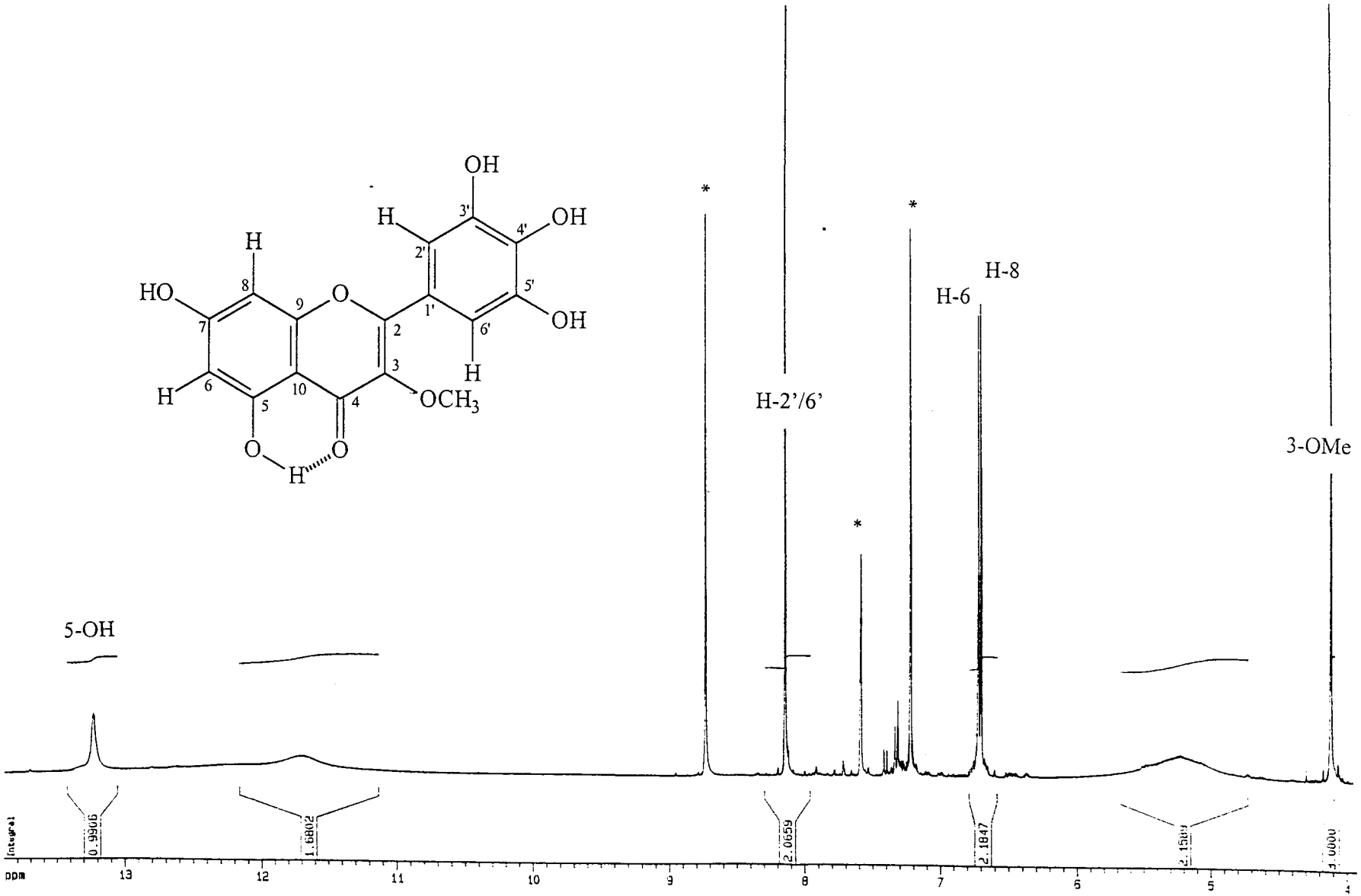
Figure 3.34: Significant NOE interactions of F-13

On this basis, **F-13** was identified as myricetin-3-*O*-methyl ether or annulatin, previously isolated from *Cleistocactus variispinus* and *Cereus jamacaru* (Cactaceae) (Burret *et al.*, 1982) and as glycosides from *Aegialitis annulata* (Plumbaginaceae) (Harborne, 1967), *Oenothera speciosa* and *O. tetragona* subsp. *glauca* (Onagraceae) (Howard *et al.*, 1972) and *Dacrycarpus dacrydioides* (Podocarpaceae) (Markham and Whitehouse, 1984). UV and MS of **F-13** showed good agreement with published data (Markham and Whitehouse, 1984). This is the first report of the unambiguous ^{1}H and ^{13}C NMR assignments.

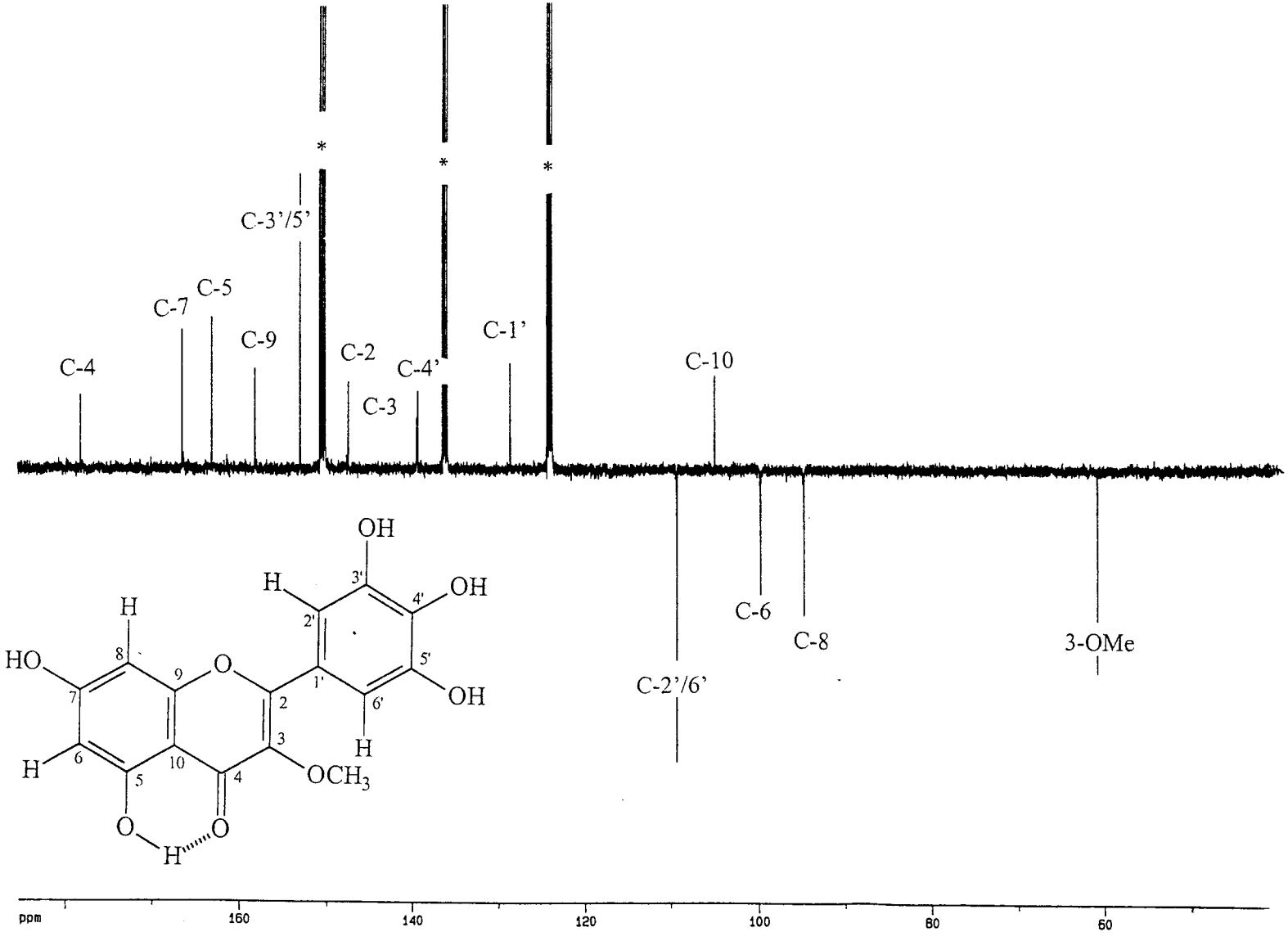
Table 3.48: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of F-13

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2	-	147.4		
3	-	139.4		
4	-	178.0		
5	-	162.9		
6	6.72 (d, 2)	99.8	162.9 (C-5), 166.3 (C-7)	94.8 (C-8), 104.9 (C-10)
7	-	166.3		
8	6.70 (d, 2)	94.8	158.0 (C-9), 166.3 (C-7)	99.8 (C-6), 104.9 (C-10)
9	-	158.0		
10	-	104.9		
1'	-	128.6		
2'/6'	8.14 (s)	109.3	128.6 (C-1'), 152.9 (C-3'/5')	139.3 (C-4'), 147.4 (C-2)
3'/5'	-	152.9		
4'	-	139.3		
3-OMe	4.11 (s)	60.8		139.4 (C-3)
5-OH	13.22 (s)			
OH	5.23 (brs), 11.74 (brs)			

All data obtained in C_5D_5N.
In parentheses coupling constant J are in Hz.



Spectrum 3.39: ^{1}H NMR (400 MHz, C_5D_5N*) of F-13



Spectrum 3.40: *J*-modulated ^{13}C NMR (100 MHz, C_5D_5N*) of F-13

3.3.8 Properties and spectral data of isolated flavonoids

F-1 [2'-Hydroxy-4,4',6'-trimethoxychalcone (flavokawain A)]

Yellow plates (*n*-hexane/EtOAc). Mp. 112° (Lit. mp. 114-115°, Hansel *et al.*, 1963). IR ν_{max} (KBr disc) cm^{-1}: 3438 (OH), 3002, 2979, 2942, 2844, 1621 (C=O), 1604, 1583, 1513, 1348, 1290, 1220, 1159, 1033, 973, 825. UV λ_{max} (MeOH) nm: Table 3.24. Found $[M]^+$ 314.1174 ($C_{18}H_{18}O_5$ requires 314.1154) HREIMS *m/z* (rel. int. %): 314 (100), 313 (86), 297 (15), 286 (11), 271 (7), 207 (42), 180 (40), 161 (17), 150 (18), 134 (52), 121 (60), 113 (15), 84 (8), 69 (7). ^1H & ^{13}C NMR: Tables 3.25 & 3.26.

F-2 (2',4'-Dihydroxy-4,6'-dimethoxychalcone)

Yellow plates (MeOH). Mp. 161-163° (Lit. mp. 158-159°, Bhardwaj *et al.*, 1982). IR ν_{max} (KBr disc) cm^{-1}: 3434 (OH), 3193 (OH), 3002, 2981, 2937, 2910, 2836, 1623 (C=O), 1604, 1573, 1511, 1342, 1292, 1259, 1222, 1209, 1168, 1114, 1024, 970, 831. UV λ_{max} (MeOH) nm: Table 3.24. Found $[M]^+$ 300.0983 ($C_{17}H_{16}O_5$ requires 300.0997) HREIMS *m/z* (rel. int. %): 300 (100), 299 (79), 283 (16), 272 (10), 257 (7), 192 (9), 167 (30), 166 (16), 161 (12), 134 (71), 121 (62), 84 (15). ^1H & ^{13}C NMR: Tables 3.25 & 3.26.

F-3 [2'-Hydroxy-4,4',6'-trimethoxy-dihydrochalcone (dihydroflavokawain A)]

Colourless plates (EtOH). Mp. 108-110°. (Lit. mp. 110-112°, Bhardwaj *et al.*, 1982). IR ν_{max} (KBr disc) cm^{-1}: 3426 (OH), 3014, 2960, 2910, 2838, 1630 (C=O), 1616 1585, 1513, 1367, 1218, 1207, 1157, 1114, 1031, 823. UV λ_{max} (MeOH) nm: Table 3.29. Found $[M]^+$ 316.1295 ($C_{18}H_{20}O_5$ requires 316.1310) HREIMS *m/z* (rel. int. %): 316 (53), 297 (7), 285 (4), 267 (5), 181 (100), 154 (29), 134 (89), 121 (38), 84 (95). ^1H & ^{13}C NMR: Tables 3.30 & 3.31.

F-4 (2',4'-Dihydroxy-4,6'-dimethoxydihydrochalcone)

Colourless needles (MeOH). Mp. 171° (Lit. mp. 175-176°, Bhardwaj *et al.*, 1982). IR ν_{max} (KBr disc) cm^{-1}: 3249 (OH), 3021, 2991, 2952, 2940, 2912, 2873, 2832, 1643 (C=O), 1629, 1610, 1567, 1513, 1297, 1243, 1197, 1164, 1110, 1031, 817, 802. UV λ_{max} (MeOH) nm: Table 3.29. Found $[M]^+$ 302.1136 ($C_{17}H_{18}O_5$ requires 302.1154) HREIMS *m/z* (rel. int. %): 302 (31), 283 (6), 271 (3), 253 (2), 228 (9), 199 (8), 167 (70), 134 (55), 121 (29), 86 (94), 84 (100). ^1H & ^{13}C NMR: Tables 3.30 & 3.31.

F-5 (4,2',4'-Trihydroxy-6'-methoxydihydrochalcone)

Pale amorphous solid. IR ν_{max} (KBr) cm^{-1}: 3332 (OH), 3008, 2923, 2854, 1631 (C=O), 1610, 1590, 1509, 1375, 1265, 1209, 1197, 1168, 1110, 842, 823. UV λ_{max} (MeOH) nm: Table 3.29. Found $[M]^+$ 288.1025 ($C_{16}H_{16}O_5$ requires 288.0997) HREIMS *m/z* (rel. int. %): 288 (69), 269 (11), 167 (100), 140 (32), 120 (76), 107 (21), 77 (8). ^1H & ^{13}C NMR: Tables 3.30 & 3.31.

F-6 [Rel-(1β,2α)-di-(2,4-dihydroxy-6-methoxy)benzoyl-rel-(3β,4α)-di-(4-methoxy)phenyl-cyclobutane (2',4'-dihydroxy-4,6'-dimethoxychalcone dimer)]

Pale amorphous solid. $[\alpha]_D^{23.5}$ +17.2° ($CHCl_3$, *c* 0.29) IR ν_{max} (film) cm^{-1}: 3409 (OH), 2935, 2836, 1623 (C=O), 1596, 1513, 1361, 1247, 1214, 1170, 1114, 1033, 829. UV λ_{max} (MeOH) nm: Table 3.35. Found $[M+H]^+$ 601.2074 ($C_{34}H_{32}O_{10}$ requires 600.1995) in FABMS. HREIMS *m/z* (rel. int. %): 300 (100), 299 (58), 240 (36), 167 (63), 161 (52), 140 (42), 134 (38), 121 (55). ^1H & ^{13}C NMR: Table 3.36.

F-7 [5,7,4'-Trimethoxyflavanone (naringenin trimethyl ether)]

Colourless prisms (*n*-hexane/EtOAc). Mp. 124° (Lit. mp. 123.5-124.5°, Kaufmann and Lam, 1967). $[\alpha]_D^{23.1}$ -4.3° ($CHCl_3$, *c* 0.234). IR ν_{max} (KBr disc) cm^{-1}: 3018, 2967, 2933, 2902, 2840, 1668 (C=O), 1610, 1573, 1517, 1457, 1261, 1213, 1159, 1108, 1068, 833, 815. UV λ_{max} (MeOH) nm: Table 3.37. Found $[M]^+$ 314.1160 ($C_{18}H_{18}O_5$ requires 314.1154) HREIMS *m/z* (rel. int. %): 314 (74), 313

(42), 286 (11), 207 (16), 180 (66), 152 (62), 137 (58), 134 (100), 121 (24), 91 (38), 65 (17). ^{1}H & ^{13}C NMR: Tables 3.38 & 3.39.

F-8 [7-Hydroxy-5,4'-dimethoxyflavanone (tsugafolin)]

Colourless prisms (EtOH). Mp. 203° (Lit. mp. 208-210°, Tanaka *et al.*, 1989). $[\alpha]_D^{19.9}$ -32.2° (Pyr., *c* 0.310) [Lit. $[\alpha]_D^{23}$ +7.0° (Pyr., *c* 0.46), Tanaka *et al.*, 1989]. IR ν_{max} (KBr disc) cm^{-1}: 3207 (OH), 3006, 2967, 2935, 2838, 1660 (C=O), 1602, 1583, 1519, 1290, 1261, 1199, 1162, 1108, 887, 840, 821. UV λ_{max} (MeOH) nm: Table 3.37. Found $[M]^+$ 300.0989 ($C_{17}H_{16}O_5$ requires 300.0997) HREIMS *m/z* (rel. int. %): 300 (52), 299 (28), 272 (4), 193 (4), 166 (25), 134 (100), 121 (26), 91 (12). ^{1}H & ^{13}C NMR: Tables 3.38 & 3.39.

F-9 [3',4',5,7-Tetrahydroxydihydroflavonol (dihydroquercetin)]

Yellow amorphous solid. $[\alpha]_D^{23.6}$ +12.7° (Acetone, *c* 0.393) [Lit. $[\alpha]_D^{24}$ +17.2° (acetone, *c* 1.04), Aft, 1961]. IR ν_{max} (KBr disc) cm^{-1}: 3432 (OH), 3087, 1629 (C=O), 1531, 1469, 1371, 1272, 1166, 1074. UV λ_{max} nm (MeOH): 290, 336 (sh); ($AlCl_3$/HCl): 311, 380; (NaOAc): 326; (NaOMe): 326. Found $[M]^+$ 304.0557 ($C_{15}H_{12}O_7$ requires 304.0583) HREIMS *m/z* (rel. int. %): 304 (44), 275 (65), 165 (25), 153 (100), 152 (28), 123 (44), 77 (10). ^{1}H NMR (400 MHz, acetone-d_6) (*J* in Hz): δ 4.98 (d, 11.5, H-2), 4.59 (d, 11.5, H-3), 5.96 (d, 2, H-6), 5.91 (d, 2, H-8), 7.05 (d, 1.8, H-2'), 6.83 (d, 8.1, H-5'), 6.89 (dd, 1.8, 8.1, H-6'), 4.75 (brs, 3-OH), 11.62 (s, 5-OH), 8.40 (brs, OH). ^{13}C (100 MHz, acetone-d_6): δ 84.6 (C-2), 73.2 (C-3), 198.2 (C-4), 164.8 (C-5), 97.0 (C-6), 167.8 (C-7), 96.1 (C-8), 164.2 (C-9), 101.6 (C-10), 129.9 (C-1'), 115.9 (C-2'), 145.7 (C-3'), 146.6 (C-4'), 115.8 (C-5'), 120.9 (C-6').

F-10 [(3',4',5,7-Tetrahydroxyflavonol (quercetin)]

Yellow amorphous solid. IR ν_{max} (film) cm^{-1}: 3208 (OH), 1655 (C=O), 1602, 1512, 1317,1164. UV λ_{max} nm Table 3.42. Found $[M]^+$ 302.0400 ($C_{15}H_{10}O_7$ requires 302.0426) HREIMS *m/z* (rel. int. %): 302 (100), 274 (12), 273 (12), 269 (8),

266 (8), 257 (7), 245 (11), 229 (8), 219 (10), 181 (22), 153 (14), 149 (14), 137 (17), 113 (12), 83 (8). ^{1}H & ^{13}C NMR: Tables 3.43 & 3.44.

F-11 [Kaempferol-3-O-rutinoside (nicotiflorin)]

Yellow amorphous solid. $[\alpha]_D^{23.5}$ +20.5° (MeOH, *c* 0.146) [Lit. $[\alpha]_D^{20}$ +4.2° (MeOH, *c* 0.67), Satake *et al.*, 1984]. IR ν_{max} (film) cm^{-1}: 3354 (OH), 2925, 1655 (C=O), 1605, 1504, 1359, 1210, 1181, 1062, 821. UV λ_{max} nm Table 3.42. $[M+H]^+$ not found ($C_{27}H_{30}O_{15}$ requires 594.1584) FABMS *m/z* (rel. int. %): 593 (32), 549 (55), 495 (45), 449 (38), 447 (35), 413 (64), 391 (47), 325 (65), 287 (100), 286 (63), 239 (63). ^{1}H & ^{13}C NMR: Tables 3.43 & 3.44.

F-12 [Myricetin-4'-O-methyl ether-3-α-L-rhamnoside (mearnsitrin)]

Pale amorphous solid. $[\alpha]_D^{23.2}$ -76.8° (MeOH, *c* 0.69). IR ν_{max} (KBr) cm^{-1}: 3427 (OH), 2964, 2935, 2850, 1654 (C=O), 1610, 1506, 1444, 1384, 1303, 1203, 1166, 1056, 1024. UV λ_{max} nm Table 3.42. Found $[M]^+$ 478.1146 ($C_{22}H_{22}O_{12}$ requires 478.1111) HREIMS *m/z* (rel. int. %): 478 (3), 332 (78), 317 (85), 297 (60), 218 (100), 142 (30), 91 (85). ^{1}H & ^{13}C NMR: Tables 3.43 & 3.44.

F-13 [Myricetin-3-O-methyl ether (annulatin)]

Yellow amorphous solid. IR ν_{max} (KBr) cm^{-1}: 3313 (OH), 3068, 2948, 2917, 2848, 1654 (C=O), 1623, 1602, 1511, 1378, 1315, 1205, 1164, 1027. UV λ_{max} nm Table 3.47. Found $[M]^+$ 332.0542 ($C_{16}H_{12}O_8$ requires 332.0532) HREIMS *m/z* (rel. int. %): 332 (100), 317 (97), 286 (37), 261 (13), 153 (12), 136 (13), 113 (8), 73 (8). ^{1}H & ^{13}C NMR: Table 3.48.

3.4 Alkaloids

3.4.1 Azaanthracene alkaloid

One azaanthracene alkaloid, coded **A-1** (CGP.12), was isolated from the petrol extract of *Cleistopholis glauca* (see Section 2.4.4) and identified as 1-aza-4-methylanthraquinone or cleistopholine (Figure 3.35).

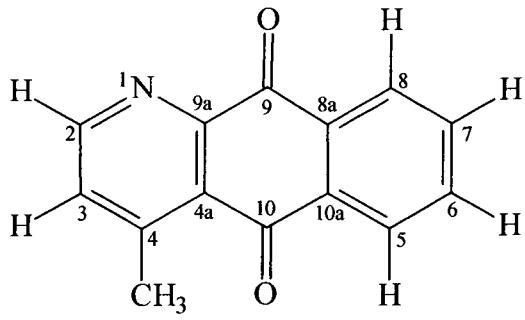


Figure 3.35: Structure of A-1

3.4.1.1 Characterisation of the isolated azaanthracene alkaloid A-1 as cleistopholine

TLC analysis revealed an intense quenching spot under short-wave UV light and an orange colour on spraying with Dragendorff's reagent, thus suggesting its possible alkaloidal structure. IR spectral data showed aromatic rings (3050, 1590, 1575 cm^{-1}) and two carbonyl functions (1685 and 1665 cm^{-1}) as expected for a quinone, while the UV spectrum showed maxima at 251, 271 (sh) and 326 nm characteristic of an anthraquinone chromophore (Williams and Fleming, 1995).

The odd-numbered molecular ion at *m/z* 223 on the HREIMS confirmed the alkaloidal structure with the molecular formula solving as $C_{14}H_9NO_2$. Major fragment ions observed at *m/z* 195 $[M\text{-}CO]^+$, *m/z* 167 $[M\text{-}2\times CO]^+$ and *m/z* 152 $[M\text{-}(2\times CO)\text{-}CH_3]^+$ confirmed the presence of two carbonyls and showed a methyl substitution. This was confirmed with a deshielded methyl resonance at δ 2.92 (s) in the ^1H NMR (Spectrum 3.41; Table 3.49). The latter further indicated the presence of an *ortho*-disubstituted aromatic ring with a multiplet at δ 7.83 (2H) and signals

(1H each) at δ 8.38 and 8.28 (ddd, J = 1.6, 2.1, 7.4 Hz) and a γ-substituted pyridine ring with signals at δ 8.91 and 7.50 (dd, J = 4.7 Hz) for an α,β pair of protons.

The structure of 1-aza-4-methylanthraquinone or cleistopholine was then suspected as a literature survey of anthraquinone alkaloids previously isolated in the Annonaceae was carried out and comparison of ^{1}H NMR assignments showed good agreement with a previous report (Tadic *et al.*, 1987). Comparison of ^{13}C NMR assignments (Spectrum 3.42; Table 3.49) also showed good agreement with the literature (Tadic *et al.*,1987). However, one quaternary carbon atom signal previously assigned as C-8a (δ 127.1) did not appear on the spectrum of **A-1**. Since only one quaternary carbon resonance (δ 134.1) remained unassigned on the latter, it was assumed to be the one for C-8a. Confirmation of this was obtained on the HMBC (Spectrum 3.43; Table 3.49) which revealed ^{3}J couplings between both H-5 (δ 8.38) and H-7 (δ 7.83) and the quaternary carbon at δ 134.1, thus identifying the C-8a resonance.

Consequently, **A-1** was identified as the known alkaloid 1-aza-4-methylanthraquinone or cleistopholine, previously isolated in the Annonaceae from *Cleistopholis patens* (Waterman and Muhammad, 1985), *Meiogyne virgata* (Tadic *et al.*, 1987), *Annona hayesii* (Rasamizafy *et al.*, 1987), *Oncodistigma monosperma* (Bou-abdallah *et al.*, 1989) and *Annona cherimolia* (Rios *et al.*, 1989). IR, UV and MS data of **A-1** showed good agreement with the literature (Waterman and Muhammad, 1985; Bracher, 1989) while the ^{13}C NMR assignments, previously uncomplete (Waterman and Muhammad, 1985) or misreported (Tadic *et al.*, 1987; Bracher, 1989), are reported here unambiguously for the first time.

Table 3.49: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of A-1

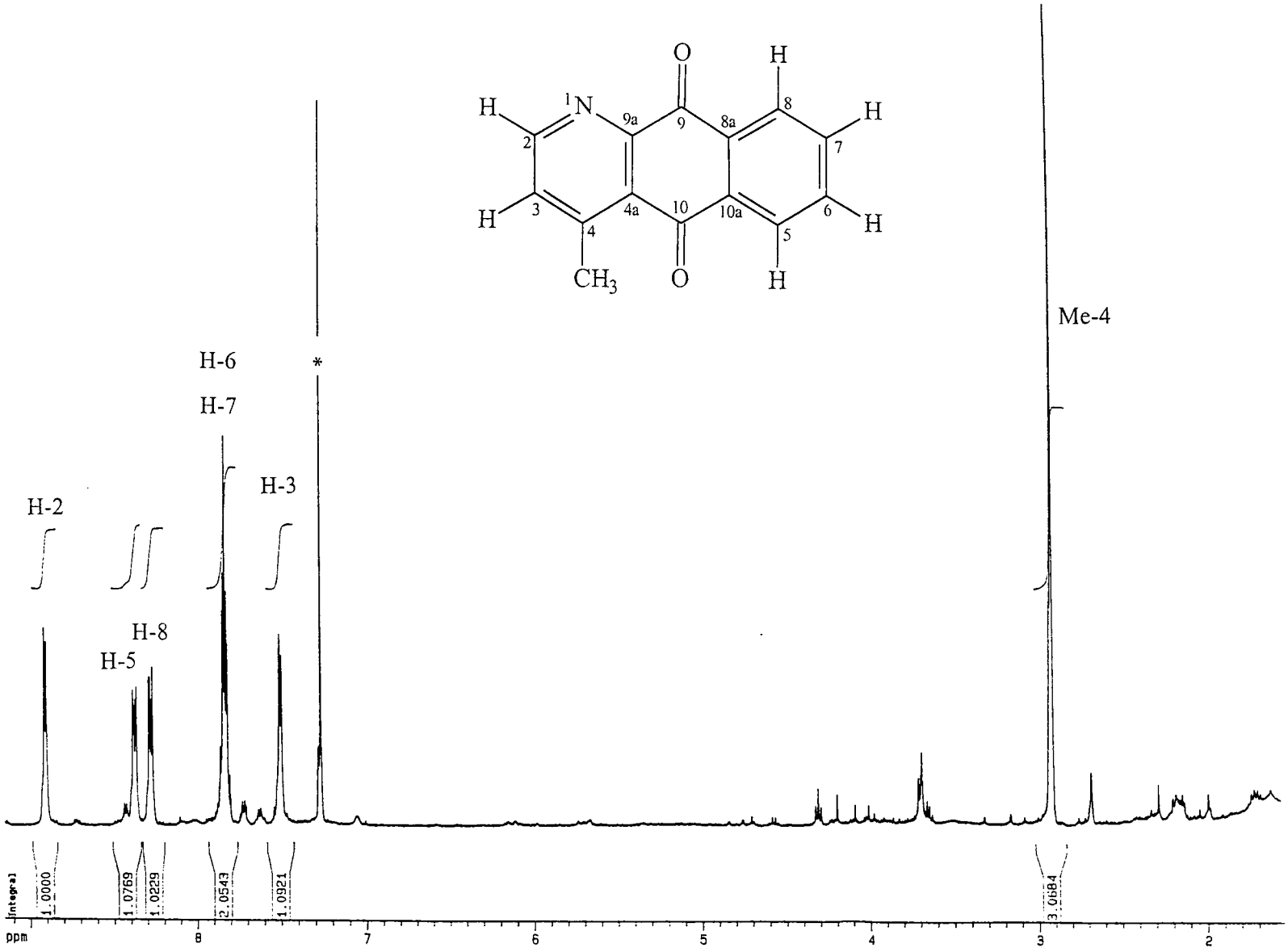
Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2	8.91 (d, 4.7)	153.6	131.4 (C-3)	150.4 (C-9a), 151.7 (C-4)
3	7.50 (d, 4.7)	131.4	153.6 (C-2)	23.1 (Me-4), 129.4 (C-4a)
4	-	151.7		
4a	-	129.4		
5	8.38 (ddd, 1.6, 2.1, 7.4)	127.6		134.1 (C-8a), 134.8 (C-7), 182.1 (C-10)
6	7.83 (m)	134.4		127.4 (C-8), 132.8 (C-10a)
7	7.83 (m)	134.8		127.6 (C-5), 134.1 (C-8a)
8	8.28 (ddd, 1.6, 2.1, 7.4)	127.4		132.8 (C-10a), 134.4 (C-6), 185.0 (C-9)
8a	-	134.1		
9	-	185.0		
9a	-	150.4		
10	-	182.1		
10a	-	132.8		
Me-4	2.92 (s)	23.1	151.7 (C-4)	129.4 (C-4a), 131.4 (C-3)

All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.

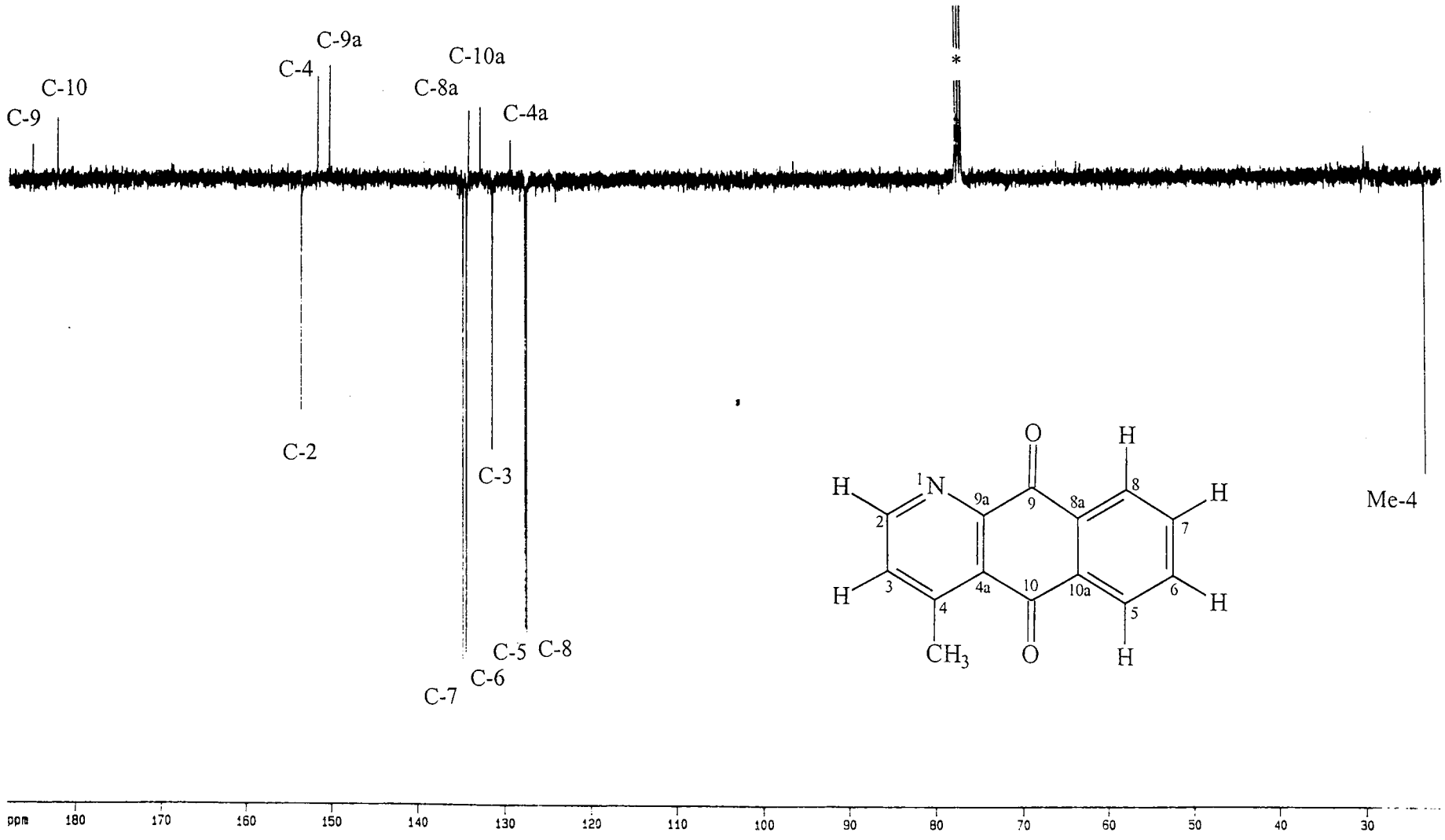
3.4.1.2 Properties and spectral data of the isolated azaanthracene alkaloid

A-1 (Cleistopholine)

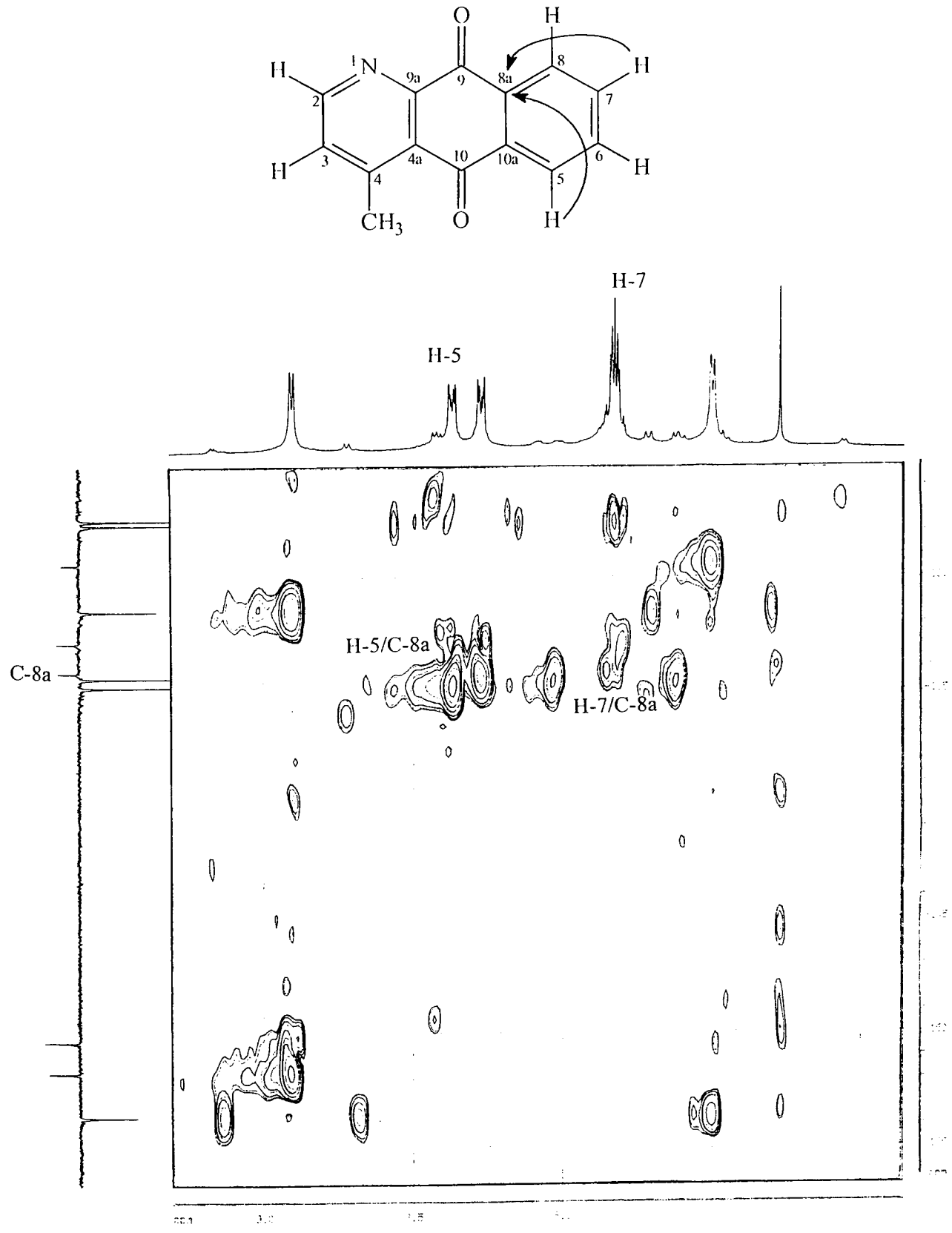
Pale yellow amorphous solid. IR ν_{max} (KBR) cm^{-1}: 3050, 2965, 2926, 2850, 1685 (C=O), 1665 (C=O), 1590, 1575, 1298, 975, 717. UV λ_{max} (EtOH) nm: 251, 271 (sh), 326. Found $[M]^{+}$ 223.0611 ($C_{14}H_9NO_2$ requires 223.0633) HREIMS *m/z* (rel. int. %): 223 (50), 195 (100), 167 (98), 152 (16), 139 (100), 76 (100). ^{1}H & ^{13}C: NMR: Table 3.49.



Spectrum 3.41: ^{1}H NMR (400 MHz, $CDCl_3$*) of A-1



Spectrum 3.42: *J*-modulated ^{13}C NMR (100 MHz, $CDCl_3$*) of A-1



Spectrum 3.43: Significant HMBC correlations (400 MHz, $CDCl_3$) of A-1

3.5 Miscellaneous compounds

Five miscellaneous compounds were isolated from the two species of *Cleistopholis* and from *Goniothalamus gardneri* (see Section 2.4.4). They included two simple aromatic compounds, coded **M-1** (CGM-4) and **M-2** (CGE-1), one neolignan coded **M-3** (CGM.5), one organic acid, coded **M-4** (CPM.1), and one acetogenin, coded **M-5** (GGP.4), identified respectively as 5-hydroxymethyl-2-furaldehyde, 4-hydroxy-3-methoxy-(*trans*)-cinnamylaldehyde (feruladehyde), *rel*-(2α,3β)-7-*O*-methyl-cedrusin, shikimic acid and *rel*-5α-hydroxymethyl-3β-eicosa-19'-en-11'-yn-tetra-hydrofuran-2-one (goniothalamusin).

3.5.1 Characterisation of isolated miscellaneous compounds

3.5.1.1 Identification of M-1 as 5-hydroxymethyl-2-furaldehyde

This compound was isolated from the methanol extract of *Cleistopholis glauca* (Figure 3.36).

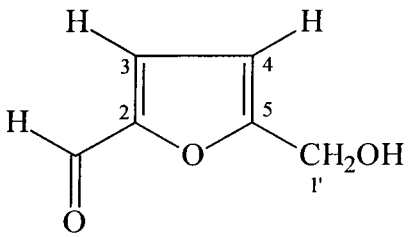


Figure 3.36: Structure of M-1

TLC analysis revealed an intense quenching spot under short-wave UV light. The IR spectrum suggested the presence of OH (3388 cm^{-1}) and α,β-unsaturated carbonyl (1674 cm^{-1}) groups (Williams and Fleming, 1995). The HREIMS established the molecular formula $C_6H_6O_3$ (4 DBE). The mass fragmentation afforded a peak at *m/z* 97 $[M\text{-}CHO]^+$, suggesting the presence of an aldehydic group. This was confirmed in the ^1H NMR with a strongly deshielded signal at δ 9.60 (s). A pair of olefinic doublets at δ 7.22 and 6.52 (1H each, J = 3.4 Hz) established the presence of a 2,5-disubstituted furan ring. A singlet at

δ 4.72 (2H) and a broad singlet at δ 2.20 (OH) revealed the presence of an allylic hydroxymethylene.

Comparison of the physical and spectral data of **M-1** showed good agreement with those previously reported for the known 5-hydroxymethyl-2-furaldehyde, which had previously been isolated from this species (Etse *et al.*, 1988) and from *Bosistoa transversa* (Rutaceae) (Auzi, 1996).

3.5.1.2 Identification of M-2 as feruladehyde

This compound was isolated from the ethyl acetate extract of *Cleistopholis glauca* (Figure 3.37).

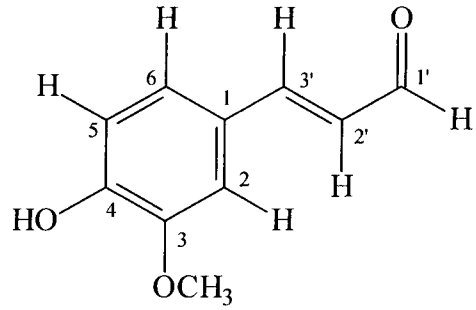


Figure 3.37: Structure of M-2

TLC analysis revealed an intense quenching spot under short-wave UV light. IR spectral data suggested the presence of OH (3371 cm^{-1}), arylmethoxyl groups (2830 cm^{-1}), aromatic rings (1599, 1586 and 1512 cm^{-1}) and α,β-unsaturated carbonyl function (1664 cm^{-1}).

A bathochromic shift observed on addition of NaOH in the UV spectrum established the presence of a phenolic chromophore (Williams and Fleming, 1995). The HREIMS yielded a molecular ion at *m/z* 178 revealing the molecular formula $C_{10}H_{10}O_3$ (6 DBE). The mass fragmentation afforded fragment ions at *m/z* 161 $[M\text{-}OH]^+$ and 147 $[M\text{-}OCH_3]^+$, accounting for the presence of phenolic and arylmethoxyl groups.

The ^1H NMR exhibited a strongly deshielded signal at δ 9.66 (d, J = 7.7 Hz), which was assigned to an aldehydic proton. The latter, coupling to an olefinic proton at δ 6.60 (dd, J = 7.7, 15.8 Hz) which in turn coupled to a further olefinic proton at δ 7.41 (d, J = 15.8 Hz), establishing the presence of a *trans*-α,β-unsaturated aldehydic chain. Signals for an ABD spin system of protons at δ 7.13 (dd, J = 1.9, 8.2 Hz), 7.07 (d, J = 1.9 Hz) and 6.97 (d, J = 8.2 Hz) indicated an aromatic ring which was tri-substituted in position C-1, C-3 and C-4. Furthermore, the presence of an hydroxyl and an arylmethoxyl group was confirmed with signals respectively at δ 6.0 and 3.96.

The placement of the α,β-unsaturated aldehydic chain, the hydroxyl and arylmethoxyl groups respectively in positions C-1, C-4 and C-3 was rationalised after comparison of the physical and spectral data of **M-2** with those reported for the known 4-hydroxy-3-methoxy-(*trans*)-cinnamylaldehyde or feruladehyde, which had previously been isolated from this species (Etse *et al.*, 1988) and from *Bosistoa transversa* (Rutaceae) (Auzi, 1996).

3.5.1.3 Identification of M-3 as rel-(2α,3β)-7-O-methylcedrusin

This compound was isolated from the methanol extract of *Cleistopholis glauca* (Figure 3.38).

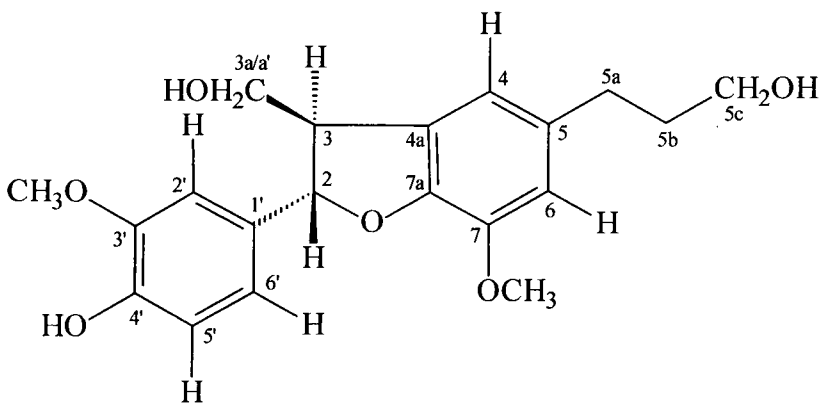


Figure 3.38: Structure of M-3

TLC analysis revealed an intense quenching spot under short-wave UV light. The IR spectrum showed hydroxyl groups (3378 cm^{-1}) and aromatic rings (1604, 1515, 1500 cm^{-1}). A bathochromic shift observed on addition of NaOH in the UV spectrum suggested the presence of a phenolic chromophore (Williams and Fleming, 1995).

The HREIMS displayed a molecular ion at *m/z* 360, corresponding to the molecular formula $C_{20}H_{24}O_6$ (9 DBE). The mass fragmentation pattern revealed peaks at *m/z* 342 $[M\text{-}H_2O]^+$ and at 330 $[M\text{-}CH_2O]^+$, 327 $[M\text{-}H_2O\text{-}CH_3]^+$, 316 $[M\text{-}CH_2O\text{-}CH_2]^+$ suggesting the presence of hydroxymethyl and arylmethoxyl groups.

The ^1H NMR (Spectrum 3.44; Table 3.50) displayed signals for protons of an ABD spin system at δ 6.95 (1H, d, J = 1.8 Hz), 6.82 (1H, dd, J = 1.8, 8.2 Hz) and 6.76 (1H, d, J = 8.1 Hz) and one singlet at δ 6.72 (2H), respectively indicating a 1',3',4'-tri-substituted aromatic A-ring and a tetra-substituted B-ring with two equivalent aromatic protons. The spectrum also showed a deshielded doublet for an oxymethine at δ 5.49 (1H, J = 6.3 Hz) coupling to a proton at δ 3.47 (dt, J = 6.2, 6.3 Hz), a multiplet for oxymethylene protons at δ 3.83/3.76 and two arylmethoxyls (δ 3.85 and 3.81). Furthermore, the presence of an *n*-propanol substitution on one aromatic ring was established with a series of coupling methylene resonances at δ 3.58 (t, J = 6.5 Hz, CH_2OH), 1.81 (tt, J = 6.5, 7.7 Hz, CH_2-CH_2OH) and 2.62 (t, J = 7.7 Hz, Ar-CH_2-CH_2-CH_2OH).

The *J*-modulated ^{13}C NMR (Spectrum 3.45; Table 3.50) revealed 20 peaks, which were made up of 7 quaternary carbons of which four were oxygenated aromatics (δ 149.2-145.4), five aromatic and two aliphatic methines at δ 89.1 (oxygenated) and 55.6, four methylenes, of which two were oxygenated (δ 65.1 and 62.4) and two arylmethoxyls (δ 56.9 and 56.5). ^1H-^{13}C correlations were examined by an HC-COBI experiment and allowed assignments to be established for the A-ring methines in C-6', 5' and 2' respectively at δ 119.9, 116.3 and 110.7, and for the B-

ring protons at δ 118.1 and 114.3. The methines at δ 89.1 and 55.6 were respectively assigned to the deshielded doublet at δ 5.49 and the proton at δ 3.47. The oxymethylenes at δ 65.1 and 62.4 were respectively assigned to the protons at δ 3.83/3.76 and 3.58 (Ar-CH_2-CH_2-CH_2OH). The remaining aliphatic methylenes were observed at δ 35.9 (CH_2-CH_2OH) and 33.0 (CH_2-CH_2-CH_2OH). The arylmethoxyls chemical shifts required them to be attached to at least one free *ortho*-position (Panichpol and Waterman, 1978). A COSY experiment revealed a coupling between the methine at δ 3.47 and the non equivalent oxymethylene protons at δ 3.83/3.76.

The structure of 2-(4'-hydroxy-3'-methoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7-methoxybenzofuran-5-propan-1-ol was rationalised from HMBC (Spectrum 3.46; Table 3.50) and NOESY experiments (Figure 3.39). The HMBC showed:

i) ^3J couplings between H-2, H-3, H-4 and H-6 and the oxygenated C-7a at δ 147.7 and a ^2J coupling between H-3 and C-4a at δ 130.1, supporting the presence of the 2,3-dihydrobenzofuran ring

ii) ^3J couplings between the oxymethylene H-3a/a' (δ 3.83/3.76) and C-2 at δ 89.1 and C-4a at 130.1, thus indicating the oxymethylene substitution in C-3.

iii) ^3J couplings between H-2 and C-2' (110.7) and C-6'(199.9), thus establishing the A-ring substitution at C-2.

iv) ^3J couplings between the methylene at δ 2.62 and the methines at δ 118.1 and 114.3 (C-4 and C-6), thus establishing the substitution of the *n*-propanol side chain in C-5 of the B-ring

v) ^3J couplings between the methoxyls at δ 3.85 and 3.81 and the oxygenated carbons respectively at δ 145.4 and 149.2.

The unambiguous placement of these methoxyls was readily achieved through a NOESY experiment which revealed cross-peaks between δ 3.85 and H-6 (7-OMe substitution) and between δ 3.81 and H-2' (3'-OMe substitution). Consequently, the remaining oxygenated position at δ 147.6 was attributed to a

phenolic OH substituent in C-4'. The C-4 and C-6 resonances were assigned on the basis of their different chemical shift relatively influenced by the 7-OMe (Wenkert *et al.*, 1976) and by comparison with assignments previously reported for a glycoside derivative of **M-3** (Kouno *et al.*, 1993).

The relative stereochemistry at C-2, C-3 was established from the NOESY experiment, which showed a correlation between H-3 (δ 3.47) and H-2' (δ 6.95), suggesting the relative configuration C-2α,3β. This was further supported by the ^{1}H chemical shifts and the *J* value (6.3 Hz) of H-2 and H-3 (Agrawal *et al.*, 1983).

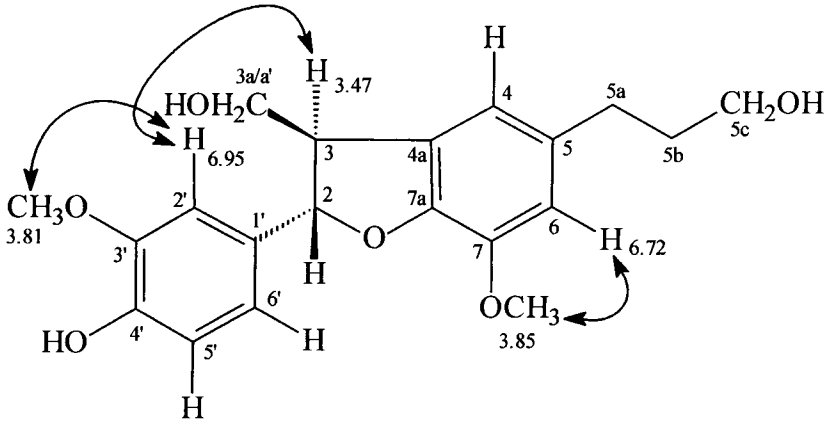


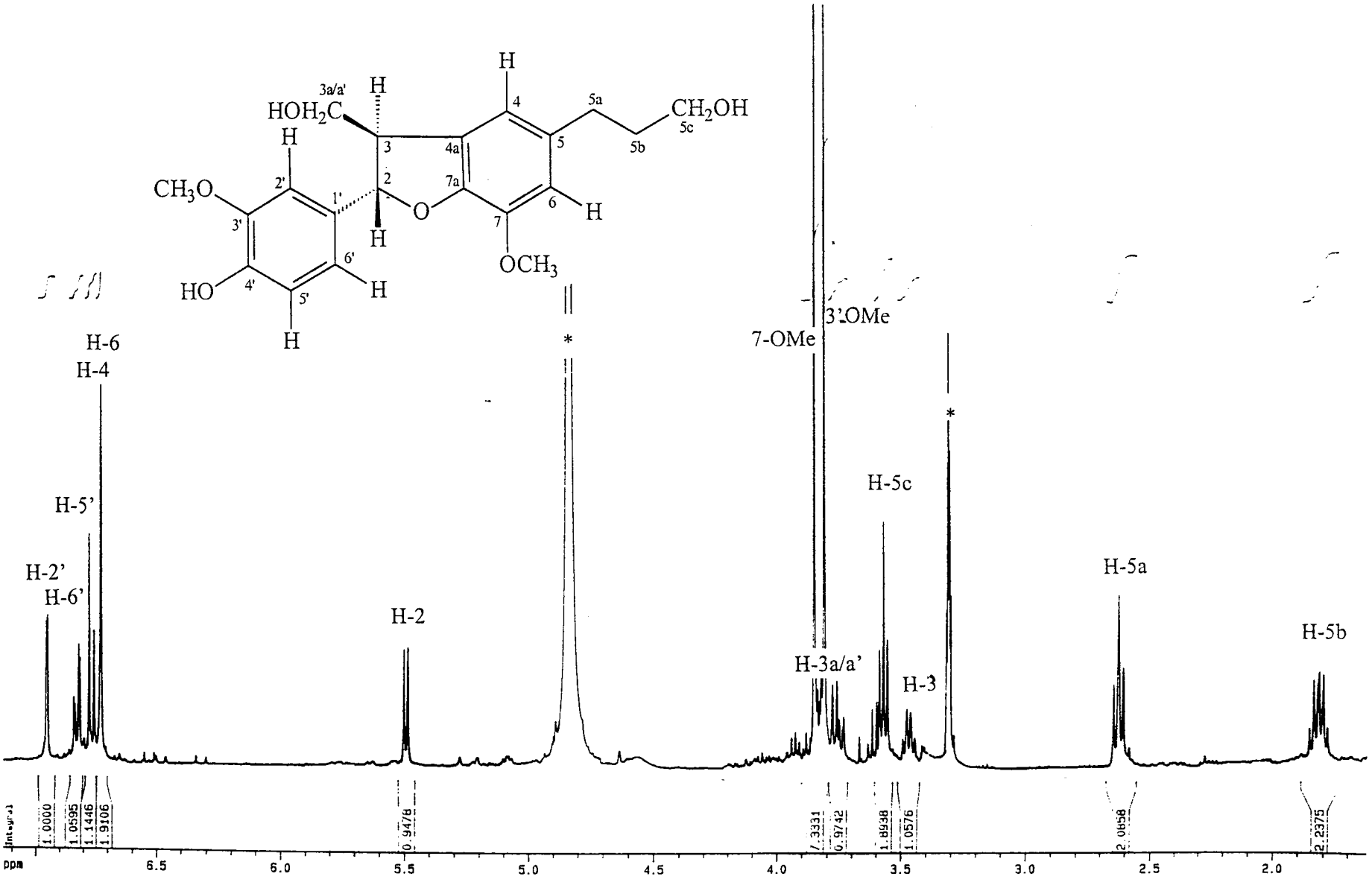
Figure 3.39: Significant NOE interactions of M-3

On the basis of physical and spectral data and by comparison with the literature, **M-3** was identified as the known dihydrobenzofuran neolignan *rel*-(2α,3β)-7-*O*-methylcedrusin or dihydrodehydrodiconiferyl alcohol, previoulsy isolated from *Cedrus deodara* (Pinaceae) (Agrawal *et al.*, 1980), *Eucommia ulmoides* (Eucommiaceae) (Deyama *et al.*, 1987) and *Licaria chrysophylla* (Lauraceae) (Da Silva *et al.*, 1989). $[\alpha]_D$, IR, UV and MS showed good agreement with the literature (Deyama *et al.*, 1987; Antus *et al.*, 1990). However, this is the first report of the complete ^{1}H, ^{13}C NMR assignments and relative stereochemistry of **M-3**, unambiguously established by means of HMBC and NOESY experiments.

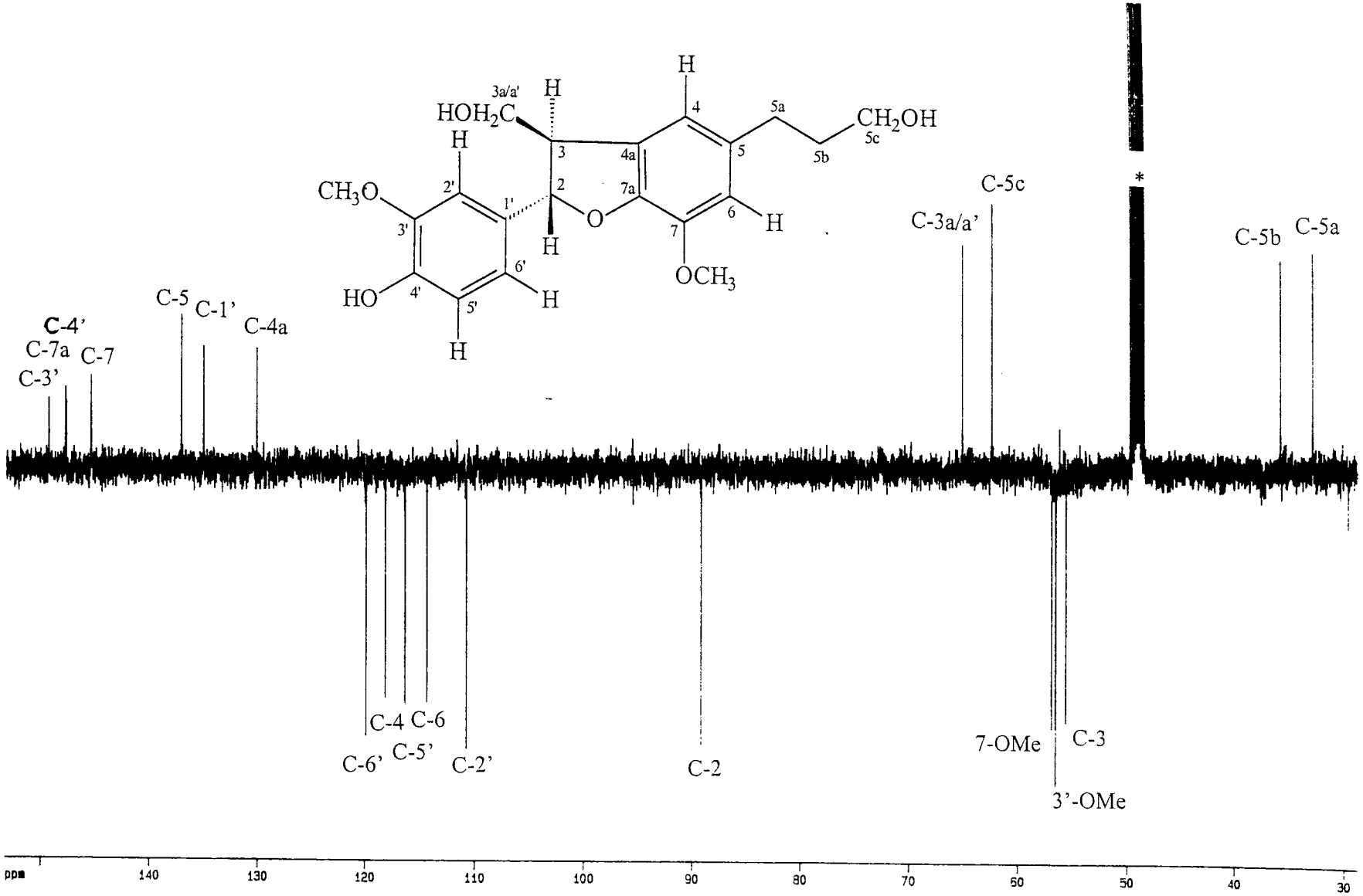
Table 3.50: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of M-3

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2	5.49 (d, 6.3)	89.1	55.6 (C-3), 135.0 (C-1')	65.1 (C-3a/a'), 110.7 (C-2'),119.9 (C-6'), 147.7 (C-7a)
3	3.47 (dt, 6.2, 6.3)	55.6	65.1 (C-3a/a'), 130.1 (C-4a)	135.0 (C-1'), 147.7 (C-7a)
3a/a'	3.83/3.76 (m)	65.1		89.1 (C-2), 130.1 (C-4a)
4	6.72 (s)	118.1		33.0 (C-5a), 55.6 (C-3), 114.3 (C-6), 147.7 (C-7a)
4a	-	130.1		
5	-	137.1		
5a	2.62 (t, 7.7)	33.0	35.9 (C-5b), 137.1 (C-5)	62.4 (C-5c), 114.3 (C-6), 118.1 (C-4)
5b	1.81 (tt, 6.5, 7.7)	35.9	33.0 (C-5a), 62.4 (C-5c)	137.1 (C-5)
5c	3.58 (t, 6.5)	62.4	35.9 (C-5b)	33.0 (C-5a)
6	6.72 (s)	114.3	145.4 (C-7)	33.0 (C-5a), 118.1 (C-4), 147.7 (C-7a)
7	-	145.4		
7a	-	147.7		
1'	-	135.0		
2'	6.95 (d, 1.8)	110.7	135.0 (C-1'), 149.2 (C-3')	89.1 (C-2), 119.9 (C-6'), 147.6 (C-4')
3'	-	149.2		
4'	-	147.6		
5'	6.76 (d, 8.1)	116.3	147.6 (C-4')	135.0 (C-1'), 149.2 (C-3')
6'	6.82 (dd, 1.8, 8.2)	119.9		89.1 (C-2), 110.7 (C-2'), 147.6 (C-4')
7-OMe	3.85 (s)	56.9		145.4 (C-7)
3'-OMe	3.81 (s)	56.5		149.2 (C-3')

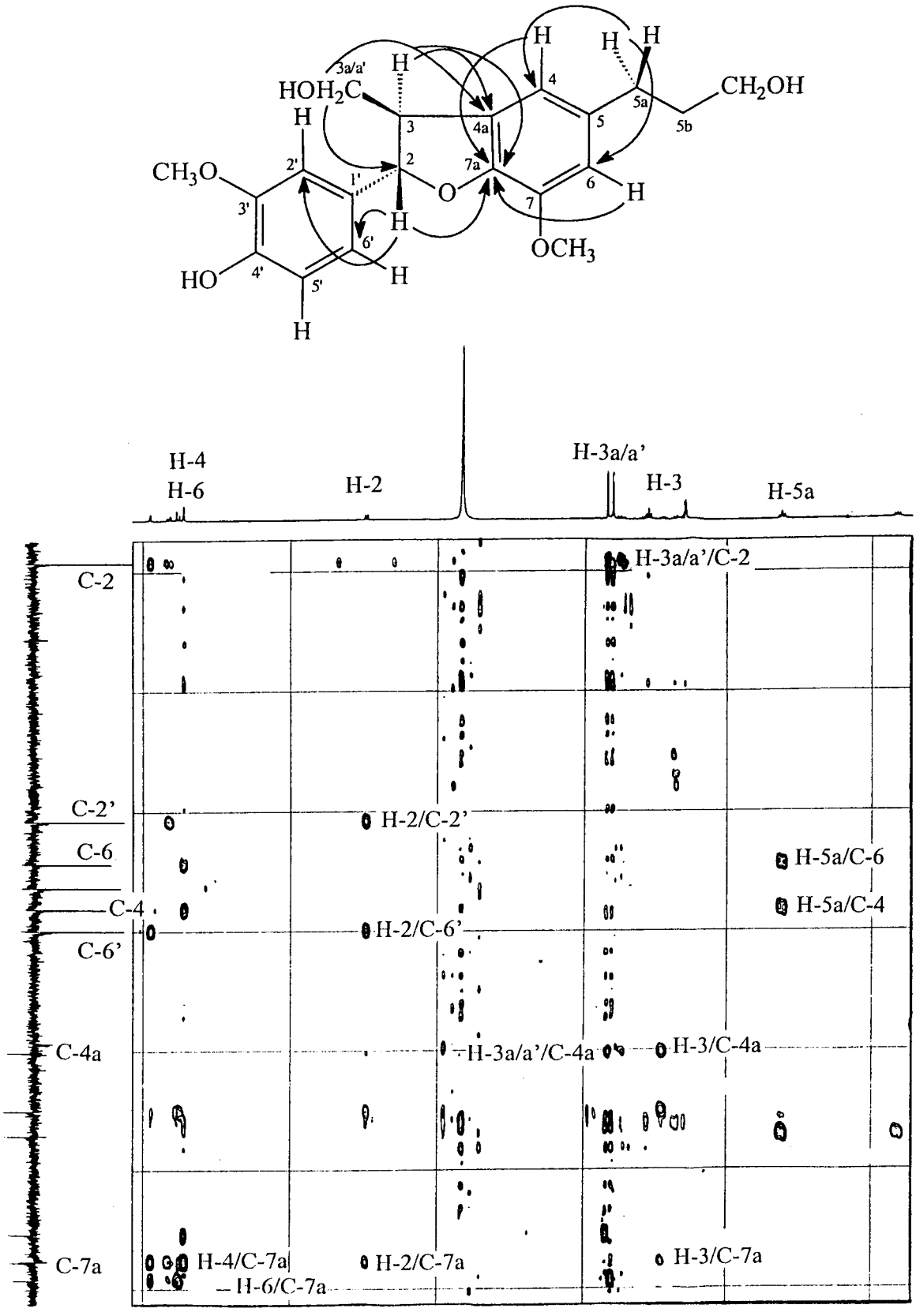
All data obtained in CD_3OD.
In parentheses coupling constant J are in Hz.



Spectrum 3.44: ^{1}H NMR (400 MHz, CD_3OD*) of M-3



Spectrum 3.45: *J*-modulated ^{13}C NMR (100 MHz, CD_3OD*) of M-3



Spectrum 3.46: Significant HMBC correlations (400 MHz, CD_3OD) of M-3

3.5.1.4 Identification of M-4 as shikimic acid

This compound was isolated from the methanol extract of *Cleistopholis patens* (Figure 3.40).

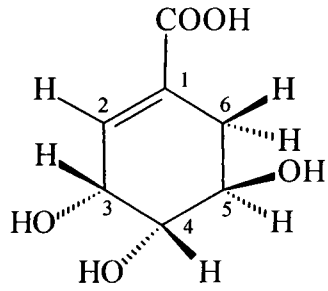


Figure 3.40: Structure of M-4

TLC analysis revealed a weakly quenching spot under short-wave UV light. The IR spectrum exhibited absorptions characteristic of hydroxyl (3434 cm^{-1}) and carbonyl groups (1643 cm^{-1}). The FABMS failed to establish a quasi-molecular ion.

The ^{1}H NMR (Spectrum 3.47; Table 3.51) comprised an olefinic proton at δ 6.49 (dd, J = 2.2, 4.3 Hz), three oxymethines at δ 4.43 (t, J = 4.3 Hz), 4.02 (ddd, J = 5.5, 7.7, 8.9 Hz) and 3.74 (dd, J = 4.3, 8.9 Hz) and a non equivalent methylene at δ 2.80 (dd, J = 5.5, 17.8 Hz) and 2.23 (ddd, J = 2.2, 7.7, 17.8 Hz). Examination of the major coupling constants showed that the olefinic proton coupled to the methine proton at δ 4.43 (J = 4.3 Hz), which in turn coupled to that at δ 3.74 (J = 4.3 Hz). The latter coupled to the third oxymethine at δ 4.02 (J = 8.9 Hz), which coupled to both protons at δ 2.80 (J = 5.5 Hz) and 2.23 (J = 7.7 Hz), showing geminal coupling to each other (J = 17.8 Hz). The spectrum further revealed a long-range coupling between the olefinic proton and the signal at δ 2.23 (J = 2.2 Hz).

The J-modulated ^{13}C NMR (Spectrum 3.48; Table 3.51), assigned by ^{1}H-^{13}C HC-COBI, revealed 7 carbons. They were one carboxylic acid carbonyl (δ 176.0), one quaternary sp^2 carbon, four methines with the olefinic methine at δ 131.9 and the three oxymethines at δ 73.2, 67.9, 67.4, a methylene resonance (δ 33.7) for protons at δ 2.80 and 2.23.

The structure of 3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid or shikimic acid was confirmed by the HMBC experiment (Spectrum 3.49; Table 3.51), which showed ^{3}J coupling between the H-6 methylene and the olefinic C-2 at δ 131.9 as well as ^{3}J coupling between H-6_{eq} and the carbonyl at δ 176.0, thus respectively establishing the cyclohexenic structure and the carboxylic acid substitution in C-1. Fragment ions in FABMS at *m/z* 156 and 138 showed agreement with published data, accounting for successive loss of H_2O and $2\times H_2O$, thereby requiring the molecular ion for *m/z* 174 and the molecular formula $C_7H_{10}O_5$ (3 DBE).

Examination of the ^{1}H NMR coupling constants values $J_{3,4}$ (4.3 Hz), $J_{4,5}$ (8.9 Hz) and $J_{5,6\beta}$ (7.7 Hz) indicated the relative configuration H-3β (quasi-equatorial), H-4β, H-5α (*trans*-diaxial) and H-6β (quasi-axial), as in the most favored half-chair conformation of shikimic acid (Hall, 1964; Talapatra *et al.*, 1989) (Figure 3.41).

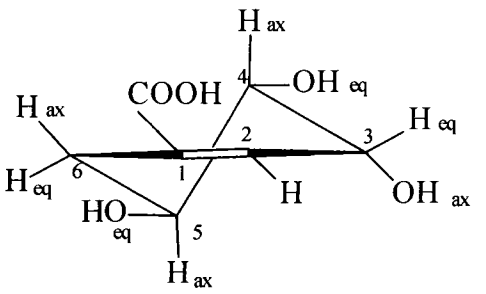


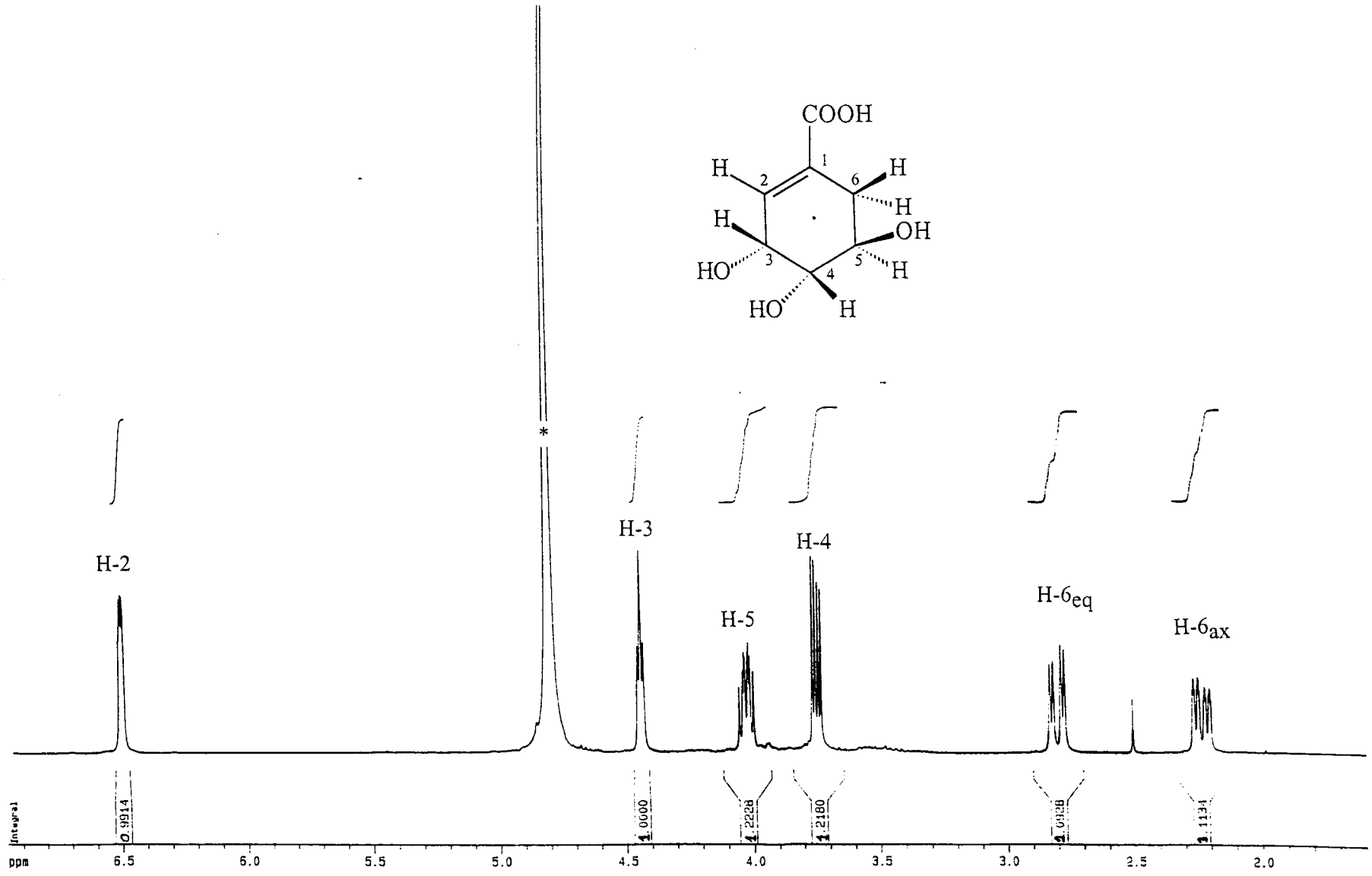
Figure 3.41: Relative stereochemistry of M-4

Consequently, **M-4** was identified as shikimic acid, previously reported from *Dendrobium fuscescens* (Orchidaceae) (Talapatra *et al.*, 1989) and from *Piptostigma fugax* (Annonaceae) (Achenbach and Schwinn, 1995). $[\alpha]_D$, IR and MS data were in good agreement with the literature (Talapatra *et al.*, 1989; Achenbach and Schwinn, 1995). This is the first report of the unambiguous ^{1}H and ^{13}C NMR assignments, previously found uncomplete or misreported (Hall, 1964; Snyder and Rapoport, 1973; Talapatra *et al.*, 1989).

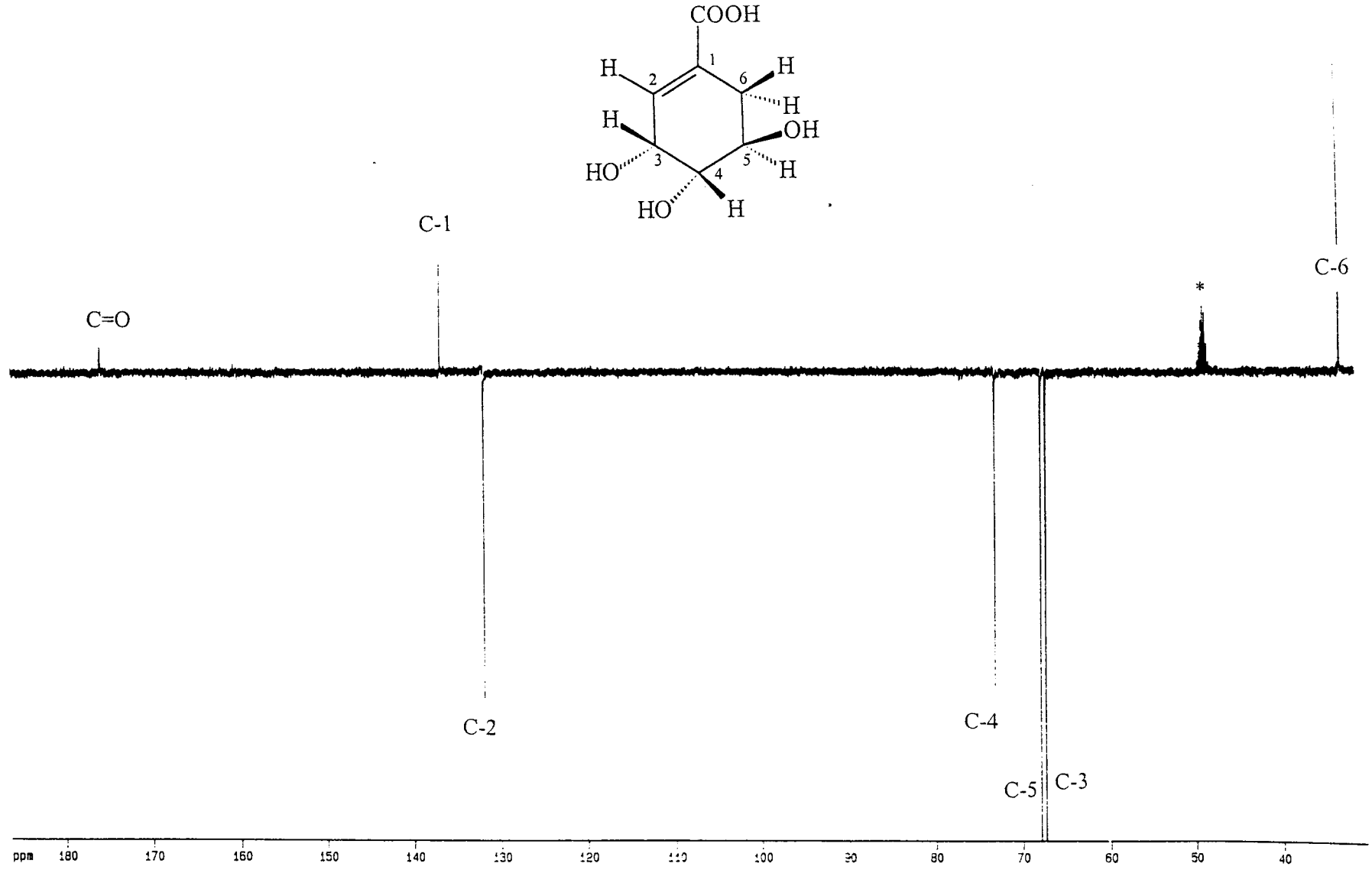
Table 3.51: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of M-4

Position	$^{\delta}H$	$^{\delta}C$ [a]	^{2}J	^{3}J
1	-	136.8		
2	6.49 (dd, 2.2, 4.3)	131.9		33.7 (C-6), 73.2 (C-4), 176.0 (C=O)
3	4.43 (t, 4.3)	67.4	73.2 (C-4), 131.9 (C-2)	67.9 (C-5), 136.8 (C-1)
4	3.74 (dd, 4.3, 8.9)	73.2	67.4 (C-3), 67.9 (C-5)	33.7 (C-6), 131.9 (C-2)
5	4.02 (ddd, 5.5, 7.7, 8.9)	67.9	73.2 (C-4)	67.4 (C-3), 136.8 (C-1)
6_{eq}	2.80 (dd, 5.5, 17.8)	33.7	67.9 (C-5), 136.8 (C-1)	73.2 (C-4), 131.9 (C-2), 176.0 (C=O)
6_{ax}	2.23 (ddd, 2.2, 7.7, 17.8)	33.7	67.9 (C-5), 136.8 (C-1)	131.9 (C-2)
C=O	-	176.0		

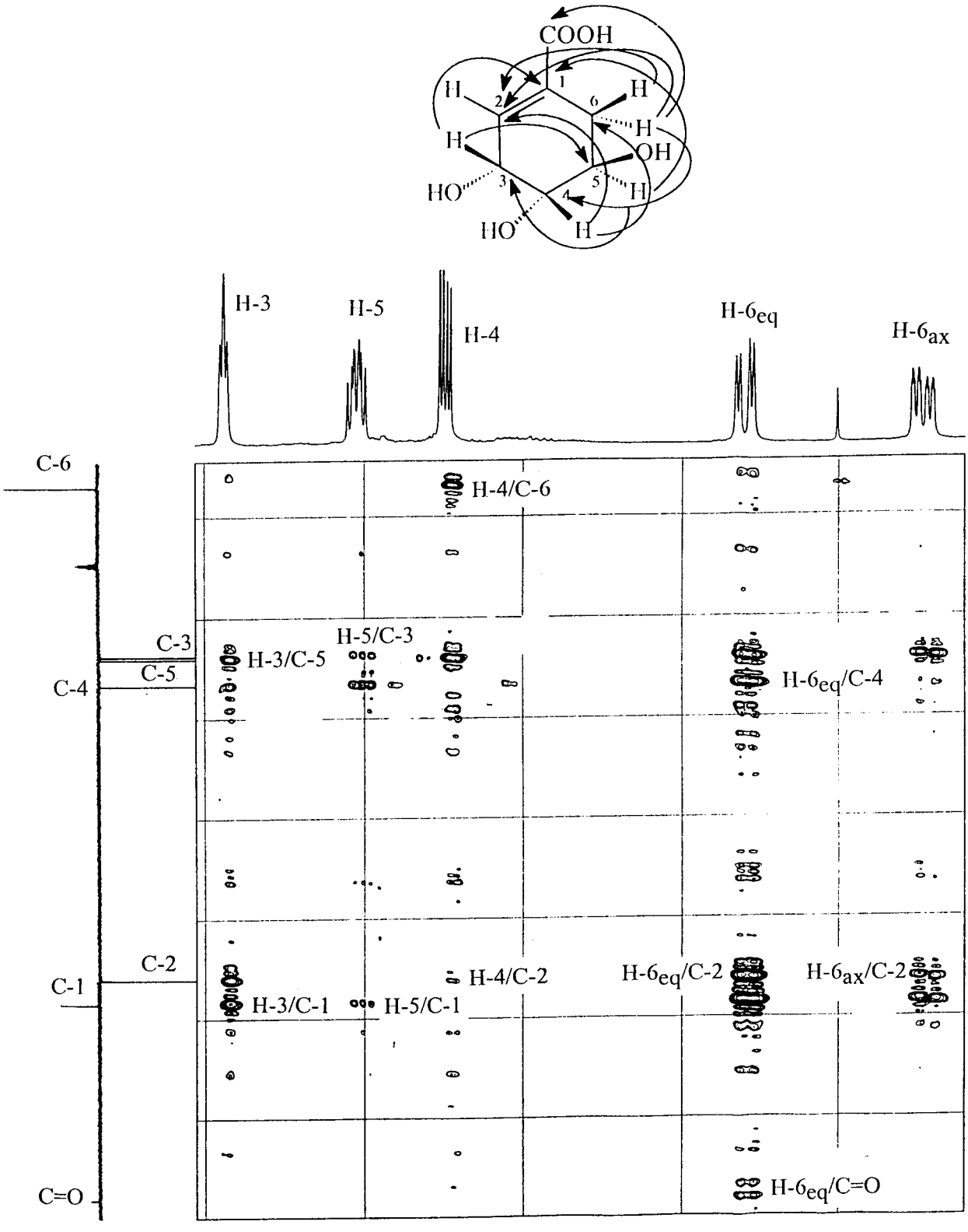
All data obtained in D_2O, a = Spectrum run in D_2O-CD_3OD.
In parentheses coupling constant *J* are in Hz.



Spectrum 3.47: ^{1}H NMR (400 MHz, D_2O*) of M-4



Spectrum 3.48: *J*-modulated ^{13}C NMR (100 MHz, D_2O-CD_3OD*) of M-4



Spectrum 3.49: Significant HMBC correlations (400 MHz, D_2O) of M-4

3.5.1.5 Identification of M-5 as goniothalamusin

This compound was isolated from the petrol extract of *Goniothalamus gardneri* (Figure 3.42).

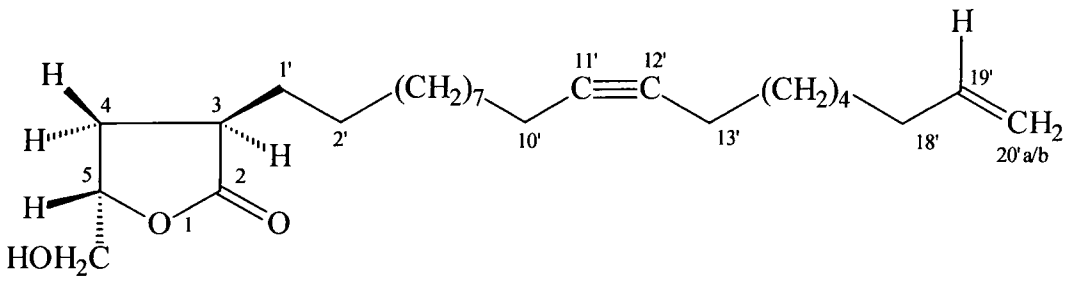


Figure 3.42: Structure of M-5

TLC analysis revealed a weakly quenching spot under short-wave UV light. The IR spectrum indicated the presence of OH (3459 cm^{-1}), $CH{=}CH_2$ groups (3077, 1643, 948 cm^{-1}) and a lactone carbonyl function (1754 cm^{-1}) (Williams and Fleming, 1995). The HREIMS yielded a molecular ion at *m/z* 390 corresponding to the molecular formula $C_{25}H_{42}O_3$ (5 DBE).

The ^1H NMR (Spectrum 3.50; Table 3.52) exhibited an olefinic methine at δ 5.79 (ddt, J = 6.6, 10, 17 Hz) adjacent to two protons at δ 4.98 (dd, J = 2.1, 17 Hz) and 4.92 (dd, J = 2.1, 10 Hz) characteristic for a vinylic group. It also showed an oxymethine at δ 4.58, two non equivalent oxymethylene protons at δ 3.86 (dd, J = 2.9, 12.3 Hz) and 3.63 (dd, J = 4.8, 12.3 Hz), a methine next to a carbonyl (δ 2.70), a broad singlet (δ 2.5) attributed to one OH group, one methylene adjacent to the vinyl group at δ 2.0 (2H, t, J = 6.6 Hz), two methylenes adjacent to another centre of unsaturation at δ 2.13 (4H, t, J = 6.2 Hz) and a series of signals at δ 2.30 (1H), 1.98 (1H), 1.83 (1H), 1.45-1.26 (25H).

The J-modulated ^{13}C NMR (Spectrum 3.51; Table 3.52), assigned by ^1H-^{13}C HC-COBI, revealed 25 carbons. It confirmed the presence of the carbonyl function (δ 180.3), olefinic methine (δ 139.1) and methylene (δ 114.3) of the vinylic group, the oxymethine (δ 78.9), the oxymethylene (δ 64.4), the methine next to the carbonyl (δ 39.7) and the methylene adjacent to the vinylic group (δ 33.8). It further

showed two equivalent quaternary carbons at δ 80.3 and 16 methylenes of which two at δ 18.8. These data were in agreement with the presence of one acetylenic bond symmetrically substituted by two methylenes (Morris, 1983).

The structure of *rel*-5α-hydroxymethyl-3β-eicosa-19'-en-11'-yn-tetrahydro-furan-2-one was established by a combination of COSY, TOCSY, HMBC and HREIMS experiments. The COSY displayed couplings between both the oxymethine H-5 (δ 4.58), the methine H-3 next to the carbonyl (δ 2.70) and the non equivalent methylene at δ 2.30/1.98. The presence of a tetrahydrofuran-2-one ring was rationalised from the number of double bond equivalents obtained in HREIMS. The TOCSY revealed correlations between H-5 and the oxymethylene at δ 3.86/3.63, thus indicating a 5-hydroxymethyl substituent, as well as correlations between H-3 and the methylene at δ 1.83/1.45, which in turn showed couplings to aliphatic methylenes at δ 1.46-1.26, thus suggesting the presence of the alkyl side chain substitution in C-3.

The HMBC (Figure 3.43; Table 3.52) displayed:

i) ^{3}J couplings between the methylene at δ 2.30/1.98 and the oxymethylene carbon (δ 64.4), the carbonyl group (δ 180.3) and the methylene at δ 31.3, thus confirming the presence of the tetrahydrofuran-2-one ring with its alkyl side chain and hydroxymethyl substituents respectively in C-3 and C-5.

ii) ^{2}J and ^{3}J couplings between the two methylenes at δ 2.13 and the quaternary carbons at δ 80.3, thus confirming the presence of the acetylenic bond with α-positions substituted by two methylenes.

iii) ^{2}J and ^{3}J couplings between the methylenes adjacent to centres of unsaturation at δ 2.0 (2H, vinyl group), 2.13 (4H, acetylenic bond) and aliphatic methylene carbons at δ 29.6-28.7, thus indicating an acetylenic alkyl chain with a terminal vinyl group.

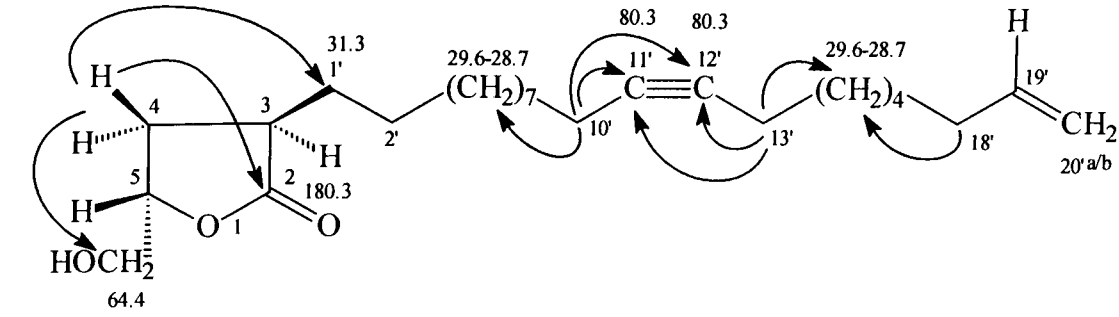
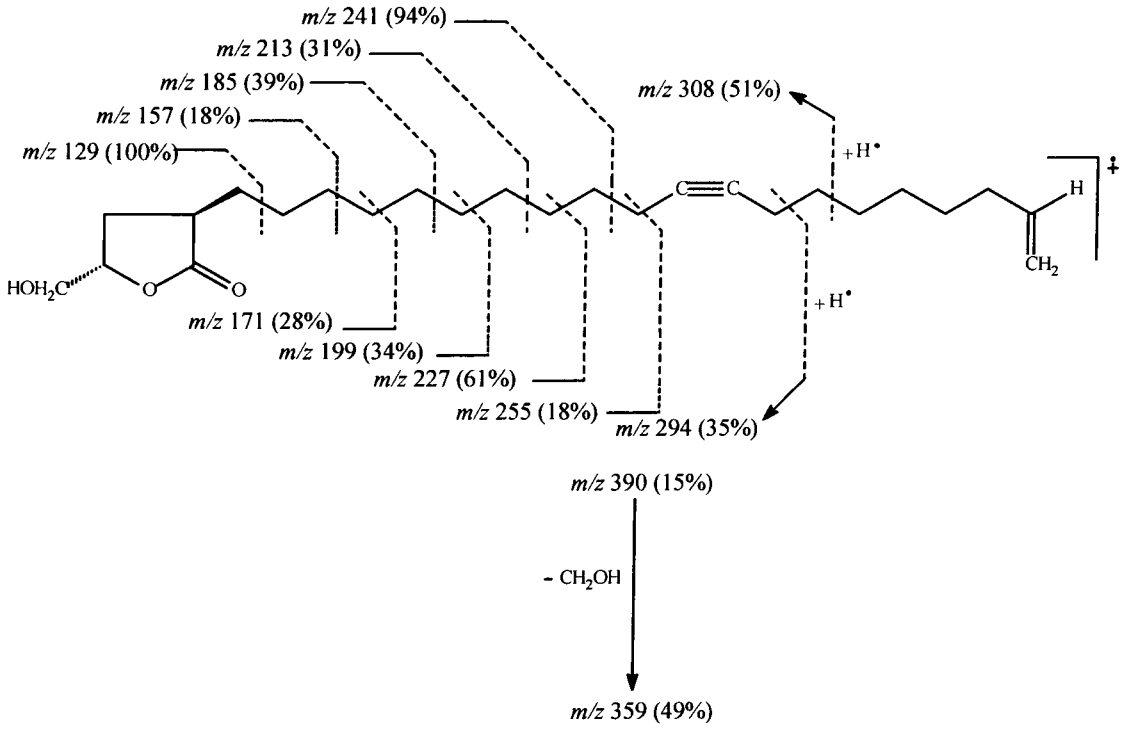


Figure 3.43: Significant HMBC correlations of M-5

The length of the alkyl chain and the position of the acetylenic bond were rationalised from the HREIMS mass fragmentation pattern (Scheme 3.18).



Scheme 3.18: Suggested mass fragmentation pattern of M-5

The relative stereochemistry of the lactone ring was established on the basis of NOE interactions (Figure 3.44).

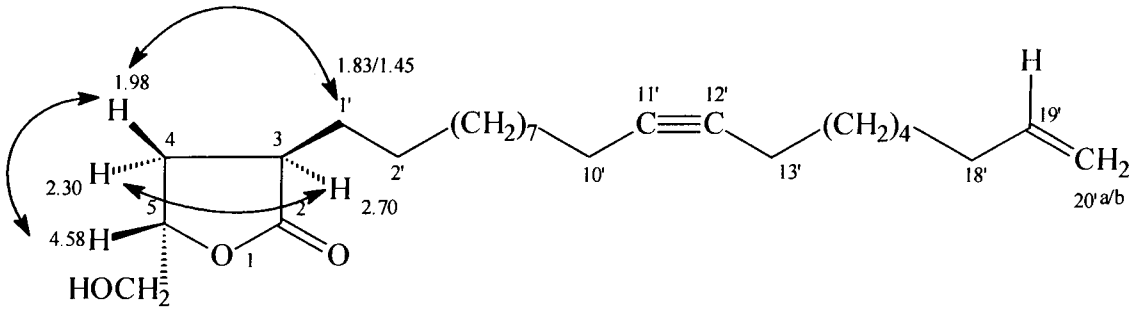


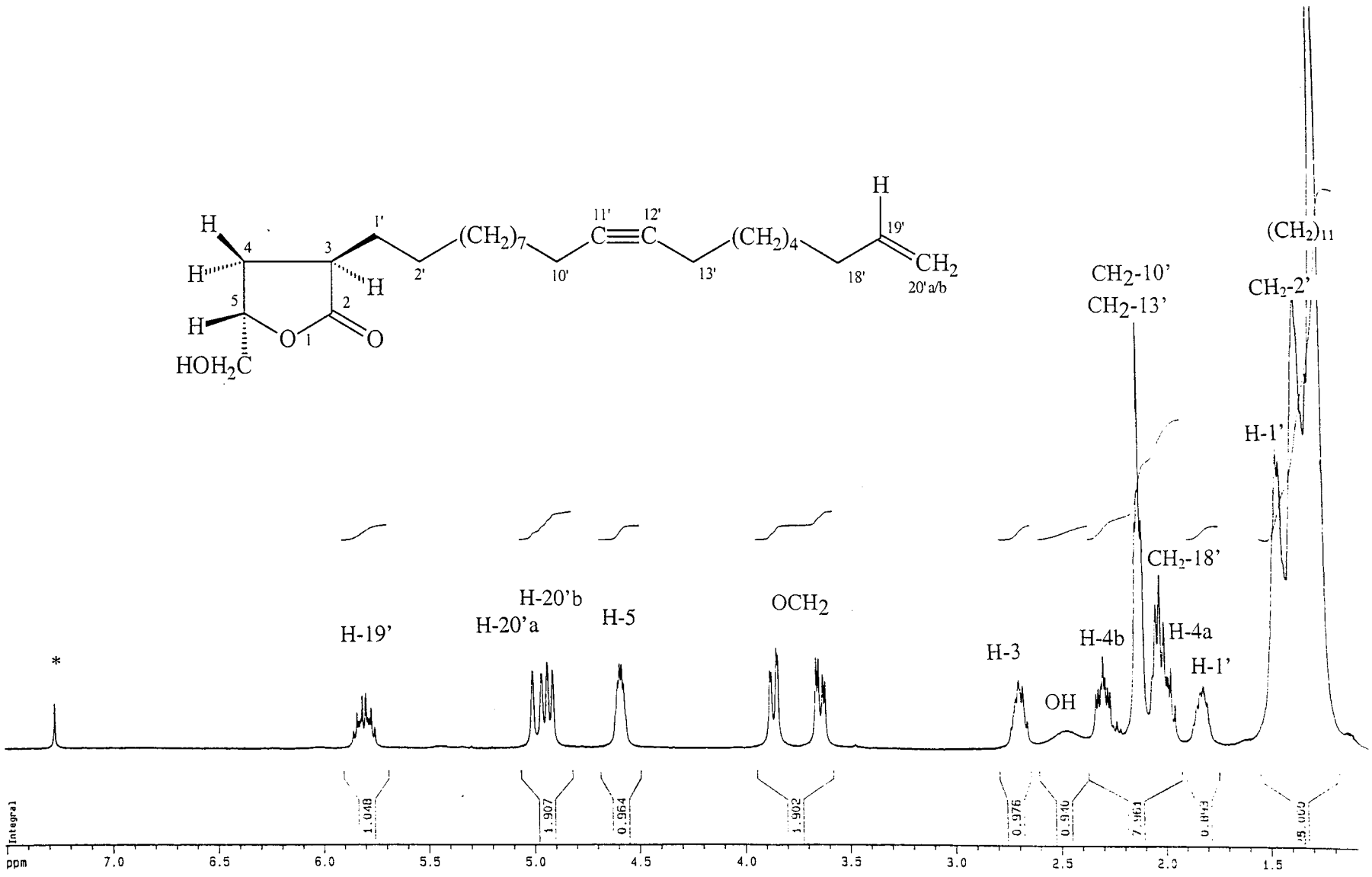
Figure 3.44: Significant NOE interactions of M-5

Consequently, **M-5** was identified as the new linear olefinic and acetylenic acetogenin *rel*-5α-hydroxymethyl-3β-eicosa-19'-en-11'-yn-tetrahydrofuran-2-one and given the trivial name goniothalamusin. Its spectral data showed similarities to a related compound named butyrolactone-1, recently isolated in the Annonaceae from *Porcelia macrocarpa* (Chaves and Roque, 1997).

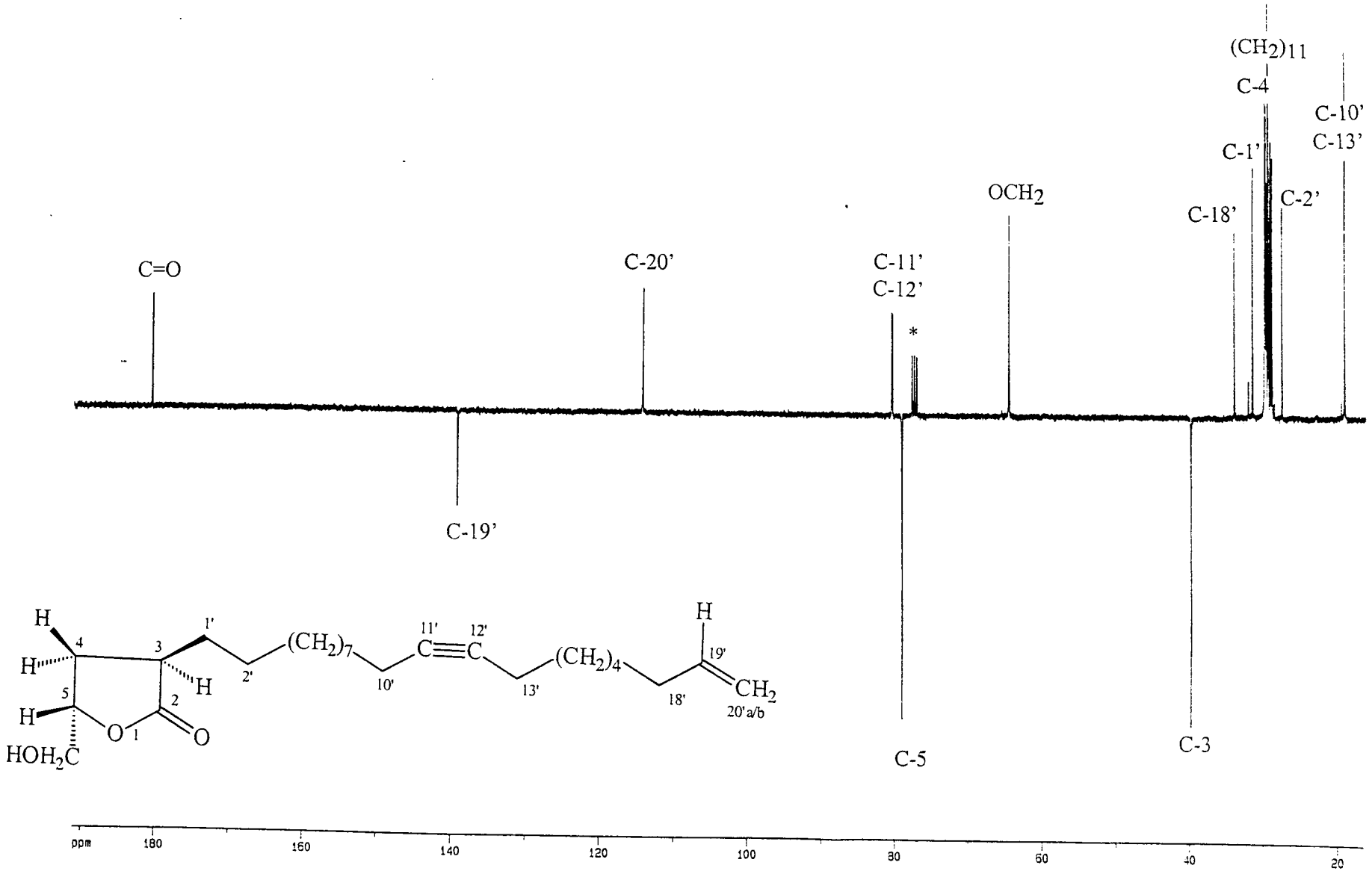
Table 3.52: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and significant HMBC correlations of M-5

Position	$^{\delta}H$	$^{\delta}C$	^{2}J	^{3}J
2	-	180.3		
3	2.70 (m)	39.7	29.7 (C-4), 31.3 (C-1'), 180.3 (C=O)	27.3 (C-2')
4	2.30/1.98 (ddd, 14.2, 9.6, 4.6)	29.7	39.7 (C-3), 78.9 (C-5)	31.3 (C-1'), 64.4 (OCH_2), 180.3 (C=O)
5	4.58 (m)	78.9	29.7 (C-4)	
1'	1.45*/1.83* (m)	31.3	27.3 (C-2'), 39.7 (C-3)	29.7 (C-4), 180.3 (C=O)
2'	1.37 (m)	27.3	29.6-28.7	29.6-28.7
3'-8'	1.46-1.26 (m)	29.6-28.7	29.6-28.7	29.6-28.7
9'	1.46-1.26 (m)	29.6-28.7	29.6-28.7	29.6-28.7, 80.3 (C-11')
10'	2.13 (t, 6.2)	18.8	29.6-28.7, 80.3 (C-11')	29.6-28.7, 80.3 (12')
11'/12'	-	80.3		
13'	2.13 (t, 6.2)	18.8	29.6-28.7, 80.3 (C-12')	29.6-28.7, 80.3 (C-11')
14'	1.46-1.26 (m)	29.6-28.7	29.6-28.7	29.6-28.7, 80.3 (12')
15'-17'	1.46-1.26 (m)	29.6-28.7	29.6-28.7	29.6-28.7
18'	2.0 (t, 6.6)	33.8	139.1 (C-19'), 29.6-28.7	114.3 (C-20') 29.6-28.7
19'	5.79 (ddt, 6.6, 10, 17)	139.1	33.8 (C-18')	
20'a	4.98 (dd, 2.1, 17)	114.3	139.1 (C-19')	33.8 (C-18')
20'b	4.92 (dd, 2.1, 10)	114.3	139.1 (C-19')	33.8 (C-18')
OCH_2	3.86 (dd, 2.9, 12.3)	64.4		29.7 (C-4)
	3 63 (dd, 4.8, 12.3)	64.4		29.7 (C-4)
OH	2.5 (brs)			

All data obtained in $CDCl_3$
In parentheses coupling constant *J* are in Hz.
Methylene signals are listed as Ha/Hb in each column
*Signals may be interchangeable.



Spectrum 3.50: ^{1}H NMR (400 MHz, $CDCl_3$*) of M-5



Spectrum 3.51: *J*-modulated ^{13}C NMR (100 MHz, $CDCl_3$*) of M-5

3.5.2 Properties and spectral data of isolated miscellaneous compounds

M-1 (5-Hydroxymethyl-2-furaldehyde)

Brown oil. IR ν_{max} (film) cm^{-1}: 3388 (OH), 2932, 2849, 1674 (C=O), 1521, 1217, 1191, 1022. UV λ_{max} (EtOH) nm: 281. Found $[M]^+$ 126.0337 ($C_6H_6O_3$ requires 126.0317) HREIMS *m/z* (rel. int. %): 126 (41), 125 (18), 109 (17), 107 (28), 97 (57), 77 (34), 51 (23). ^1H (400 MHz, $CDCl_3$) (*J* in Hz): δ 7.22 (d, 3.4, H-3), 6.52 (d, 3.4, H-4), 4.72 (s, H-1'), 2.20 (brs, OH), 9.60 (s, CHO). ^{13}C (100 MHz, $CDCl_3$): δ 152.7 (C-2), 122.7 (C-3), 110.2 (C-4), 160.7 (C-5), 57.9 (C-1'), 177.8 (CHO).

M-2 [4-Hydroxy-3-methoxy-(trans)-cinnamylaldehyde (feruladehyde)]

Brown oil. IR ν_{max} (film) cm^{-1}: 3371 (OH), 2937, 2830 (OMe), 1664 (C=O), 1620, 1599, 1586, 1512, 1286, 1208, 1132, 1030, 970, 811. UV λ_{max} (EtOH) nm: 224, 250, 301, 340. UV λ_{max} (EtOH+NaOH) nm: 254, 412. Found $[M]^+$ 178.0635 ($C_{10}H_{10}O_3$ requires 178.0630) HREIMS *m/z* (rel. int. %): 178 (100), 177 (35), 161 (33), 153 (71), 151 (81), 147 (43), 136 (56), 107 (59), 77 (99), 63 (30). ^1H (400 MHz, $CDCl_3$) (*J* in Hz): δ 7.07 (d, 1.9, H-2), 6.97 (d, 8.2, H-5), 7.13 (dd, 1.9, 8.2, H-6), 9.66 (d, 7.7, H-1'), 6.60 (dd, 7.7, 15.8, H-2'), 7.41 (d, 15.8, H-3'), 3.96 (s, 3-OMe), 6.0 (brs, 4-OH). ^{13}C (100 MHz, $CDCl_3$): δ 126.9 (C-1), 115.2 (C-2), 149.1 (C-3), 147.2 (C-4), 109.7 (C-5), 124.3 (C-6), 193.8 (C-1'), 126.7 (C-2'), 153.2 (C-3'), 56.2 (3-OMe).

M-3 [Rel-(2α,3β)-7-O-methylcedrusin]

Pale amorphous solid. $[\alpha]_D^{22}$ +18.3° (MeOH, *c* 0.22) [Lit. $[\alpha]_D^{25}$ +5.5° (MeOH, *c* 0.18), Deyama *et al.*, 1987]. IR ν_{max} (film) cm^{-1}: 3378 (OH), 2935, 2881, 1604, 1515, 1500, 1272, 1211, 1141, 1033. UV λ_{max} (MeOH) nm: 228, 278, 285, 313. UV λ_{max} (MeOH+NaOH) nm: 246, 287, 355. Found $[M]^+$ 360.1538 ($C_{20}H_{24}O_6$ requires 360.1573) HREIMS *m/z* (rel. int. %): 360 (63), 342 (100), 330 (39), 327 (31), 316 (18), 275 (24), 162 (44), 150 (41), 113 (34), 55 (27). ^1H & ^{13}C NMR: Table 3.50.

M-4 (Shikimic acid)

Colourless amorphous solid. $[\alpha]_D^{24}$ -146.8° (H_2O, *c* 0.218) [Lit. $[\alpha]_D$ -157° (H_2O, *c* 1.3) Achenbach and Schwinn, 1995). IR ν_{max} (film) cm^{-1}: 3434 (OH), 1698, 1643 (C=O), 1540, 1396, 1151. UV λ_{max} (MeOH) nm: 265. $[M+H]^+$ not found ($C_7H_{10}O_5$ requires 174.0528) FABMS *m/z* (rel. int. %): 156 (80), 155 (98), 138 (100), 109 (83). ^1H & ^{13}C NMR: Table 3.51.

M-5 [Rel-5α-hydroxymethyl-3β-eicosa-19'-en-11'-yn-tetrahydrofuran-2-one (goniothalamusin)]

Amorphous solid. $[\alpha]_D^{23.5}$ +14.6° ($CHCl_3$, *c* 0.206). IR ν_{max} (KBr) cm^{-1}: 3459 (OH), 3077, 2931, 2919, 2850, 1754 (C=O), 1643, 1469, 1367, 1272, 1176, 1078, 1047, 948. UV λ_{max} (EtOH) nm: 212. Found $[M]^+$ 390.3237 ($C_{25}H_{42}O_3$ requires 390.3134) HREIMS *m/z* (rel. int. %): 390 (15), 359 (49), 341 (16), 308 (51), 294 (35), 255 (18), 241 (94), 227 (61), 213 (31), 199 (34), 185 (39), 171 (28), 157 (18), 129 (100). ^1H & ^{13}C NMR: Table 3.52.

3.6 Discussion

This phytochemical study examined five species of Annonaceae, *Cleistopholis glauca*, *Cleistopholis patens*, *Piptostigma fasciculata*, *Goniothalamus thwaitesii* and *Goniothalamus gardneri*. Neither *Piptostigma fasciculata* nor the two species of *Goniothalamus* had been previously investigated.

Secondary metabolites isolated were oligosaccharides, terpenes, flavonoids, one alkaloid and miscellaneous compounds. The significance of the occurrence of these constituents in the family/genus/species or plant part is discussed below for each species.

3.6.1 *Cleistopholis glauca* Pierre ex Engl. & Diels

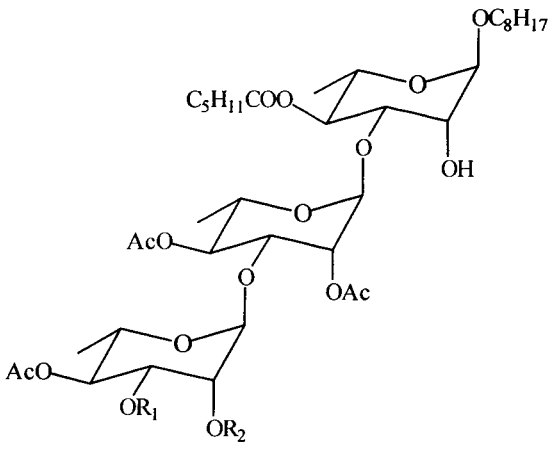
Previous phytochemical investigations on this species have been carried out on stem bark samples from Cameroon. Compounds isolated included partially acetylated 1-*O*-dodecanyl tri- and tetrarhamnosides **(20)** to **(25)**, acyclic and monocyclic sesquiterpene methyl esters **(27)** to **(32)**, β-sitosterol **(45)**, ferulaldehyde **(46)** and 5-hydroxymethyl-2-furaldehyde **(47)** (see Section 1.4.3).

In this study, further investigation of the stem bark afforded in addition to the partially acetylated 1-*O*-dodecanyl tri- and tetrarhamnosides **(20** or **OS-4)**, **(22** or **OS-9)** and **(24** or **OS-10)**, two new tetrarhamnosides, namely cleistetroside-6 **(OS-6)** and cleistetroside-7 **(OS-8)**. In addition to the acyclic sesquiterpene methyl esters **(27** or **S-2)** and **(28** or **S-3)**, the stem bark also yielded the new acyclic sesquiterpene methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate **(S-1)**. Further compounds included the very common flavonol quercetin **(F-10)** and its dihydro-analogue dihydroquercetin **(F-11)**, the azaanthracene alkaloid cleistopholine **(A-1)** and the neolignan *rel*-(2α,3β)-7-O-methylcedrusin **(M-3)** (Figure 3.48).

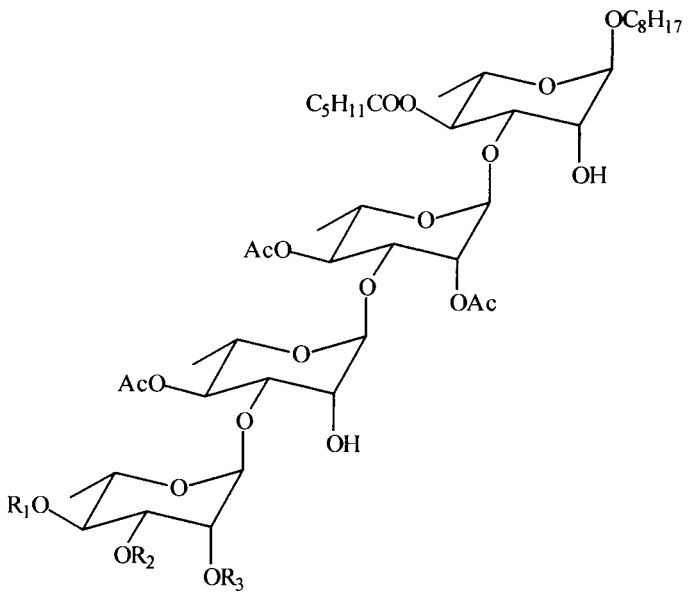
The presence of cleistetroside-6 **(OS-6)** and cleistetroside-7 **(OS-8)** is in conformity to what was already known for the species. These two new compounds are typical 1-*O*-dodecanyl tetrarhamnosides, showing a different acetylation pattern on the last rhamnose unit compared to already isolated oligosaccharides. Related derivatives (mezzettiaside-2 to -7, **(165)** to **(170)**) have already been reported in the family from the stem bark of the Malaysian species *Mezzettia leptopoda* (Powell *et al.*, 1990; Etse *et al.*, 1991) (Figure 3.45). This suggests a close similarity between the two species and may be of chemotaxonomic significance. However, it is also possible that such oligosaccharides occur quite widely in the family but have, to date, largely been overlooked.

Methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate **(S-1)** is biogenetically related to the other acyclic farnesoic acid methyl esters already isolated from the stem bark of *C. glauca*, the root and stem bark samples of *C. patens* from Ghana and the stem bark sample of *C. patens* from Sierra Leone. The proposed biogenetic pathway for sesquiterpenes **S-1** to **S-3** is shown in Scheme 3.19 (Etse *et al.*, 1988). This demonstrates a close affinity between species of *Cleistopholis*. Furthermore, the occurrence of **S-1** in the family is noteworthy since only a few farnesane sesquiterpenes have been isolated so far, predominantly from essential oils (Ekundayo and Ogutimein, 1987; Ekundayo *et al.*, 1988).

Quercetin **(F-10)** and dihydroquercetin **(F-9)** represent the first flavonoids isolated in the genus. Quercetin has previously been isolated in the family from *Annona glabra*, *A. senegalensis* and *Asimina triloba* (Leboeuf *et al.*, 1982). However, this is the first occurrence of dihydroquercetin in the Annonaceae. Their anticipated biosynthetic origin is shown in Scheme 3.20.

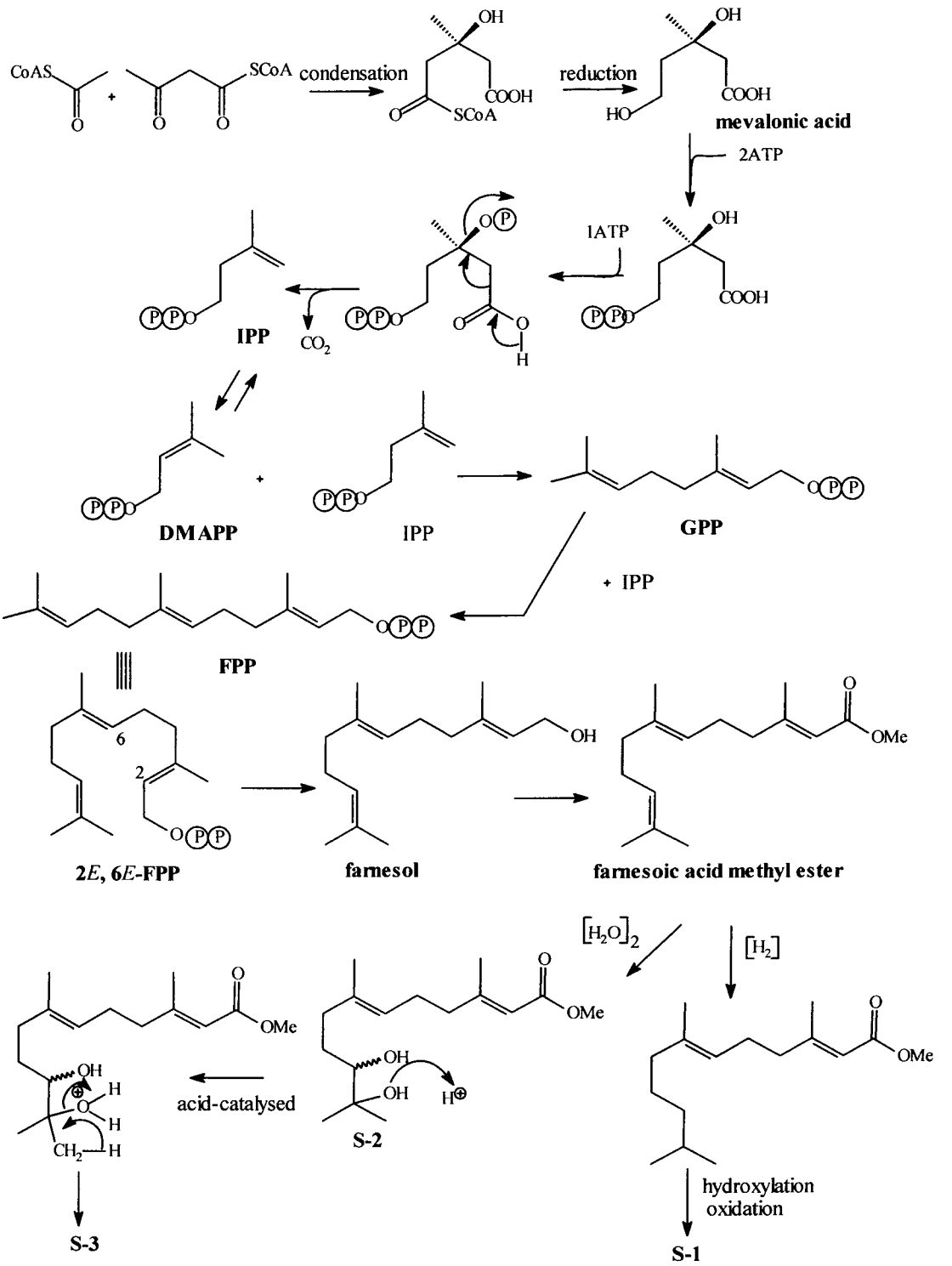


Compound	R_1	R_2
(165)	H	Ac
(166)	Ac	H
(167)	H	H



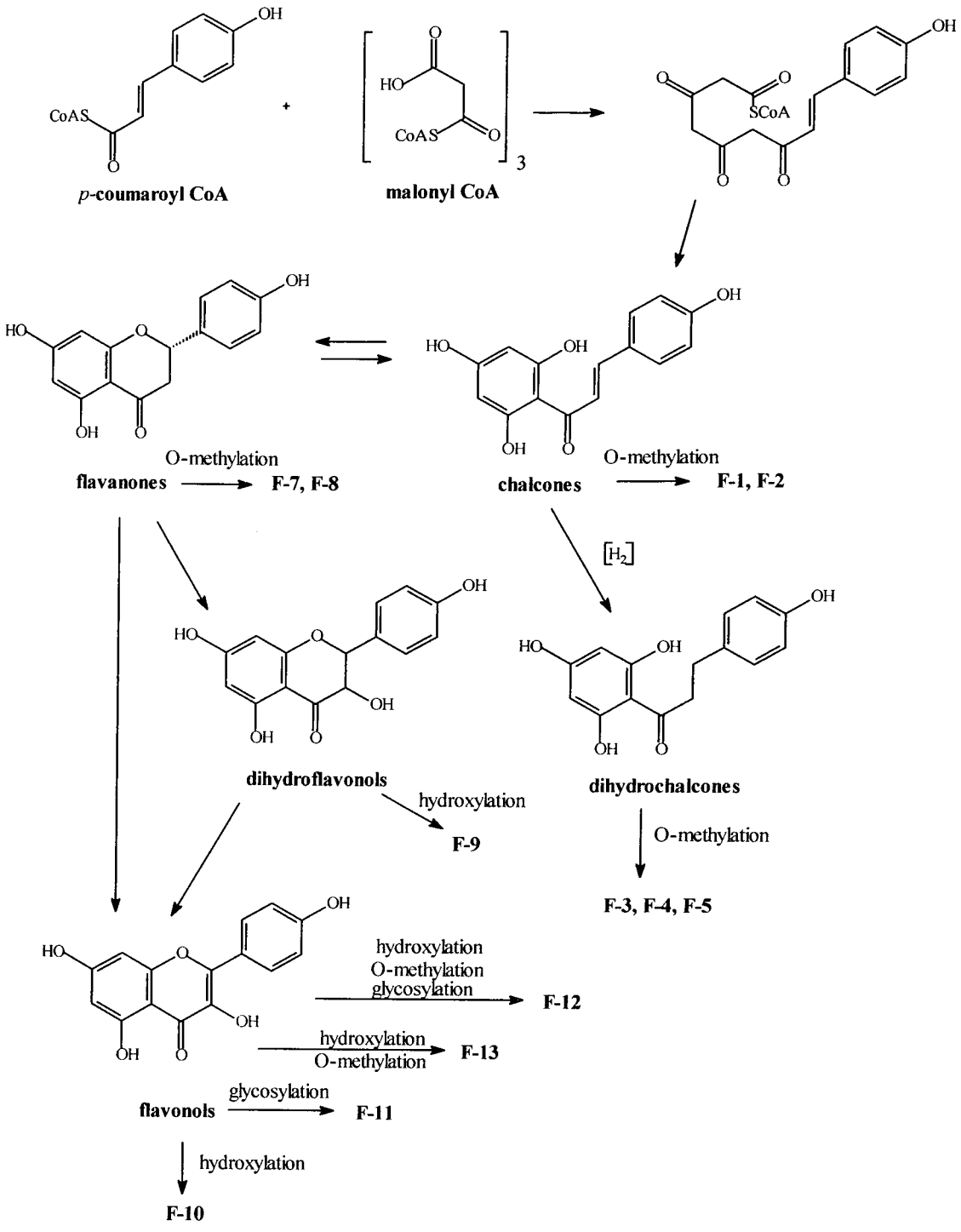
Compound	R_1	R_2	R_3
(168)	H	Ac	H
(169)	H	H	Ac
(170)	H	H	H

Figure 3.45: Structures of the oligosaccharides from *Mezzettia leptopoda*



IPP = Isopentenylpyrophosphate, **DMAPP** = Dimethylallylpyrophosphate, **GPP** = Geranylpyrophosphate, **FPP** = Farnesylpyrophosphate

Scheme 3.19: Suggested biogenetic pathway for S-1 to S-3

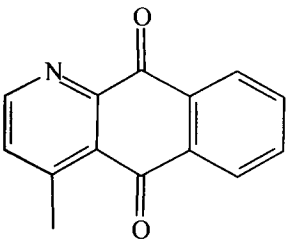


Scheme 3.20: Suggested biogenetic pathway for F-1 to F-5 and F-7 to F-13

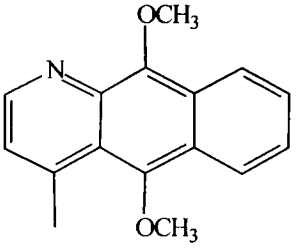
Cleistopholine **(A-1** or **42)** has been previously isolated from the roots of *C. patens*, again showing the affinity between species of *Cleistopholis*. Its presence in *C. glauca* is significant since early investigations had revealed no trace of alkaloids. Only a few azaanthracene alkaloids have been recorded so far in nature and those isolated appear exclusive to the Annonaceae (Figure 3.46; Table 3.53). **A-1** may be related biogenetically to the aporphinoids through a catabolic pathway (Tadic *et al.*, 1987) (Scheme 3.21).

Table 3.53: Azaanthracenes previously isolated in the Annonaceae

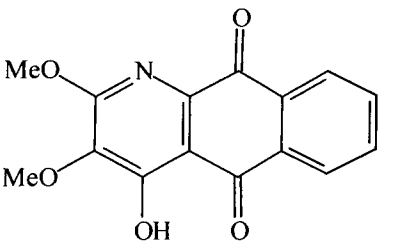
Isolated azaanthracenes	Species (references)
Cleistopholine **(42)**	*Cleistopholis patens* (Waterman and Muhammad, 1985)
	Meiogyne virgata (Tadic *et al.*, 1987)
	Annona hayesii (Rasamizafy *et al.*, 1987)
	Oncodistigma monosperma (Bou-abdallah *et al.*, 1989)
	Annona cherimolia (Rios *et al.*, 1989)
Annopholine **(171)**	*Annona hayesii* (Rasamizafi *et al.*, 1987)
Scorazanone **(128)**	*Goniothalamus scortechinii* (Din *et al.*, 1990)



(42)

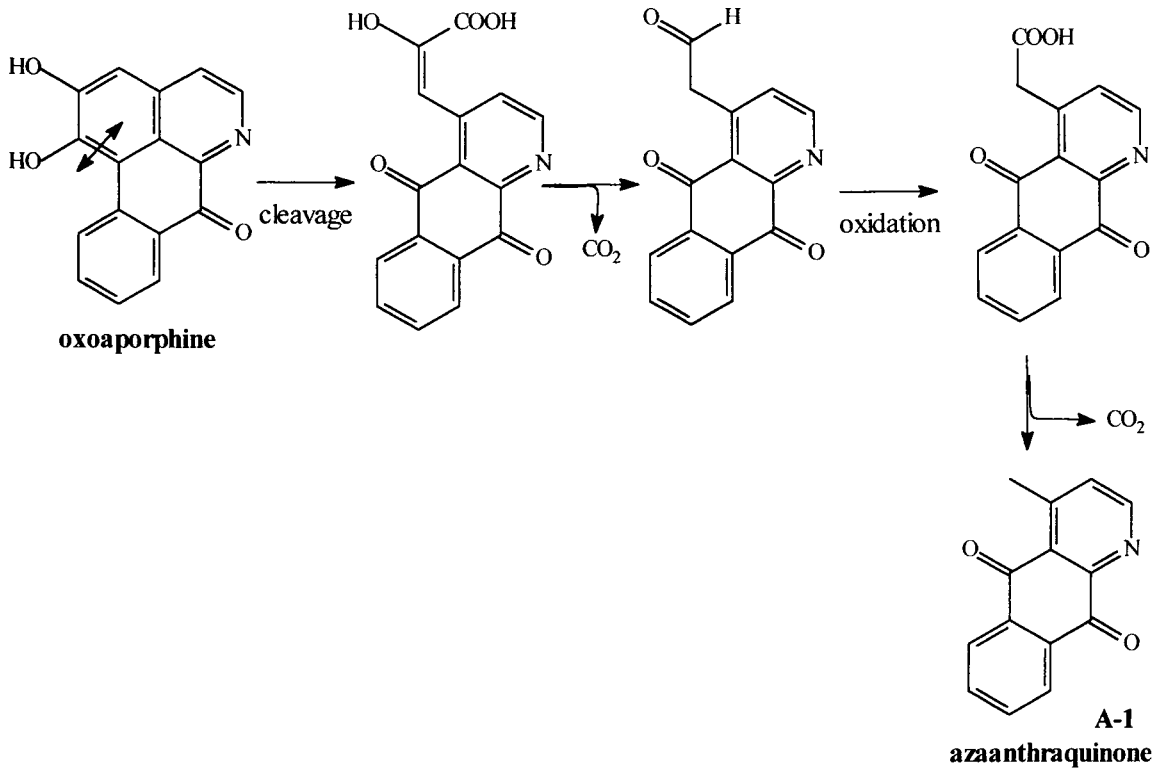


(171)



(128)

Figure 3.46: Structures of the azaanthracene alkaloids in the Annonaceae

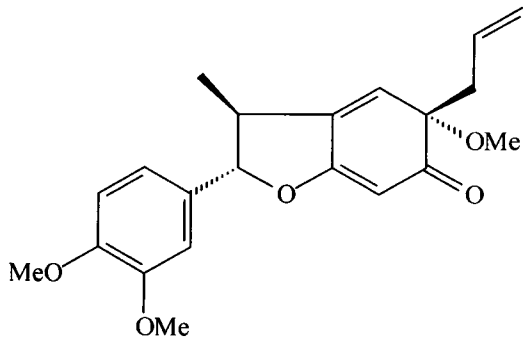


Scheme 3.21: Suggested biogenetic pathway for A-1

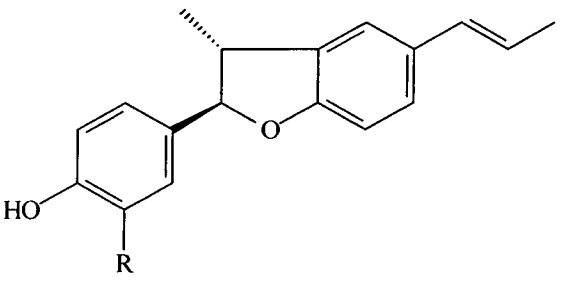
The dihydrobenzofuran neolignan *rel*-(2α,3β)-7-O-methylcedrusin **(M-3)** has been previoulsy isolated from *Cedrus deodara* (Pinaceae) (Agrawal *et al.,* 1980), *Eucommia ulmoides* (Eucommiaceae) (Deyama *et al.,* 1987) and *Licaria chrysophylla* (Lauraceae) (Da Silva *et al.*, 1989). It is reported for the first time in the family and represents the first neolignan isolated from *Cleistopholis.* Its occurrence in *C. glauca* may be of chemotaxonomic value since lignan-type compounds are rare in the Annonaceae and more frequently encountered in the neighbouring Magnoliaceae, Myristicaceae, Eupomatiaceae and Lauraceae (Gottlieb, 1978). However, neolignans isolated so far in the Annonaceae appear to be exclusively dihydrobenzofuran derivatives. Thus, the isolation of **M-3** from *C. glauca* conforms with what is already known (Figure 3.47; Table 3.54). The proposed biosynthetic pathway for **M-3** is shown in Scheme 3.22.

Table 3.54: Neolignans previously isolated in the Annonaceae

Isolated neolignans	Species (references)
(2S,3S,5S)-2,3-dihydro-2-(3,4-dimethoxyphenyl)-3-methyl-6-oxo-5-methoxyallylbenzofuran **(172)**	*Duguetia surinamensis* (Gottlieb, 1978)
(2R,3R)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-methyl-5-(*E*)-propenylbenzofuran **(173)**	*Alphonsea mollis* (Ning *et al.*, 1994)
Conocarpan **(174)**	*Alphonsea mollis* (Ning *et al.*, 1994)

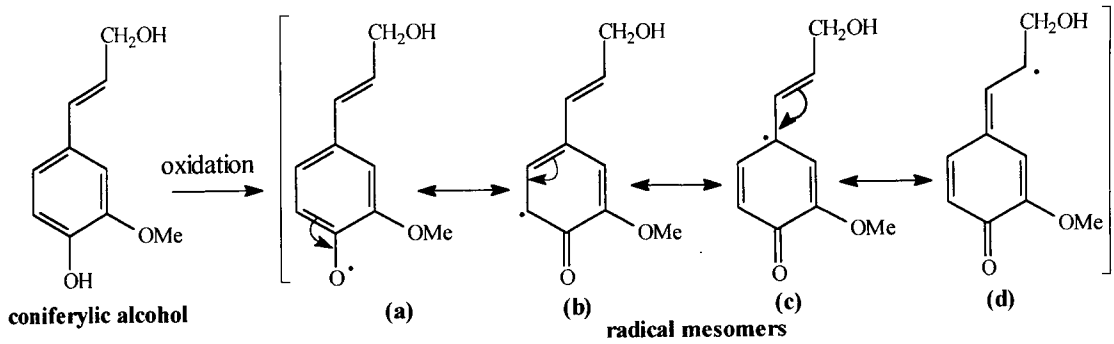


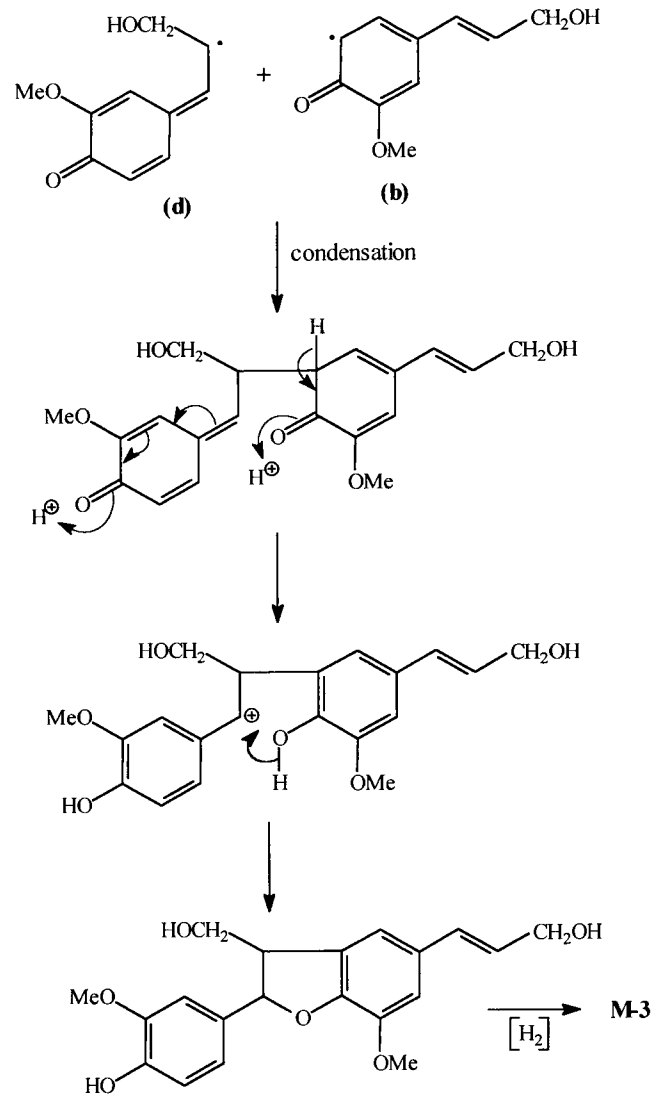
(172)



Compound	R
(173)	OMe
(174)	H

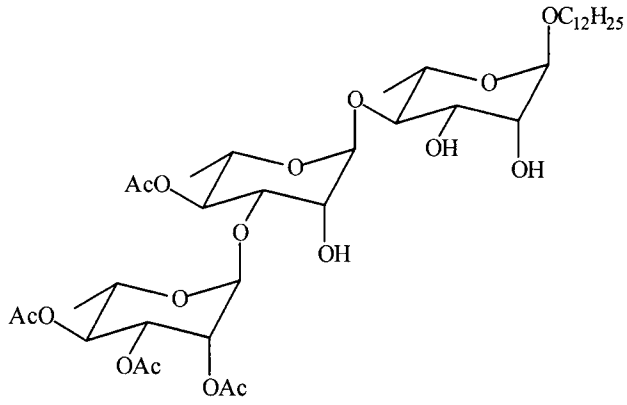
Figure 3.47: Structures of the neolignans in the Annonaceae



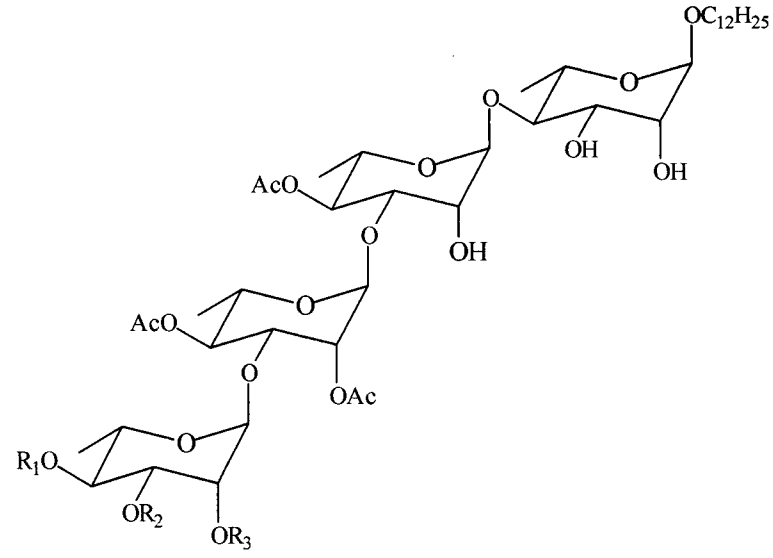


Scheme 3.22: Suggested biogenetic pathway for M-3

Oligosaccharides



OS-4

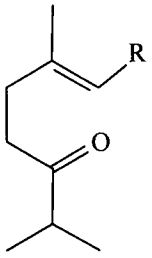


Compound	R_1	R_2	R_3
OS-6*	Ac	H	Ac
OS-8*	Ac	Ac	Ac
OS-9	H	H	Ac
OS-10	H	H	H

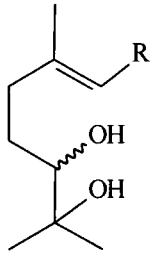
* New compounds

Figure 3.48: Structures of the secondary metabolites isolated from the stem bark of *Cleistopholis glauca*

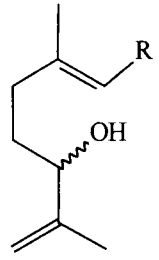
Sesquiterpenes



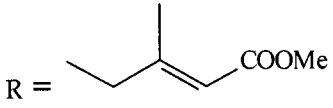
S-1*



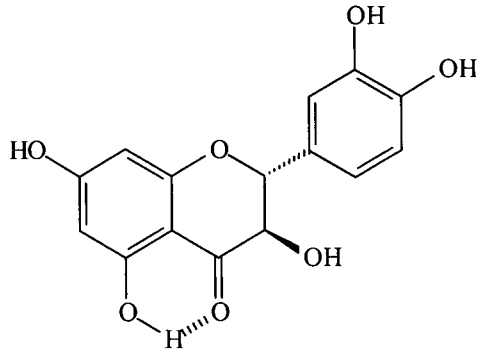
S-2



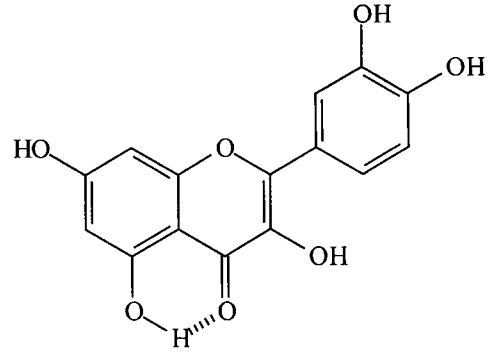
S-3



Flavonoids

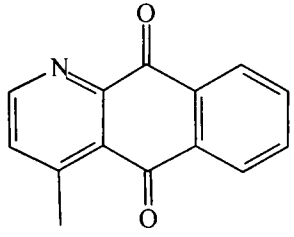


F-9



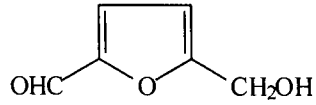
F-10

Alkaloids

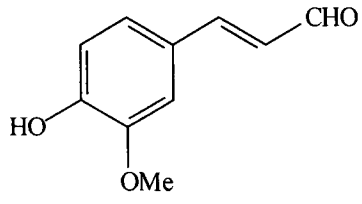


A-1

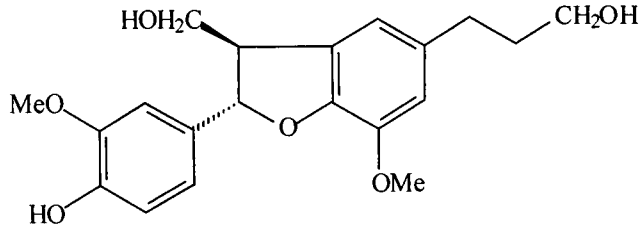
Miscellaneous



M-1



M-2



M-3

* New compounds

Figure 3.48 (cont.): Structures of the secondary metabolites isolated from the stem bark of *Cleistopholis glauca*

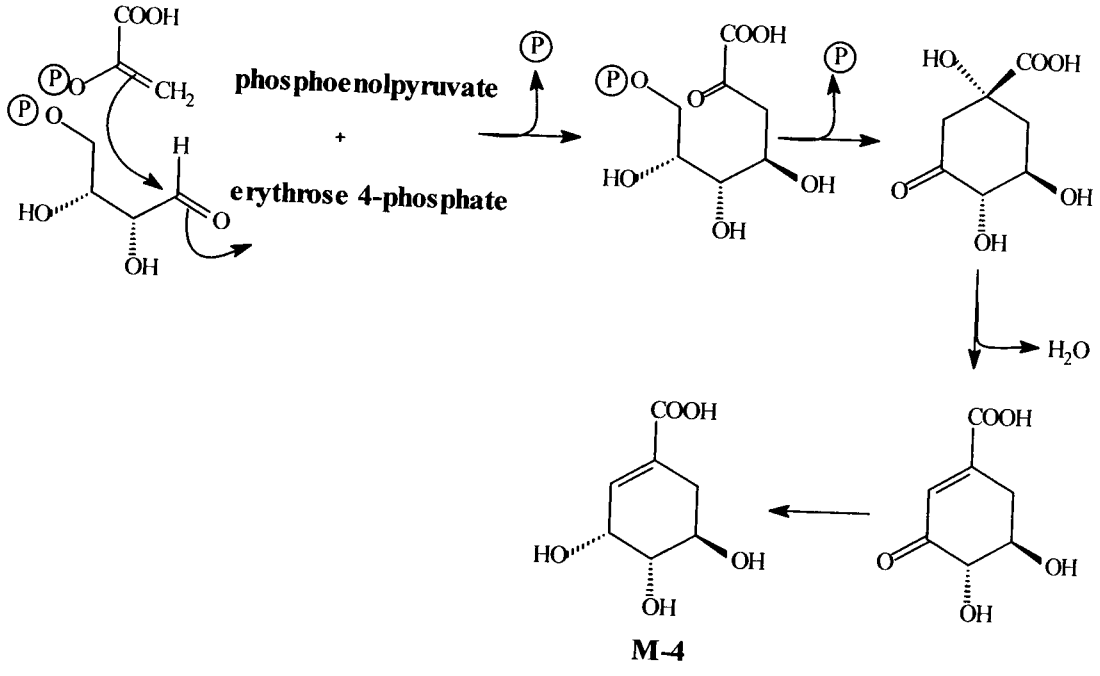
3.6.2 *Cleistopholis patens* (Benth.) Engl. & Diels

Previous phytochemical investigations carried out on this species have been focused on root and stem bark samples from Ghana and Sierra Leone. Compounds isolated included acyclic and monocyclic sesquiterpene methyl esters **(27)**, **(30)** and **(31)**, oxoaporphines **(37)** and **(38)**, naphthyridine alkaloids **(39)** to **(41)**, cleistopholine **(42)**, onychine **(43)** and 3-methoxysampangine **(44)** (see Section 1.4.3). No phytochemical work had been reported on the leaves.

In this study the leaves of *C. patens* from Cameroon afforded three new partially acetylated 1-*O*-dodecanyl trirhamnosides, cleistrioside-2 **(OS-1)**, cleistrioside-3 **(OS-2)** and cleistrioside-4 **(OS-3)**. It also yielded the known tetrarhamnosides, cleistetroside-1 **(21** or **OS-5)**, cleistetroside-2 **(23** or **OS-7)** and the new cleistetroside-6 **(OS-6)**. Shikimic acid **(M-4)** was also isolated (Figure 3.4).

Cleistrioside-2 **(OS-1)**, cleistrioside-3 **(OS-2)** and cleistrioside-4 **(OS-3)** are related to the trirhamnoside **(20** or **OS-4)** isolated from *C. glauca.* However they show a different acetylation pattern and a different type of interglycosidic link between the first and the second rhamnose unit. Cleistetroside-6 **(OS-6)** is a new tetrarhamnoside which was isolated also from the stem bark of *C. glauca* (see Section 3.5.1). This represents the first report of the isolation of such oligosaccharides in *C. patens*. However, it is in conformity with what is already common in the genus and further supports the close affinity between species of *Cleistopholis*.

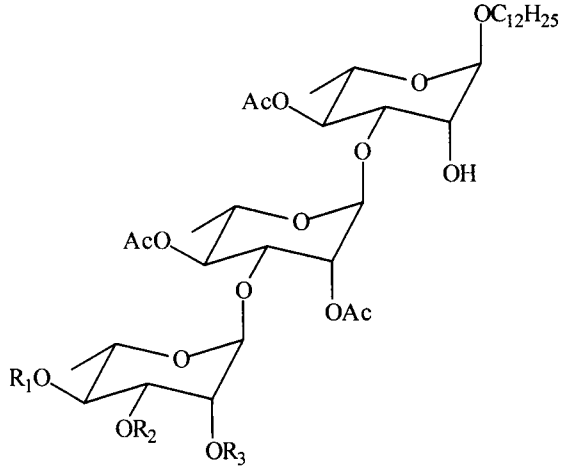
This is the first report of shikimic acid **(M-4)** in the genus. Although shikimic acid is a very important metabolic intermediate, it has rarely been isolated. It was reported in *Dendrobium fuscescens* (Orchidaceae) (Talapatra *et al.*, 1989) and in the Annonaceae from *Piptostigma fugax* (Achenbach and Schwinn, 1995). Its biogenesis is described in Scheme 3.23.



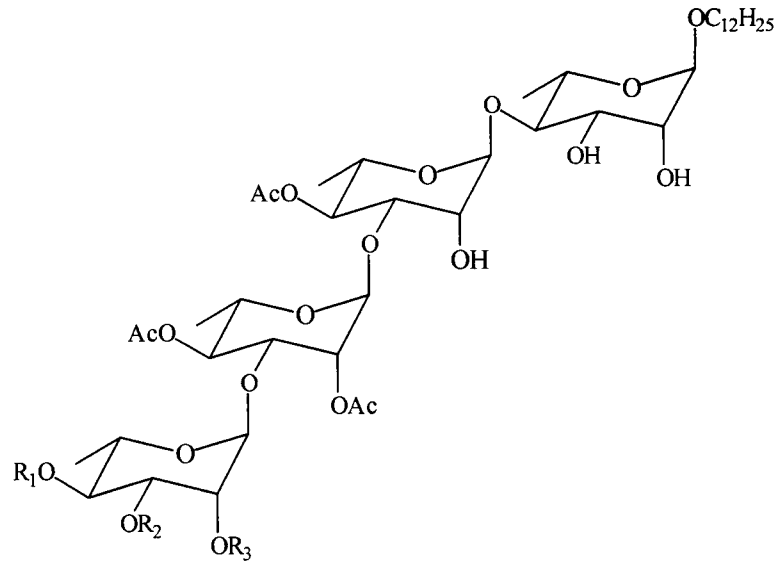
M-4

Scheme 3.23: Suggested biogenetic pathway for M-4

Oligosaccharides

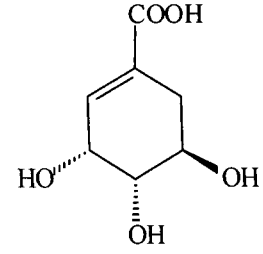


Compound	R_1	R_2	R_3
OS-1*	Ac	Ac	H
OS-2*	Ac	H	Ac
OS-3*	Ac	H	H



Compound	R_1	R_2	R_3
OS-5	Ac	Ac	H
OS-6*	Ac	H	Ac
OS-7	Ac	H	H

Miscellaneous



M-4

* New compounds

Figure 3.49: Structures of the secondary metabolites isolated from the leaves of *Cleistopholis patens*

3.6.3 *Piptostigma fasciculata* (De Wild.) Boutique

The only species in the genus which has been studied, *Piptostigma fugax*, yielded sesquiterpenes, triterpenes, steroids, isoquinoline and azafluorenone alkaloids, nitrogen compounds and shikimate-derived metabolites (see Section 1.5.2).

In this study the leaves of *P. fasciculata* from Ghana, afforded the sesquiterpene β-caryophyllene-4,5-oxide **(S-4)**, the acyclic diterpene (2*E*,7ξ,11ξ)-phytol **(D-1)** and the flavonol glycoside nicotiflorin **(F-11)** (Figure 3.51).

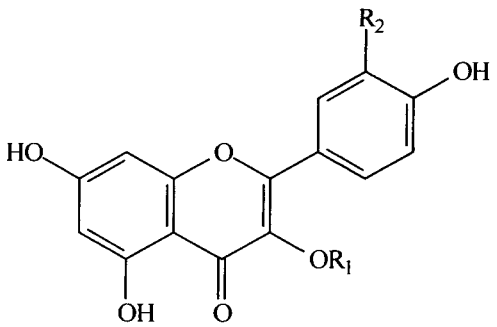
β-Caryophyllene-4,5-oxide **(S-4)** occurs commonly in the family, for example in *Xylopia fusca*, *Polyalthia jenkinsii*, *Annona reticulata* (Etse, 1986), *Artabotrys stenopetalus*, *Neostenanthera hamata* (Fleischer, 1997), *Oxymitra velutina* (Achenbach and Hemrich, 1991) and as a constituent of the essential oils of *Uvaria chamae* (Ogutimein *et al.*, 1989), *Cleistopholis patens* (Ekundayo and Ogutimein, 1987; Ekundayo *et al.*, 1988) and *Xylopia nitida* (Fournier *et al.*, 1993). Its occurrence is of interest since it has a caryophyllane skeleton in contrast to the sesquiterpenes encountered in the genus, which are cadinane and guaiane derivatives. Its biogenetic route is shown in Scheme 3.24.

Trans-Phytol **(D-1)** has been previously isolated from marine algae, *Ulva*, *Scenedesmus* and *Chlorella* species (Chlorophyceae) (Iwata and Sakurai, 1963), *Fucus vesiculosus* (Phaeophyceae), *Phormidium luridum* (Cyanophyceae) (De Souza and Nes, 1969) and *Gracilaria andersoniana* (Rodophyceae) (Sims and Pettus, 1976). It has also been reported in *Fatsia japonica* (Araliaceae) (Suga and Aoki, 1974), *Tetragonia tetragonoides* (Aizoaceae) (Aoki *et al.*, 1982) and *Artemisia annua* (Compositae) (Brown, 1994). Its occurrence in the Annonaceae is noteworthy since it has an acyclic skeleton in contrast to the diterpenes encountered in the family, which are clerodane, trachylobane, kolavane and predominantly kaurane derivatives. **(D-1)** is the first diterpene isolated from the genus. Its biogenesis is shown in Scheme 3.24.

Nicotiflorin **(F-11)** has been previously reported in the family and its occurrence is of importance since only a few flavonoid glycosides have been isolated so far (Figure 3.50; Table 3.55). It is the first flavonol glycoside to be isolated from the genus. Its biogenesis is described in Scheme 3.20.

Table 3.55: Flavonoids glycosides previously isolated in the Annonaceae

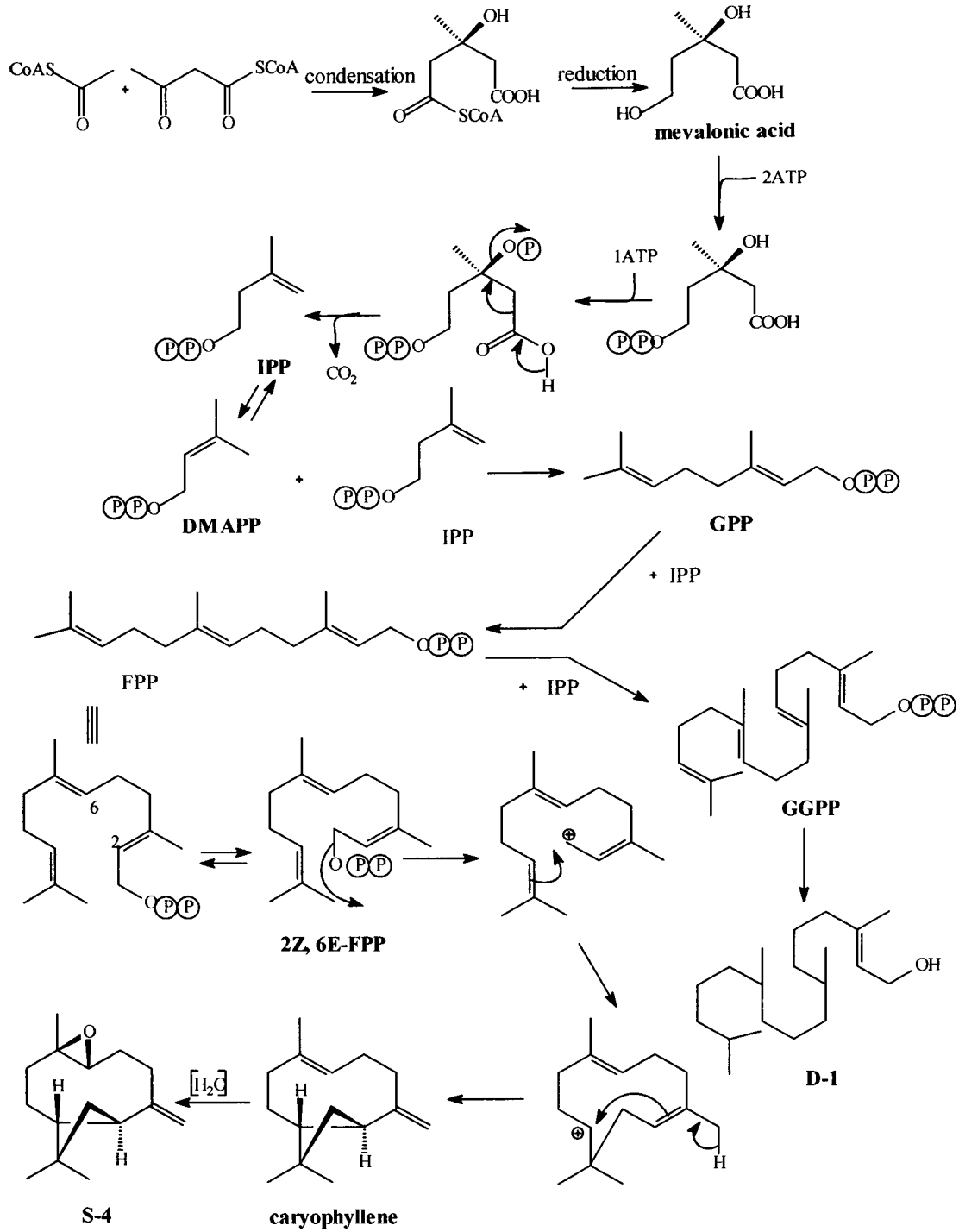
Isolated flavonoid glycosides	Species (references)
Quercetrin **(175)**	*Annona senegalensis* (Leboeuf *et al.*, 1982)
Rutin **(176)**	*Annona senegalensis* (Leboeuf *et al.*, 1982)
	Cananga latifolia (Leboeuf *et al.*, 1982)
Nicotiflorin **(177)**	*Cananga latifolia* (Siv and Paris, 1972)



Compound	R_1	R_2
(175)	Rha*	OH
(176)	Rha-glc**	OH
(177)	Rha-glc	H

Figure 3.50: Structures of the flavonoid glycosides in the Annonaceae

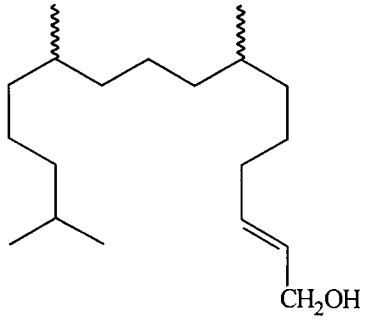
*Rha = rhamnosyl
**Rha-glc = rhamnoglucosyl



IPP = Isopentenylpyrophosphate, **DMAPP** = Dimethylallylpyrophosphate, **GPP** = Geranylpyrophosphate, **GGPP** = Geranylgeranylpyrophosphate, **FPP** = Farnesylpyrophosphate

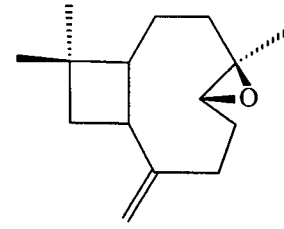
Scheme 3.24: Suggested biogenetic pathway for S-4 and D-1

Diterpenes



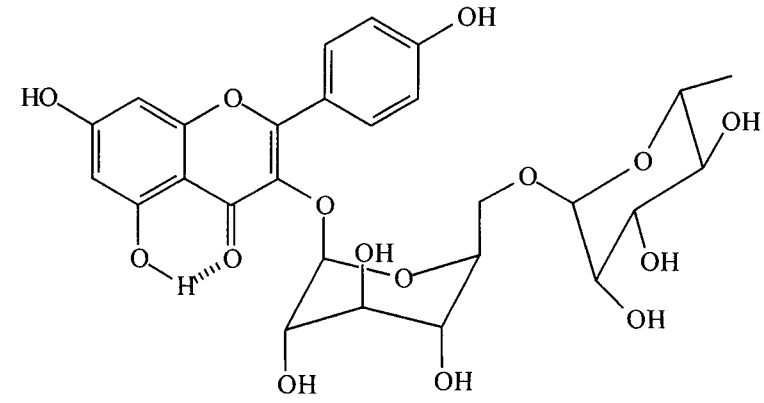
D-1

Sesquiterpenes



S-4

Flavonoids



F-11

Figure 3.51: Structures of the secondary metabolites isolated from the leaves of *Piptostigma fasciculata*

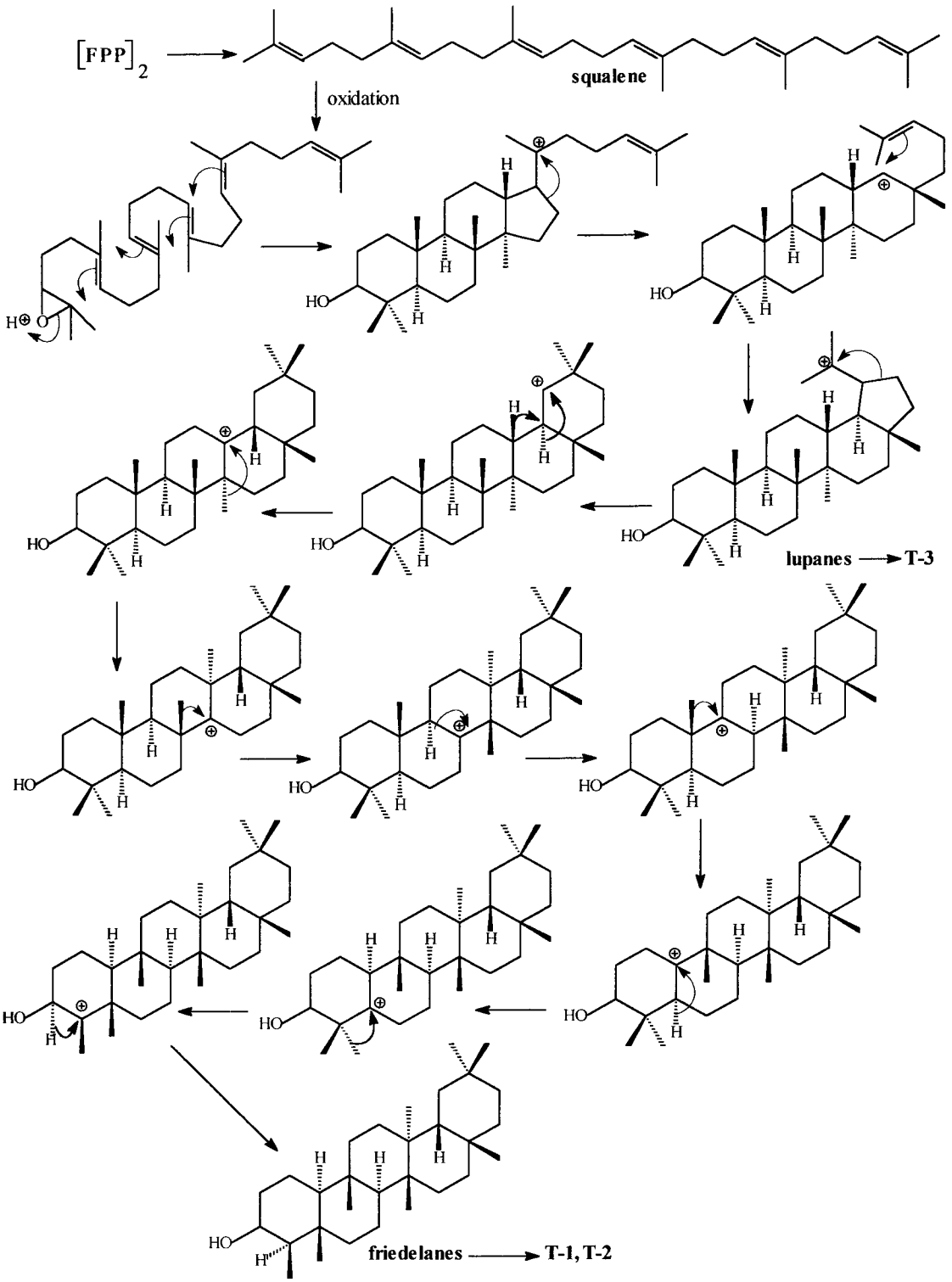
3.6.4 *Goniothalamus thwaitesii* Hook. f. & Thoms.

Previous studies on the genus *Goniothalamus* have yielded mainly acetogenins, isoquinoline alkaloids and styryl-lactones (see Section 1.6.3). No previous phytochemical investigation has been carried out on *G. thwaitesii*.

In this study the aerial parts, from Sri-Lanka, afforded the two friedelane triterpenes, friedelinol **(T-1)** and friedelin **(T-2)**, the lupane betulinic acid **(T-3)**, the flavonol glycoside, mearnsitrin **(F-12)** and the flavonol-3-*O*-methyl ether, annulatin **(F-13)** (Figure 3.52).

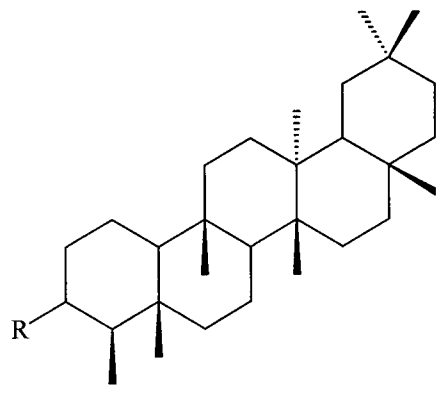
The occurrence of even common triterpenes is of interest as they have rarely been reported in the family. Friedelin **(T-2)** has previously been reported from *Annona squamosa* (Bhaumik *et al.*, 1979). However, it is the first isolation of friedelinol **(T-1)** and betulinic acid **(T-3)** in the family and the first report of the occurrence of triterpenes in *Goniothalamus*. Their biogenetic route is described in Scheme 3.25.

The occurrence of flavonoids is also of interest since the only flavonoid previously isolated from the genus is the flavanone pinocembrin **(84)**. Mearnsitrin **(F-12)** has been previously isolated from *Acacia mearnsii* (Leguminosae) (Mackenzie, 1969). Annulatin **(F-13)** has been previously isolated as an aglycone from *Cleistocactus variispinus* and *Cereus jamacaru* (Cactaceae) (Burret *et al.*, 1982) and as a glycoside from *Aegialitis annulata* (Plumbaginaceae) (Harborne, 1967), *Oenothera speciosa*, *O. tetragona* subsp. *glauca* (Onagraceae) (Howard *et al.*, 1972) and *Dacrycarpus dacrydioides* (Podocarpaceae) (Markham and Whitehouse, 1984). They are both reported for the first time in the Annonaceae where flavonoid glycosides and flavonol-3-*O*-methyl ethers are not very common (Leboeuf *et al.*, 1982). Furthermore, in contrast to most of the flavonoids isolated from the family, which lack B-ring substitution, **F-12** and **F-13** show a highly oxygenated B-ring. Their biosynthetic route is described in Scheme 3.20.



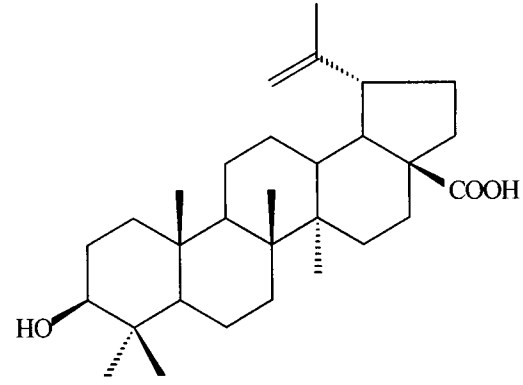
Scheme 3.25: Suggested biogenetic pathway for T-1 to T-3

Triterpenes



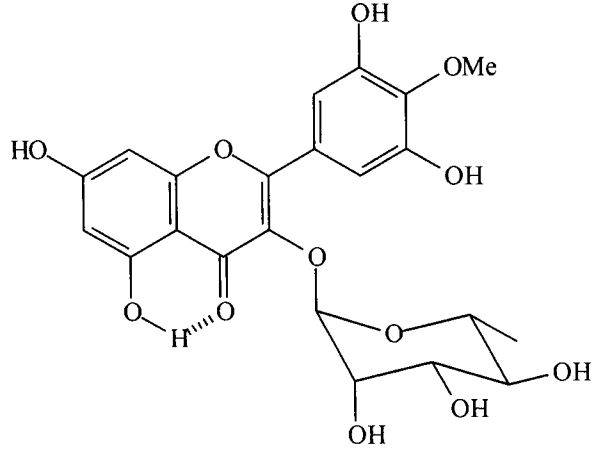
T-1 (R = ·····OH)

T-2 (R = =O)

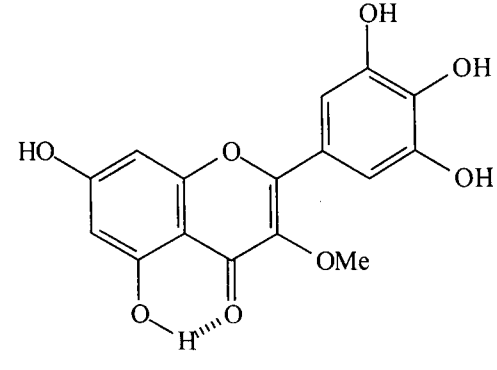


T-3

Flavonoids



F-12



F-13

Figure 3.52: Structures of the secondary metabolites isolated from the aerial parts of *Goniothalamus thwaitesii*

3.6.5 *Goniothalamus gardneri* Hook. f. & Thoms.

No records on the chemistry of this species were found. In this study the aerial parts, from Sri-Lanka, afforded chalcones [flavokawain A **(F-1)** and 2',4'-dihydroxy-4,6'-dimethoxychalcone **(F-2)**], dihydrochalcones [dihydroflavokawain A **(F-3)**, 2',4'-dihydroxy-4,6'-dimethoxydihydrochalcone **(F-4)** and 4,2',4'-trihydroxy-6'-methoxy-dihydrochalcone **(F-5)**], the chalcone dimer [2',4'-dihydroxy-4,6'-dimethoxy-chalcone dimer **(F-6)**], flavanones [naringenin trimethyl ether **(F-7)** and tsugafolin **(F-8)**] and the new acetogenin goniothalamusin **(M-5)** (Figure 3.54).

G. gardneri appears to produce predominantly flavonoids, encountered as aglycones of different types. Chalcones were isolated together with their corresponding dihydrochalcones and flavanones. Flavokawain A **(F-1)** has previously been reported in *Xanthorrhoea preissii* (Xanthorrhoeaceae) (Birch and Ryan, unpublished results-see Birch and Hextall, 1955), *Piper methysticum* (Piperaceae) (Hansel *et al.*, 1963) and *Dahlia tenuicaulis* (Compositae) (Lam and Wrang, 1975). Both 2',4'-dihydroxy-4,6'-dimethoxychalcone **(F-2)** and dihydroflavokawain A **(F-3)** have only been synthesised (Braz Filho *et al.*, 1980; Bhardwaj *et al.*, 1982) and thus are recorded as natural products for the first time.

2',4'-Dihydroxy-4,6'-dimethoxydihydrochalcone **(F-4)** is a common constituent of *Iryanthera* species of the related Myristicaceae (Braz Filho *et al.*, 1980; Garzon *et al.*, 1987; Conserva *et al.*, 1990a/b; Kawanishi *et al.*, 1990). 4,2',4'-Trihydroxy-6'-methoxydihydrochalcone **(F-5)** has previously been isolated from *Coptis japonica* var. *dissecta* (Ranunculaceae) (Mizuno *et al.*, 1987).

2',4'-Dihydroxy-4,6'-dimethoxychalcone dimer **(F-6)** is an artefact which could have been produced as a result of a photochemical reaction involving the chalcone **F-2** (Toda *et al.*, 1998).

Naringenin trimethyl ether **(F-7)** has been found in *Dahlia tenuicaulis* and *Dahlia lehmanni* (Compositae) (Kaufmann and Lam, 1967; Lam and Wrang, 1975)

while tsugafolin **(F-8)** has been reported from *Tsuga diversifolia* (Pinaceae) (Tanaka *et al.*, 1989). None of these flavonoids have previously been found in the Annonaceae. The biogenesis of the flavonoids isolated is described in Scheme 3.20.

Many acetogenins have been reported in the Annonaceae, predominantly in the genera *Annona*, *Asimina*, *Rollinia*, *Uvaria* and *Goniothalamus* (Zafra-polo *et al.*, 1998). Thus, the occurrence of goniothalamusin **(M-5)** in *G. gardneri* appears conform to what is already known on the chemistry of the genus. Goniothalamusin **(M-5)** is a new linear olefinic and acetylenic acetogenin with a C_{25} skeleton, lacking oxygenated substituents on its alkyl chain and with a saturated γ-hydroxymethyl-γ-lactone terminal ring. All these characteristics are of interest since linear acetogenins form only a small group among annonaceous acetogenins, which are mainly mono- and bistetrahydrofuran derivatives. Furthermore, among the linear acetogenins, very few are olefinic and/or acetylenic without hydroxyl groups on the alkyl chain (Zafra-polo *et al.*, 1998) (Figure 3.49; Table 3.56).

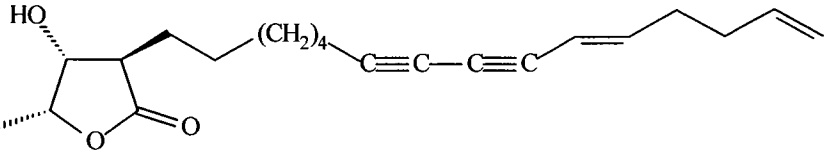
Goniothalamusin **(M-5)** is related to butyrolactone-1 **(181)** and butyro-lactone-2 **(182)**, two "non-classical" linear acetylenic acetogenins recently isolated from *Porcelia macrocarpa*, and which have a C_{25} chain instead of the usual C_{35}/C_{37} skeleton and a saturated β-hydroxy γ-methyl-γ-lactone ring. Butyrolactone-1 **(181)** with its acetylenic/olefinic chain is the closest related compound to goniothalamusin **(M-5)**. However in the case of the latter, the structure of a new type of γ-lactone moiety bearing an hydroxylated γ-methyl substituent was identified. Goniothalamusin **(M-5)** represents the third linear acetogenin encountered so far in the genus, along with donhexocin **(85)** and giganin **(86)**, but the only acetylenic and olefinic acetogenin without oxygenated substituents. Biogenetically **M-5** may derived from a C_{22} fatty acid unsaturated in C-13 after condensation with pyruvic acid (Etse and Waterman, 1986; Chaves and Roque, 1997) (Scheme 3.53).

It is also of interest to mention that neither styryl-lactones nor alkaloids were found in the species studied in contrast to what was common in *Goniothalamus*.

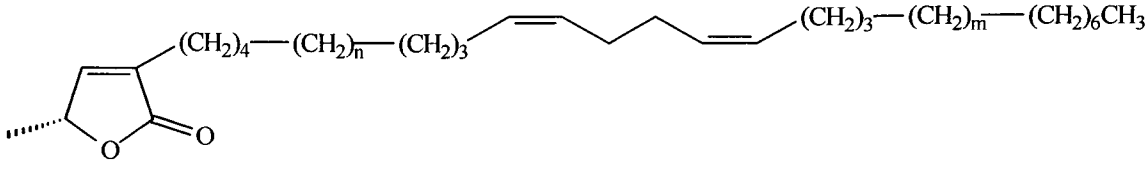
Table 3.56: Olefinic and/or acetylenic non hydroxylated linear acetogenins previously isolated in the Annonaceae

Isolated acetogenins	Species (references)
Sapranthin **(178)**	*Sapranthus palanga* (Etse and Waterman, 1986)*
Muridienin-1 **(179)**	*Annona muricata* (Gleye *et al.*, 1996)
Muridienin-2 **(180)**	*Annona muricata* (Gleye *et al.*, 1996)
Butyrolactone-1 **(181)**	*Porcelia macrocarpa* (Chaves and Roque, 1997)
Butyrolactone-2 **(182)**	*Porcelia macrocarpa* (Chaves and Roque, 1997)

* originally identified as an "acetylenic lactone".

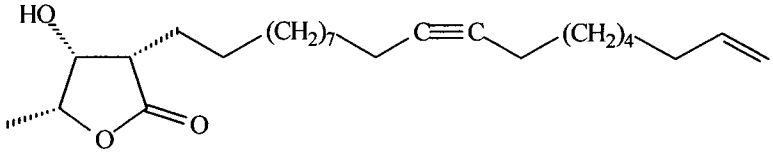


(178)

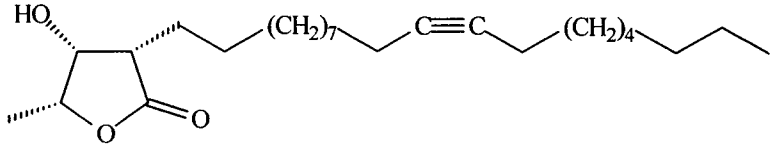


(179) n = 3, m = 4

(180) n = 5, m = 4

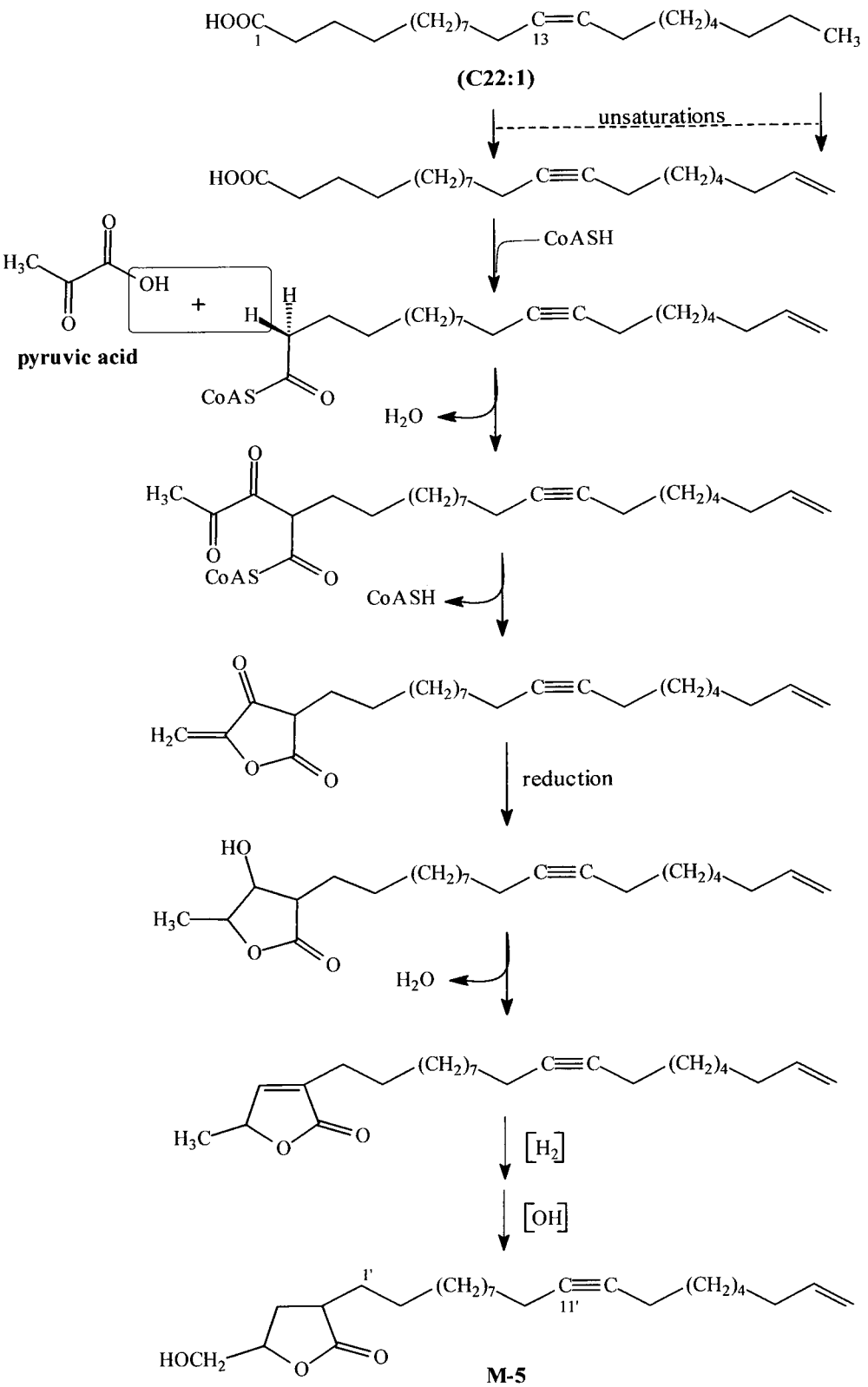


(181)



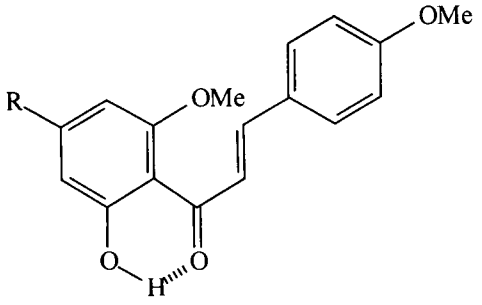
(182)

Figure 3.53: Structures of the olefinic and/or acetylenic non hydroxylated linear acetogenins in the Annonaceae

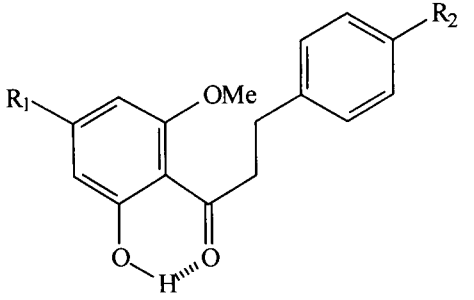


Scheme 3.26: Suggested biogenetic pathway for M-5

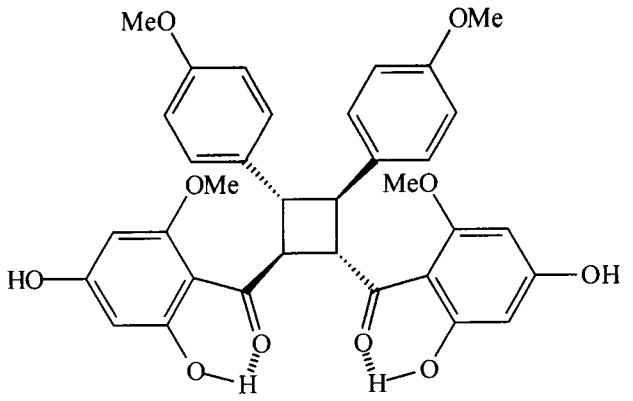
Flavonoids



F-1 (R = OMe)
F-2** (R = OH)

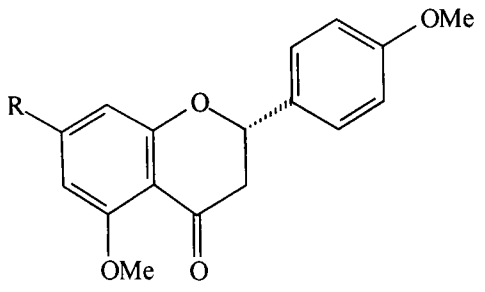


F-3** (R_1 = OMe, R_2 = OMe)
F-4 (R_1 = OH, R_2 = OMe)
F-5 (R_1 = OH, R_2 = OH)

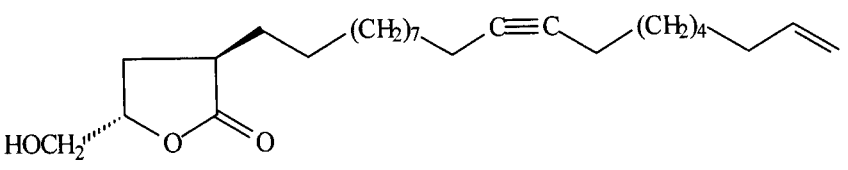


F-6*

Acetogenin



F-7 (R = OMe)
F-8 (R = OH)



M-5*

* New compounds / ** New natural products

Figure 3.54: Structures of the secondary metabolites isolated from the aerial parts of *Goniothalamus gardneri*

Chapter 4:

Pharmacological Results and Discussion

4.1 Bradykinin receptor affinity studies

4.1.1 Preliminary screenings

The crude detannified EtOH extracts of *Cleistopholis glauca* and *C. patens* were initially screened at a final concentration of 0.5 mg/ml. Specific binding values of 41.5 and 59.8% obtained respectively for the two species showed a potential bradykinin receptor affinity worthy of further investigation.

4.1.2 Bioassay-guided fractionation

4.1.2.1 Cleistopholis glauca

Most of the activity of the stem bark of *C. glauca* resided in the EtOAc extract (specific binding of 3.3%). This extract was subjected to VLC, eluting with petrol/EtOAc and EtOAc/MeOH mixtures of increasing polarity. Among fractions tested, activity was detected in the fractions eluted with:

i) 100% EtOAc (specific binding of 36.5%)

ii) 3% EtOAc in MeOH (specific binding of 57.3%)

iii) 20% EtOAc in MeOH (specific binding of 8.4%).

TLC examination of these fractions revealed the presence of six non UV-active greenish-brown spots on spraying with vanillin-H_2SO_4 reagent, followed by heating for a few minutes.

Purification of fraction (i) (4 spots) by flash chromatography, eluting with $CHCl_3$/EtOAc/MeOH (82:9:9), led to the isolation of CGE.22 (72.2 mg) identified as the oligosaccharide cleistetroside-6 **(OS-6)** (see Sections 2.4.4 & 3.1.1). Repeated attempts to separate the three other spots form this fraction did not succeed. However, two of them were found again in a VLC fraction from the petrol extract and were successfully separated by flash chromatography, eluting with $CHCl_3$/EtOAc/MeOH (85:7.5:7.5), to afford CGP.24 (293 mg) and CGP.25 (44.1 mg) identified respectively as cleistrioside-1 **(OS-4)** and cleistetroside-7 **(OS-8)**

(see Sections 2.4.4 & 3.1.1). The third spot present in fraction (i) was found again in fraction (ii).

Purification of fraction (ii) (2 spots) by flash chromatography, eluting with $CHCl_3$/EtOAc/MeOH (78:11:11), led to the isolation of CGE.23 (38 mg) also present in fraction (i) and identified as the oligosaccharide cleistetroside-3 **(OS-9)** (see Sections 2.4.4 & 3.1.1). The second spot was identified as a mixture of two oligosaccharides which could not be separated.

Purification of fraction (iii) (2 spots) by gel filtration, eluting with $CHCl_3$, led to the isolation of CGE.21 (335.7 mg) identified as the oligosaccharide cleistetroside-4 **(OS-10)** (see Sections 2.4.4 & 3.1.1). The second spot was identified as the mixture of oligosaccharides described above.

4.1.2.2 Cleistopholis patens

Most of the activity of the leaves of *C. patens* resided in the *n*-hexane (specific binding of 42.6%) and EtOAc extracts (specific binding of 27.2%). The EtOAc extract was subjected to VLC, eluting with petrol/EtOAc and EtOAc/MeOH mixtures of increasing polarity. Among fractions tested, activity was detected in the fractions eluted with:

i) 65% EtOAc in petrol (specific binding of 58.7%)

ii) 85% EtOAc in petrol (specific binding of 27.8%)

iii) 90% EtOAc in petrol (specific binding of 25.3%)

iv) 2% MeOH in EtOAc (specific binding of 9.5%)

v) 20% MeOH in EtOAc (specific binding of 34.6%).

TLC examination of these fractions revealed the presence of again six non UV-active greenish-brown spots on spraying with vanillin-H_2SO_4 reagent, followed by heating for a few minutes.

Purification of fraction (i) (2 spots) by gel filtration, eluting with $CHCl_3$, followed by flash chromatography, eluting with $CHCl_3$/EtOAc/MeOH (91:4.5:4.5), led to the isolation of CPE.6 (158.6 mg) and CPE.7 (40.6 mg) identified as the oligosaccharides cleistrioside-2 **(OS-1)** and cleistrioside-3 **(OS-2)** (see Sections 2.4.4 & 3.1.1).

Purification of fraction (ii) (3 spots) by gel filtration, eluting with $CHCl_3$, followed by flash chromatography, eluting with $CHCl_3$/EtOAc/MeOH (85:7.5:7.5), led to the isolation of CPE.8 (36.3 mg) identified as the oligosaccharide cleistrioside-4 **(OS-3)** (see Sections 2.4.4 & 3.1.1). The remaining spots were found again in fraction (iii).

Purification of fraction (iii) (2 spots) by gel filtration, eluting with $CHCl_3$, followed by flash chromatography, eluting with $CHCl_3$/EtOAc/MeOH (83:8.5:8.5), led to the isolation of CPE.9 (87.2 mg) identified as the oligosaccharide cleistetroside-6 **(OS-6)** (see Sections 2.4.4 & 3.1.1). The second spot was found again in fraction (iv).

Purification of fraction (iv) (1 spot) by gel filtration, eluting with $CHCl_3$, led to the isolation of CPE.1 (2.5 g) identified as the oligosaccharide cleistetroside-1 **(OS-5)** (see Sections 2.4.4 & 3.1.1).

Purification of fraction (v) (2 spots) by gel filtration, eluting with $CHCl_3$, followed by flash chromatography, eluting with $CHCl_3$/MeOH (9:1), led to the isolation of CPE.4 (224.5 mg) identified as the oligosaccharide cleistetroside-2 **(OS-7)** (see Sections 2.4.4 & 3.1.1). The second spot was identified as CPE.1 **(OS-5)**.

All oligosaccharides isolated were found again in the *n*-hexane extract.

4.1.3 Results and Discussion

Preliminary results showed that extracts of *Cleistopholis,* tested at a final concentration of 0.5 mg/ml, had the ability to lower the specific binding of radiolabelled bradykinin to its B_2 receptors. This was rationalised by compounds present in each extract inhibiting specific binding of the ligand. After fractionation, low values of specific binding were found in fractions which contained only oligosaccharides. This suggested that these compounds were responsible for the inhibition of specific binding.

Competitive binding studies were carried out for some of the pure oligosaccharides at different concentrations (see Section 2.6.3). This revealed in most cases IC_{50} values associated with a high error. One reasonable inhibition curve was obtained for compound **OS-7** which showed an IC_{50} of 64.1μM $\pm$ 23 (Figure 4.1). This was considered of low binding affinity compared to non-peptide and peptide compounds of synthetic origin which have already been developed (see Section 1.7.1.2). Furthermore, in view of the errors associated with the remaining oligosaccharides, it was recognised that this value may not be reliable.

4.2 Further work

The *in vitro* binding assay performed was only a preliminary screen for the search of potential BK antagonists. Further work to evaluate the binding affinity of the compounds isolated should be directed again by using a method which affords reliable and reproducible IC_{50} values. On the basis of results obtained further experiments could include:

i) conducting secondary screens on isolated tissues to study antagonistic/agonists effects.

ii) modifying the structures of the compounds to study structure activity relationships in the case of antagonistic properties.

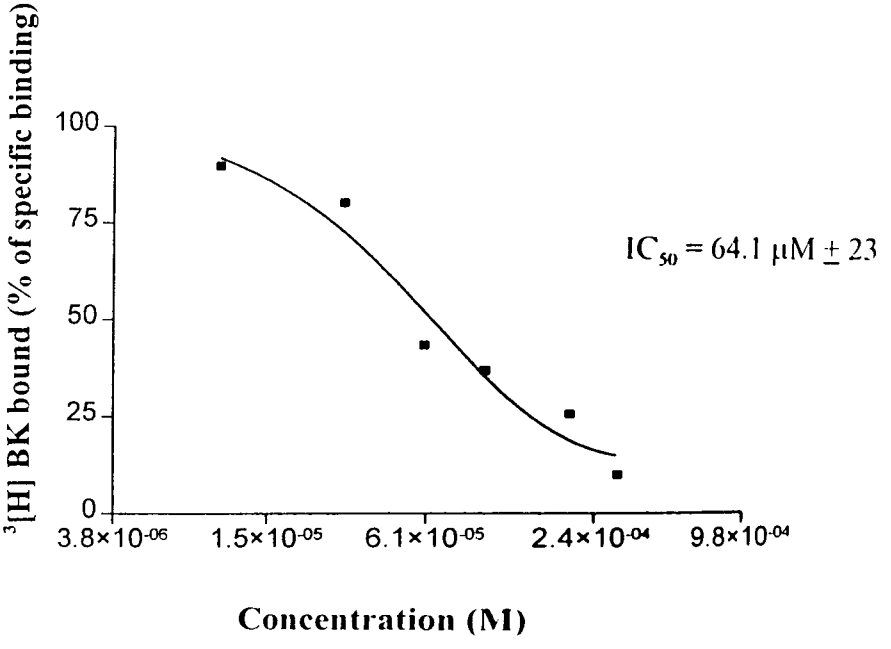


Figure 4.1: Competition binding curve of ^{3}[H] BK to B_2 receptors of the guinea-pig ileum in the presence of OS-7

Publications/Communications

1. Seidel, V., Bailleul, F., Waterman, P. G. (1998). *Two terpenes and a flavonol glycoside from Piptostigma fasciculata. Biochem. Syst. Ecol.*, in the press.

2. Seidel, V., Bailleul, F., Waterman, P. G. (1998). *Partially acetylated tri- and tetrarhamnoside dodecanyl ether derivatives from Cleistopholis patens. Phytochemistry,* submitted.

3. Seidel, V., Bailleul, F., Waterman, P. G. *Partially acetylated tri- and tetrarhamnoside derivatives from two species of the genus Cleistopholis (Annonaceae).* A short lecture given at the Young Scientists PSE Symposium, Future trends in Phytochemistry, Rolduc, Kerkrade, The Netherlands, 12th May 1998.

4. Seidel, V., Bailleul, F., Waterman, P. G. *Partially acetylated tri- and tetrarhamnoside derivatives from two species of the genus Cleistopholis (Annonaceae).* An oral presentation given at the Research Seminar, University of Strathclyde, Glasgow, 25th March 1998.

5. Seidel, V., Bailleul, F., Waterman, P. G. *Phytochemical investigation of Cleistopholis glauca (Annonaceae).* A poster and an oral presentation given at the Research Day, University of Strathclyde, Glasgow, 7th May 1997.

Under preparation

6. Seidel, V., Bailleul, F., Waterman, P. G. *Chemistry in the Annonaceae: Novel constituents from the stem bark of Cleistopholis glauca.*

7. Seidel, V., Bailleul, F., Waterman, P. G. *Flavonoids from Goniothalamus gardneri and Goniothalamus thwaitesii.*

8. Seidel, V., Bailleul, F., Waterman, P. G. *Goniothalamusin, a new linear acetogenin from Goniothalamus gardneri.*

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