SYNTHESIS OF STEROIDAL ANALOGUES OF PROSTACYCLIN

Thesis presented by

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Abstract

This body of work is concerned with the development of synthetic routes to steroidal analogues of prostacyclin.

Chapter one, the introduction, gives a concise historical account of prostaglandins beginning with their discovery in the 1930's to their biosynthesis from fatty acid precursors and structure elucidation in the 1960's.

The major routes of metabolic inactivation of prostaglandins are discussed.

Prostacyclin, the most recent addition to the family of prostaglandins, is introduced and the chemical and metabolic instability of this prostanoid highlighted.

The particular selection of reported analogues of prostacyclin is presented in an attempt to illustrate how structural modification of prostacyclin has led to congeners with improved hydrolytic and metabolic stability profiles. Where structural change has also resulted in an improvement in potency and/or selectivity of action attention is drawn to this.

The increased complexity in structures of the biologically active prostacyclin analogues is a feature of this selection which culminates in tetracyclic non-steroidal analogues.

The synthesis of steroidal analogues of prostaglandins containing an intact steroidal nucleus is reviewed.

The concept of steroidal prostacyclin analogues, the central trust of this research, is introduced and the analogy explained by reference to three classes, type I-III, of proposed steroidal prostacyclin analogues.

Chapter two, the discussion, highlights the problems of the original synthetic approach to 5α-androstan-16-one (**56**) and details the alternative synthetic strategies pursued in an attempt to obtain this essential ketone (**56**) in acceptable yield.

Ketalisation of (**56**) afforded the fungal substrate which is dihydroxylated by *Calonectria decora* and hydrolysed to give 6α,12β-dihydroxy-5α-androstan-16-one (**54**). This microbiologically derived product occupies a pivotal position in the synthesis of the steroidal prostacyclin analogues.

The use of the keto-steroid (**56**) as a model for establishing the amenabilty of the C-16 position to the Wittig olefination is discussed and the rationale behind the exploration of various strategies for introducing C-16 alkylidene and substituted alkylidene side chains into (**56**) is discussed. Once reaction conditions were established, the microbiologically derived dihydroxy ketone (**54**) was transformed to the type III steroidal prostacyclin analogue, 16(4-carboxybutylidene)-5α-androstan-6α,12β-diol (**129**). Derivatisation of (**129**) to the methyl ester (**130**) is followed by nonselective oxidation to afford the corresponding 6,12-dione ester (**132**). Selective reduction of (**132**) gave to the corresponding 12-ol-6-one (**133**).

Although constraints of time did not allow the fulfilment of all research objectives valuable ground work has been laid to support future research.

An outline of the synthetic routes to other proposed steroidal prostacyclin analogues is briefly discussed.

This chapter also includes a brief report on the results of preliminary biological studies on analogue (**129**), which has been shown to have similar potency to prostacyclin as an inhibitor of collagen-induced platelet aggregation in human and pig whole blood.

A discussion of proposed development work leading to a series of type III steroidal analogues based on lead compound (**129**) closes this chapter.

Chapter three catalogues the experimental procedures and the appendix contains the details of the pharmacological evaluation of analogue (**129**).

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Dr. T.M. Healey for his advice, encouragement and unstinting support throughout the course of my work.

I am grateful to Professor D.N. Kirk, Honorary Curator of the Steroid Reference Collection, Chemistry Department, Queen Mary College, University of London, for the supply of an authentic sample of 6α,12β-dihydroxy-5α-androstan-16-one.

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To Candice and Gary

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1.0 Introduction

1.1 Discovery of eicosanoids

The scientific origins of eicosanoids can be traced back to the 1930's. The observation in 1930 by Kurzrok and Lieb that human semen induced contraction or relaxation of the human uterus[1] marked the discovery of eicosanoids.

A short time later, Goldblatt in England and von Euler in Sweden extended this work. They showed that the active substance in extracts from sheep vesicular glands and human seminal fluid was also a vasodepressant and could stimulate various smooth muscle preparations [2,3].

During the course of purification it became clear that the active principle was freely soluble in lipid solvents and appeared to have the properties of an unsaturated hydroxylic fatty acid. Believing that the new factor was produced in the prostate, von Euler named it prostaglandin [4,5].

Further research on prostaglandin proceeded at a slow pace. Confirmation of its fatty acid nature was achieved by Bergstrom[6] in 1949. In 1957 Bergstrom and Sjovall succeeded in isolating a crystalline prostaglandin factor from freeze-dried sheep prostate gland[7]. The hint of the presence of at least one other active acidic substance in the extracts of sheep prostate glands was soon confirmed by the isolation of a second crystalline lipid-soluble factor with pharmacological activity on both intestinal strips and rabbit blood pressure [8,9].

Later, additional compounds having similar biological activities were isolated and it soon became apparent that prostaglandin was not a single substance but instead a family of closely related compounds.

1.2 Structure elucidation of prostaglandins.

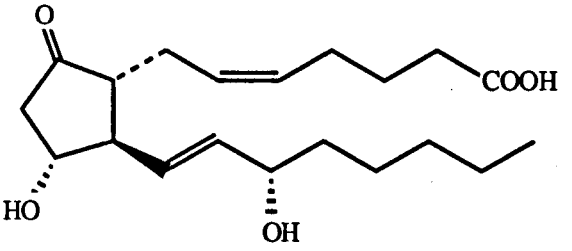
The structure of prostaglandins was elucidated by Bergstrom and co-workers in a series of remarkable investigations which culminated in the delineation of the structure of the whole family of new prostaglandin substances by 1966.

Bergstrom relied mainly upon modern instrumental and ultramicroanalytical techniques during his investigations. The prostaglandins and their derivatives were degraded by chemical methods and their products separated by gas chromatography. Mass spectroscopy was extensively used both on the parent compounds and their degradation products for obtaining structural information [10-19].

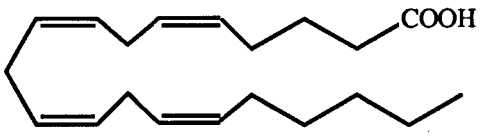
The technique of X-ray crystallography was employed to finally confirm the structure of prostaglandins and to determine their absolute configurations and conformations [20-29]. These studies showed that, in the crystalline state, a conformational characteristic of active prostaglandins is the hairpin alignment of the carboxyl (C-1 through C-7) and the ω (C-13 through C-20) side chains.

1.3 Precursors of eicosanoids.

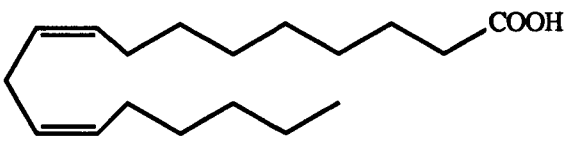
The knowledge of the structure of prostaglandins led to the recognition of their kinship with the essential fatty acids and in 1964 the hypothesis that essential fatty acids could serve as precursors of prostaglandins was substantiated when Bergstrom and co-workers and van Dorp and associates independently achieved the biosynthesis of prostaglandin E_2 [PGE_2 (**1**)] from arachidonic acid (**2**) [30,31].



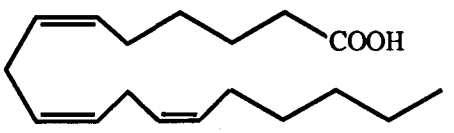
PGE_2 (**1**)



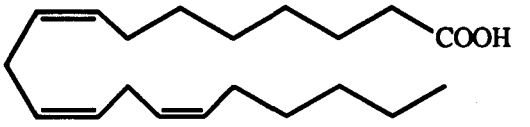
Arachidonic acid (2)



Linoleic acid (3)

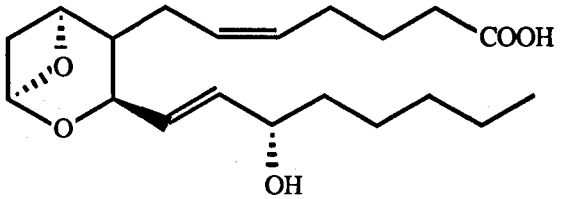


γ-Linolenic acid (4)

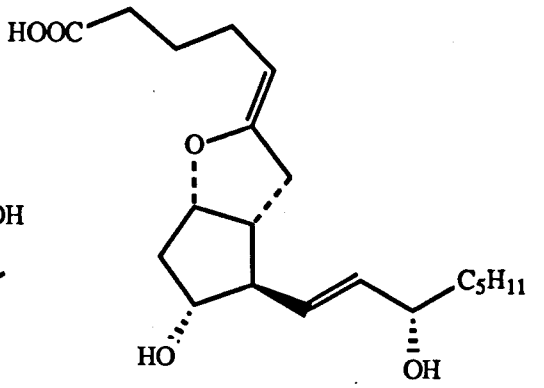


Dihomo-γ-linolenic acid (5)

Related experiments, performed earlier, established that arachidonic acid (2) is biosynthesised from the essential fatty acid, linoleic acid (3). This 18-carbon fatty acid is converted by mammals, *via* γ-linolenic acid (4) to dihomo-γ-linolenic acid (5) and arachidonic acid (2) by chain elongation and desaturation [32-34]. Although a number of 20-carbon polyunsaturated fatty acids can be bioconverted to prostaglandins, arachidonic acid (2) appears to be utilised most frequently, *in vivo*, presumably because of its greater abundance.

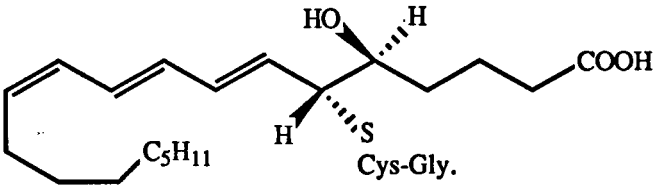


Thromboxane A_2 (6)



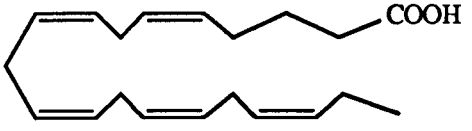
Prostacyclin (7)

With the discovery of thromboxane A_2 [TxA_2 (**6**)] [35], prostacyclin [PGI_2 (**7**)] [36], and the leukotrienes [37], for example leukotriene D_4 [LTD_4 (**8**)], came



Leukotriene D_4 (**8**)

the realisation that the classically known prostaglandins constitute only a fraction of the physiologically active products of arachidonate metabolism. The families of thromboxanes, leukotrienes, and related compounds are collectively called eicosanoids because they are derived from 20-carbon essential fatty acids that contain three, four, or five double bonds; 8,11,14-eicosatrienoic acid [dihomo-γ-linolenic acid (**5**)], 5,8,11,14-eicosatetraeonic acid [arachidonic acid (**2**)] and 5,8,11,14,17-eicosapentaenoic [timnodonic acid (**9**)] [38-45].



Timnodonic acid (**9**)

In man arachidonic acid (**2**) is the most abundant of the eicosanoid precursors and it is biosynthetically derived from dietary linoleic acid [9,12-octadecadienoic acid (**3**)] or is ingested as a dietary constituent.

Arachidonic acid (**2**) serves as a substrate for several enzyme systems, including cyclo-oxygenase or one of the several lipoxygenases, which rapidly metabolise arachidonic acid to oxygenated products[46]. Among the metabolites of the cyclo-oxygenase pathway are thromboxane A_2 (**6**) and prostacyclin (**7**) which are formed in blood platelets and vascular endothelial cells respectively [35,36].

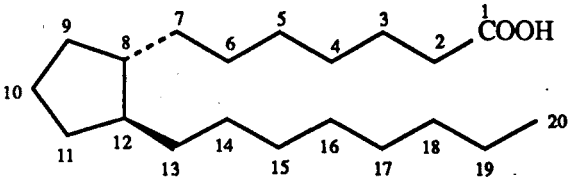
The pharmacodynamic effects of these two eicosanoids are complementary. Thromboxane A_2 (**6**) is a powerful platelet aggregant and vasoconstrictor and conversely prostacyclin (**7**) is a powerful platelet antiaggregant and vasodilator [35,47,48]. Thus PGI_2 (**7**) and TxA_2 (**6**) represent, in biological terms, the opposite poles of the general homeostatic mechanism for regulation of platelet aggregability, *in vivo*. Physiologically, this is significant because, unless a subtle balance between the production of these two substances is maintained pathophysiological states may result. A decrease in the formation of TxA_2 (**6**) should lead to an increased bleeding time and inhibition of thrombus formation, whereas, inhibition or loss of prostacyclin (**7**) formation should be propitious for a prothrombotic state.

The metabolites of arachidonic acid in the 5-lipoxygenase pathway are called leukotrienes. These substances have been implicated in a number of inflammatory processes and it is now generally accepted that leukotriene D_4 (**8**) is part of the mixture known as the slow-reacting substance of anaphylaxis (SRS-A)[37,49].

1.4 Definition, structure, and nomenclature of prostaglandins.

Prostaglandins are oxygenated derivatives of 20-carbon fatty acids, principally arachidonic acid (**2**) [50].

Chemically, the prostaglandins may be regarded as substituted derivatives of prostanoic acid (**10**) [51], and are classified according to the substitution pattern on the cyclopentane ring.



Prostanoic acid (**10**)

Currently ten types, designated A-J, are known (*see* structures in Figure 1). Prostaglandins E_2 (**1**) and $F_{2\alpha}$ (**15**), the first to be discovered were so named because they partitioned into ether and phosphate buffer (Swedish fosfat) respectively[52], whereas prostaglandins A_2 (**11**) and B_2 (**12**) were so named because they can be produced by treatment of PGE_2 (**1**) with acid and base respectively [53]. Prostaglandin G_2 (**16**) and H_2 (**17**) are exceptional in that they both have the same endoperoxide ring structure, differing only at C-15, having hydroperoxy and hydroxy groups respectively.

Prostaglandins are classified into three series according to the number of double bonds in the side chains. The position and number of sites of unsaturation are indicated by a numerical subscript in the name of the prostaglandin (for example PGE_2) and also reflects the fatty acid precursor. Thus the 1-series have a *trans*-13-double bond and are derived from 8, 11, 14-eicosatrienoic acid (**5**), the 2-series *cis*-5 and *trans*-13-double bonds and are derived from arachidonic acid (**2**),and the 3-series *cis*-5, *trans*-13 and *cis*-17-double bonds and are derived from 5, 8, 11, 14, 17-eicosapentaenoic acid (**9**) [46].

The E-Z system [54], which is based on the priorities of groups in the Cahn-Ingold-Prelog convention is also used to describe the stereochemistry of these double bonds.

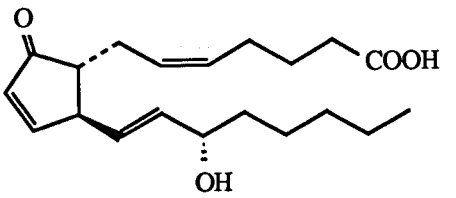
The stereochemistry of the substituents on the 5-membered cyclopentane ring is designated β or α depending on whether the substituent is above or below the plane of the ring. Thus, the carboxylic acid side chain at C-8 is directed α, while the ω chain is oriented β and the two side chains are *trans* to each other.

Prostaglandins of the E-series are characterised by the presence of a carbonyl group at the 9-position and differ in this respect form the F-series which contain a hydroxy group at the C-9 position instead of a carbonyl. The inclusion of a Greek symbol in the name of the prostaglandin [for example $PGF_{2\alpha}$ (**15**)] indicates

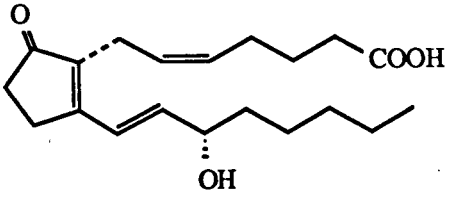
the stereochemistry of the C-9 hydroxy group. Thus the C-9 hydroxy group in the F-prostaglandins is in the α-configuration.

To avoid confusion, the absolute configurations of the asymmetric centres in the side chains at C-8 and C-12 are designated as R or S according to the Cahn-Ingold-Prelog convention [55]. Thus the C-15 hydroxy group of natural prostaglandins is therefore assigned the S-configuration.

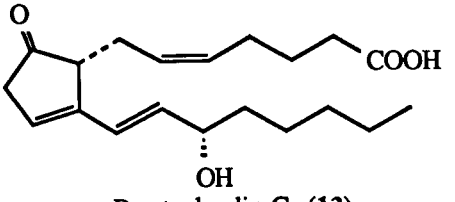
In application of the above stereochemical considerations, the systematic chemical name for, as an example, prostaglandin $F_{2\alpha}$ **(15)** is 9α,11α,15(S)-trihydroxy-5Z,13E-prostadienoic acid.



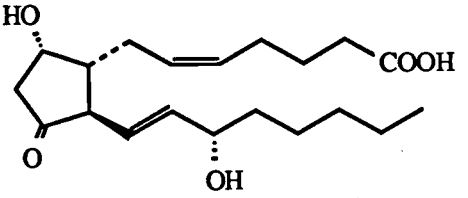
Prostaglandin A_2 (11)



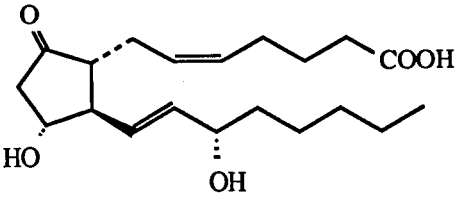
Prostaglandin B_2 (12)



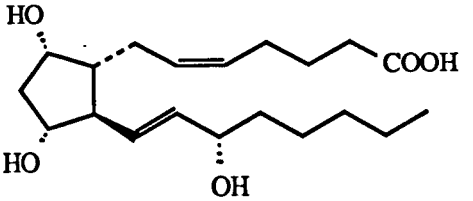
Prostaglandin C_2 (13)



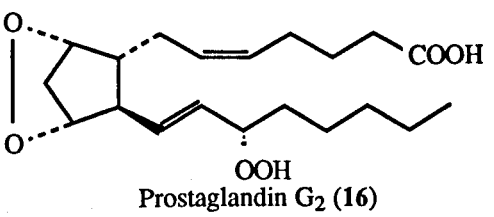
Prostaglandin D_2 (14)



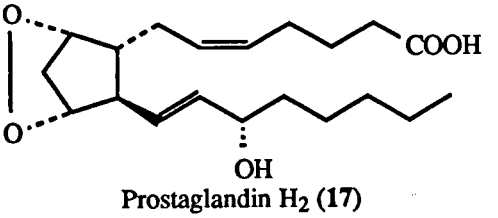
Prostaglandin E_2 (1)



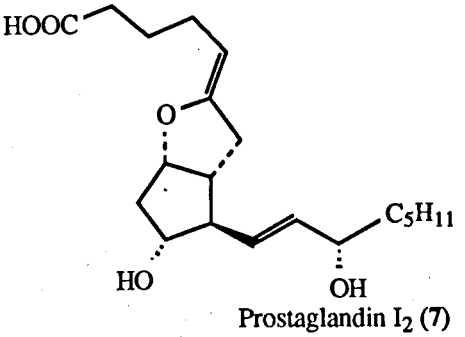
Prostaglandin $F_{2\alpha}$ (15)



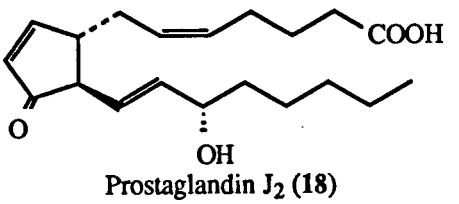
Prostaglandin G_2 (16)



Prostaglandin H_2 (17)



Prostaglandin I_2 (7)



Prostaglandin J_2 (18)

Figure 1. Structures of prostaglandins.

1.5 Biosynthesis and metabolism of prostaglandins.

The biosynthesis of prostaglandins from essential fatty acids constituents of membrane phospholipids is well documented [31]. The prostaglandin endoperoxide, PGH_2 (**17**), occupies a pivotal position in the biosynthetic pathway leading to the other prostaglandins and thromboxanes (*see* Figure 2).

The short duration of action of prostaglandins reflects their rapid metabolic inactivation[56]. The enzymic breakdown consists of three major modes of attack. Occuring most readily is the dehydrogenation at C-15 followed by reduction of the Δ^{13}-double bond to give the corresponding 13,14-dihydro-15-oxo-product. These two enzymic transformations are so rapid that a single pass of the prostaglandin through the lungs converts more than 90% of it to this essentially inactive metabolite. The lungs are a rich source of both 15-hydroxyprostaglandin dehydrogenase and the Δ^{13}-reductase enzyme and because of the unique position of the lungs, between the venous and arterial circulation, the pulmonary circulation constitutes an important barrier through which many prostaglandins which have potent physiological actions cannot normally pass intact and are thus prevented from reaching target organs *via* the arterial circulation.

A notable exception in this respect is prostaglandin I_2 (**7**) which is not inactivated on passage through the pulmonary circulation [57]. Indeed, the lungs continuously generate PGI_2 (**7**) and release it into the circulation [58].

Also occuring rapidly is β-oxidation of the carboxylic acid side chain. This process removes sequentially C-1, C-2 and C-3, C-4 of the side chain in the classical manner of β-oxidation. The tetranor metabolites are the primary products of this sequence although dinor metabolites also may be isolated.

The final important metabolic change occurs at the C-20 or C-19 position to give a C-20 carboxylic acid or a C-19 hydroxy metabolite respectively. Major

metabolites of PGE_2 or $PGF_{2\alpha}$ in humans are the corresponding 2,3,4,5-tetranor-13,14-dihydro-15-oxo-20-carboxylic acids arising from these oxidative changes.

Knowledge of these metabolic pathways of inactivation of prostaglandins has proved extremely valuable for the development of the synthesis of prostaglandin analogues.

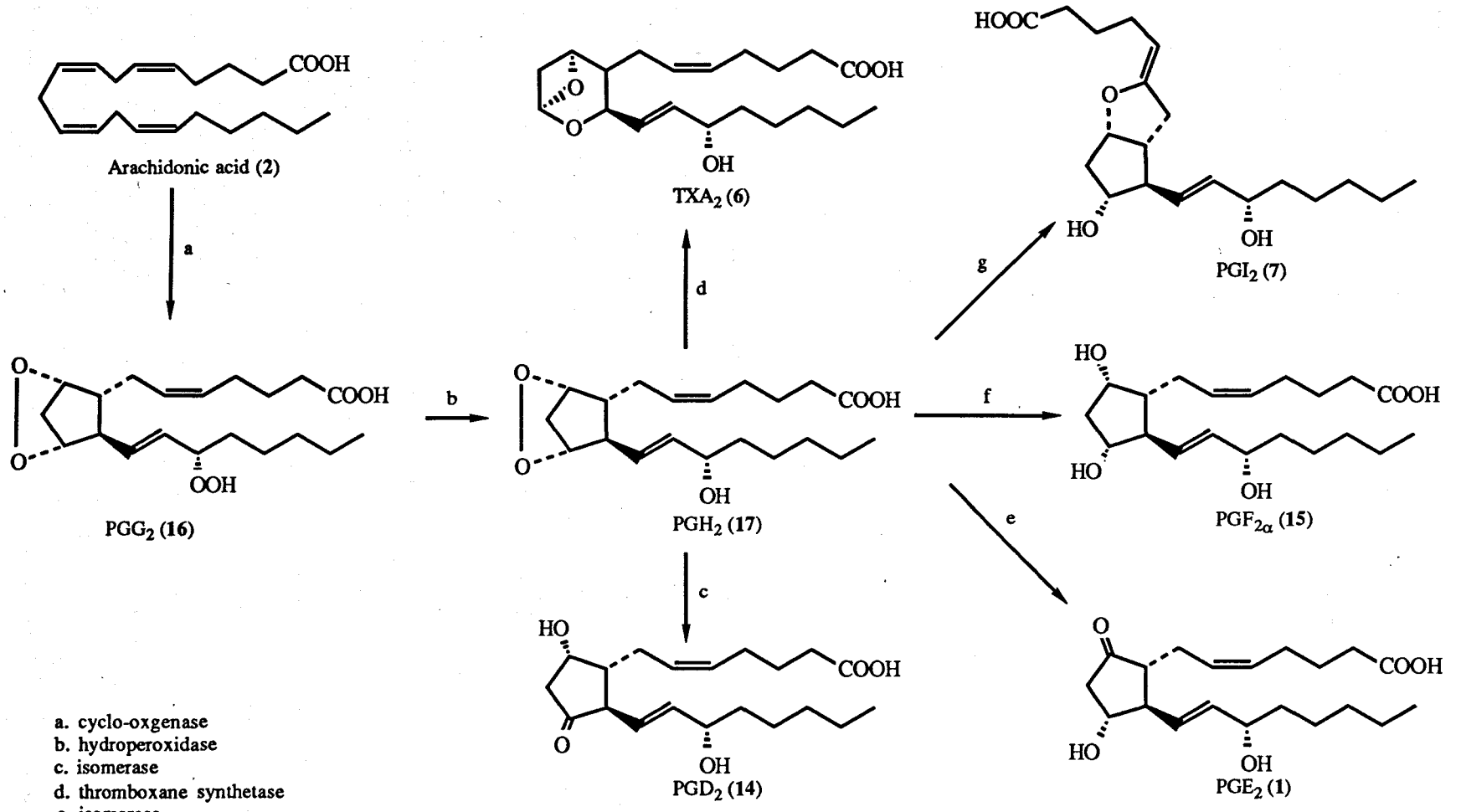
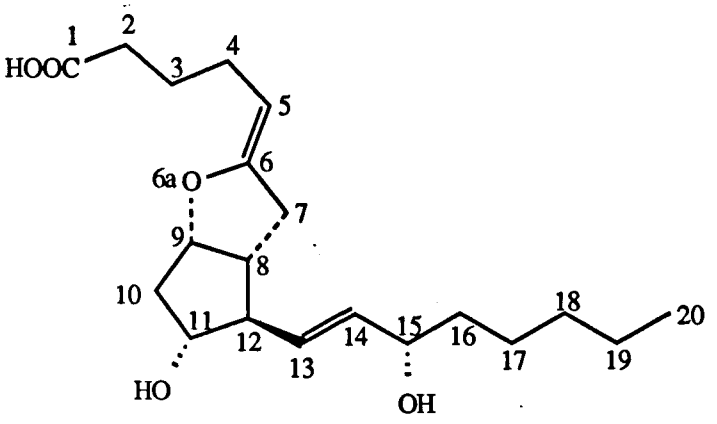


Figure 2. Biosynthesis of prostaglandins.

1.6 Prostacyclin.

Prostacyclin (7), a metabolite of arachidonic acid (2) via the PG endoperoxide PGH_2 (17) is the most recently discovered addition to the prostaglandin family [36].



Prostaglandin I_2 (7)

Although the mechanism of its biosynthesis is not clearly understood the final ring-closure step in its formation is thought to involve initial polarisation of the endoperoxide bridge in such a manner that the oxygen atom attached to C-9 acquires a partial positive charge followed by opening of the endoperoxide bridge as a result of nucleophilic attack by the Δ^5-double bond to form PGI_2 (7) [59] [*see* Figure 3].

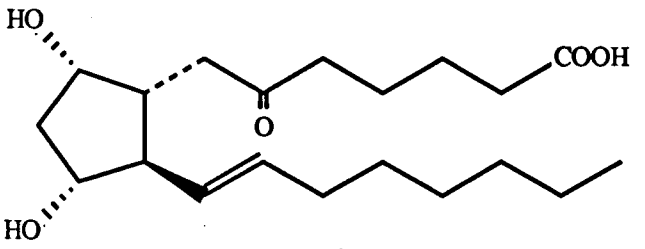
Prostacyclin (7) is a potent vasodilator and inhibitor of platelet aggregation which is produced by the vascular endothelium [36,47,48]. This substance (7) has also been shown to inhibit gastric acid secretion and to exert a cytoprotective role in the gastric mucosa. This ability to protect the gastrointestinal mucosa against injury from a variety of noxious agents is independent of its gastric anti-secretory activity. PGI_2 also stimulates fibrinolysis [60,61].

Due to these pharmacological properties of prostacyclin (7) speculations have been made as to the potential therapeutic areas in which this prostaglandin (7) might be used. These include gastric ulceration, myocardial infarction, peripheral

vascular diseases, organ transplantation, charcoal haemoperfusion, renal dialysis, and cardiopulmonary by-pass procedures; in short, in situations in which platelet activation has been suggested to occur [61,62,63].

However, shortly after the discovery of prostacyclin (**7**), it was soon realised that it did not represent an ideal therapeutic agent since its use in humans is severely limited by cardiovascular effects[64] and the compound is unstable and orally inactive.

Failure to achieve the full therapeutic potential envisioned for PGI_2 (**7**) has been due, in part, to its inherent chemical instability. The labile enol-ether function of prostacyclin is rapidly hydrolysed at physiological pH converting it to the biologically inactive compound, 6-oxo-$PGF_{1\alpha}$ (**19**).



(19)

The half-life of PGI_2 (**7**) at physiological pH is 1.6 minutes[65] (*in vitro*) at 37 °C. One might think that the instability of PGI_2 (**7**) would have prevented its clinical application. However, this property is being used to advantage in one of the first medical applications proposed for this substance. The anti-aggregatory properties of PGI_2 are beneficial in preserving platelet numbers and preventing the formation of potentially dangerous microaggregates during extracorporeal procedures[66] where the continued infusion of PGI_2 (**7**) compensates for its short half-life. The short half-life of PGI_2 (**7**) also permits prompt resumption of aggregatory properties once the infusion of the compound (**7**) is discontinued.

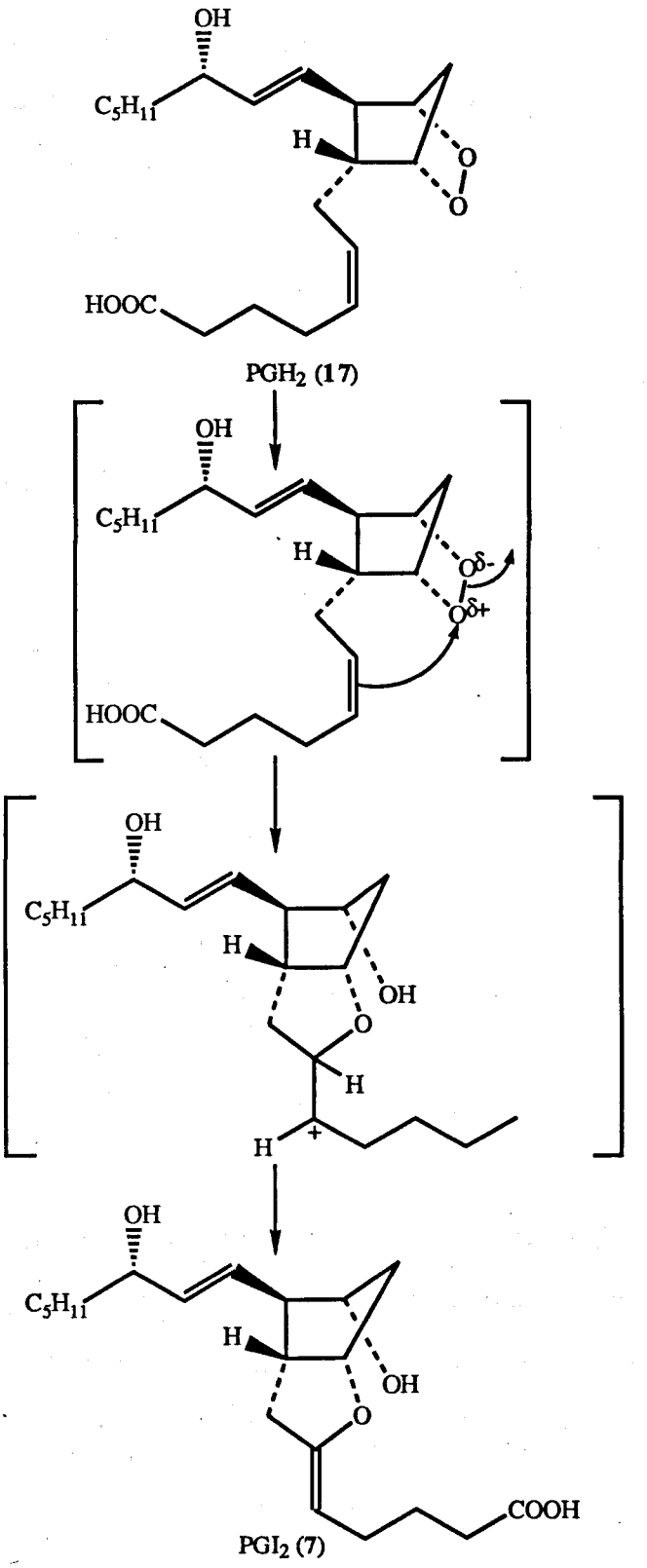
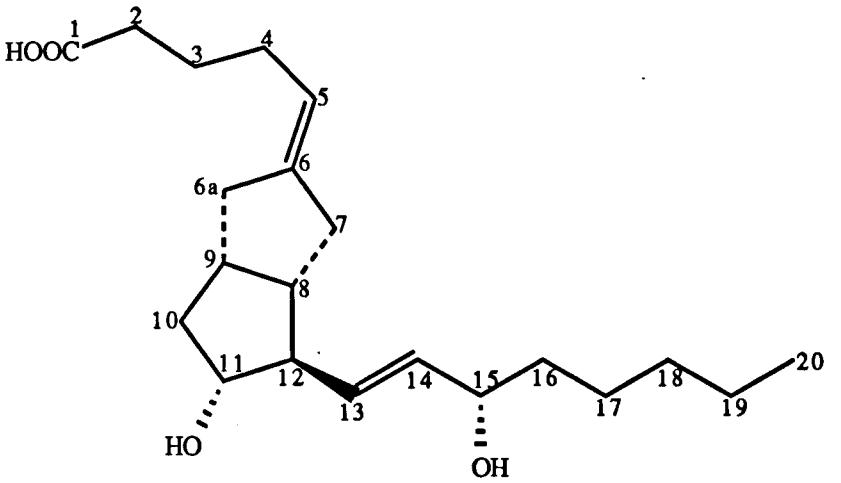


Figure 3. Proposed mechanism of final ring-closure step in prostacyclin biosynthesis.

1.7 Reported prostacyclin analogues.

The therapeutic potential of prostacyclin (**7**) coupled with its short biological half-life, as a result of the chemical instability of the molecule, has led to intensive research aimed at producing analogues with improved hydrolytic stability profiles. Much of the synthetic activity in this area has centred on the stabilisation of, or the replacement of, the labile enol-ether linkage by more stable chemical entities. Analogues in which the 6a-oxygen in (**7**) has been replaced by other heteroatoms (N, S) have been synthesised [67,68]. Both azaprostacyclin and thiaprostacyclin were more stable to hydrolysis and retained biological profiles similar to prostacyclin (**7**).

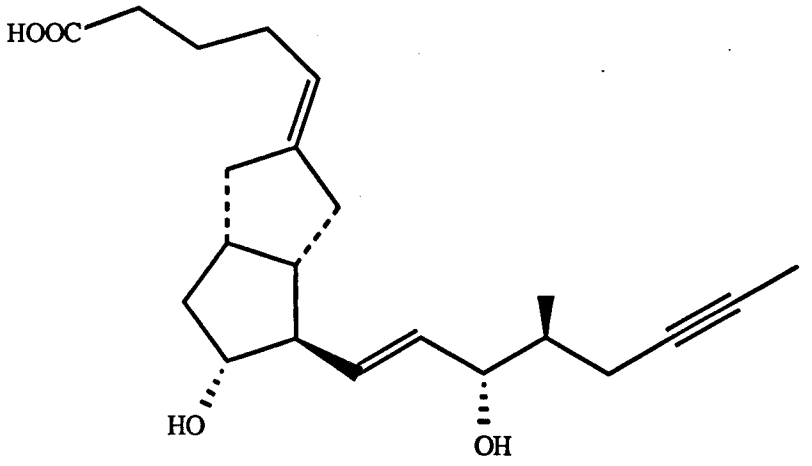


(20)

The synthesis of the carbon analogue, 5-(E)-6a-carba-PGI_2 [carbacyclin (**20**)] overcame the problem of the hydrolytic instability of the parent compound (**7**). Although this analogue (**20**) has retained the platelet anti-aggregatory property of prostacyclin (**7**) the potency has been markedly reduced [0.03 times the activity of (**7**)][69, 70].

Despite the improved chemical stability of these prostacyclin analogues their biological half-lives were still of short duration . Rapid metabolic inactivation occurred *via* the classical routes of prostaglandin catabolism; C-15 hydroxyl oxidation, reduction of the Δ^{13}-double bond and β-oxidation of the carboxyl side chain.

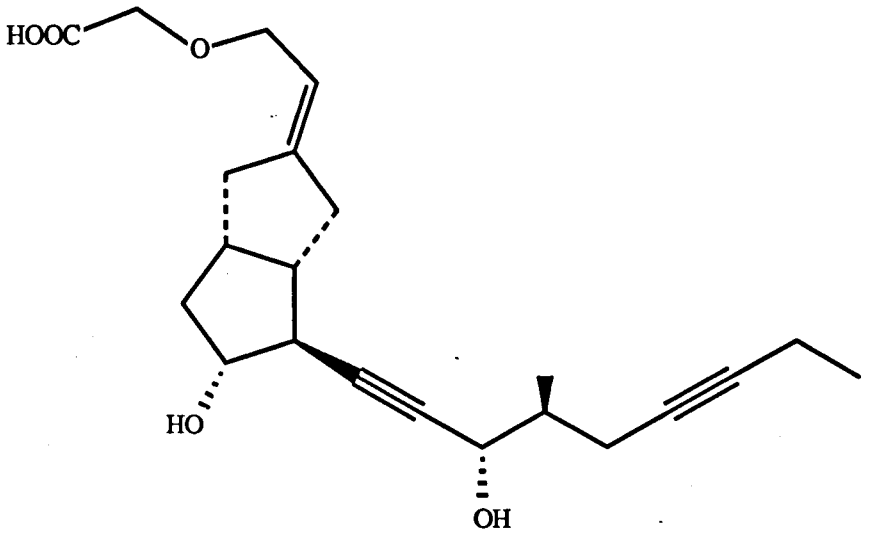
The emphasis of the chemical effort was then directed to improving the metabolic stability of these chemically stable PGI_2 (**7**) analogues. Structural modification of the carboxyl and ω side chains of these analogues, at or near the sites of enzymic metabolic attack, was behind the philosophy of enhancing metabolic stability .



Iloprost (**21**)

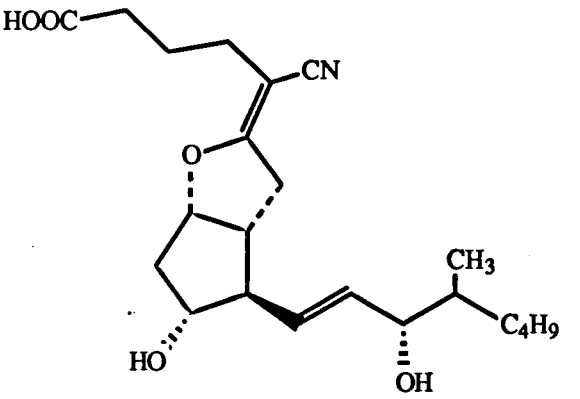
In addition to the replacement of the 6a-oxygen atom of (**7**) by a methylene group, the introduction of a C-16 methyl and a C-18, C-19 alkynyl groups afforded iloprost (**21**) [71]. The ω chain modifications achieved the intended retardation of enzymic C-15 hydroxyl oxidation and other oxidative changes at C-19 and C-20. Iloprost (**21**) is the first reported example of a stable analogue of similar potency to prostacyclin (**7**). In contrast to the natural PGI_2 (**7**), iloprost is orally active in man with a biological half-life of 20-30 minutes, and shows inhibition of *ex vivo* adenosine diphosphate (ADP)-induced platelet aggregation as well as vasodilatory effects[72].

This relatively short duration of action after oral application is due to rapid metabolism of iloprost (**21**), primarily by β-oxidation of the carboxyl side chain[73].



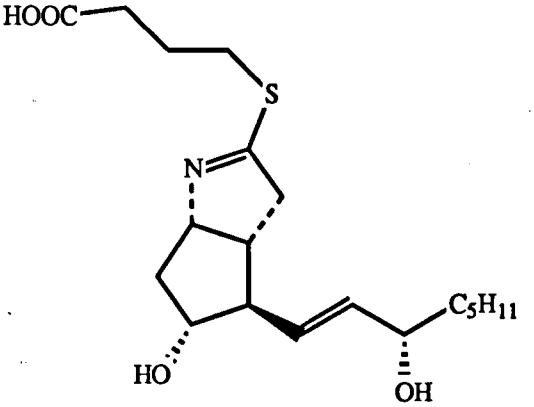
Cicaprost (**22**)

In an attempt to further impede the metabolic inactivation of iloprost (**21**), by β-oxidation of its carboxyl side chain, the molecule was further modified. The introduction of an oxygen atom in the 3-position of the carboxyl side chain prevented β-oxidation. This substitution, however, resulted in a decrease in intrinsic activity and further modification in the molecule became necessary to obtain a compound with at least equal potency to that of iloprost. The decrease in intrinsic activity was compensated for by the introduction of an additional methyl group at C-20 and the conversion of the Δ^{13}-double bond to a triple bond to give cicaprost (**22**), the 16(S)-methyl diastereomer[72]. This compound (**22**) is more potent than either PGI_2 (**7**) or iloprost (**21**) as an inhibitor of platelet aggregation and as a hypotensive agent [74]. It also demonstrates selectivity for PGI_2 receptors [75].



Nileprost (**23**)

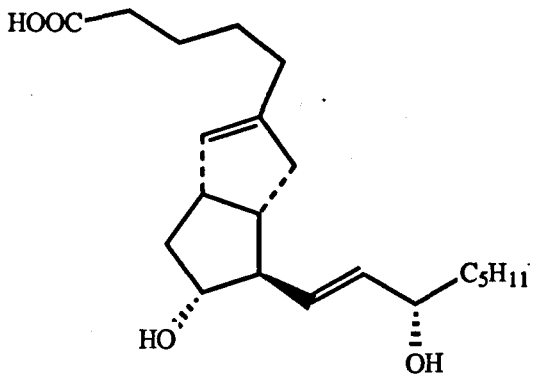
In nileprost (**23**) the labile enol-ether function of PGI_2 (**7**) has been stabilised by the introduction at C-5 of the cyano group. Both electron withdrawal and resonance effects are thought to be involved in increasing the stability of the heterocycle towards hydrolytic cleavage. The introduction of the C-16 methyl group protects against C-15 hydroxyl oxidation and Δ^{13}-double bond reduction [73]. Nileprost has been shown to be orally active and to have similar cytoprotective properties, but only moderate gastric antisecretory activity relative to E-prostaglandins. Nileprost (**23**) is also anti-enteropooling (inhibits the accumulation of fluid in the small intestines) and can actually prevent diarrhoea induced by E-prostaglandins [76].



HOE - 892 (**24**)

The replacement of the acid-labile enol-ether function of PGI_2 (**7**) with a β-thia-imino group resulted in HOE-892 (**24**). Unlike PGI_2 which is chemically

unstable particularly at low pH and has, *in vivo*, a very short half-life, HOE-892 (**24**) is chemically stable with an *in vivo* half-life of several hours [77]. Because of its high resistance to the low pH of the gastric contents, it is more effective than PGI_2 (**7**) when given orally. The cytoprotective effect of HOE-892 (**24**) is about 3 times longer in duration than that of PGI_2 (**7**). In addition HOE-892 (**24**) does not cause enteropooling.



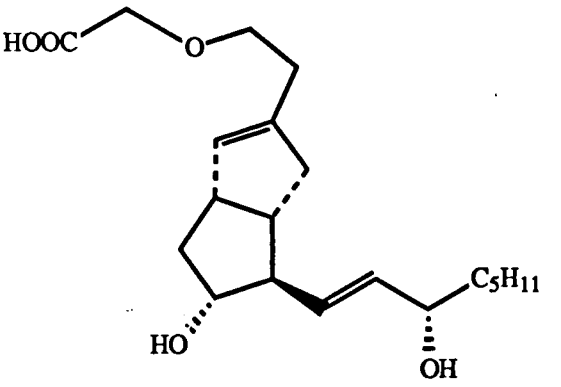
Isocarbacyclin (**25**)

Isocarbacyclin (**25**) is isomeric with carbacyclin (**20**), where the exocyclic double bond has been moved to the C-6,6a endocyclic position. Chemically isocarbacyclin (**25**) is more stable than PGI_2 (**7**).

Preliminary biological results indicate that isocarbacyclin (**25**) is one-tenth as potent as prostacyclin (**7**) as an inhibitor of ADP-induced platelet aggregation in rabbit platelet rich plasma (PRP)[78].

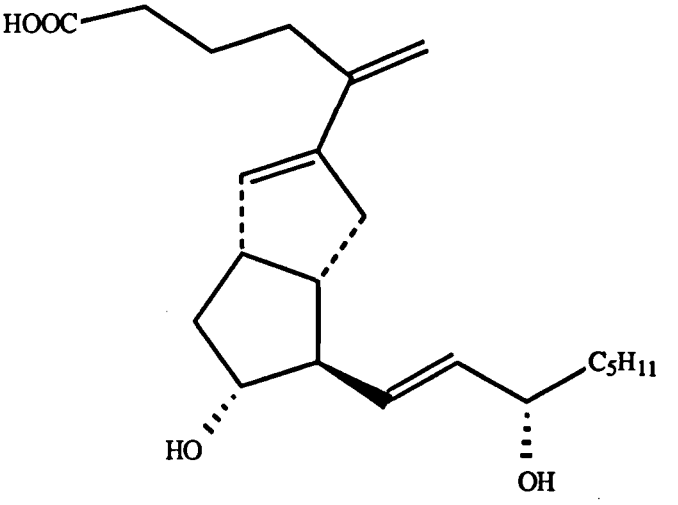
Metabolically, isocarbacyclin (**25**) is still susceptible to oxidative enzymic degradation at the carboxyl and ω side chains [79].

With a view to synthesising a biologically more stable isocarbacyclin analogue, the C-3 methylene group was replaced by an oxygen atom, giving 3-oxa-isocarbacyclin (**26**) which is more resistant to β-oxidation of the carboxyl side chain.



3 - Oxa - isocarbacyclin (**26**)

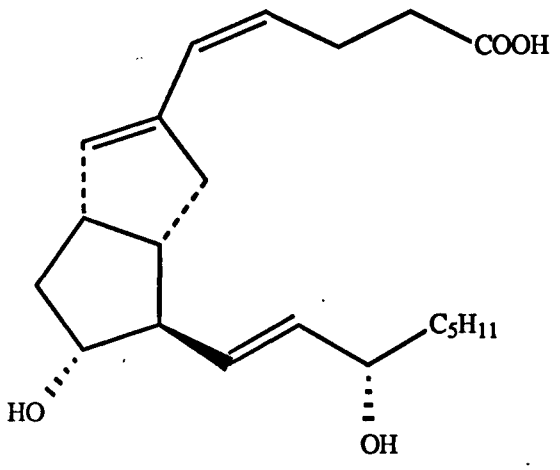
Analogue (**26**) was found to be a more potent inhibitor of ADP-induced platelet aggregation than carbacyclin (**20**) and in an *in vitro* experimental model, using liver homogenates, 3-oxa-isocarbacyclin (**26**) was found to be more stable to β-oxidation than isocarbacyclin (**25**) [79].



5 - methyleneisocarbacyclin (**27**)

Alternatively the potency of isocarbacyclin (**25**) was improved by the introduction of an exo-methylene group at the C-5 position. The resulting analogue, 5-methyleneisocarbacyclin (**27**)[80] is considered to show similar π-resonance between the conjugated double bonds as occurs between the Δ^5-double bond and the lone pair of electrons on the ring oxygen in prostacyclin (**7**).

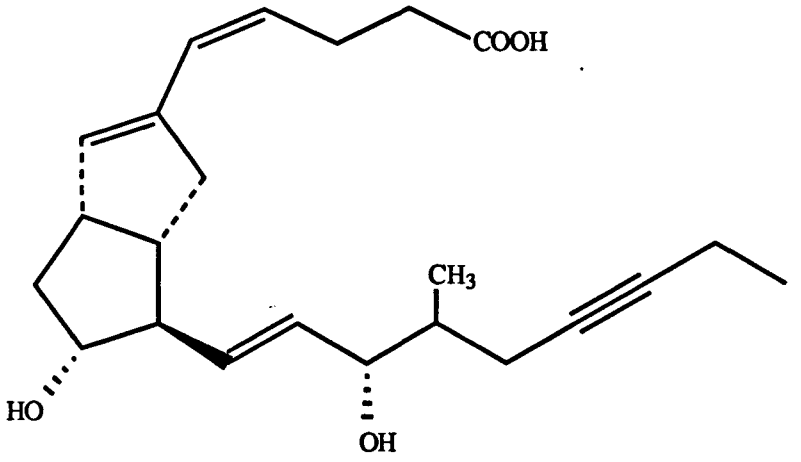
Analogue (27) was found to be equipotent to PGI_2 (7) as an inhibitor of ADP-induced platelet aggregation in human platelet-rich plasma (PRP) and 5 times as potent as prostacyclin in the same test using rabbit PRP [80].



(Z) - 4,5 - didehydroisocarbacyclin (28)

In analogue (28), the (Z)-4,5-dehydro derivative of isocarbacyclin (25), the π-resonance of the *trans*-dienic system is considered similar to that found in PGI_2 (7). This analogue (28), although shown to be more potent than isocarbacyclin (25) was metabolically unstable, despite the fact that the Δ^4-double bond retards β-oxidation [81].

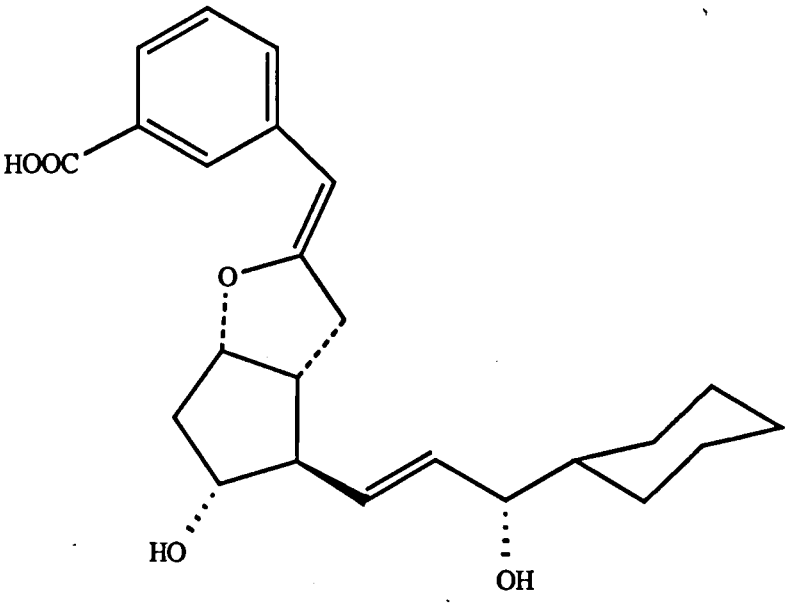
Cognisant of the fact that a long duration of action in an intravenously and orally active prostacyclin analogue would facilitate its clinical application, attention was then centred on increasing the duration of action of (28) by modification of the ω chain. Conversion of the C-18, C-19 single bond into a triple bond, introduction of two further methyl groups at C-16 and C-20 and selectively synthesising the pure 16(S)-methyl diastereomer gave the isocarbacyclin analogue KP-10614 (29) with high oral and intravenous activity [82].



KP-10614 (**29**)

KP-10614 (**29**) is a potent inhibitor of ADP-induced platelet aggregation in human PRP. In this test KP-10614 (**29**) was shown to be twice as potent as iloprost (**21**) and thirty times as active as isocarbacyclin (**25**) [82].

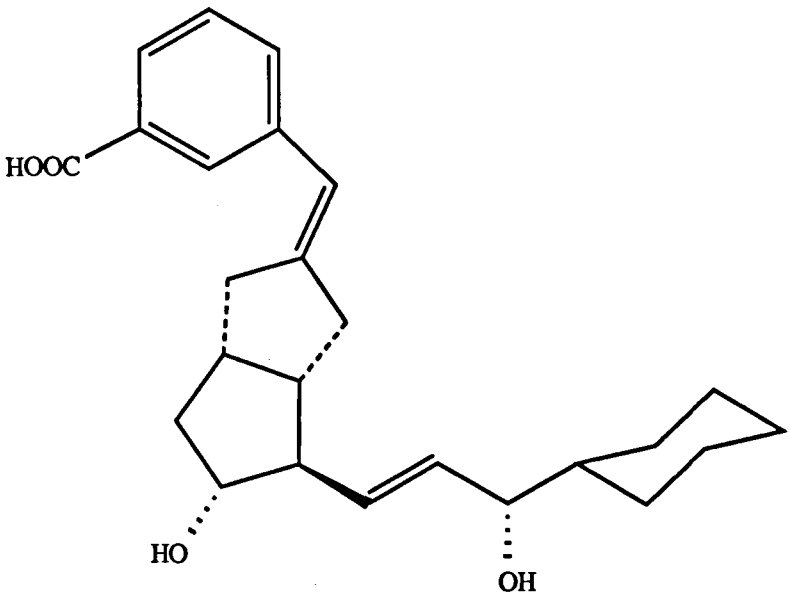
The inhibitory activity on *ex vivo* platelet aggregation (ADP-induced) and the hypotensive effect of compound (**29**) were tested following intravenous administration in anaesthetised rats. As an inhibitor of platelet aggregation compound (**29**) was shown to be 56 times more potent than isocarbacyclin (**25**) and 48 times more potent than iloprost (**21**) [82]. The hypotensive effect of compound (**29**) was shown to be 11.4 times greater than isocarbacyclin (**25**) and 7.9 times greater than iloprost (**21**) [82]. Although these tests showed that compound (**29**) has a much greater separation of inhibiting platelet aggregation from the hypotensive effect than either isocarbacyclin (**25**) or iloprost (**21**), it (**29**) is still a potent hypotensive agent.



CG-4203 (**30**)

In analogue (**30**), the C-2 to C-4 and C-16 to C-20 units of prostacyclin (**7**) have been replaced by a benzenoid and a cyclohexyl residue respectively.

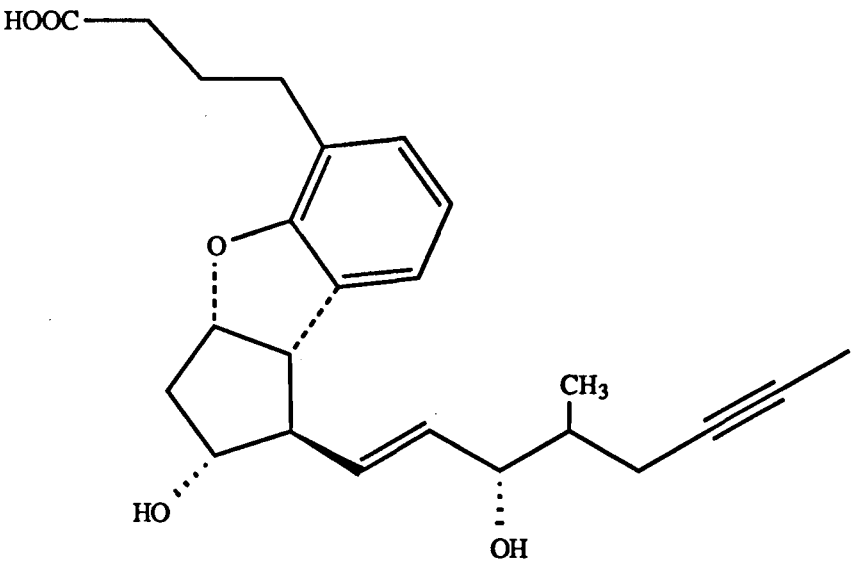
CG-4203 (**30**) is more resistant to hydrolysis than PGI_2 (**7**) and this increased hydrolytic stability is thought to be due to the stabilisation of the enol-ether linkage by extending the conjugation of the Δ^5-double bond. Also by replacing the aliphatic carbons (C-2 to C-4) of PGI_2 (**7**) with aromatic ones this analogue (**30**) is less prone to β-oxidation and is metabolically more stable. The C-15 cyclohexyl residue also makes a contribution to the improved metabolic stability by blocking metabolic attack by the 15-hydroxyprostaglandin dehydrogenase enzyme[83].



CG-4305 (**31**)

Analogue (**31**), CG-4305, differs from (**30**) by having a 6a-methylene instead of a 6a-oxygen. This analogue, lacking the enol-ether moiety, is resistant to hydrolysis.

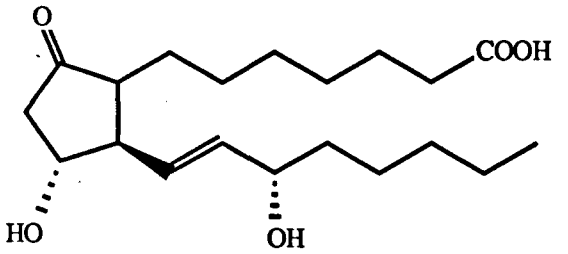
Both analogues, CG-4203 (**30**) and CG-4305 (**31**), were shown to be more potent than PGI_2 (**7**) in their ability to inhibit ADP-induced platelet aggregation in anaesthetised rats. As an inhibitor of arachidonic-induced platelet aggregation in human PRP compound CG-4203 appeared to have a greater intrinsic activity than CG-4305 [83].



TRK-100 (**32**)

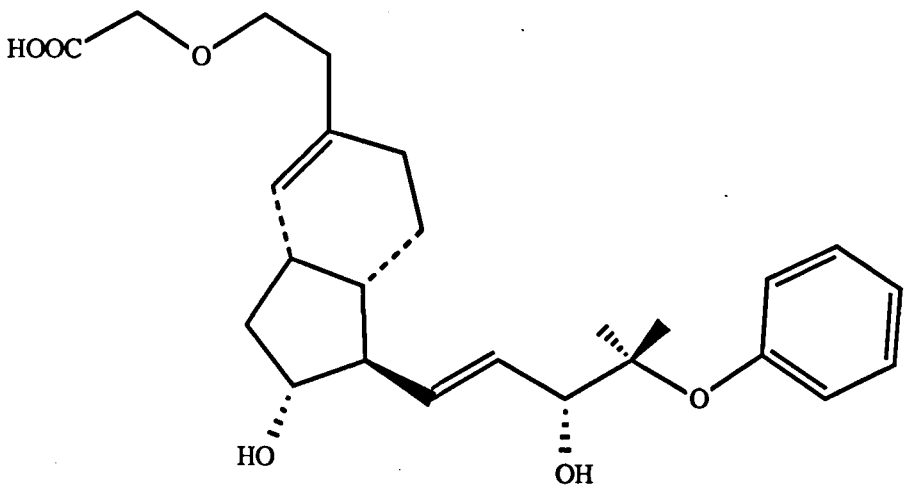
The aromatisation of C-5, C-6 and C-7 of PGI_2 (**7**), brought about by fusion of the benzene ring to the furan ring of PGI_2 (**7**) effectively converts the labile enol-ether functionality of PGI_2 into the stable aryl-ether moiety which is resistant to hydrolysis. In addition, the introduction of the C-16 methyl and the C-18, C-19 triple bond generates TRK-100 (**32**) which is also metabolically stable.

TRK-100 (**32**) was shown, *in vitro*, to be a potent inhibitor of ADP-induced platelet aggregation using human PRP. The inhibitory activity of TRK-100 (**32**) was 0.5 times that of PGI_2 (**7**) and 8 times that of PGE_1 (**33**). There was a marked tendency for platelet clumps to disaggregate following secondary aggregation in the presence of TRK-100 (**32**) at final concentrations higher than 1 ng/mL. This activity was similar to PGI_2 (**7**) and more than 30 times that of PGE_1 (**33**).



PGE_1 (**33**)

TRK-100 (**32**) was shown to induce disaggregation of a pre-existing thrombus in the microcirculation of the hamster cheek pouch. A dose-dependent response was obtained following oral administration of the drug at concentrations of 50-200 μg/Kg. It was also reported that TRK-100 (**32**) did not show the hypotensive properties of PGI_2 (**7**) [84], although no experimental evidence was quoted in support of this claim.



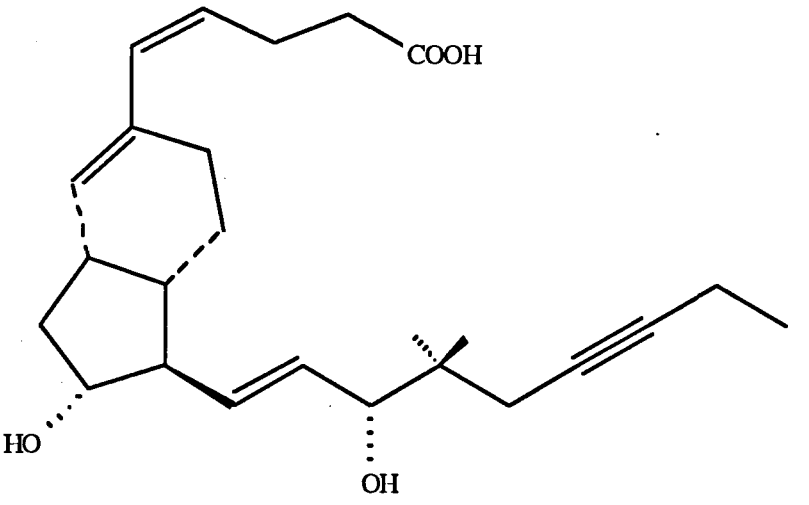
(**34**)

Analogue (**34**) may be considered to be derived from PGI_2 (**7**) as a result of the following modifications to its structure: replacement of the C-3 methylene group by an oxygen atom; substitution of the 6a-oxygen by a methylene group; the isomerisation of the Δ^5-double bond to the $\Delta^{6(6a)}$-position with concomittant expansion of the cyclopentane ring; introduction of 2 methyl groups at the C-16 position and the replacement of the *n*-butyl terminus by a phenoxy group.

Alternatively (**34**) may be regarded as a homoisocarbacyclin analogue [*see* isocarbacyclin (**25**)].

Such a structural transformation of PGI_2 (**7**), as expected, led to an analogue (**34**) that is both chemically and metabolically stable.

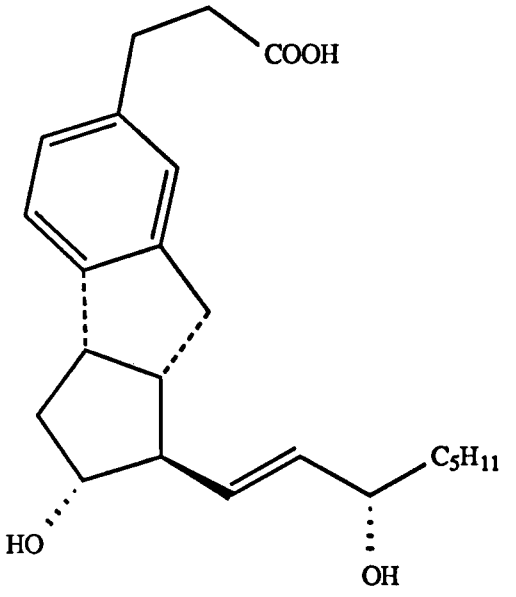
Preliminary biological examination indicated that the 3-oxa-analogue (**34**) had potent and long-lasting antiulcer activities. However it did not inhibit platelet aggregation of rabbit PRP at a concentration of 3×10^{-6} M. Thus analogue (**34**) was shown to have good seperation of antiulcer activity from inhibitory activity in platelet aggregation.



(**35**)

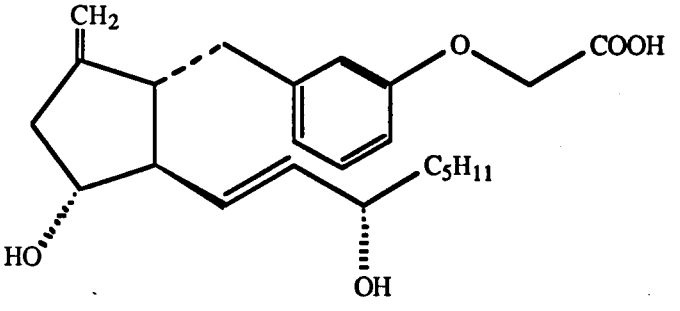
Analogue (**35**) is, in effect, a derivative of (Z)-4,5-dehydroisohomocarbacyclin [*see* (Z)-4,5-dehydroisocarbacyclin (**28**)] in which 2 methyl groups have been introduced at C-16 and the *n*-butyl terminus has been extended by a C-20 methyl substitution and a C-18, C-19 triple bond introduced. Compound (**35**) is both chemically and metabolically stable.

This conjugated *trans*-dienic analogue (**35**) appears to have a biological profile similar to PGI_2 (**7**) [85].



(36)

Introduction of an aromatic ring to 6a-carba-PGI_2 or carbacyclin (**20**) led to analogue (**36**). Owing to the incorporation of C-5, C-6, and C-6a of carbacyclin (**20**) into the fused aromatic system the sp^2 character of C-5 and C-6 of carbacyclin is retained. Delocalisation of the π-electrons effectively introduces a Δ^4-double bond which is known to impart a resistance to β-oxidation of the carboxyl side chain [81]. However this aromatic PGI_2 analogue (**36**) did not show any PGI_2-like biological properties [86].

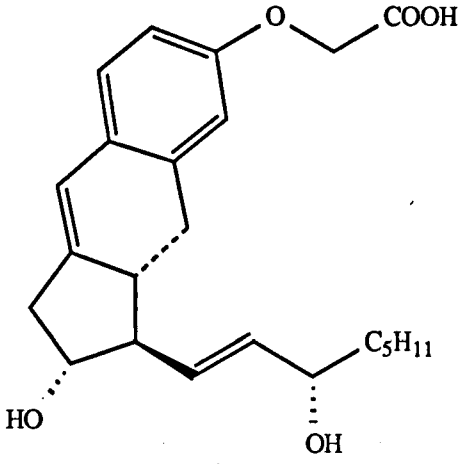


(37)

Recently it was shown that the interphenylene-9-methylene prostaglandin (**37**), though much less potent than PGI_2(**7**), had certain prostacyclin-like activities [87].

Compound (37) is an inhibitor of ADP-induced platelet aggregation and a vasodepressant. It is orally active; its oral activity has been prolonged by the use of the 1-amide and 1-methyl ester derivatives [87].

It was hypothesised that by making the interphenylene compound (37) more rigid by joining the aromatic ring to the cyclopentane ring, the PGI_2-type activity might be enhanced. This led to the synthesis of PGI_2 analogues (38), (39), and (40).



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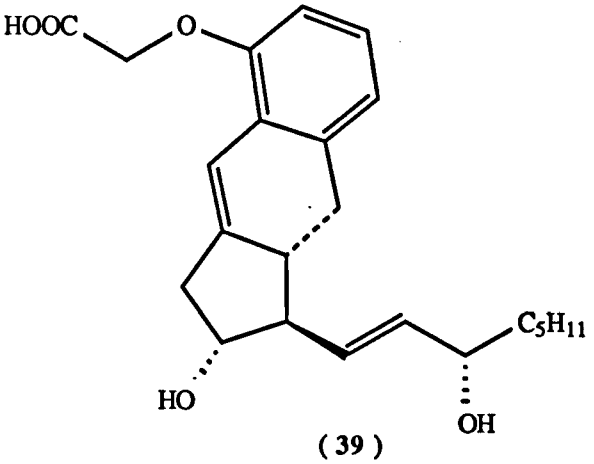

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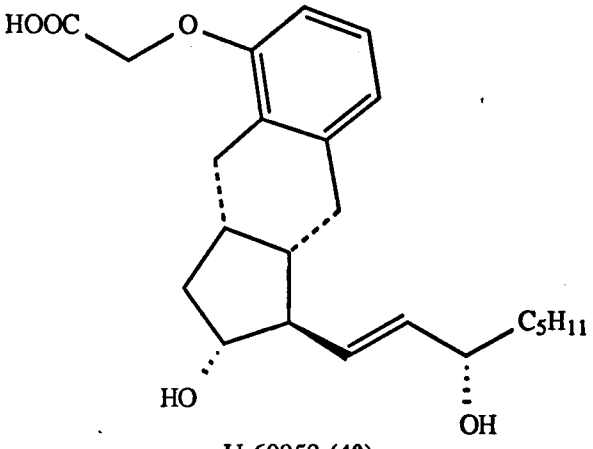


U-60959 (40)

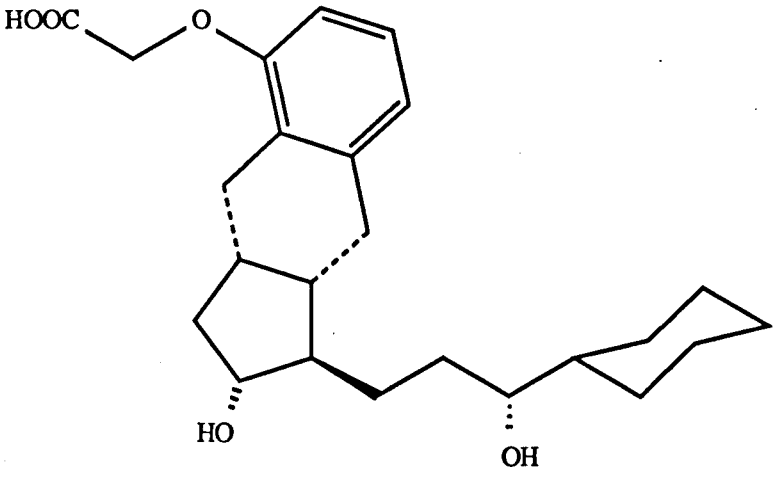
Included in the molecular framework of (38), (39), and (40) are structural features which increase the resistance of the carboxyl side chain to β-oxidation. In

addition analogue (**40**) differs from (**38**) and (**39**) in that it lacks a double bond exocyclic to the cyclopentane residue.

While compound (**38**) did not exhibit any prostacyclin-type activity, (**39**) and (**40**) were potent inhibitors of platelet aggregation. In particular (**40**), which can be considered a homocarbacyclin analogue with a fused aromatic ring, is twice as active as carbacyclin (**20**) as an inhibitor of platelet aggregation [86, 87].

Further biological evaluation of U-60959 (**40**) revealed that it was also an effective gastric cytoprotective agent and a weak inhibitor of gastric acid secretion [88].

More recent structural modification of the ω side chain of U-60959 (**40**) has led to the synthesis of the 13,14-dihydro-15-cyclohexyl analogue (**41**)[89,90].

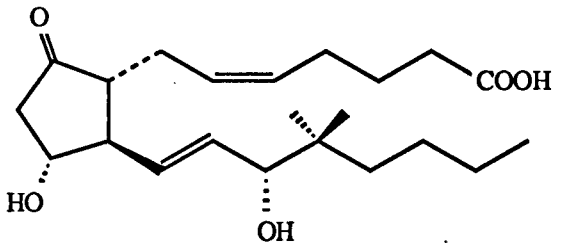


U-68215 (**41**)

The moderate decrease in intrinsic activity brought about by reduction of the Δ^{13}-double bond is more than compensated for by the replacement of the *n*-pentyl terminus with a cyclohexyl residue.

When given by the intravenous route of administration U-68215 (**41**) is equipotent with prostacyclin (**7**) on platelets and blood pressure. Given orally, (**41**) is an extremely potent cytoprotective and gastric antisecretory agent. Orally in rats,

analogue (**41**) is about 140 times as active as (**40**) at inhibiting gastric acid secretion, being effective at μg/kg concentrations.



dm PGE_2 (**42**)

When compared to 16,16-dimethyl PGE_2 (**42**) [*dm*PGE_2], U-68215 (**41**) was almost as cytoprotective for the stomach and equally cytoprotective for the intestines. It (**41**) was three times more potent than (**42**) as a gastric antisecretory agent. In contrast to *dm*PGE_2, analogue U-68215 (**41**) was not enteropooling, diarrhoeagenic nor uterotonic even at doses 100 times the antiulcer dose. Like PGI_2 (**7**), U-68215 exhibits blood pressure lowering effects, but this is seen at oral doses 150 and 5 times its cytoprotective and antisecretory doses respectively.

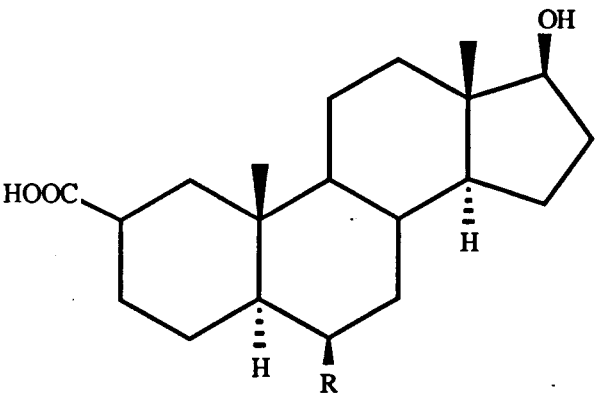
U-68215 (**41**) is a long acting agent. When given at equipotent doses for gastric cytoprotection its effects lasted twice as long as that of *dm*PGE_2 (**42**). This could be due to increased inherent activity of the molecule and/or to a greater resistance to enzymic degradation [91].

Consistently retained in the structures of most of these PGI_2 analogues are the 1-carboxy, the C-11 and C-15 hydroxyl groups and the unsaturation at C-5, C-6 as well as the stereochemistry of the parent compound. These features are a necessary safeguard for the retention of biological activity.

This particular selection of PGI_2 analogues has been presented not only to illustrate how structural changes to PGI_2 (**7**) affect its chemical stability, metabolic stability and pharmacological profiles, but also because many of these features may be incorporated into the architecture of steroidal analogues of prostacyclin (**7**).

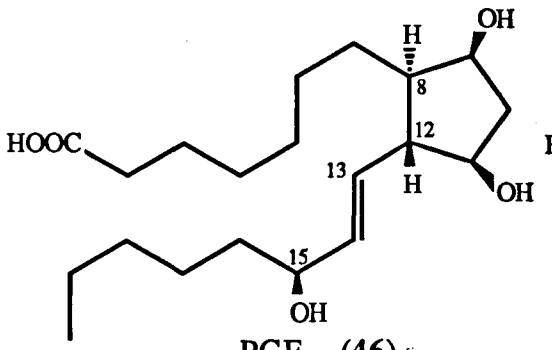
1.8 Steroidal analogues of prostaglandins.

A review of the literature revealed a singular publication on the subject in which the intact steroidal nucleus was retained in the analogues. Venton and Counsell[92] in 1975 reported the synthesis of 3 steroid carboxylic acids (**43,44,45**) as steroidal analogues of $PGF_{1\alpha}$ (**46**).

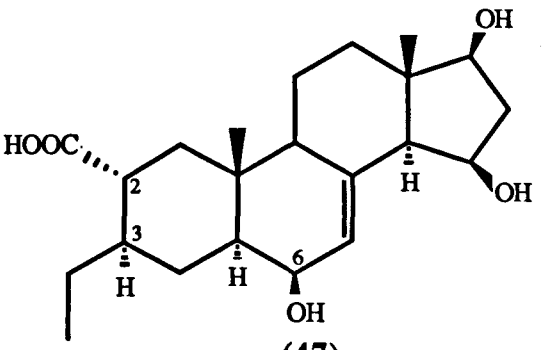


(**43**) R = H, 2α-COOH
(**44**) R = H, 2β-COOH
(**45**) R = OH, 2α-COOH

This analogy was developed from examination of molecular models which revealed that prostaglandins can assume a conformation similar to that of another important class of lipids - the steroids. $PGF_{1\alpha}$ (**46**) with its closely packed side chains was shown to be capable of assuming a conformation similar to that represented by the hypothetical steroid structure (**47**).



$PGF_{1\alpha}$ (46)



(47)

Preliminary biological evaluation[92] showed that (43), (44), and (45) possessed no significant prostaglandin-like activity in standard platelet aggregation, rat blood pressure and 15-hydroxyprostaglandin dehydrogenase assays. However compound (43) demonstrated weakly specific PGE_2-stimulated smooth muscle antagonism when compared with antagonism of bradykinin and acetylcholine induced contractions. The antagonism of PGE_2-stimulated smooth muscle contraction was shown to be due to non-competitive inhibition.

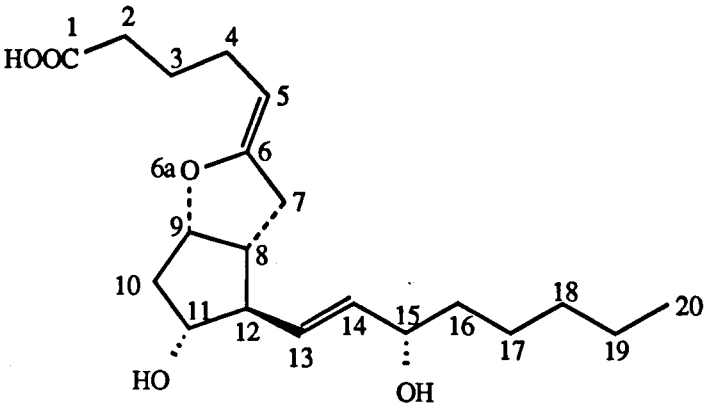
The total loss of of inherent flexibility of the $PGF_{1\alpha}$-mimetic side chains of (43), (44),and (45) as a result of their incorporation into the rigid steroidal nucleus may be the major contributory factor to the disappointing biological profiles of these steroidal analogues.

Perhaps a less rigid or semi-rigid steroidal analogue, in which the prostaglandin-mimetic side chains are instead substituents on nuclear carbons and in which the salient features of the prostaglandins with the appropriate stereochemistry are incorporated into the steroidal nucleus, may stand a better chance of safeguarding biological properties.

Alternatively, steroidal analogues in which one of the prostaglandin-mimetic side chains is a substituent on a nuclear (steroid) carbon and the other is incorporated into the steroidal nucleus together with closely mimetic sites of

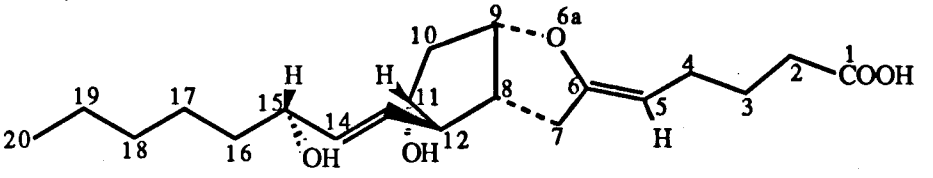
oxygenation and unsaturation are also considered as candidates which may retain biological activity.

1.9 Concept of a steroidal prostacyclin analogue.



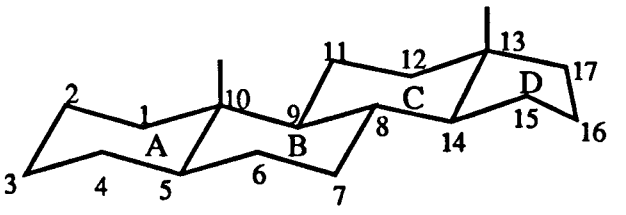
Prostaglandin I_2 (7)

Although not immediately obvious from the 2D-representation of (7), inspection of molecular models has revealed that the inherent flexibility of the carboxyl and ω side chains at C-6 and C-12 respectively, allows the side chains to assume an anti-parallel alignment. An attempt is made to illustrate this in structure (7a) as viewed from C-1 (through C-6, C-7, C-8, and C-12) to C-20.



(7a)

Similarly, the introduction of PGI_2-mimetic side chains into suitable positions in the steroidal nucleus will allow a similar anti-parallel arrangement of these chains to be attained.



(48)

In such steroidal analogues, the 5α-androstane nucleus (**48**) forms the backbone of the molecule with the PGI_2-mimetic side chains built on at the C-12 and C-16, and C-6 and C-16 positions respectively.

The D-ring in (**48**) may be considered to simulate the 5-membered heterocycle of PGI_2 (**7**) [in which the 6a-oxygen has been replaced by a methylene group] and the C-ring in (**48**) is representative of the cyclopentane ring in PGI_2 (**7**) which has been expanded [as in the very potent homoisocarbacyclin analogues (**34**) and (**35**) pages 27 and 28].

The introduction of hydroxy groups in C-6 and C-12 positions of (**48**) will serve to mimic the C-11 and C-15 hydroxy groups of PGI_2 (**7**).

1.10 Objective of research.

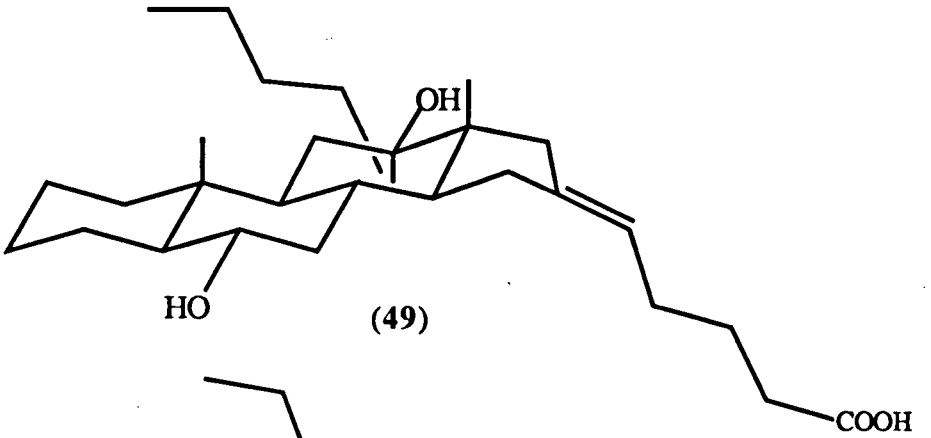
The objective of this research is to investigate the development of synthetic routes to proposed steroidal analogues of prostacyclin (**7**).

1.11 Proposed steroidal analogues of prostacyclin.

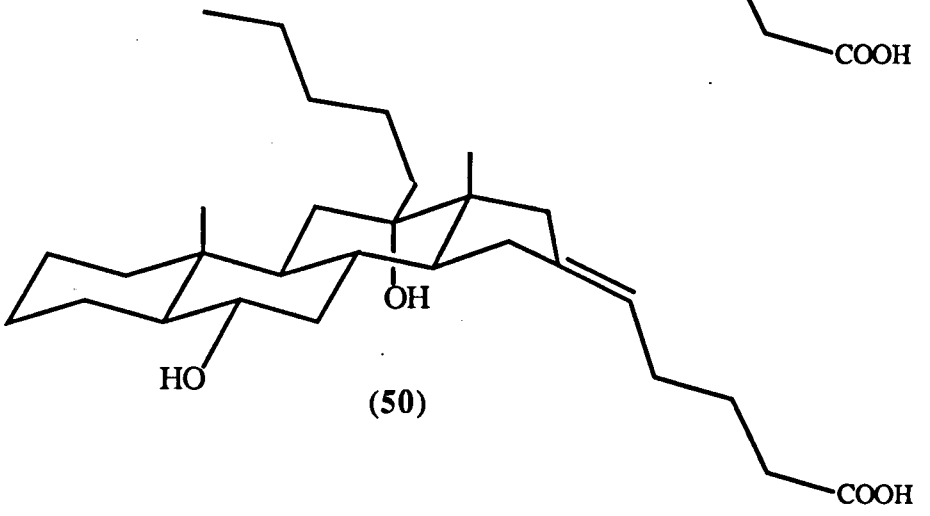
The proposed steroidal analogues are classified into three groups on the basis of the positions in the 5α-androstane nucleus (**48**) in which the PGI_2-mimetic side chains are incorporated. Hereinafter these are referred to as type I, II, and III analogues.

1.11.1 Type I steroidal analogues.

These are exemplified by (**49**) and (**50**) in which the PGI_2-mimetic carboxyl and ω side chains are substituents at the C-16 and C-12 positions of the 5α-androstane (**48**) nucleus.



(**49**)



(**50**)

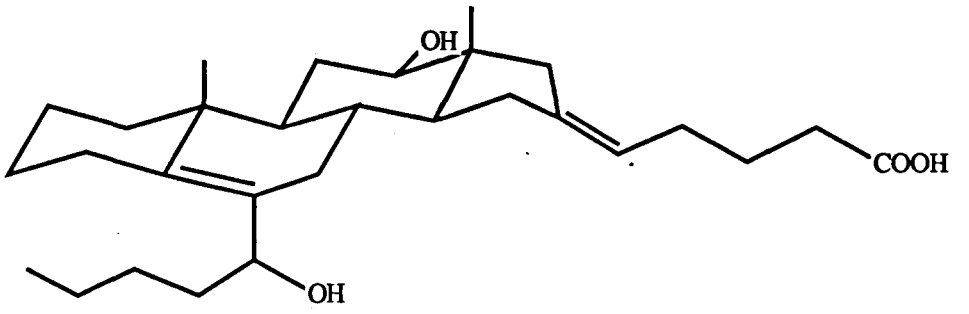
The absence of the analogous PGI_2-Δ^{13}-double bond in (**49**) and (**50**) is not seen as a structural deficiency that would seriously compromise the attainment of PGI_2-agonist or PGI_2-antagonist activity, since a similar deficiency found in the tetracyclic PGI_2 analogue (**41**) is accompanied by only a moderate decrease in PGI_2-like activity [89, 90].

The presence of the quaternary C-13 in (**49**) and (**50**) is expected to perform a similar role as the C-16 methyl and the C-16 dimethyl groups in substituted

PGI_2 analogues [**(21)**-**(23)**, **(29)**, **(32)**, **(34)**, **(35)**], that is, to retard metabolic oxidation of the adjacent hydroxy function.

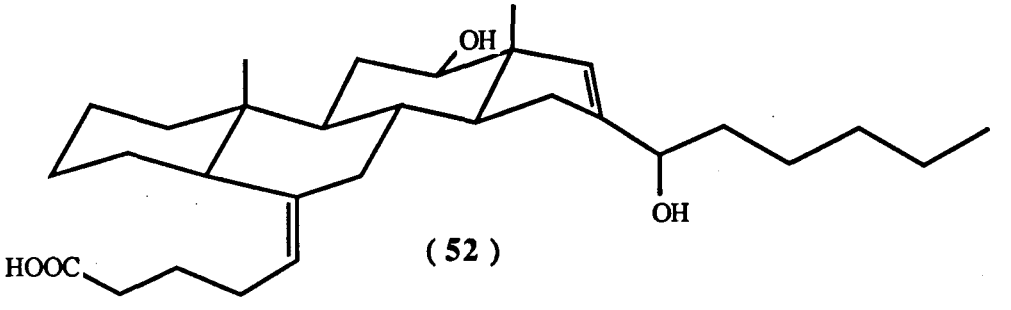
1.11.2 Type II steroidal analogues.

In **(51)** the PGI_2-mimetic ω and carboxyl side chains are carried on the C-6 and C-16 positions of the 5α-androstane **(48)** nucleus.



(51)

The tetrasubstituted endocyclic Δ^5-double bond is mimetic of the Δ^{13}-double bond in PGI_2 (7) and to achieve an anti-parallel arrangement of side chains in **(51)** the exocyclic Δ^{16}-double bond must be of the (Z)-configuration.



(52)

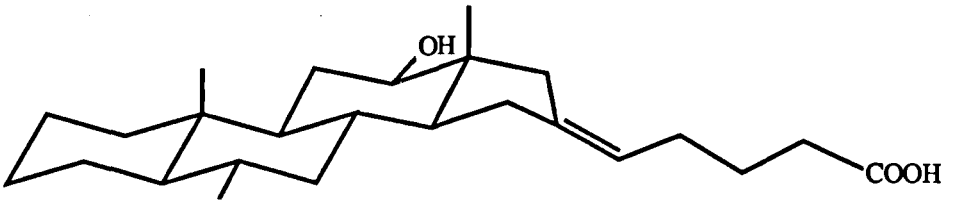
Analogue **(52)** differs from **(51)** in that the the positions of the PGI_2-mimetic side chains in the 5α-androstane nucleus **(48)** are reversed. In **(52)**, the analogous PGI_2-Δ^{13}-double bond is the trisubstituted endocyclic Δ^{16}-double bond

and the exocyclic C-6 double bond is mimetic of the Δ^5-double bond of PGI_2 (**7**). The (Z)-configuration of the double bond exocyclic to ring-B in (**52**) ensures that an anti-parallel arrangement of the two side chains is attained. In (**52**), the 6-membered B ring, which carries the PGI_2-mimetic carboxyl side chain is similar to that of the homocarbacyclin-type analogues [(**34**), (**35**)] which have potent antiulcer properties [91].

The 12β-OH group in (**51**) and (**52**) mimics the C-11 hydroxyl group in PGI_2 (**7**) and the increased substitution of the double bonds in (**51**) and (**52**) which are mimetic of the PGI_2-Δ^{13}-double bond may retard metabolic reduction.

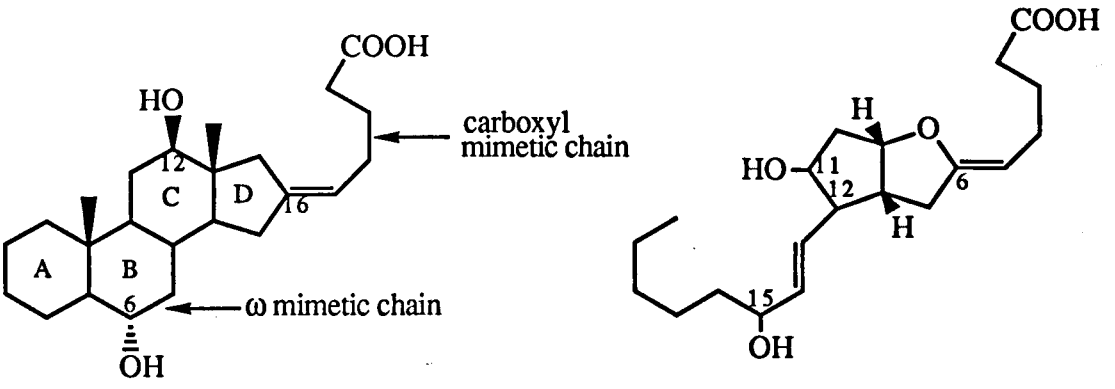
The anticipated increase in metabolic stability coupled with the anti-parallel arrangement of the side chains in (**51**) and (**52**) may safeguard and augment biological activity.

1.11.3 Type III steroidal analogue.



(**53**)

Analogue (**53**) differs from (**49**)-(**52**) in that the PGI_2-mimetic ω chain is not a nuclear substituent but rather is now part of the steroidal nucleus. Analogue (**53**) contains structural features mimetic of the carboxyl and ω side chains as well as the hydroxy groups at C-11 and C-15 of prostacyclin (**7**) [*see* figure 4].



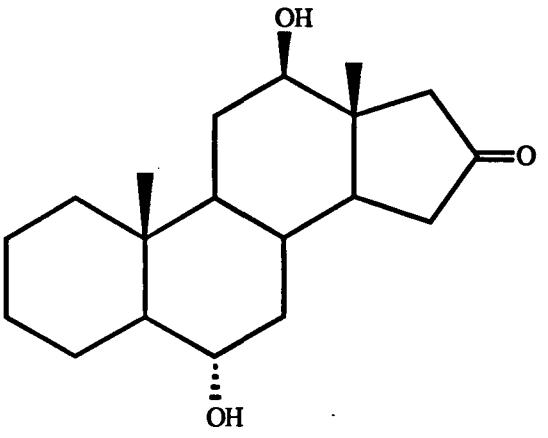
(53) Prostacyclin

Figure 4.

In figure 4, the carboxyl mimetic chain originates from C-16 of the steroidal nucleus and the ω mimetic chain is part of the steroidal nucleus (rings B and A, C-7, C-6, C-5, C-4, C-3, C-2, C-1). Steroidal rings C and D are mimetic of the cyclopentane and furan rings of prostacyclin. The unsaturation present in the ω chain of prostacyclin means that C-12, C-13, C-14 and C-15 are in the same plane and a line drawn through the C-11 and C-15 hydroxy groups intersects at approximately right angles with a line drawn through the C-5, C-6 double bond in the carboxyl chain. A similar arrangement is found in analogue (**53**) because of the *trans* fusion of the B and C rings in the steroidal nucleus along with the exocyclic olefinic substituent at C-16.

The unsaturation found in the ω chain of prostacyclin may be important for alignment of hydroxy groups at the receptor surface and this configuration is mimicked in analogue (**53**) by the native shape of the steroidal nucleus. As the ω mimetic chain is rigid there is no conformational mobility in this part of the molecule. Additionally, the mimetic hydroxy groups are both open to hydrogen bonding interactions with the receptor.

Central to the synthesis of these steroidal analogues is the microbiologically derived 6α, 12β-dihydroxy-5α-androstan-16-one (**54**)



(**54**)

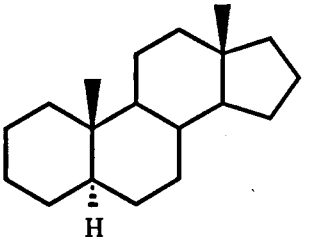
The particular oxygenation pattern of (**54**) provides suitable handles *via* which the PGI_2-mimetic side chains may be introduced at the C-6, C-12, and C-16 positions of the steroidal nucleus. In general, Wittig condensations at the C-16 keto and the potential C-6 keto function and a Grignard reaction at the potential C-12 keto function will serve to introduce these mimetic side chains.

2.0 Discussion

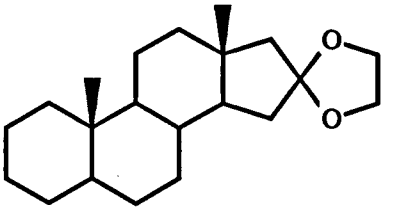
2.1 Original approach to 5α-androstan-16-one (56).

Hydroxylation by fungal cultures is a well documented method for the production of steroids that are not readily prepared by chemical methods.

The fungus, *Calonectria decora,* is reported to regiospecifically and stereospecifically introduce the 6α- and 12β- hydroxy groups into the 5α-androstane nucleus (**48**) when incubated with 16,16-ethylenedioxy-5α-androstane (**55**) as substrate.



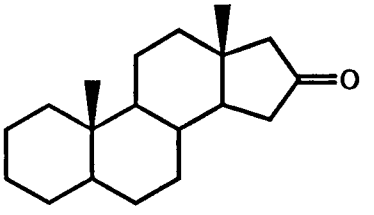
(48)



(55)

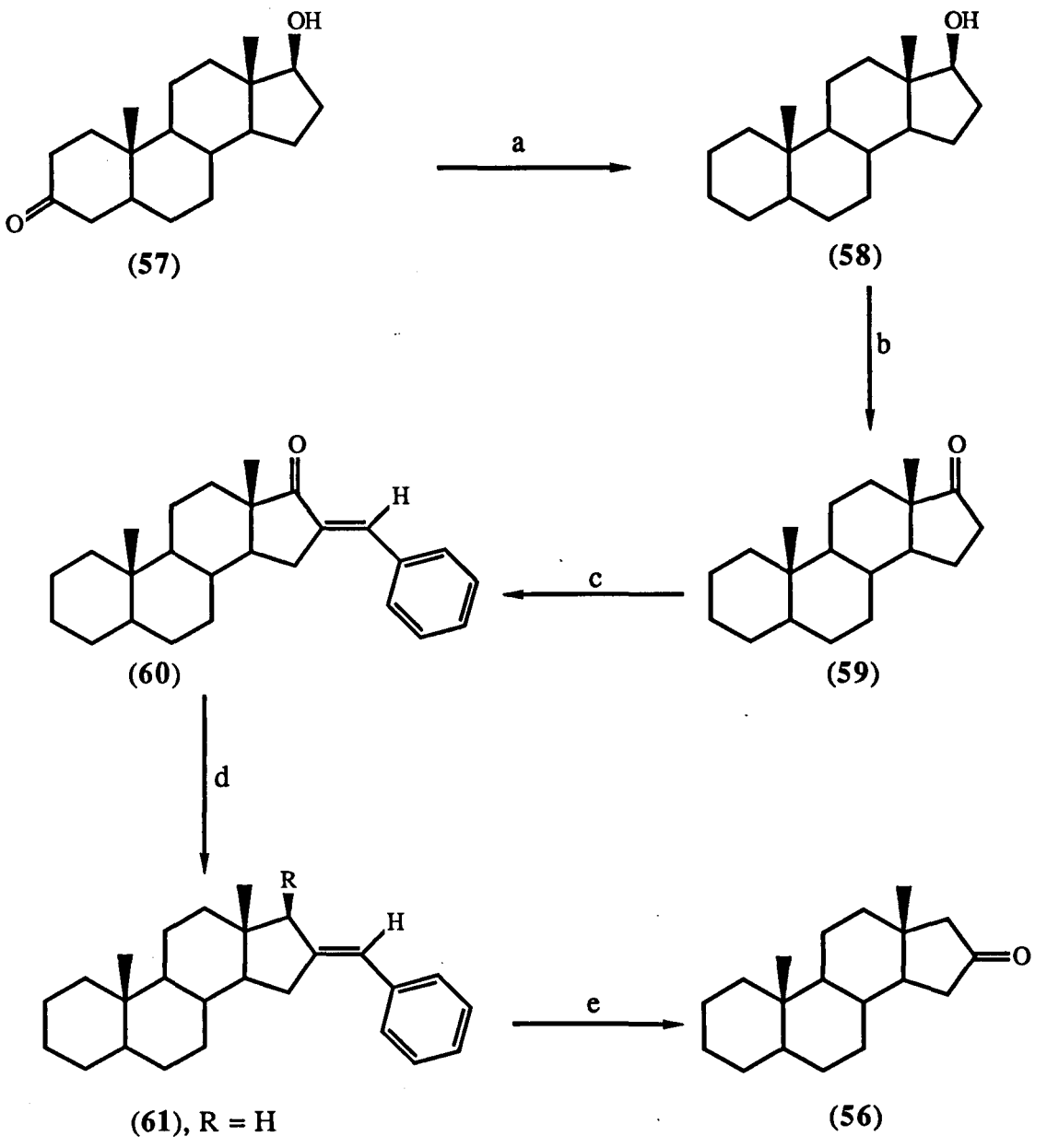
The oxygenation pattern in (**55**) was shown to be an important influence in directing hydroxylation to the desired sites of the steroid (**48**) nucleus [93,94,95].

The dioxygenated steroid (**55**) was conveniently prepared by ketalisation of the corresponding steroid ketone, 5α-androstan-16-one (**56**) [96].



(56)

However, compound (**56**), an uncommon keto-steroid, was not commercially available and had to be prepared by preliminary synthesis (Scheme 1) from the commercially available 17β-hydroxy-5α-androstan-3-one (**57**)[97].



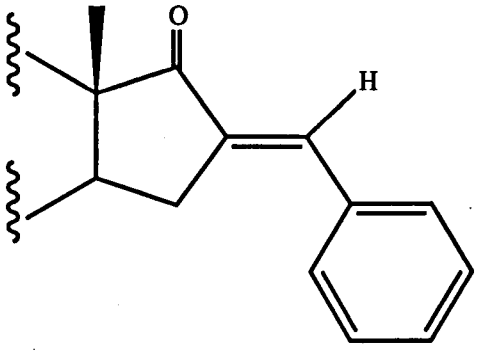
Scheme 1. Reagents: *a*, $NH_2NH_2.H_2O$ / KOH / Digol; *b*, H_2CrO_4-Me_2CO; *c*, PhCHO / KOH / EtOH; *d*, $LiAlH_4$ / $AlCl_3$; *e*, O_3-AcOH / Zn.

17β-Hydroxy-5α-androstan-3-one (**57**) was readily converted to 5α-androstan-17β-ol (**58**) by the Huang-Minlon reduction of the 3-keto functionality. The 17α-H of (**58**), like that of (**57**), resonates as a triplet (δ 3.62, J 8.39 Hz) in the ^1H NMR spectrum: this triplet, in effect, results from an overlapping double doublet

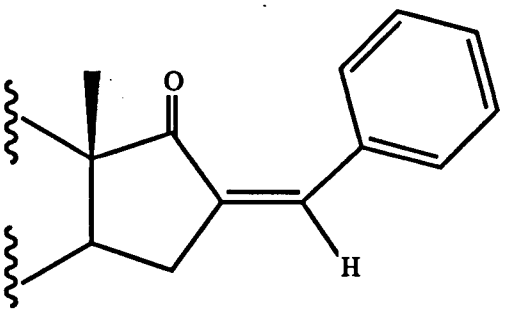
due to the coupling of the 17α-H with the two 16 -H's.

Jones oxidation of the sterol (**58**) afforded the corresponding ketone (**59**) in 90% yield. Reaction of (**59**) with benzaldehyde in ethanolic KOH gave the keto - benzylidene steroid (**60**) as the Aldol condensation product. The position of introduction of the benzylidene substituent is unequivocal because in the 17-ketone (**59**) only the C-16 bears α-hydrogens.

Since reactions in which unsymmetrically substituted double bonds are formed always carry the possibility of generating geometric isomers, a mixture of 16(E)- and 16(Z)-benzylidene-5α-androsta-17-one was expected as the product composition. In this case, however, only the 16(E)-isomer (**60**) was formed; no trace of the (Z)-isomer (**62**) was detected.



16(E)-isomer (**60**)



16(Z)-isomer (**62**)

Its exclusive formation is explained on the basis of its greater stability; in the (E)-isomer (**60**) the conjugated double bond system has a *transoid* arrangement which allows a greater degree of coplanarity and overlap of the π-orbitals, thereby extending the conjugation and enhancing stability.

In the (Z)-isomer (**62**) a steric interaction between the *ortho* hydrogens of the benzene ring and the carbonyl oxygen is unavoidable in the planar conformation and the benzene ring is forced out of coplanarity with the rest of the π-bond system.

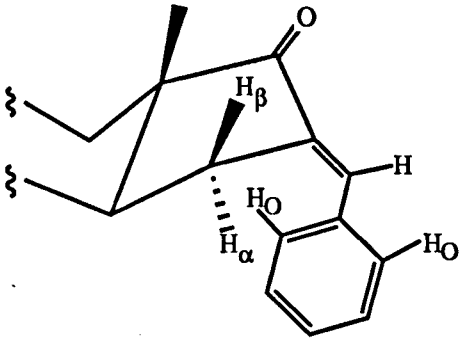
The non-bonded repulsive interaction of the π-clouds of the carbonyl group and the benzene ring will also compromise coplanarity [98].

The tentative assignment of the (E)-configuration to (**60**) was supported by several lines of spectral evidence. The strong C = C double bond stretch absorption (1630 cm^{-1}) in the infrared spectrum of (**60**) infers the presence of an exocyclic α, β-unsaturated ketone (an arylidene ketone) in which the double bond is of the (E)-configuration; this absorption band has been shown to be absent in the spectra of (Z)-α, β-unsaturated ketones (arylidene ketones) [98, 99].

Also in the ^1H NMR spectrum of (**60**) the vinyl proton is buried in the δ 7.38-7.55 region of the aromatic protons. This observation may be explained on the basis that in the (E)-isomer, the vinylic-H, being *cis* to the 17-carbonyl is deshielded due to the paramagnetic anisotropy of the carbonyl group and comes to resonance in the aromatic region.

It is noteworthy that the vinylic-H of the (Z)-isomer of exocyclic α, β-unsaturated ketones resonates upfield of this region [98, 99].

Unequivocal assignment of the (E)-configuration to the 16-benzylidene function of (**60**) was confirmed by studying the Nuclear Overhauser Enhancement (NOE) effects;



(**60**)

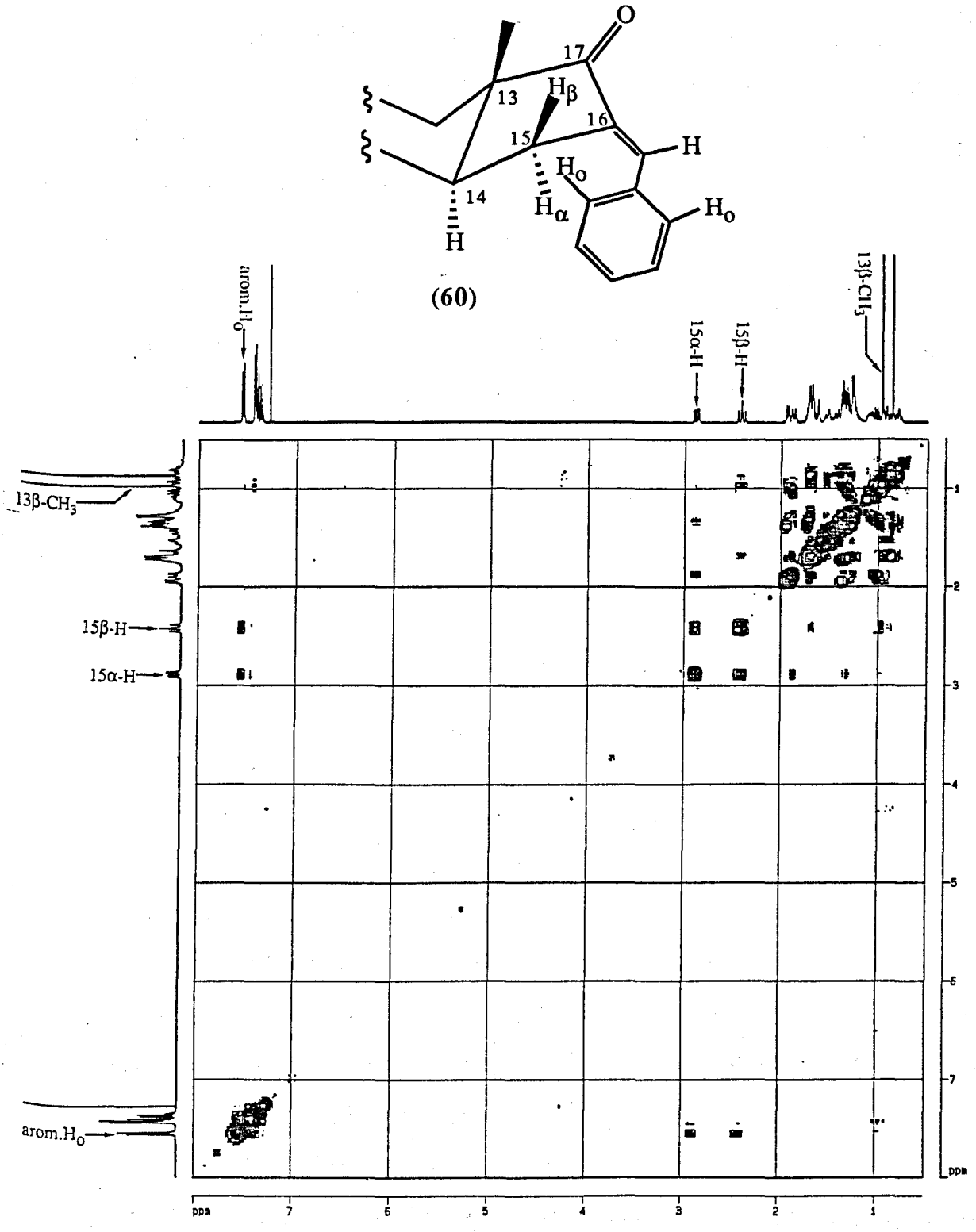
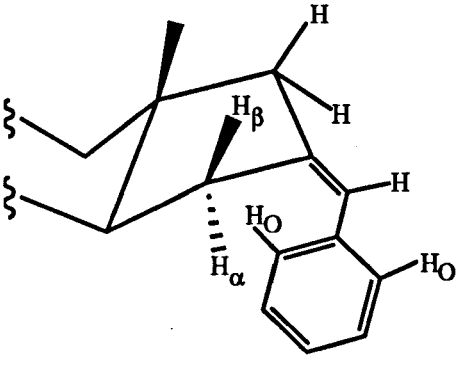


Figure 5. NOESY spectrum (400 MHz, $CDCl_3$) of 16(E)-benzylidene-5α-androstan-17-one (**60**).

In the NOSEY spectrum of (**60**) [Figure 5] the strong NOE's between the 15α-H (δ 2.88), the 15β-H (δ 2.41), and the aromatic-H_o's (δ 7.56) requires that the 16-exocyclic double bond be of the (E)-configuration. A strong NOE is also observed between the 15β-H (δ 2.41) and the 13β-CH_3 (δ 0.96).

Deoxygenation of the 17-keto-benzylidene steroid (**60**) was effected using the $LiAlH_4$-$AlCl_3$ reagent to give the 16(E)-benzylidene-5α-androstane (**61**) as the major product (75%). The conversion of the 17-keto function to the 17-methylene group removes the desheilding influence on the vinylic-H which now comes to resonance upfield (δ 6.37) of the aromatic protons as a broad singlet and exhibits an NOE interaction with the 17-H's [(δ 2.28), see Figure 6].



(**61**)

Additional NOE's observed between the vinylic-H and the aromatic-H_o's and not the 15-H's restricts the exocyclic double bond to the (E)-configuration. Further confirmation of this assignment is provided by the observed NOE's between the aromatic-H_o's and the 15-H's and not the 17-H's.

The minor product, isolated in 23% yield, from the reaction mixture of the mixed hydride reduction of (**60**) was 16(E)-benzylidene-5α-androstan-17β-ol [(**61a**), mp 199-200 °C (from acetone), lit.[97] mp 198-199 °C (from EtOH)]; the synthesis of (**61a**) was reported to have been accomplished by $NaBH_4$ reduction of (**60**) [97].

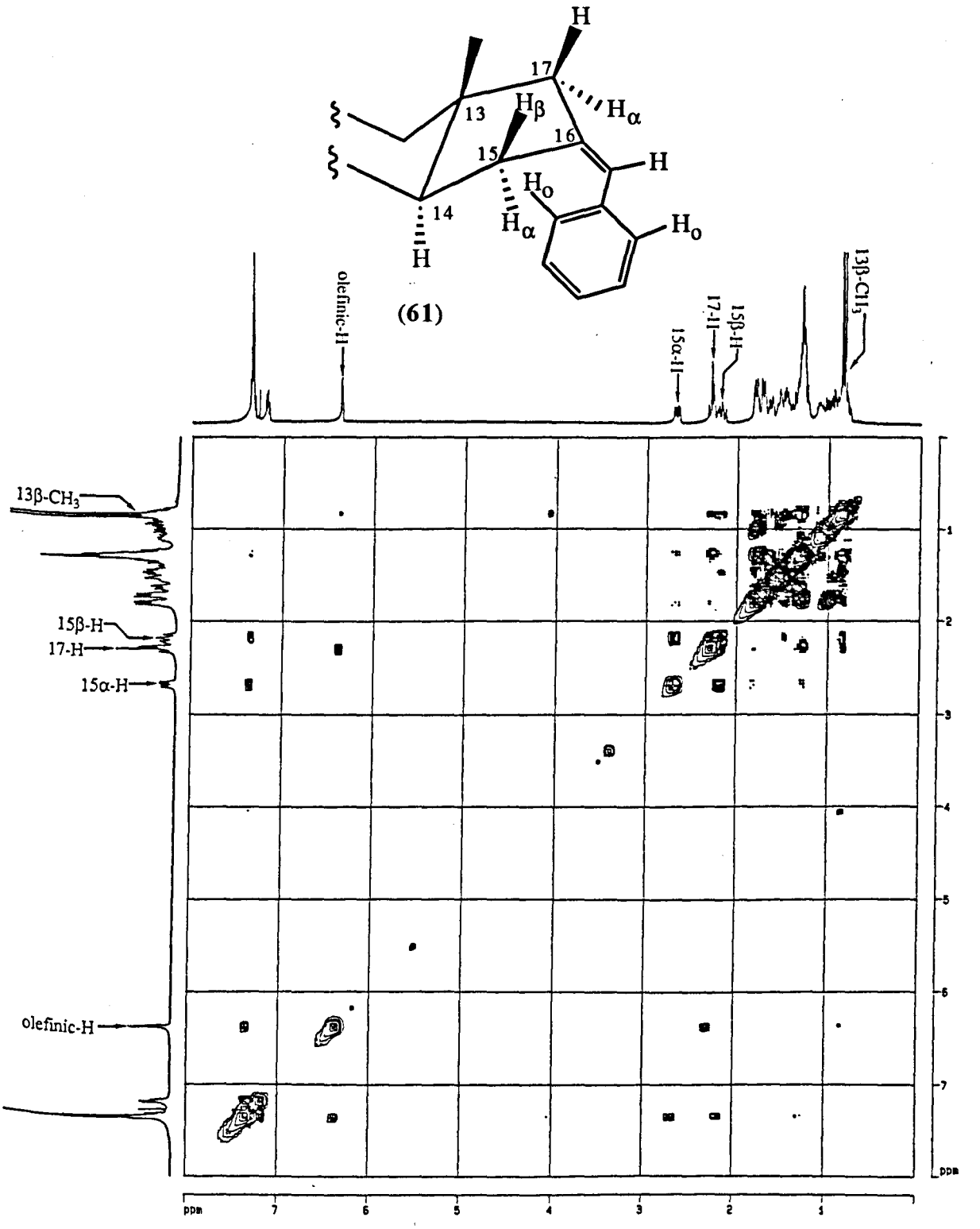


Figure 6. NOESY spectrum (400 MHz, $CDCl_3$) of 16(E)-benzylidene-5α-androstane (**61**).

At variance with our findings, Bridgeman *et al* [97] reported the quantitative conversion of (**60**) to (**61**), in 96% yield, with no mention of (**61a**) being formed.

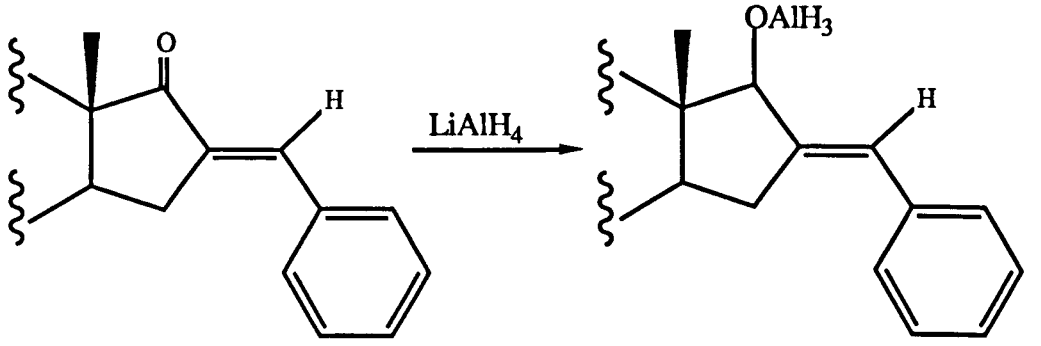
Brewster *et al* reported the preparation and use of the mixed hydride reagent for the dihydro-de-oxo-bisubstitution of ketones. These researchers reacted aluminium chloride and lithium aluminium hydride (in a stoichiometric molar ratio of 3:1) in sodium-dried ether to give the mixed hydride reagent formulated as dichloroaluminium hydride ($AlCl_2H$) [100]:

$$3\,AlCl_3 \quad + \quad LiAlH_4 \quad = \quad LiCl \quad + \quad 4\,AlCl_2H.$$

However, in a modified procedure, Bridgeman *et al* [97] claimed that when an appreciable excess of hydride was used for reagent synthesis [$AlCl_3$:$LiAlH_4$ (1.6 : 1)] a quantitative yield (96%) of the mixed hydride reduction product (**61**) was obtained.

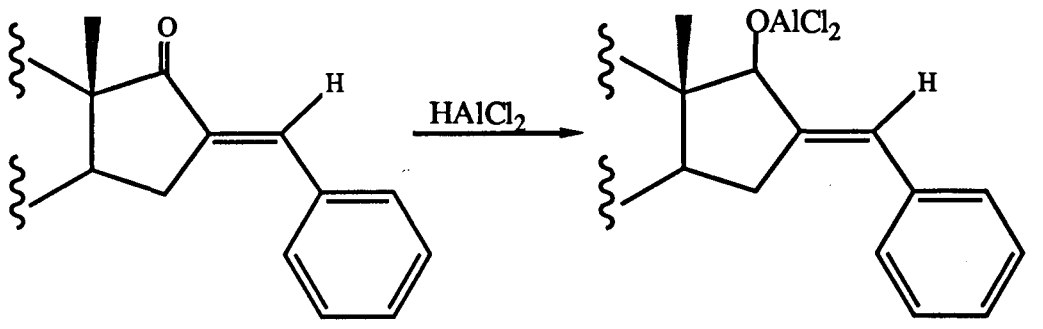
In our experience, using Bridgeman's [97] ratio of reactants for synthesis of the mixed hydride reagent, only a moderate yield (75%) of (**61**)was achieved. In addition the by-product (**61a**) was formed in 23% yield.

The formation of (**61a**) is thought to proceed by way of the excess hydride rapidly converting a fraction of the benzylidene ketone (**60**) to the intermediate alkoxy aluminium hydride (**63**):



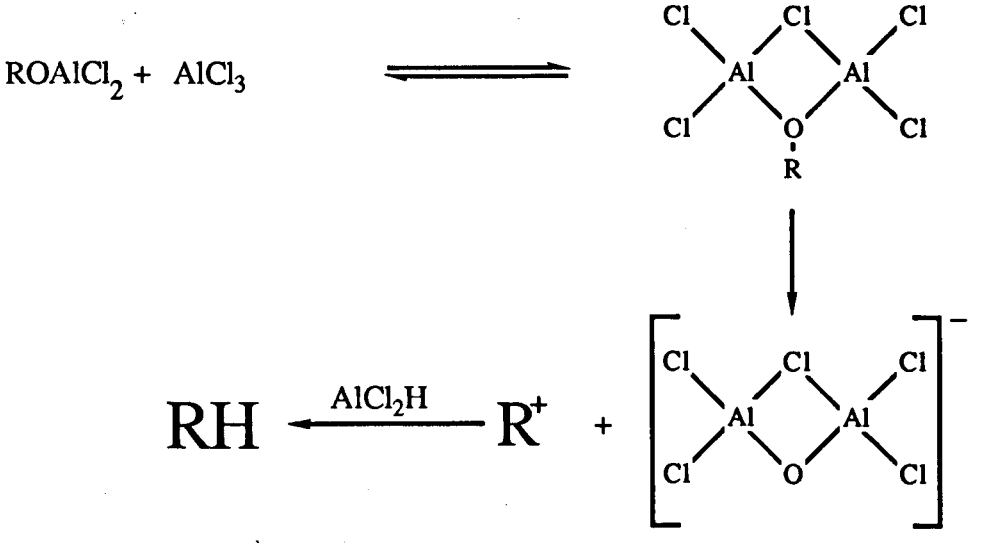
(**60**) (**63**)

which is more resistant to hydrogenolysis than the corresponding dichloroaluminium alkoxide (**64**) which is formed between (**60**) and dichloroaluminium hydride:

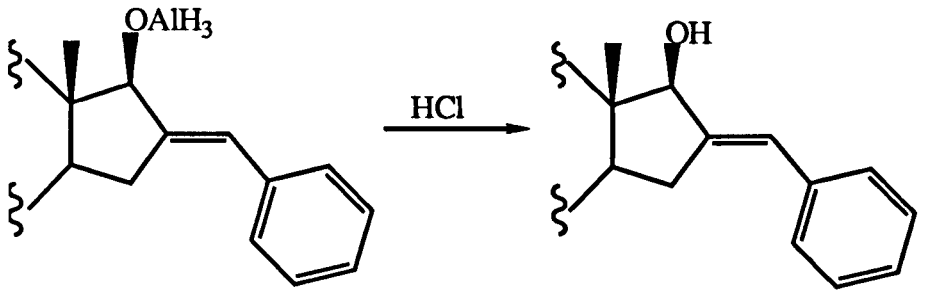


(60) **(64)**

It has been postulated in the literature that the dichloroaluminium alkoxide ($ROAlCl_2$) undergoes ionisation, which is assisted by preceded coordination of the oxygen atom with a second molecule of Lewis acid ($AlCl_3$), to give a carbonium ion which is then reduced to give the deoxygenated product [100] :



The intermediate alkoxy aluminium hydride (**63**) may be resistant to this assisted ionisation facilitated by aluminium chloride and results in the formation of (**61a**) during work-up with aqueous acid:

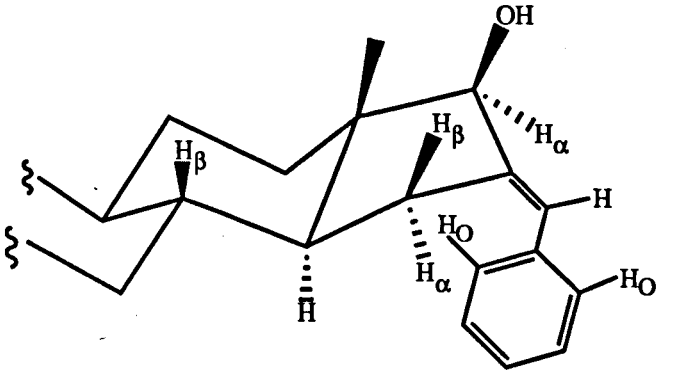


(63) **(61a)**

Although the Huang-Minlon reduction was used to effect the deoxygenation of (**57**) to (**58**) [see Scheme 1, page 44] it was not employed with this substrate (**60**) because it has been shown to give a poor yield (20%) of the desired product (**61**)[97].

The appearance in the ^1H NMR spectrum of (**61a**) [Figure 7] of the 17α-H and the olefinic-H as a broad ill-defined triplet (δ 4.06) and as an ill-resolved quartet (δ 6.52) respectively was shown in the COSY spectrum of (**61a**) [Figure 8] to be due to long range couplings; the 17α-H shows long range coupling with the olefinic-H and the 15β-H (δ 2.12-2.22) and the olefinic-H shows long range coupling to the 17α-H, the 15β-H and the 15α-H (δ 2.66).

The stereochemistry of the exocyclic double bond and the 17-OH group of (**61a**) was established to be of the (E)-configuration and of the β-orientation respectively on the basis of the observed NOE's (Figure 9).



(61a)

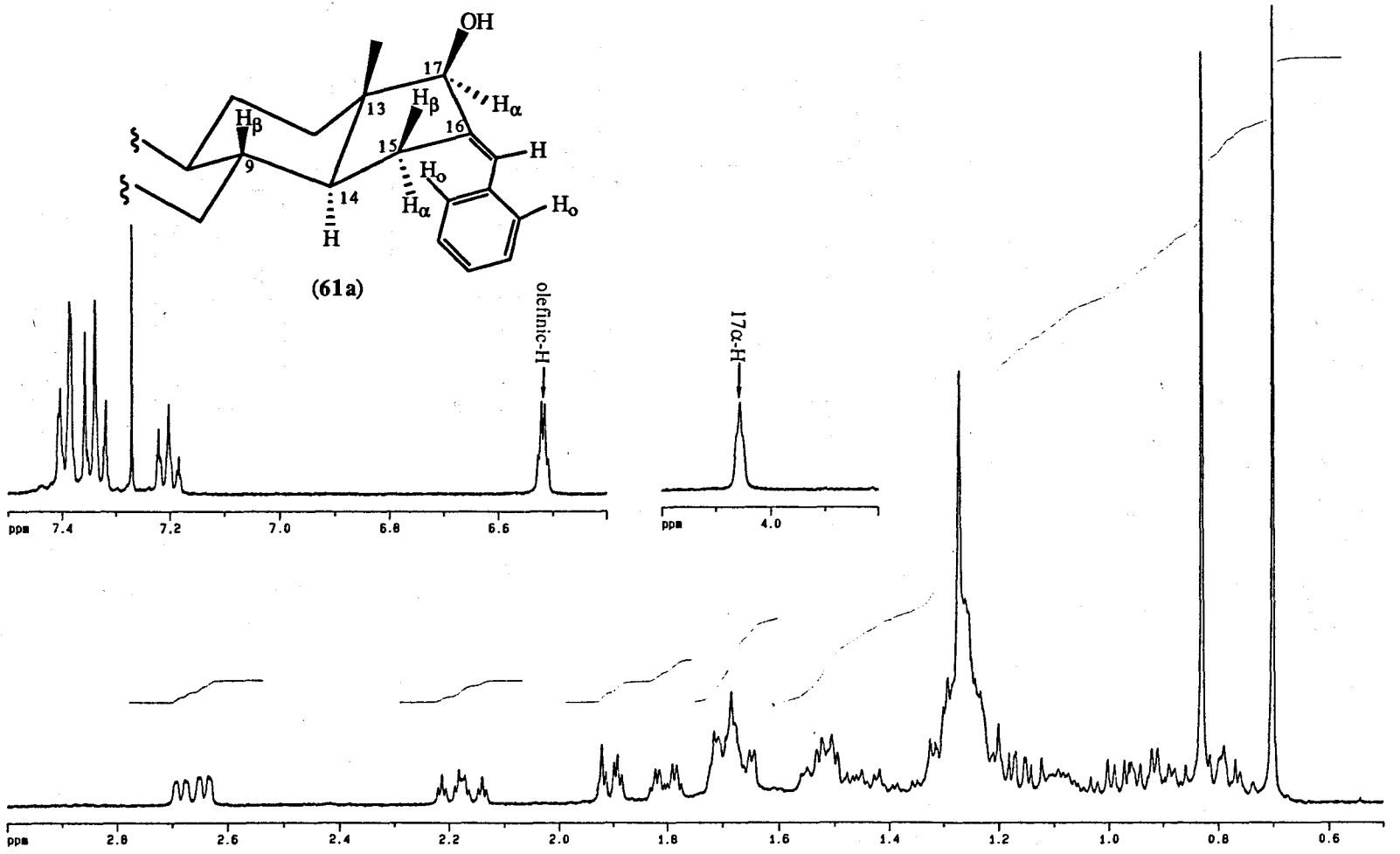


Figure 7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 16(E)-benzylidene-5α-androstan-17β-ol (**61a**).

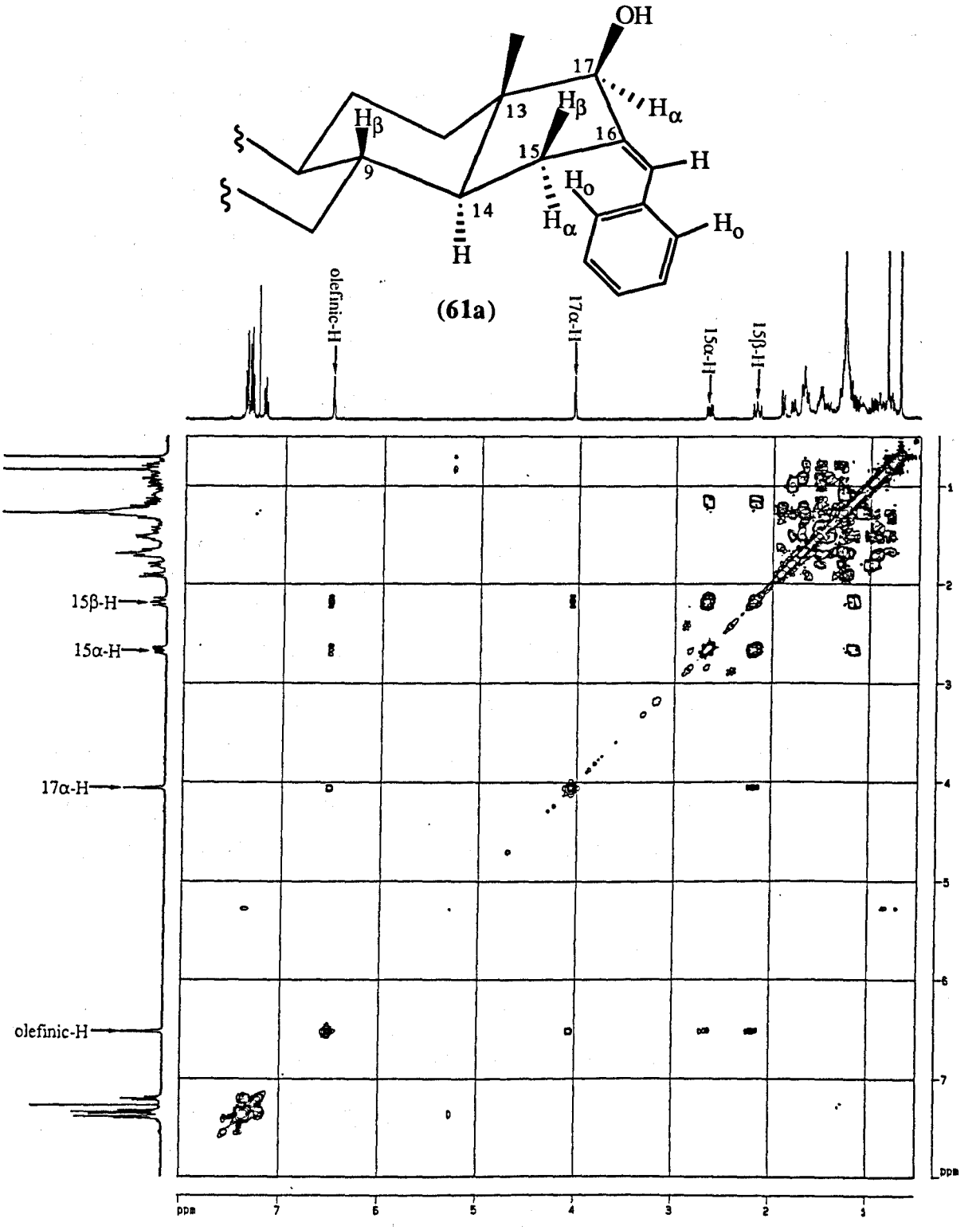


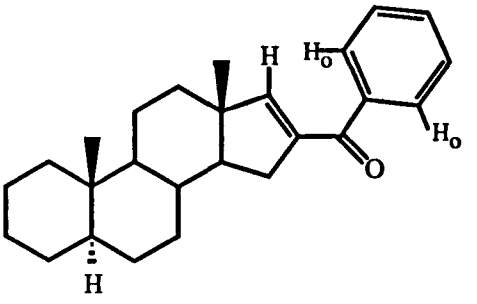
Figure 8. COSY spectrum (400 MHz, $CDCl_3$) of 16(E)-benzylidene-5α-androstan-17β-ol (**61a**).

The observed NOE's between the aromatic-H's and the 15α-H, the 15β-H and the olefinic-H, and not the carbinolic 17-H requires the exocyclic double bond to be of the (E)-configuration.

The NOE's between the 13β-CH_3 (δ 0.70) and the 15β-H (δ 2.12-2.22) and not the 15α-H (δ 2.66) confirm their orientations. Also the weak NOE observed between the 13β-CH_3 and the 17-H (δ 4.06) and the absence of an NOE between the 17-H and the 15β-H require that the 17-H is of the α-configuration. Therefore the 17-OH group is confirmed in the β-orientation.

Ozonolysis of (**61**) was followed by reductive hydrolysis with glacial acetic acid and zinc powder to afford 5α-androstan-16-one (**56**) in poor yield.

In the above sequence of reactions (Scheme 1, page 44) the ozonisation step was responsible for the low overall yield of (**56**) as steps **a**) to **d**) led to the desired products in reproducibly high yields. The ozone output from the laboratory ozoniser (Tack Model-L5), used initially, was low and erratic. Attempts to drive step **e**) to completion by employing extended reaction times led to the formation in moderate yields (61%) of a product (**65**) which, on the basis of accurate mass spectral data, was shown to be isomeric with 16(E)-benzylidene-5α-androstan-17-one (**60**).



16-Benzoyl-5α-androst-16-ene (**65**)

Following the interpretation of results from a series of NMR experiments (COSY, NOESY, HMBC, HMQC) the above structure was assigned to product (**65**).

A number of candidate structures, isomeric with (**65**) [*see* Figure 10] were

considered and then discounted as none of them were wholly consistent with the spectral data gathered.

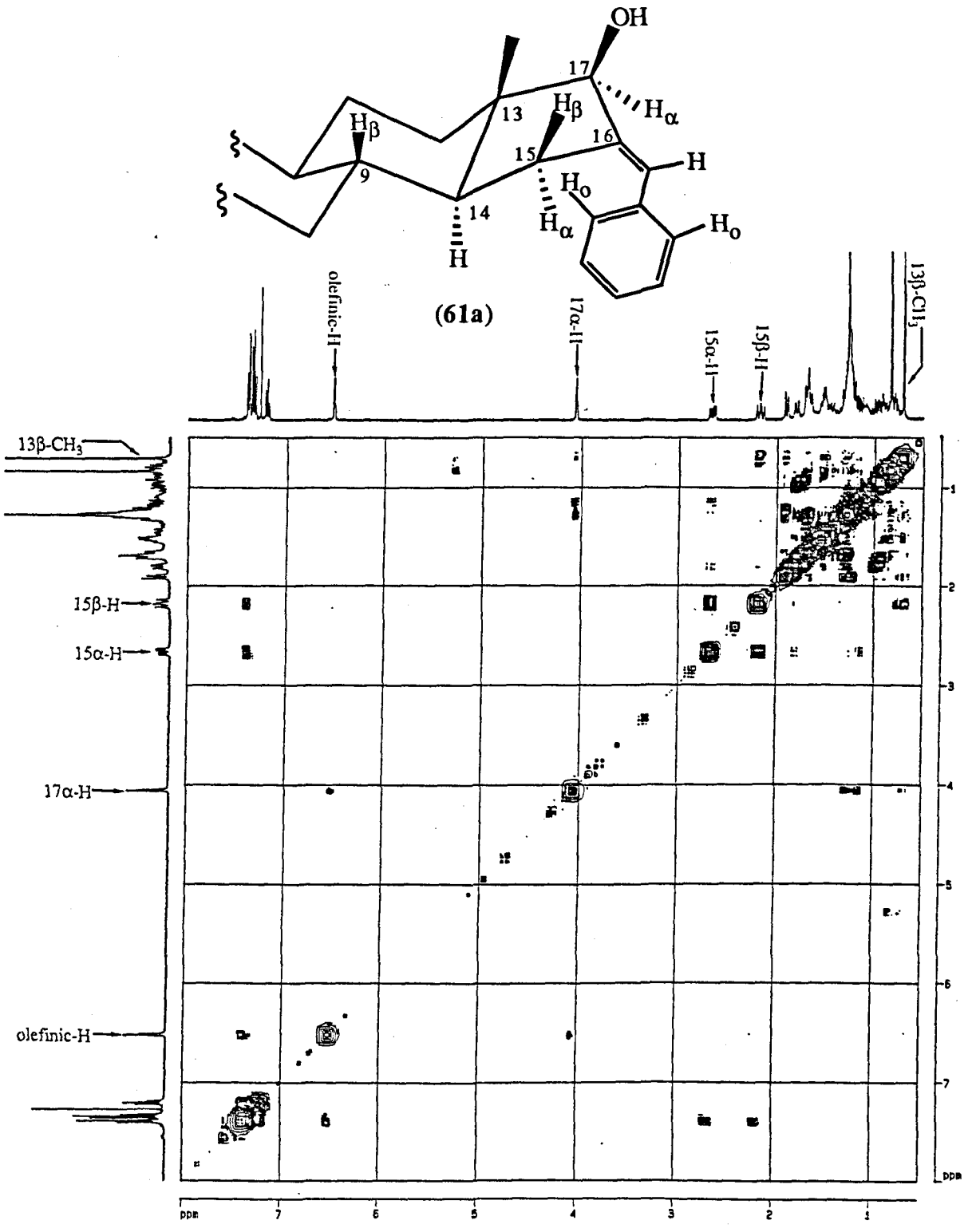
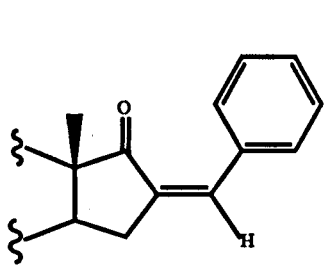
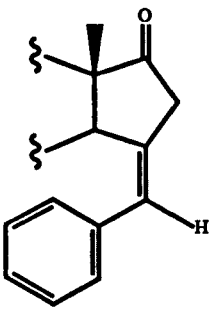


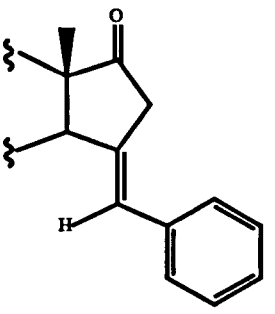
Figure 9. NOESY spectrum (400 MHz, $CDCl_3$) of 16(E)-benzylidene-5α-androstan-17β-ol (**61a**).



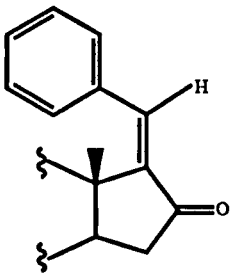
(62)



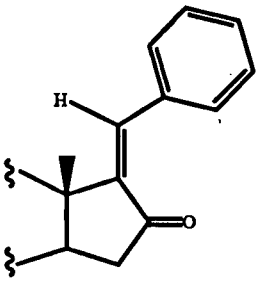
(66)



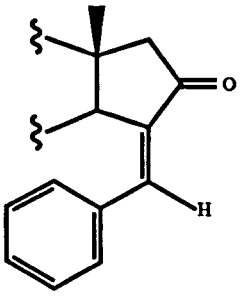
(67)



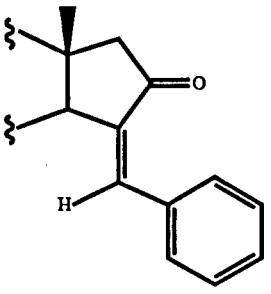
(68)



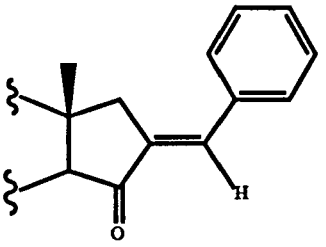
(69)



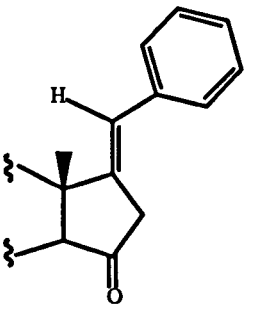
(70)



(71)

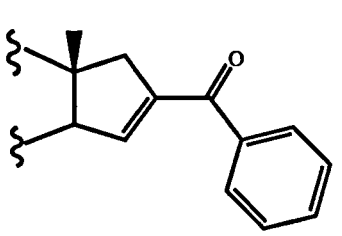


(72)

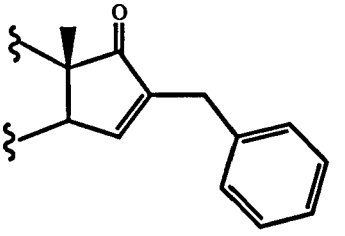


(73)

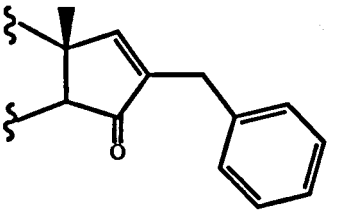
Figure 10: Structural isomers of 16-benzoyl-5α-androst-16-ene (**65**).



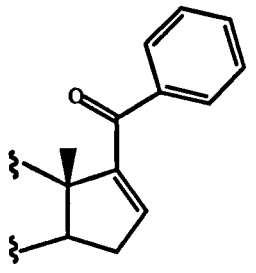
(74)



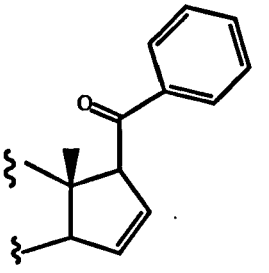
(75)



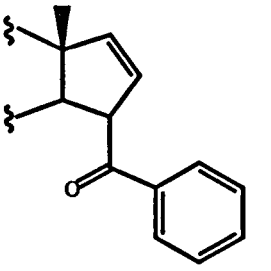
(76)



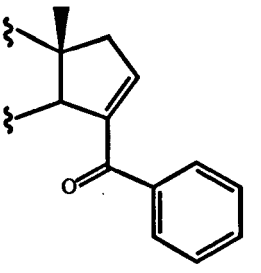
(77)



(78)



(79)



(80)

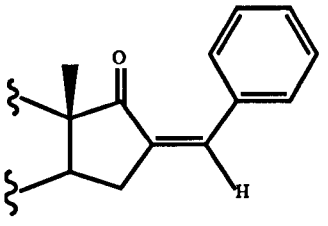
Figure 10: Continued

Examination of the ^1H NMR spectrum of **(65)** [Figure 11] revealed a one-proton double doublet centred at δ 2.27 (J 17.6, 14.14 Hz), a one-proton double doublet centred at δ 2.71 (J 17.6, 4.42 Hz), and a sharp one-proton singlet at δ 6.79 which were assigned to the 15β-H, the 15α-H, and the olefinic-H respectively (*see* later).

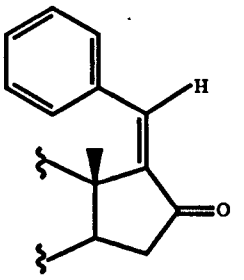
On the basis of the sharp unperturbed one-proton olefinic signal δ 6.79 and the absence of any coupling to other protons (see COSY spectrum, Figure 12), proposed structures (**77**)-(**80**) were rejected.

Structures (**74**)-(**76**) could not account for the one-proton double doublets at (δ 2.27, 2.71) and were disallowed. The same is true of structures (**66**), (**67**), and (**70**)-(**73**) which were not further entertained.

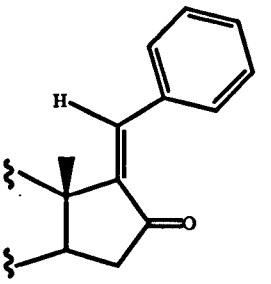
The remaining structures (**62**), (**68**), and (**69**) short-listed below were worthy of further consideration.



(**62**)



(**68**)



(**69**)

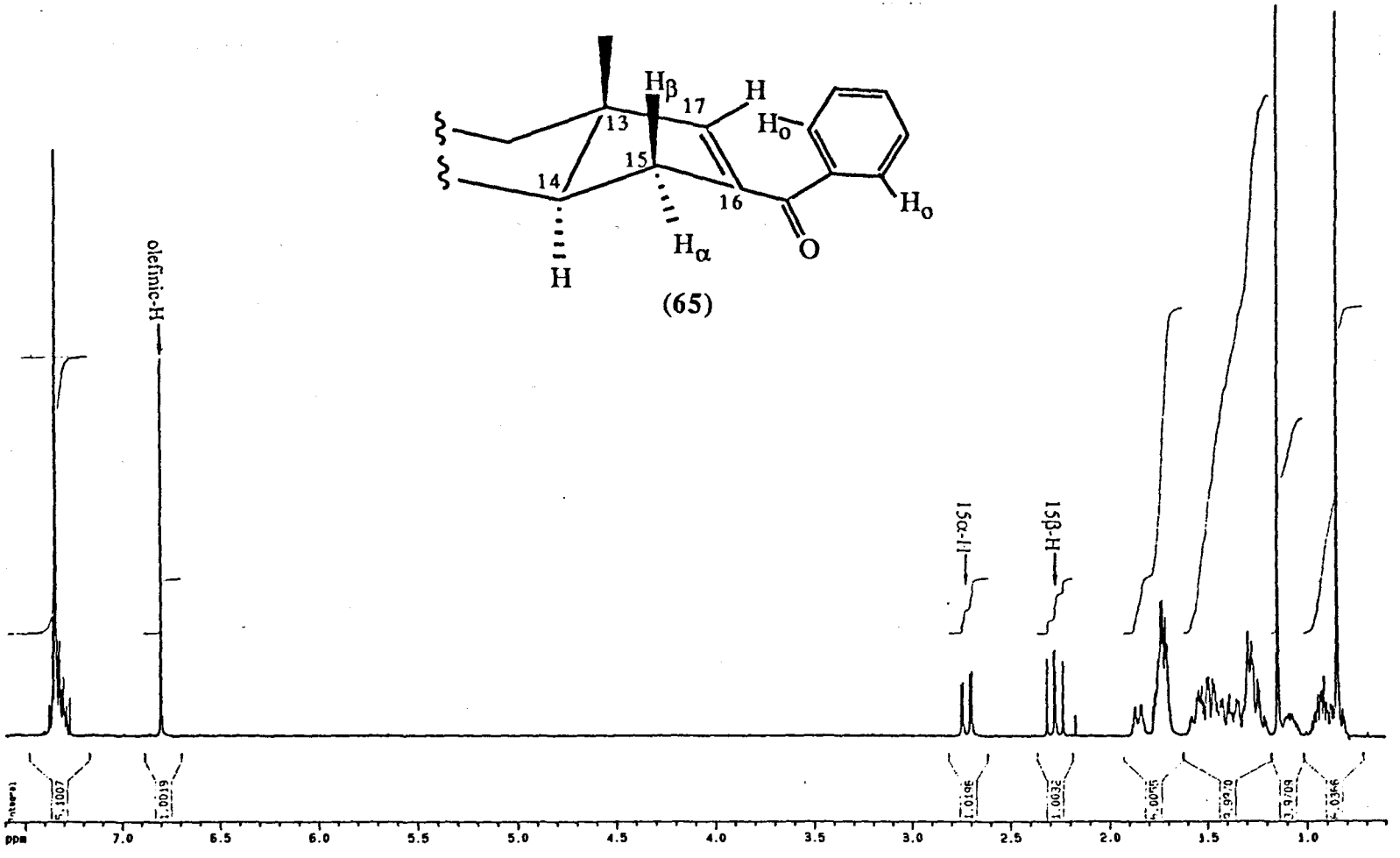
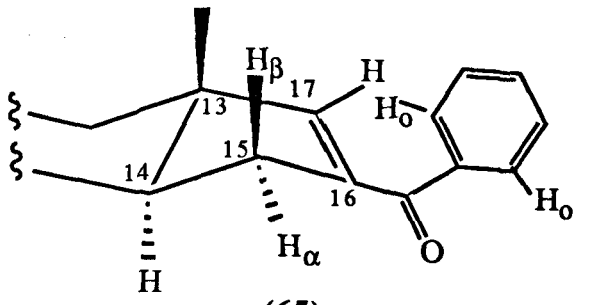


Figure 11. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 16-benzoyl-5α-androst-16-ene (**65**).



(65)

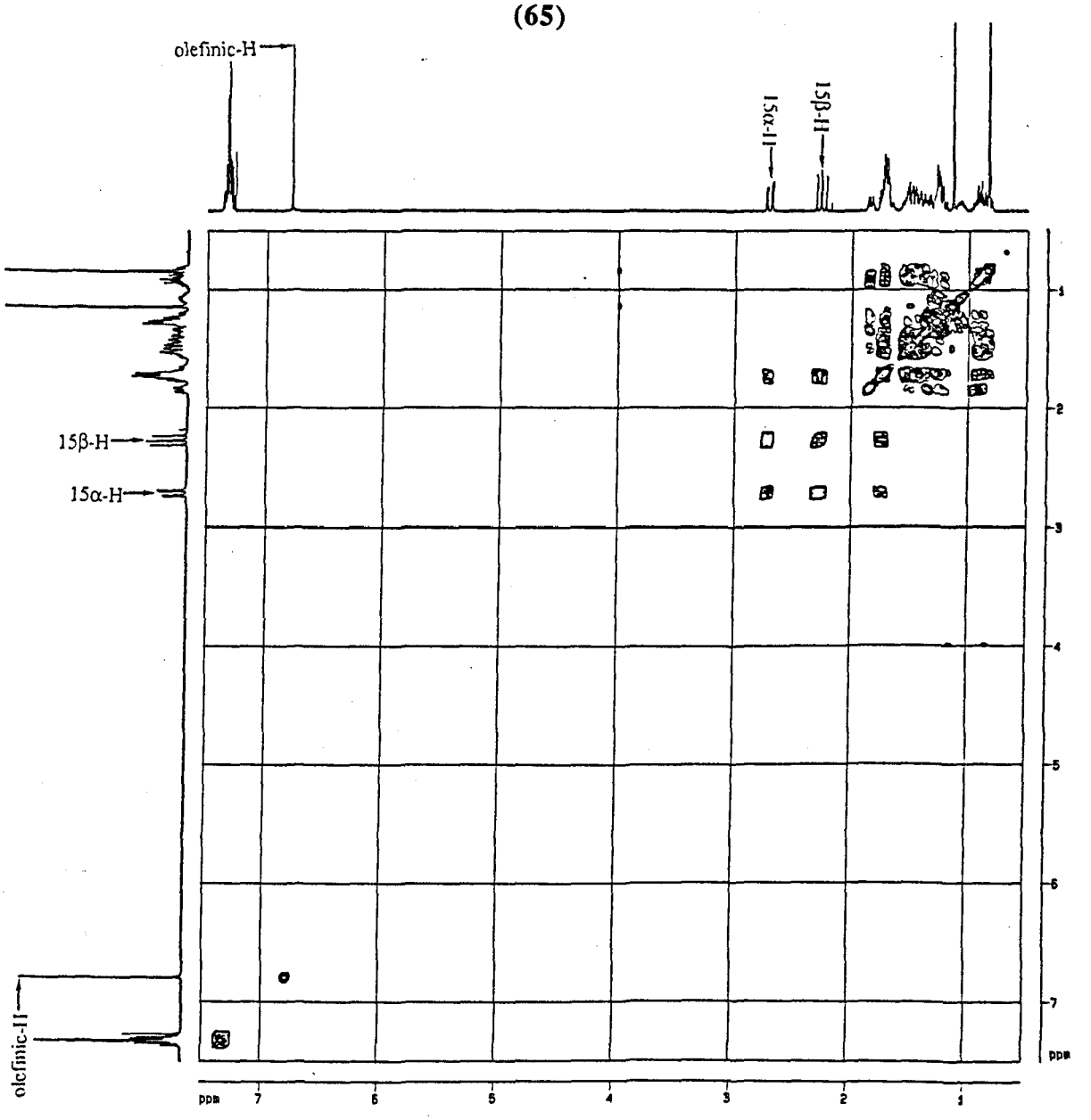


Figure 12. COSY spectrum (400 MHz, $CDCl_3$) of 16-benzoyl-5α-androst-16-ene (**65**).

A study of the NOESY spectrum of (**65**) [Figure 13] showed a strong NOE between the 13β-CH_3 and the olefinic-H, which excluded (**62**) as likely.

Although no NOE's were observed between the 13β-CH_3 and the aromatic-H's, an expectation with structures (**68**) and (**69**), they were retained until further spectral evidence for their rejection was found; this was provided by studying the HMBC spectrum of (**65**) [Figure 14] which illustrates the connectivities of carbon-13 and hydrogen atoms in the molecules, by examining the long range heteronuclear couplings between these atoms over two and three bonds (^2J, $^3J \sim 7Hz$).

Long range couplings between the olefinic-H and the carbonyl carbon (^3J), the olefinic-H and the quaternary C-13 (^3J), the olefinic-H and the *ipso* alkenic carbon (C-16) [^2J], the 15-methylene protons and the carbonyl carbon (^2J), and the 15-methylene protons and the tertiary C-14 (^2J) were satisfied by structures (**68**) and (**69**).

However the additional long range couplings observed between the 18-protons and the alkenic =C-H carbon (C-17) [^3J], the olefinic-H and C-15 (^3J), and between the olefinic-H and the tertiary C-14 (^3J) could not have been accounted for by structures (**68**) and (**69**) since this would have required couplings over four and/or five bonds. Structures (**68**) and (**69**) were therefore rejected.

On the other hand, structure (**65**) , in addition to accounting for all the long range couplings justified by (**68**) and (**69**) it can also accommodate all the additional couplings not accounted by (**68**) and (**69**) since in (**65**) these couplings occur over three bonds.

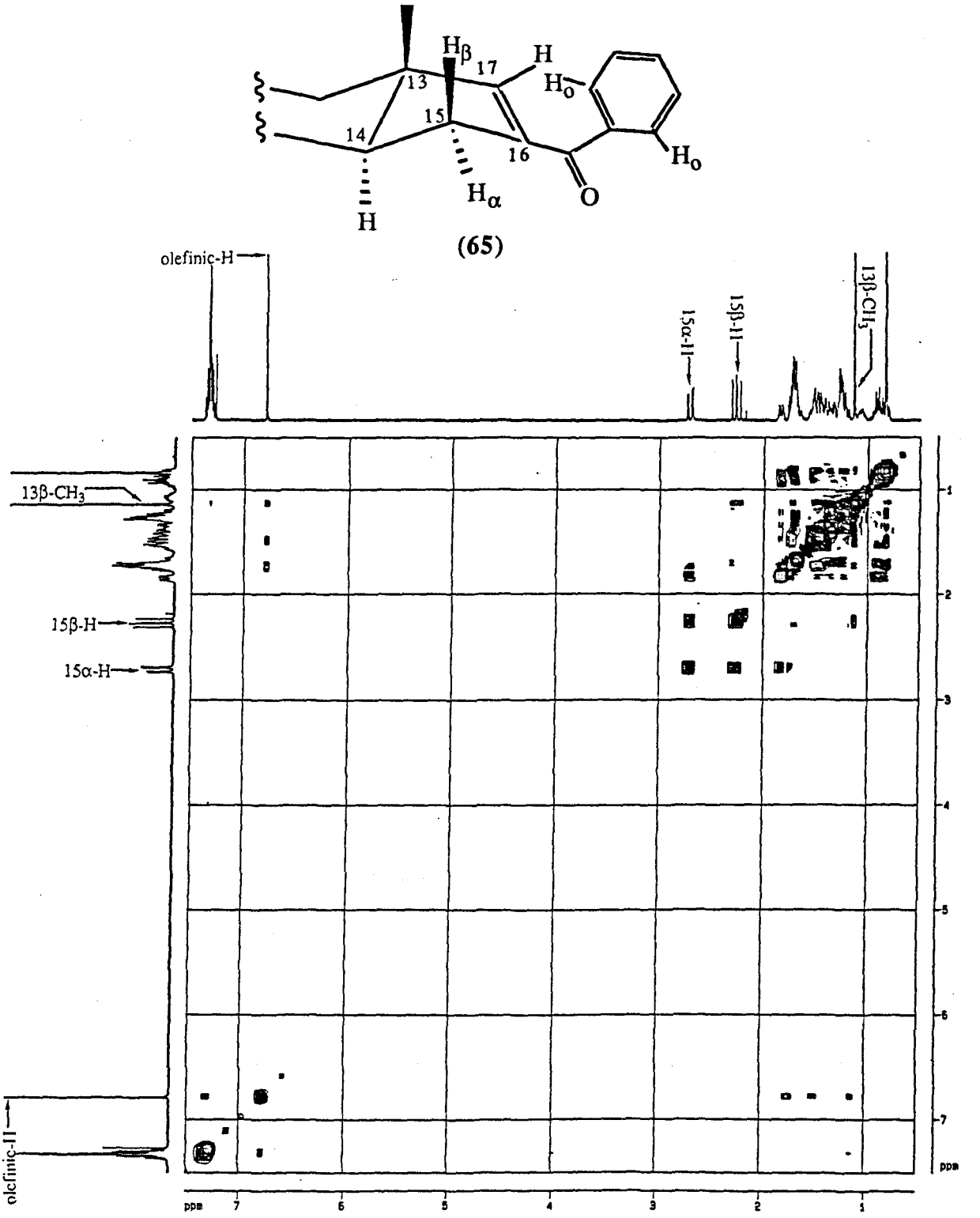


Figure 13. NOESY spectrum (400 MHz, $CDCl_3$) of 16-benzoyl-5α-androst-16-ene (**65**).

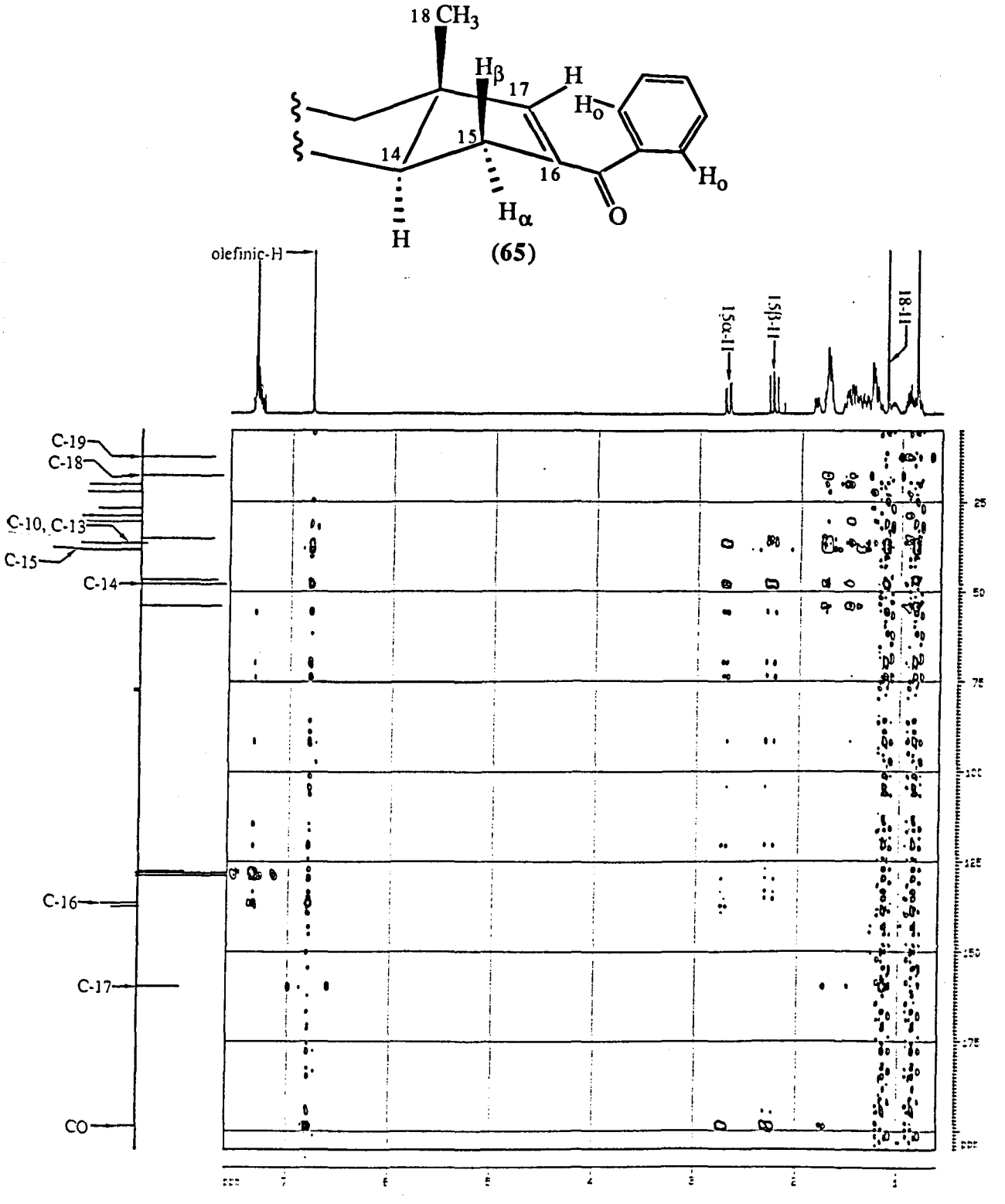


Figure 14. HMBC spectrum (400 MHz, $CDCl_3$) of 16-benzoyl-5α-androst-16-ene (**65**).

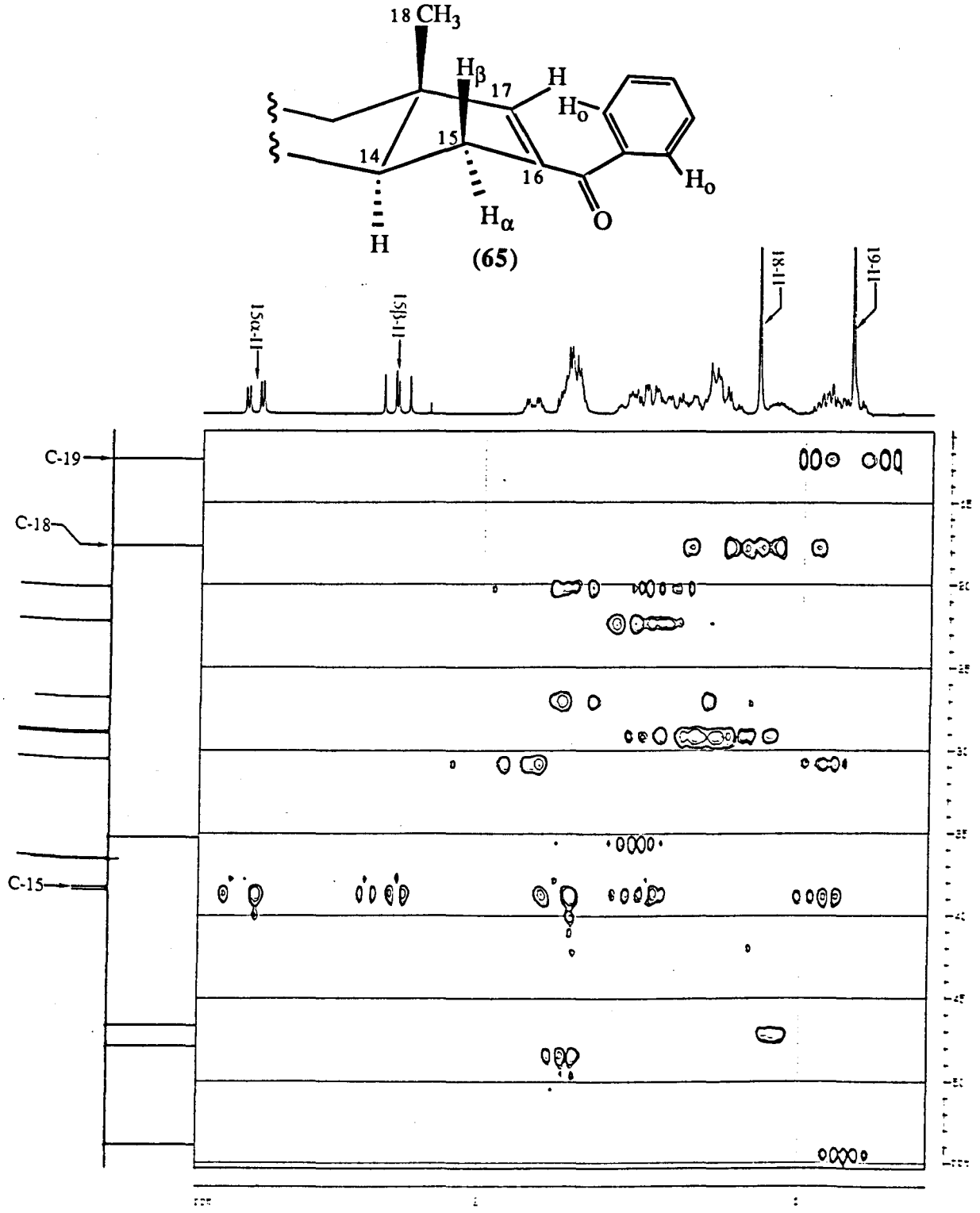
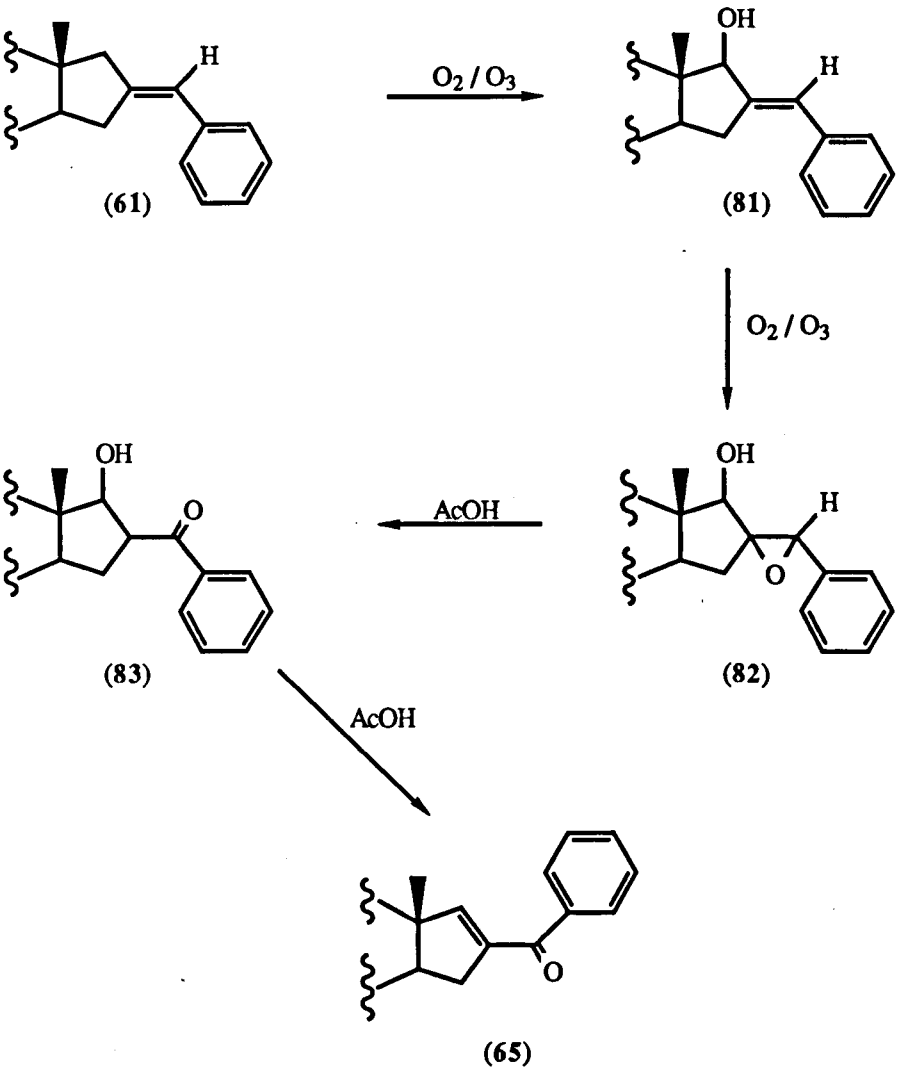


Figure 15. HMQC spectrum (400 MHz, $CDCl_3$) of 16-benzoyl-5α-androst-16-ene (**65**).

The HMQC spectrum of (**65**) [Figure 15], used for identifying the direct C-H couplings (and hence direct C-H bonding) was a useful adjunct in assigning δ_C to C-18, C-19, C-10 and C-13, C-14, C-15 and C-17, which were 17.63, 12.35, 36.49 and 36.57 (not necessarily in that order), 47.86, 38.44 or 38.27, and 159.68 respectively.

The unexpected formation of (**65**) during ozonolysis of (**61**) may have occurred by the reaction pathway proposed in scheme 2 :



Scheme 2. Proposed mechanism for formation of (**65**).

The low O_3/O_2 ratio output by the portable laboratory ozonizer (Model-L5) may have favoured initial allylic oxidation followed by epoxidation of the olefinic double bond to give (**82**). Epoxide rearrangement to the β-hydroxy ketone (**83**) and its subsequent dehydration would afford (**65**).

Support for the above proposal is provided by literature citations of allylic oxidation and epoxide formation during the ozonisation of hindered olefins[101-104]. Such epoxides have been shown to undergo rearrangement to ketones[105-110].

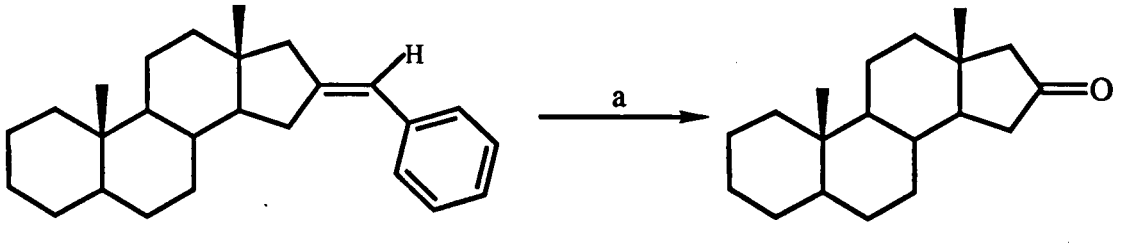
The lability of the β-hydroxy ketone system (**83**) is understandable, since under acid conditions, there is a strong driving force for elimination of the 17-hydroxy group, as water, to give the more stable α, β -unsaturated ketone (**65**).

Because of the poor yields of 5α-androstan-16-one (**56**) obtained in the above (Scheme 1), alternative synthetic strategies to (**56**) were pursued with an aim to improve the yield of this essential ketone (**56**).

2.2 Alternative synthetic approaches to 5α-androstan-16-one (56).

2.2.1 Approach 1.

In the first approach (Scheme 3) 16(E)-benzylidene-5α-androstane (**61**) was subjected to the Lemieux-von Rudloff oxidation:



(**61**) (**56**)

Scheme 3. Reagents: *a*, KIO_4 - $KMnO_4$ / Pyridine.

The yield of (**56**) was disappointingly low and a large proportion of the starting material (**61**) was recovered.

The permanganate-periodate oxidation of olefins soluble in slightly alkaline aqueous media was developed by Lemieux and von Rudloff[111]. With some slight modifications, the procedure was adapted to the oxidation of the hydrophobic 16-benzylidene steroid (**61**). Pyridine was chosen as solvent since it was capable of dissolving potassium periodate and the substrate (**61**) and is miscible with the catalytic amount of potassium permanganate which is added in aqueous solution. Pyridine also maintains the optimum pH of 8.0-8.5 necessary for reaction[112].

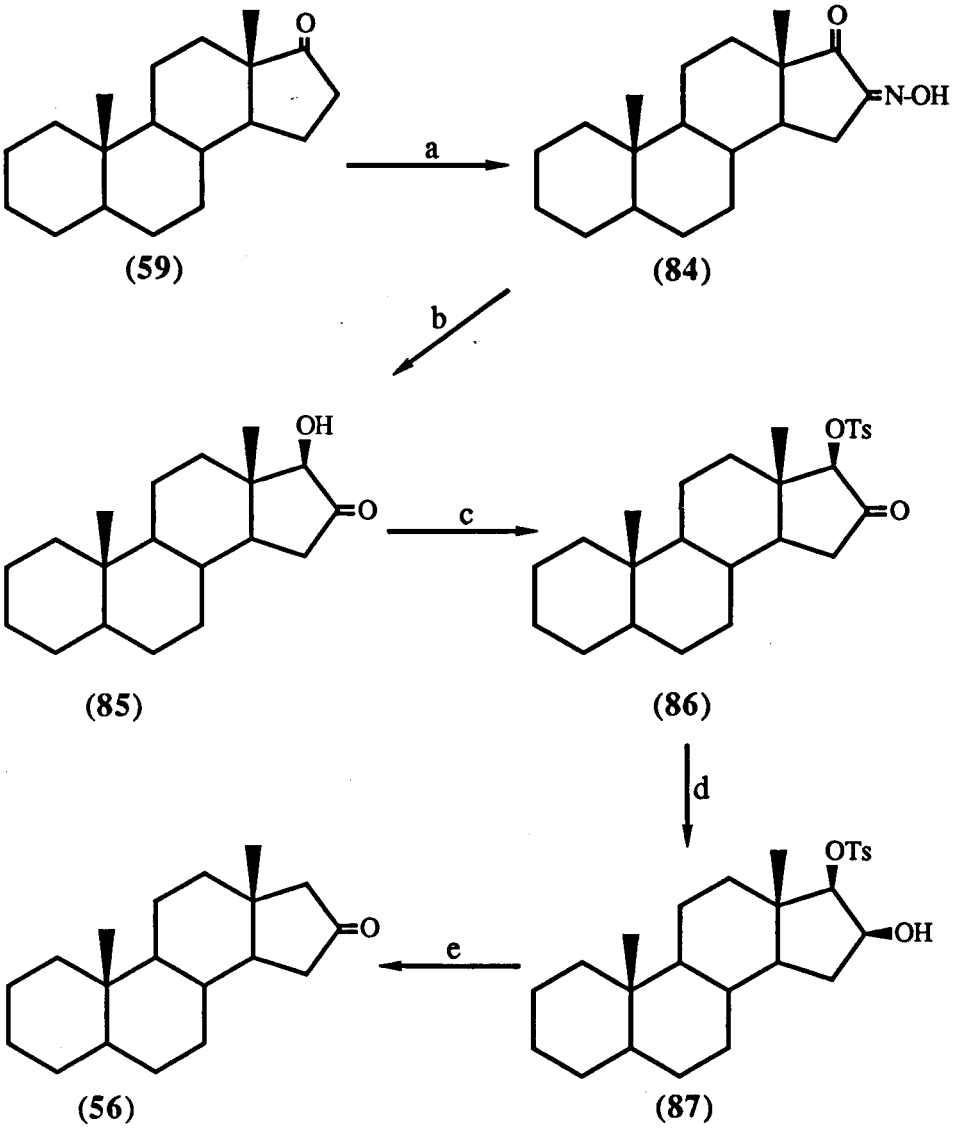
Permanganate has been shown to oxidise olefinic double bonds to a mixture of α-hydroxy ketones, and *vic*-diols which are rapidly cleaved by periodate[113] to products which may be further oxidised by permanganate[111].

In this procedure permanganate is regenerated by the action of periodate *in situ*, hence the use of catalytic quantities of permanganate.

The discharging of the purple permanganate colour of the reaction medium and its replacement with a pinkish colouration together with the formation of brown precipitate, assumed to be manganese dioxide, seems to suggest that the failure of the reagent to convert more of the starting material (**61**) to the product (**56**) may be due to the rapid reduction of the permanganate beyond the manganate stage, by the exocyclic olefin (**61**), more quickly than re-oxidation by periodate, thus stopping further permanganate-olefin reaction[113].

2.2.2 Approach 2.

In the second approach (Scheme 4), the method described by Varech and Jacques[114] was followed:

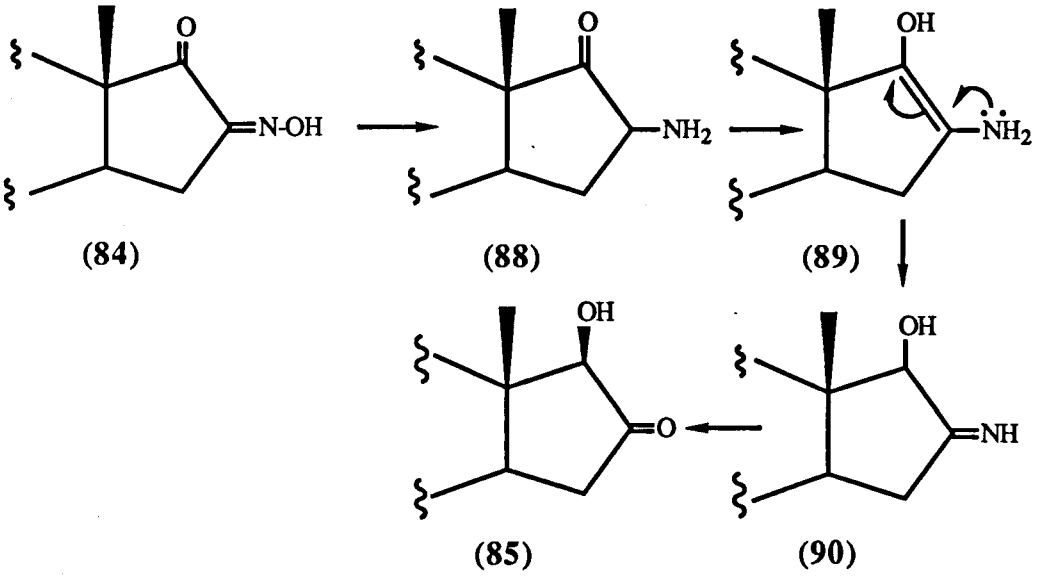


Scheme 4. Reagents: *a*, KOBu^t - Bu^{t}OH - isoamyl nitrite; *b*, Zn powder - AcOH - H_2O; *c*, *p* -TsCl - pyridine; *d*, $NaBH_4$ - CH_3OH - C_5H_5N; *e*, NaOH - CH_3OCH_2OH reflux.

In the conversion of (**59**) to (**84**), a slight modification to the literature procedure was made. Instead of generating the tertiary alkoxide, *in situ*, by reacting potassium metal with anhydrous *t*-butanol, an equivalent amount of commercially

available KOBu^t was used. A slight increase over the literature yield for (**84**) [68%][114] was obtained (77%).

The zinc-acetic acid reduction of the 16-hydroxyimino-17-keto steroid (**84**) to 17β-hydroxy-16-ketone (**85**) has been reported to proceed through the 16-amino-17-ketone (**88**), followed by tautomerisation and subsequent hydrolysis of the 16-imino compound (**90**) to (**85**)[115] (Scheme 5):



Scheme 5. Reductive hydrolysis of (**84**)

Tosylation of the α-ketol (**85**) proceeded smoothly in anhydrous pyridine to give the 17β-tosyloxy-5α-androstan-16-one (**86**) in 91% yield, which was reduced using $NaBH_4$ in a mixture of anhydrous methanol and anhydrous pyridine to give 17β-tosyloxy-5α-androstan-16β-ol (**87**) in 71% yield.

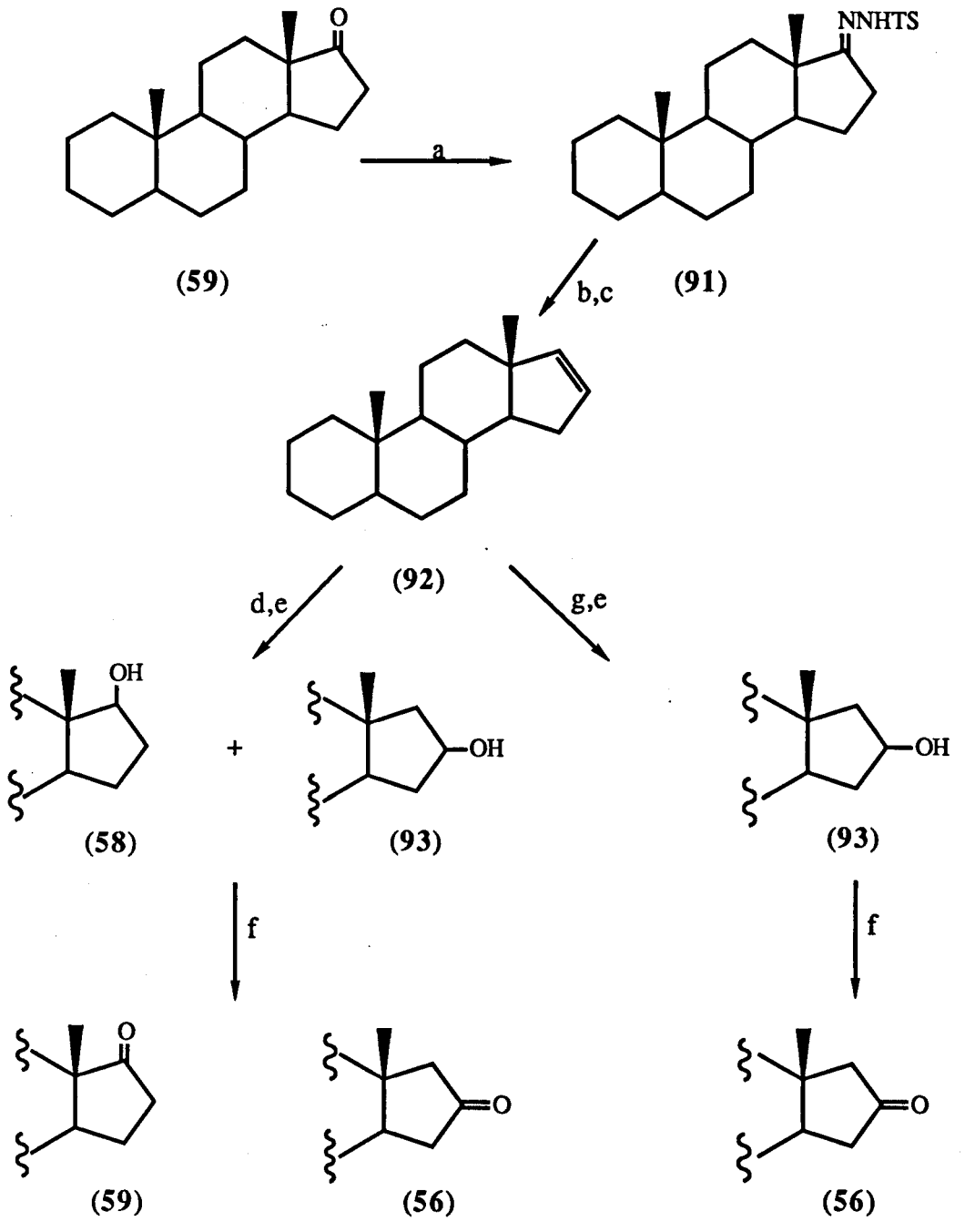
The formation of the 16β-hydroxy product (**87**) is consistent with the principle of steric approach control reported for the reduction of hindered ketones by $NaBH_4$. The presence of the bulky 17β-tosyl and 13β-CH_3 groups adjacent to the C-16 ketone effectively blocks approach of the hydride reducing agent from the β-face of the steroid resulting in the generation of the β-hydroxy group.

The treatment of (**87**) with base (NaOH) in refluxing methoxymethanol led to formation of 5α-androstan-16-one (**56**) in 67% yield.

The *anti*-configuration of the 16α-hydrogen and the 17β-tosyl groups facilitates base catalysed elimination to the intermediate enol which then tautomerises to the ketone (**56**).

2.2.3 Approach 3.

In the third approach (Scheme 6) to 5α-androstan-16-one (**56**), the condensation of 5α-androstan-17-one (**59**) with *p*-toluenesulphonyl hydrazide in absolute alcohol led to the corresponding tosyl hydrazone (**91**) in 47% yield.



Scheme 6. Reagents: *a*, NH_2NHTs; *b*, 1.4 M ethereal MeLi; *c*, H_2O; *d*, 1.0 M BH_3 - THF; *e*, $NaOH / H_2O_2$; *f*, Jones Reagent; *g*, 0.5 M 9 BBN.

The decomposition of (**91**) with methyl lithium followed by aqueous work-up afforded 5α-androst-16-ene (**92**) in 78% yield [118,119].

The conversion of (**92**) to (**56**) proceeded by hydroxylation and then oxidation.

Hydroboration, followed by oxidation and hydrolysis provided the route to the intermediate alcohol(s).

The use of BH_3-THF complex as the hydroborating agent led to a mixture of 5α-androstan-16-one (**56**) and 5α-androstan-17-one (**59**), in low yield (16%), following Jones oxidation of the crude mixture of intermediate alcohols [(**58**), (**93**)].

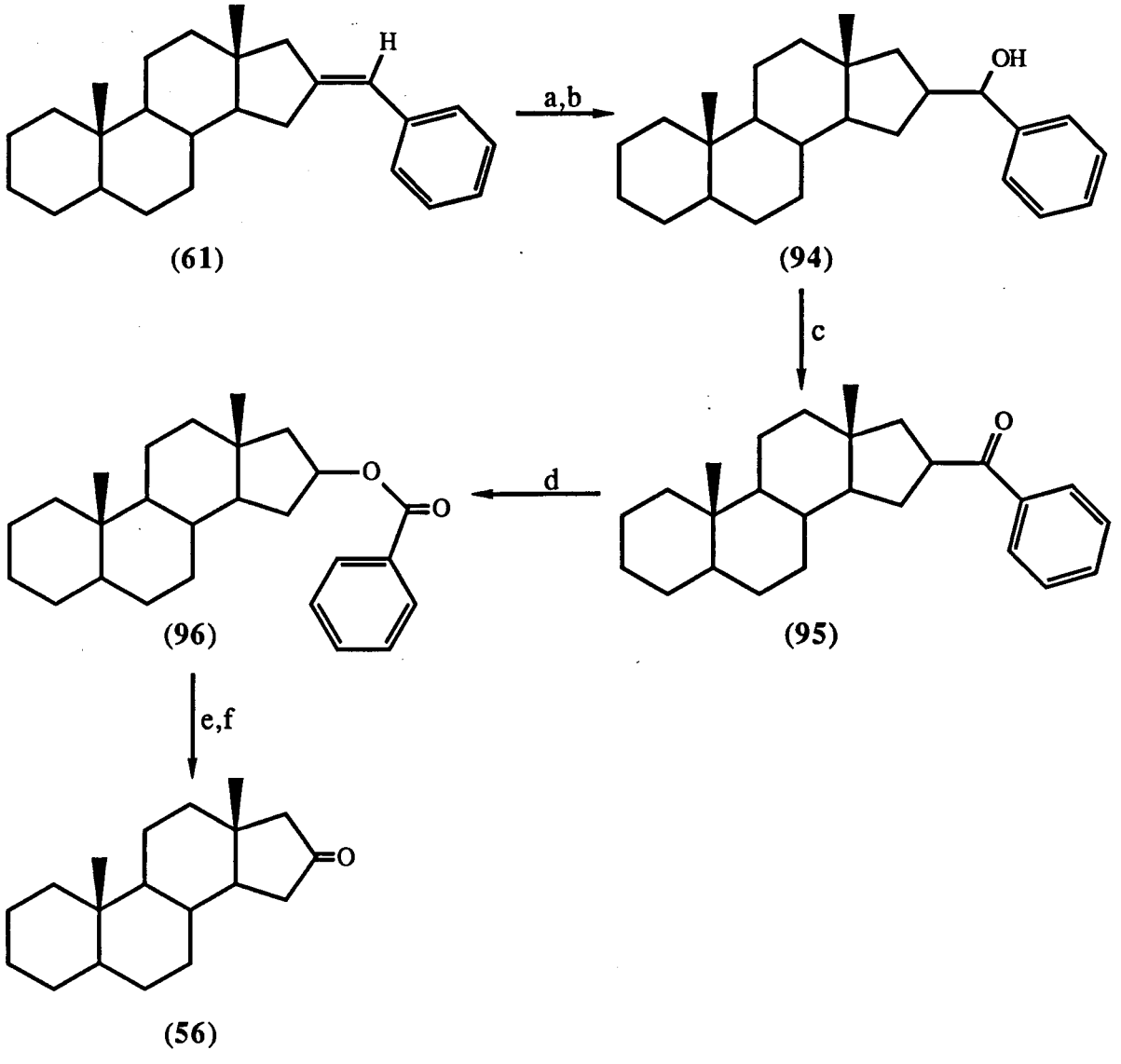
However 9-borobicyclo-[3.3.1]-nonane (9-BBN), a more selective hydroborating agent, gave the desired ketone (**56**), albeit in low yield (10%).

Hydroboration/oxidation provides a valuable synthetic tool for the *anti*-Markovnikov hydration of olefins [120]. For disubstituted olefins such as (**92**), hydroboration using BH_3-THF complex shows little selectivity and results in the formation of equiproportional amounts of each of the possible isomeric alcohols [121]. For this reason it was decided to use 9-BBN as the hydroborating agent after the initial attempt with the BH_3-THF complex afforded an isomeric mixture of the C-16 and C-17 alcohols [(**58**), (**93**)]; this was established by ^1H NMR studies on the corresponding ketones [(**56**), (**59**)] generated by oxidation with Jones reagent. The 13β-CH_3 groups in the 16- and 17-ketones resonate at δ 0.87 and 0.86 respectively[122].

The bulky nature of 9-BBN[123] was expected to allow selective introduction of the boron atom at C-16. Such an introduction at C-17 seemed unlikely due to the presence of the angular methyl group at C-13. Selective reaction did occur at C-16 but the reaction was sluggish and the yield of 5α-androstan-16-ol (**93**) low. The rigid nature of the steroid olefin (**92**) along with the proximal presence of the angular methyl at C-13 and the axial C-14 hydrogen may have hindered optimal alignment of the boron-hydrogen bond with the $\Delta^{16,17}$-double bond which is reported to be necessary for the transfer of the boron and hydrogen atoms *via* a four centred transition state [120,124].

2.2.4 Approach 4.

In a novel synthetic strategy (Scheme 7), the fourth approach to 5α-androstan-16-one (**56**), BH_3-THF complex was used to selectively hydroborate 16(E)-benzylidene-5α-androstane (**61**).



Scheme 7. Reagents: *a*, BH_3 - THF; *b*, NaOH / H_2O_2; *c*, Jones Reagent H_2CrO_4; *d*, *m*-CPBA; *e*, NaOH / MeOH; *f*, Jones Reagent H_2CrO_4.

Oxidative cleavage of the intermediate organoboranes with alkaline peroxide afforded the expected product (**94**) in 70% yield.

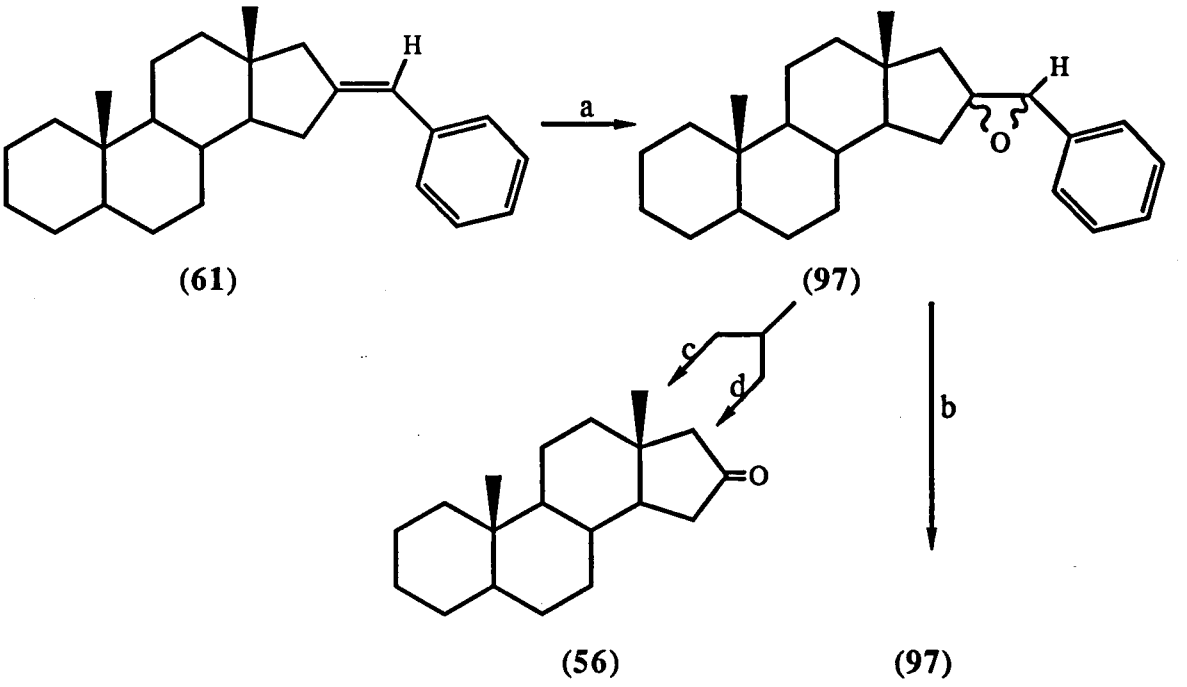
The generation of an asymmetric centre in (**94**) was expected to yield a mixture of diastereomers, but ^1H NMR studies did not confirm the presence of a two-component mixture.

Oxidation of (**94**) using Jones reagent generated the steroid phenone (**95**) in 92% yield. Baeyer-Villiger oxidation of (**95**) gave the 5α-androstan-16-yl benzoate (**96**) which was hydrolysed and the resulting alcohol oxidised to the desired ketone (**56**) in low yield (19.16%).

It is worthy of note that the hydroboration of (**61**) was also carried out using 9-BBN and a similar result was obtained.

2.2.5 Approach 5.

In the fifth approach (Scheme 8) to 5α-androstan-16-one (**56**) oxidation of the 16(E)-benzylidene steroid (**61**) with *m*-CPBA in dry CH_2Cl_2 at room temperature afforded the epoxide (**97**) in quantitative yield.



Scheme 8. Reagents: *a*, *m*-CPBA; *b*, 0.3 N NaOH / 85% DMSO; *c*, periodic acid / H_2O / acetone; *d*, dry ethereal periodic acid.

That this was a mixture of α- and β-isomers was evident from the ^1H NMR spectrum in which the epoxide-methine proton signal was duplicated[125]; integration of these signals afforded the isomer ratio.

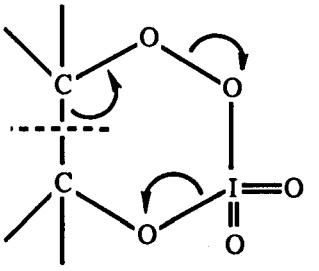
Epoxide opening of (**97**) to the corresponding *vic*-diol[126,127] and its (*vic*-diol) cleavage with lead tetra-acetate to the desired ketone (**56**) was envisaged. However attempts to generate the *vic*-diol failed; with 0.3 N NaOH in 85% DMSO, the starting material was recovered unchanged; with aqueous periodic acid in acetone 5α-androstan-16-one (**56**) was produced.

The latter observation prompted the use of a seldom reported method for the direct conversion of an epoxide to a carbonyl compound [128]. Using a saturated solution of periodic acid in sodium-dried ether under nitrogen, the epoxide (**97**) was converted into (**56**) in 40% yield; Fieser and Fieser[128] explained that since periodic

acid is a hydrate (H_5IO_6), the reagent can be used to effect hydrolytic cleavage of an epoxide.

The periodic acid cleavage of epoxides to carbonyl compounds in aqueous media has been reported by Nagarkatti and Ashley[129] The suggestion by Ashley *et al* [129] that this unusual oxidation does not necessarily proceed by an intermediate *vic*-diol, formed as a result of the cleavage of the epoxide in acid media, was confirmed by performing the reactions in solutions buffered at pH 7.2.

They suggested that a possible mechanism for the oxidation could be visualised as the formation of a periodate complex of the epoxide and subsequent cleavage as illustrated below [129].



During the investigation of the alternative approaches to 5α-androstan-16-one (**56**), a more efficient ozoniser became available. This led to a re-investigation of the original method of synthesis of the 16-ketone (**56**) [*see* scheme 1. page 44] and the use of the efficient ozoniser generated the desired product (**56**) in high yield (92%) and rendered the other approaches redundant for our purposes.

A number NMR experiments were performed on (**56**) to confirm some assignments made.

The assignment of the methyl resonance at δ 0.86 to the 13β-CH_3 group, initially based on the deshielding effect of the of the 16-carbonyl was confirmed by studying the HMBC spectrum of (**56**) [Figure 16]. This revealed strong ^3J couplings to the deshielded 17-methylene protons (α to the CO). Such correlations are not observed with the 15-methylene protons since they would require couplings

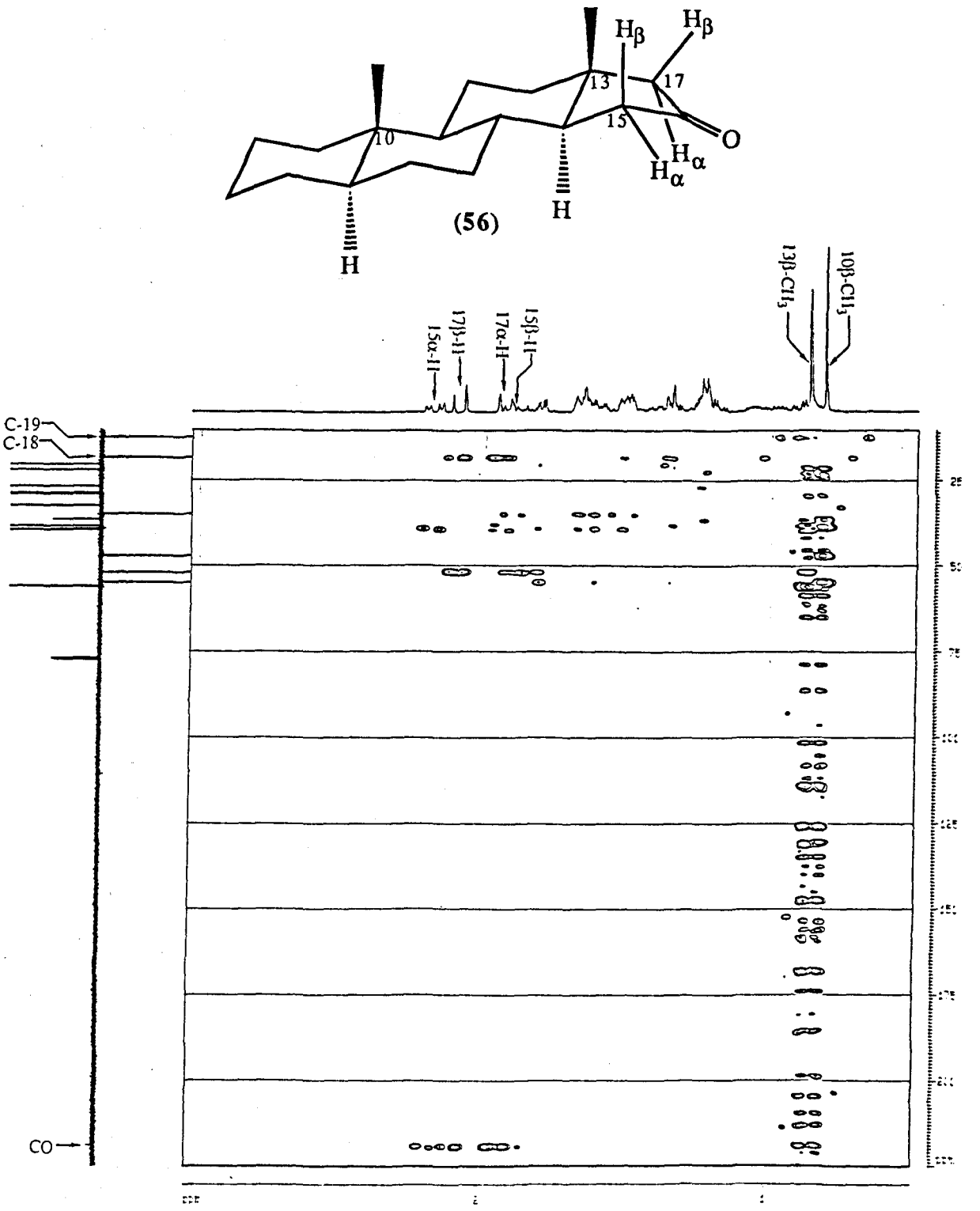


Figure 16. HMBC spectrum (400 MHz, $CDCl_3$) of 5α-androstan-16-one (**56**).

over four and/or five bonds. The 10β-CH_3, therefore, resonates at δ 0.81.

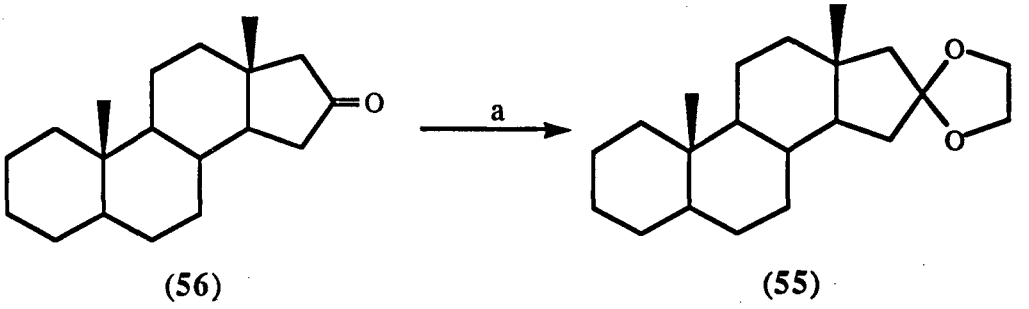
The 17-methylene protons resonate as broad doublets centred at δ 1.94 and 2.09 (J 16.92 Hz). The downfield signal (δ 2.09) has been assigned to the 17β-H since this proton, being more in the plane of the CO group, experiences a greater deshielding effect. In addition, NOE experiments showed effects between the 13β-CH_3 and the 17-proton signal at δ 2.09 and not δ 1.94.

On the other hand, the 15-methylene protons resonate as broad double doublets centred at δ 1.89 and 2.18. Such signal multiplicity results from their mutual geminal couplings, as well as couplings to the 14α-H.

The assignment of the downfield signal (δ 2.18) to the 15α-H has been made on the basis of its vicinal coupling constant (J_{ae}) to the 14α-H, being 6.88 Hz, compared to that of the 15β-H, where J_{aa} is 13.24 Hz.

The HMBC spectrum of (**56**) [Figure 16] has also been used in the assignment of carbon resonances. For example, the tertiary carbon resonance at δ 51.79 which shows ^2J and ^3J couplings to the 15- and 17-protons respectively and a ^3J coupling to the 18-protons must be C-14.

The conversion of the steroid ketone (**56**) to the corresponding 16,16-ethylenedioxy derivative (**55**) [Scheme 9] was achieved by refluxing a solution of (**56**) in sodium-dried benzene with freshly distilled ethylene glycol and triethyl orthoformate in the presence of *p*-toluenesulphonic acid as catalyst [96].



(**56**) (**55**)

Scheme 9. Reagents: *a*, $HO(CH_2)_2OH$ / $HC(OC_2H_5)_3$ / TsOH, benzene-reflux.

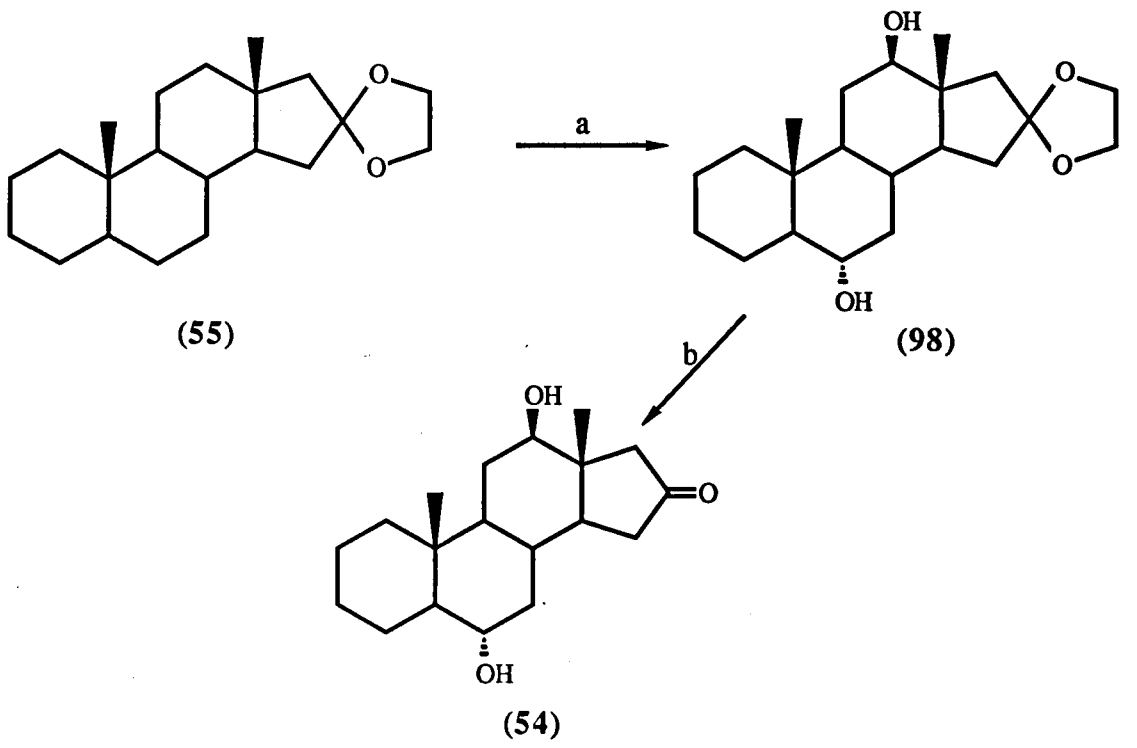
The characteristic peak at 99 *a.m.u* for ethylene ketals was observed in the mass spectrum of (55) [96]. However, in this case it was not the base peak [96].

2.3 Steroid dihydroxylation with *Calonectria decora.*

The fungus, *Calonectria decora*, was used to regioselectively and stereospecifically introduce hydroxyl functions at C-6 and C-12 positions of (55).

Initial attempts at the incubation of 16,16-ethylenedioxy-5α-androstane (55), with the fungus, *Calonectria decora*, followed by work-up of the fermentation medium and hydrolysis of the crude intermediate diol (98) gave 6α,12β-dihydroxy-5α-androstan-16-one (54) in poor yield (19.10%) [Scheme 10].

Product (54) isolation was complicated by problems of emulsification during solvent extraction. Attempts at emulsion cracking using brine were unsuccessful. Although dilute hydrochloric acid achieved this, the final volumes for solvent extraction were considerably increased and exceedingly large volumes of organic solvent (CH_2Cl_2) were needed for extraction of (54). After drying of the organic extract and evaporation of the CH_2Cl_2 the residual DMSO proved difficult to remove from the product (54). The recommended short-path distillation[131,132] on a steam bath under reduced pressure did not achieve the removal of DMSO as bumping was difficult to control and the use of higher temperatures led to charring and loss of material.



Scheme 10. Reagents: *a*, *Calonectria decora* / 25 °C; *b*,TsOH-H_2O-$(CH_3)_2CO$.

In an attempt to circumvent these isolation problems it was decided to use a more volatile solvent to introduce the substrate (**55**) into the broth culture. However when ethanol was employed as solvent, hydroxylation of (**55**) did not take place and the starting material was recovered as the hydrolysate (**56**).

It was observed that the change of solvent from DMSO to EtOH was accompanied by a marked turbidity to the broth culture as the solution of the fungal substrate (**55**) was added. This apparent inability of EtOH to hold the fungal substrate (**55**) in solution , after dilution with the broth culture, is perhaps the cause for failure of hydroxylation.

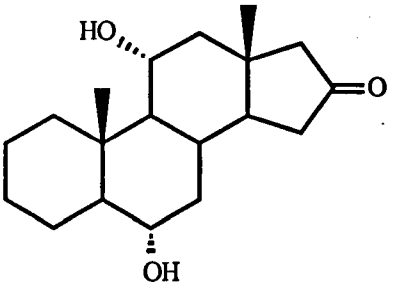
By modification of the work-up procedure, the original problems of emulsification and those associated with entrainment of DMSO in the product were resolved. In summary, the inclusion of a freeze-drying step in the work-up procedure stripped the product of DMSO. The freeze-dried residue was taken up in a minimum volume of water to give a suspension of (**54**) which was diluted with acetone and

extracted with CH_2Cl_2. This led to an improvement in the recovery of (**54**), with yields of about 40% being achieved consistently.

It is worthy of note, that the nutrient medium was always adjusted to pH 5.5 before sterilisation.

At this pH, unless the dihydroxylation of (**55**) is very rapid and/or the hydrolysis of (**55**) is very slow, some 16-ketone (**56**) will be produced during the incubation period.

Calonectria decora, has been shown to dihydroxylate (**56**) differently, though inefficiently, with the introduction of a 6α- and 11α- hydroxyl groups to give (**99**) [94].



(**99**)

On the basis of ^1H NMR ($CDCl_3$) studies this product (**99**) was not a contaminant of (**54**).

The 18-H, 19-H, 6-H, and 11-H of 6α,11α-dihydroxy-5α-androstan-16-one (**99**) resonate at δ 0.94 (s), 1.04 (s), 3.4 (sx), and 4.0 (sx) respectively[131] (parentheses contain signal multiplicity).

In the ^1H NMR spectrum of (**54**) the 18-H, 19-H, 6-H, and 12-H resonate at δ 0.88 (s), 0.84 (s), 3.4 (sx), and 3.6 (q) respectively. This is in agreement with literature reports for (**54**)[93]: δ 0.89 (s, 18-H), 0.85 (s, 19-H), 3.45 (sx, 6-H), and 3.65 (q, 12-H).

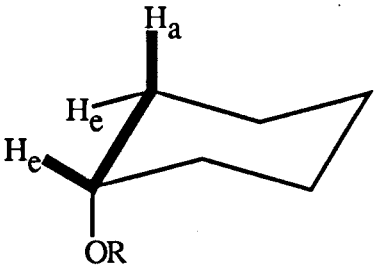
The appearance of the 12α-H resonance of (**54**) as a four-line pattern is consistent with its vicinal couplings to the 11-methylene protons. No additional

vicinal coupling of the 12α-H is observed since C-13 is quaternary in nature.

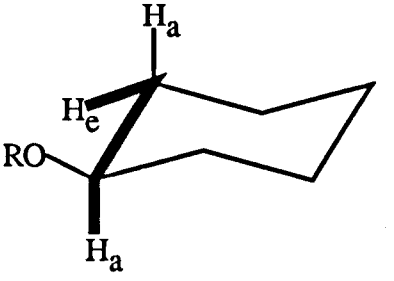
However in (**99**) the 11β-H is vicinally coupled to both 12-protons and the 9-proton which is responsible for the six-line pattern observed.

The equatorial configuration of the C-6 and C-12 hydroxyl groups in (**54**) was confirmed by examination of the axial-equatorial coupling constants (J_{ae}) of the associated carbinolic protons.

The dependence of J_{HH} (vicinal) on the electronegativity of a substituent (**R**) has been investigated by a number of workers [132-135]; in the fragment, -CH-CH(R)-, it has been shown that increases in the electronegativity of (**R**) causes a decrease in J_{HH}. Additionally, the magnitude of this effect on J_{HH} shows a stereochemical dependence. Bhacca and Williams[136] have shown that for a number of hydroxy- and acetoxy-steroids (partial structures (**100**) and (**101**); R = H or Ac) this *cis*-coupling constant J_{ea} (2.5-3.2 Hz) in the axially substituted compound (**100**) is invariably less than the *cis*-coupling constant J_{ae} (4.5-5.5 Hz) in the equatorially substituted compound (**101**).



(**100**)



(**101**)

The finding that the *cis*-coupling constants (J_{ae}) for the 6- and 12-carbinolic protons of (**54**) were 4.46 and 4.54 Hz respectively, confirms that the C-6 and C-12 hydroxyl groups are equatorially orientated.

Now that procedures had been validated for the production of 6α,12β-dihydroxy-5α-androstan-16-one (54) attention was turned to the introduction in (54)

of the side chains mimetic of the prostacyclin molecule (**7**) [see structures (**49**), (**50**), (**51**), (**52**), and (**53**) on pages 37-39].

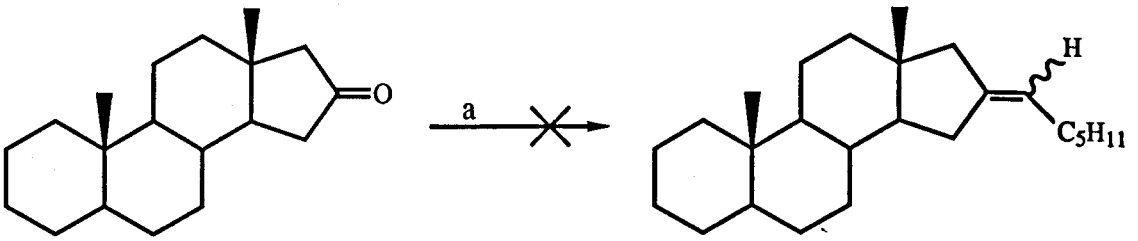
The introduction, by way of the Wittig olefination, of the hexylidene and the 4-carboxybutylidene side chains into the 16-position of (**54**) were the initial synthetic challenges to engage our efforts.

2.4 Wittig olefinations.

The Wittig olefin synthesis is a two-stage process involving the reaction of an alkylidenetriphenylphosphorane with a carbonyl compound to give a betaine which eliminates triphenylphosphine oxide to form an olefin (usually a mixture of E- and Z- isomers).

To conserve on valuable stocks of (**54**) it was decided to establish the reactivity of the model keto-steroid, 5α-androstan-16-one (**56**), towards the Wittig olefination[137,138] using long chain ylids derived from base treatment of alkyl and substituted alkyl triphenylphosphonium halides.

The attempted condensation of 5α-androstan-16-one (**56**) with hexyl triphenylphosphorane in refluxing THF was unsuccessful (Scheme 11).



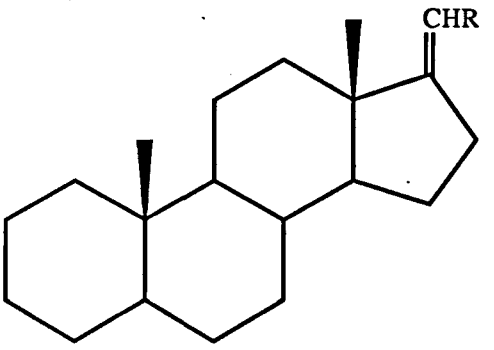
(**56**) (**102**)

Scheme 11. Reagents: *a*, $(C_6H_5)_3P^+CH_2(CH_2)_4CH_3Br^-$ / *n*-BuLi-THF, reflux.

The failure of the reaction under these conditions suggested perhaps the low inherent reactivity of the 16-ketone augmented by the steric interaction of the

proximal 13β-angular methyl group and the long chain of the ylid which may have hindered formation of the intermediate betaine [139]. However, in the light of literature reports by Fetizon *et al* [140], the suggested reasons for reaction failure with the 16-ketone seemed an unlikely explanation.

Fetizon *et al* [140] employed the Wittig olefination to prepare a series of exocyclic steroid alkenes, [17-alkylidene-5α-androstanes **(103)**-**(107)**] from 5α-androstan-17-one **(59)** [a more sterically hindered ketone than **(56)**] and the appropriate alkyltriphenylphosphorane.



(103) R = -H
(104) R = -CH_2
(105) R = -CH_2CH_3
(106) R = -$CH_2CH_2CH_3$
(107) R = -$CH(CH_3)_2$

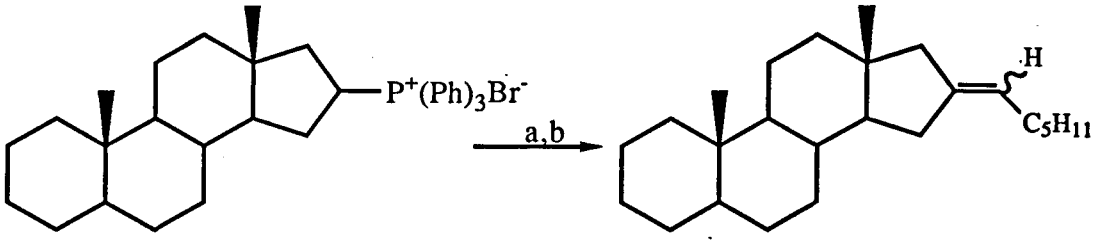
Their results showed that as the length of the *n*-alkyl chain in the alkyl triphenylphosphonium bromide increased from C_1 through C_2, C_3, to C_4, the yields of the 17-alkylidene-5α-androstanes [**(103)**-**(107)**] fell off from 98%, through 86%, 33% to 34% respectively.

A notable exception was the synthesis of 17-isobutylene-5α-androstane **(107)** which was achieved in 78% yield.

The latter result, taken in conjunction with that of the straight chain isomer **(106)**, suggests that chain length may be more critical to yields than chain branching. This observation, being more pertinent to our failed reaction with the 16-ketone **(56)** and

n-hexylidenetriphenylphosphorane, led us to pursue an alternative synthetic strategy for the introduction of the alkylidene side chain into the 16-position of (**56**).

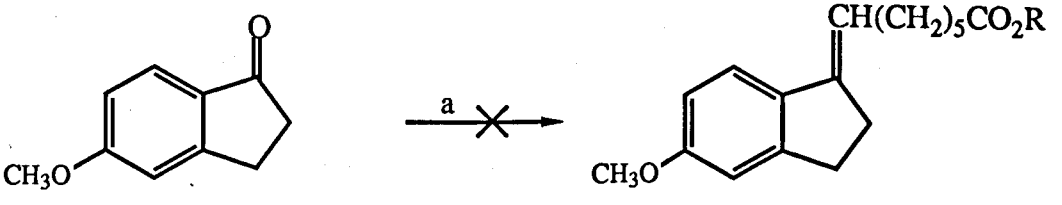
This approach involved a change in the nature of the participating reactants. Instead of using 5α-androstan-16-one (**56**) it was proposed that the corresponding steroidal phosphonium bromide (**108**) would be prepared and its condensation with a more reactive carbonyl compound, *n*-hexanal, would be attempted (Scheme 12, proposed).



(**108**) (**102**)

Scheme 12. Reagents: *a*, *n*-BuLi; *b*, $CH_3(CH_2)_4CHO$ / THF.

A similar approach is reported in the literature by Taub *et al* [141]. They had encountered similar problems of unreactivity in attempting the Wittig condensation of 5-methoxyindanone (**109**) with ylids derived from $(Ph)_3P^+(CH_2)_6CO_2RBr^-$ (where R = CH_3 or H) [Scheme 13].



(**109**) (**110**)

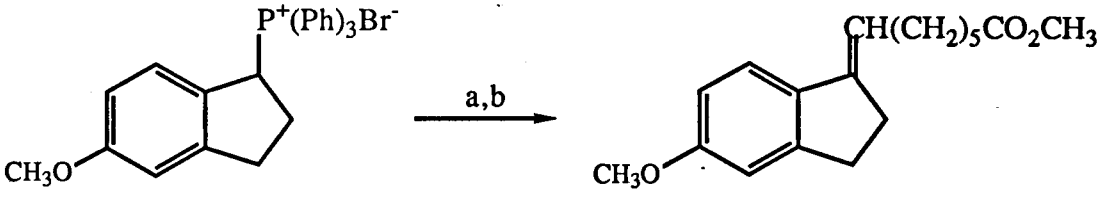
Scheme 13. Reagents: *a*, $(Ph)_3P^+CH_2(CH_2)_5CO_2RBr^-$ (R = CH_3, H).

Although attempts at reaction were unsuccessful no explanation was put forward to account for this. However they did achieve reaction when using ethylidene triphenylphosphorane, a shorter chain ylid:

$$(C_6H_5)_3P{=}CHCH_3$$

Presumably the longer chain ylids sterically hindered reaction at the ketonic centre.

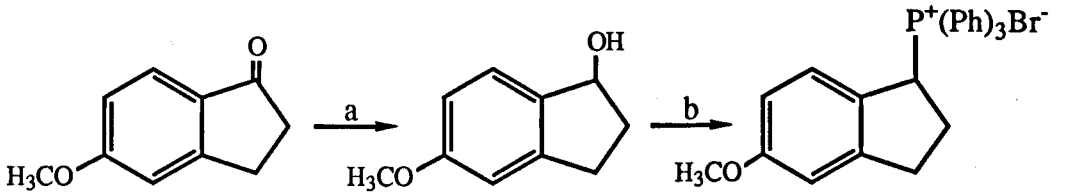
Using the alternative synthetic strategy Taub *et al* [141] achieved successful condensation and produced the olefin [(**110**), R = CH_3] in 70% yield (Scheme 14).



(111) **(110)**

Scheme 14. Reagents: *a*, $KOBu^t$ / DMSO; *b*, $CH_3CO_2(CH_2)_5CHO$.

The reported method of preparation of (**111**) is shown (Scheme 15).

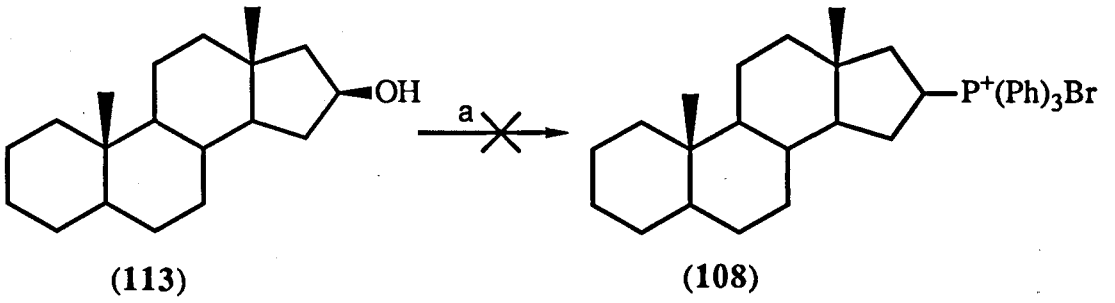


(109) **(112)** **(111)**

Scheme 15. Reagents: *a*, $NaBH_4$-EtOH; *b*, $(Ph)_3P^+HBr^-$ / CH_2Cl_2, 25 °C.

In order to investigate this alternative synthetic strategy, as a resolution to the problems, it was necessary to prepare the steroidal phosphonium bromide (**108**).

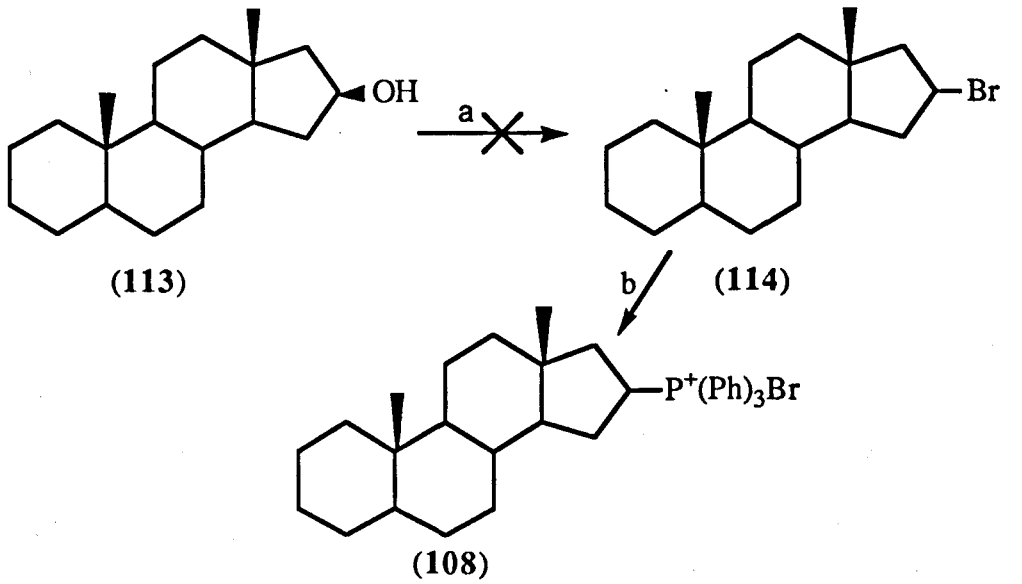
This involved reacting 5α-androstan-16β-ol (**113**), obtained from $NaBH_4$-isopropanol reduction[142,143] of 5α-androstan-16-one (**56**), with triphenylphosphine hydrobromide (Scheme 16).



(**113**) (**108**)

Scheme 16. Reagents: *a*, $(C_6H_5)_3P.HBr$ - $CHCl_3$, reflux.

Failure to achieve the (**113**) to (**108**) conversion led to further attempts at preparing (**108**), *via* a different sequence of reactions (Scheme 17).



(**113**) (**114**) (**108**)

Scheme 17. Reagents: *a*, $(C_6H_5)_3P.Br_2$ - DMF, reflux, 6h; $(C_6H_5)_3P$, pressure, 20-40 °C, 36-40h [144,145].

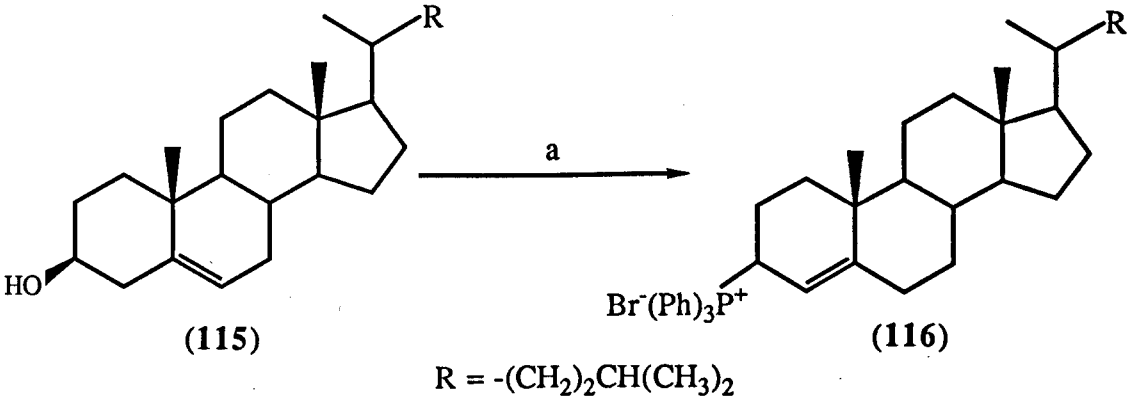
However, the proposed scheme of reactions was abandoned when the attempted reaction of (**113**) with $(C_6H_5)_3P.Br_2$ in refluxing DMF [146] failed to give the

desired 16-bromosteroid (**114**). Instead, an isomeric mixture of steroid olefins, with identical R_f to that of androst-16-ene (**92**), was obtained. GC-MS analysis showed each of them to have an *a.m.u.* of 258, which is consistent with an androstene.

In retrospect, it is thought that the reaction did occur, but the resulting 16-bromo-5α-androstane (**114**) subsequently underwent DMF-induced elimination [147] to give a mixture of Δ^{15}- and Δ^{16}-androstenes. Perhaps DMF [146] was a poor choice of solvent and reaction time may have been too long. However, constraints of time did not permit the experimental variation of these parameters.

In comparison with our sterol (**113**), Taub's methoxyindanol (**112**) is the inherently more reactive benzylic alcohol [148] which is accessible to attack by $(C_6H_5)_3P.HBr$ from above and below the plane of the ring system. So, in view of the anticipated isomerisation of the Δ^5- to the Δ^4-double bond of cholest-5-en-3β-ol (**115**) under acidic conditions in chloroform, it was decided to test the hypothesis that an activated alcohol may be necessary for the formation of the steroidal triphenylphosphonium bromide.

The reaction of (**115**) with $(C_6H_5)_3P.HBr$ in refluxing chloroform led to (**116**) in 82% yield (Scheme 18), for which satisfactory elemental analysis was obtained.



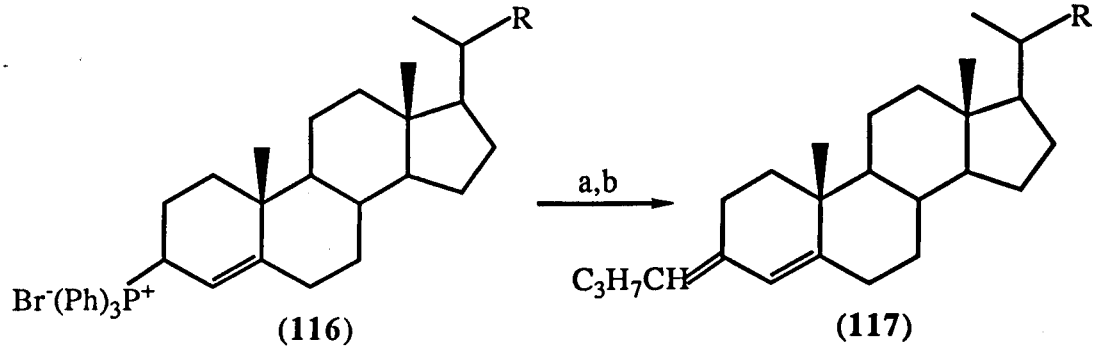
Scheme 18. Reagents: *a*, $(Ph)_3P.HBr$ / $CHCl_3$, reflux, 16 h.

The results of a number of NMR experiments on (**116**) confirms that, in (**116**), the double bond is in the Δ^4-position.

The COSY spectrum of (**116**) showed that the 4-olefinic proton is coupled to the 3-proton. Also NOE effects are observed between the 4-olefinic proton and the 3- and the aromatic-H's of (**116**). Additional NOE's are also observed between a deshielded methylene group and the aromatic-H's; this is assumed to be the 2-methylene function.

On the basis of the above, activation of the secondary alcohol seems a prerequisite for reaction to occur.

For the sake of completeness, the reactivity of (**116**) to the Wittig olefination was established by the synthesis of 3-butylidene-cholest-4-ene (**117**), as an isomeric mixture, in 28 % yield (Scheme 19). The required molecular ion of 424 *a.m.u.* for each isomer of the E/Z mixture was detected by GC-MS studies of (**117**).



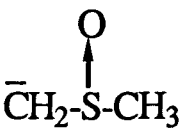
(**116**) (**117**)

R = $-(CH_2)_2CH(CH_3)_2$

Scheme 19. Reagents: *a*, 2N BuLi in hexane / THF; *b*, *n*-butanal / THF, r.t., 3h.

2.5 Use of methyl sulphinyl carbanion as base in Wittig olefinations.

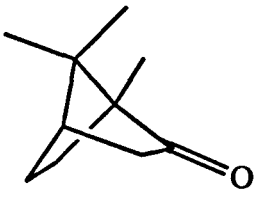
Corey and co-workers[149] have shown that when the methyl sulphinyl carbanion (**118**) [formed by the reaction of sodium hydride and DMSO] is used as base and DMSO as the solvent, the Wittig olefination proceeds readily.



(118)

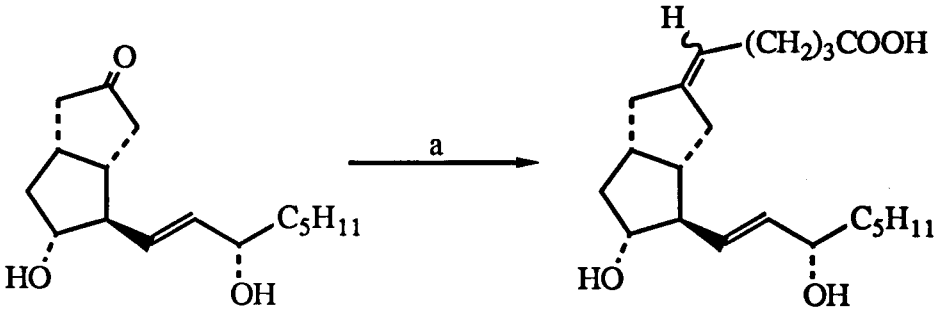
By this method methylene cyclohexane was obtained in 86% yield from cyclohexanone, as compared to the yield of 35-40% using butyl lithium as base and ether as the solvent [150]

Camphor (**118a**), the ketone group of which is sterically hindered readily afforded the methylene derivative in DMSO [149].



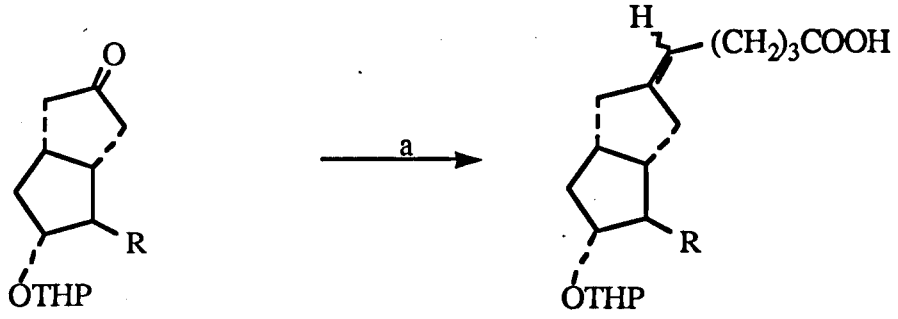
(118a)

Subsequently, in the field of prostaglandin total synthesis, Morton and Konishi[151,152] achieved successful Wittig condensations with long chain ylids (present in 6 molar excess) with cyclopentanone systems using DMSO as solvent and methyl sulphinyl carbanion as base (Schemes 20 and 21).



(119) **(120, 121, 83%)**

Scheme 20. Reagents: *a*, NaH, DMSO, $(Ph)_3P{=}CH(CH_2)_3COONa$, 35 °C.



(122) **(123)**

R = $-CH_2OC(CH_3)_2OCH_3$

Scheme 21. Reagents: *a*, NaH, DMSO, $(Ph)_3P{=}CH(CH_2)_3COONa$, 35 °C.

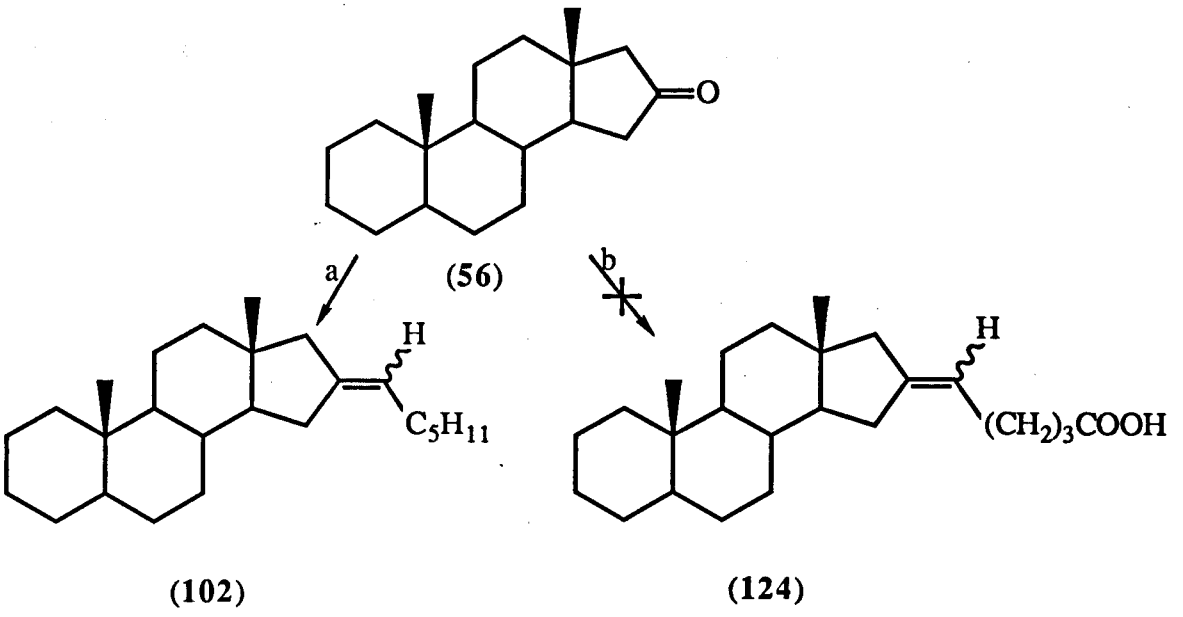
These results are in agreement with other published reports by Kojima and Sakai[153] and Nicolaou *et al* [154].

Therefore, it was felt that the use of the methyl sulphinyl carbanion as base and DMSO as solvent would facilitate the Wittig reactions designed to introduce PGI_2-mimetic side chains into the 16-position of the steroid nucleus (*see* steroidal analogues).

After establishing the correct conditions for generating the base these expectations were realised.

Initial attempts at preparing **(124)** under the aforementioned conditions failed, and although **(102)** was obtained under the same conditions the yield (5%) was

very poor (Scheme 22).



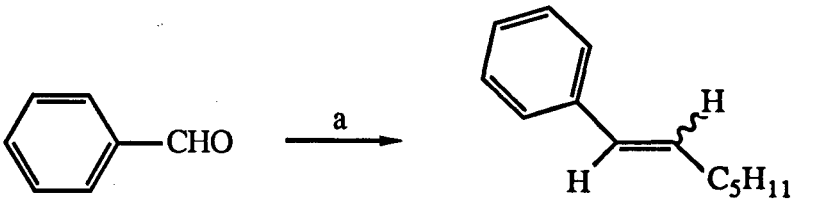
(56) **(102)** **(124)**

Scheme 22. Reagents: *a*,NaH, DMSO, $(Ph_3P{=}CHC_5H_{11}$, 35 °C; *b*, NaH, DMSO, $(Ph)_3P{=}CH(CH_2)_3COONa$, 35 °C.

In conjunction with the low yield of **(102)**, the observation that the colour (pale orange) of the ylid solution, obtained in both cases, was not the intense red-orange referred to in the literature suggested that the method of preparation of the base (methyl sulphinyl carbanion) might have led to its decomposition. This involved reacting dry powdered sodium hydride with an excess of anhydrous dimethyl sulphoxide (DMSO) at 70 °C under nitrogen until the evolution of hydrogen was complete (45-60 min.) and resulted in the generation of a viscous tarry solution **(METHOD I)** [149].

However, when the base was prepared at 55 °C [155] a green translucent solution was obtained which gave the intense red-orange ylid solution with the quaternary phosphonium bromides (*n*-hexyl-, and 4-carboxybutyltriphenyl phosphonium bromides) **[METHOD II]**.

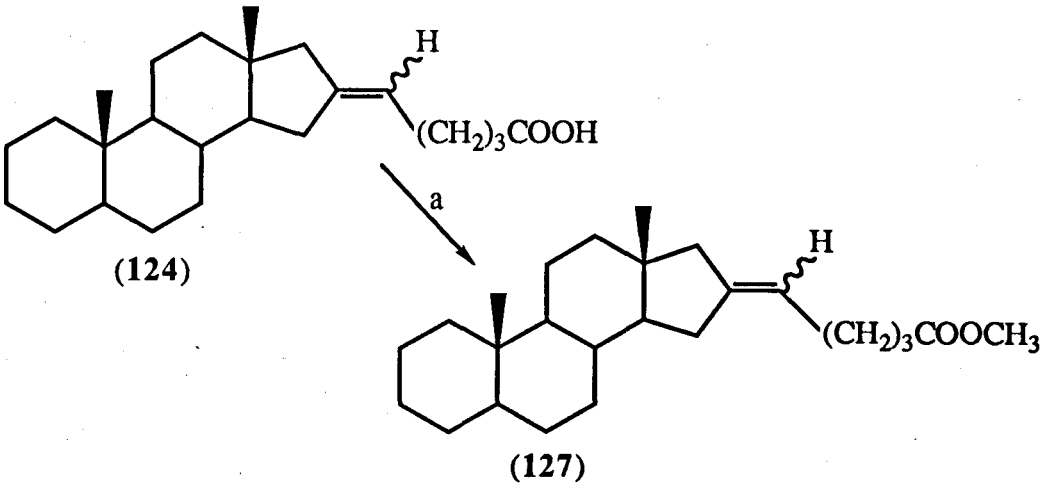
As a test, the resulting ylid from n-hexyltriphenylphosphonium bromide and methyl sulphinyl carbanion (**METHOD II**) was reacted with freshly distilled benzaldehyde (**125**) and yielded a mixture of geometric isomers of 1-heptenyl benzene (**126**) [Scheme 23].



(125) **(126)**

Scheme 23. Reagents: *a*, NaH, DMSO, $(Ph)_3P{=}CHC_5H_{11}$, r.t.

Using **METHOD II** for base generation, the reactions outlined in Scheme 22 were repeated at 55 °C. This time, however, (**102**) and (**124**) were obtained as mixtures of geometric isomers in yields of 71% and 47% respectively; the yield of (**124**) being expressed in terms of the methyl ester derivative (**127**), prepared by reacting (**124**) with ethereal diazomethane (Scheme 24).



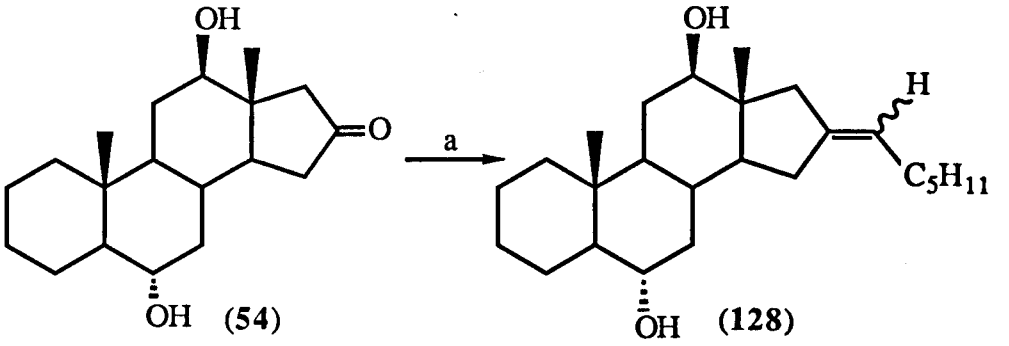
(124) **(127)**

Scheme 24. Reagents: *a*, ethereal diazomethane (CH_2N_2), r.t.

Having established the conditions for successful Wittig olefination at the 16-position of the model steroid (**56**) attention was then turned to transforming the microbiologically derived 6α, 12β-dihydroxy-5α-androstan-16-one (**54**) into the proposed steroidal prostacyclin analogues (**49, 50, 51, 52, 53**).

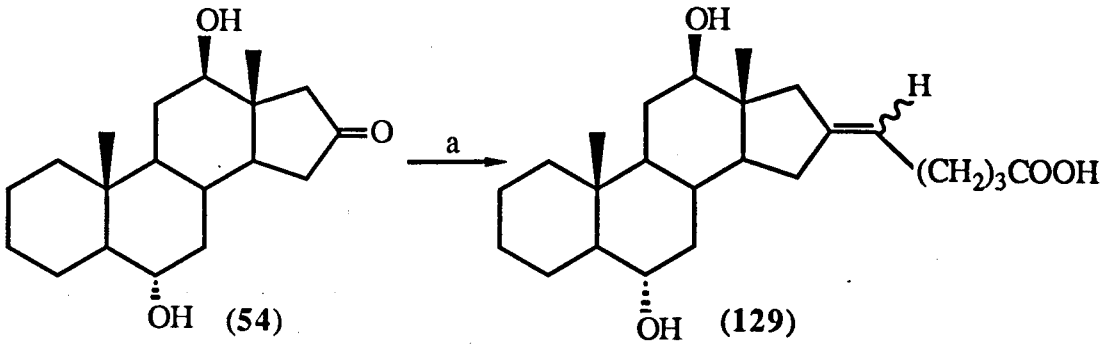
2.6 Synthesis of steroidal prostacyclin analogues.

Reaction of (**54**) with the ylid derived from *n*-hexyl triphenylphosphonium bromide yielded (**128**) as a mixture of geometric isomers (Scheme 25)



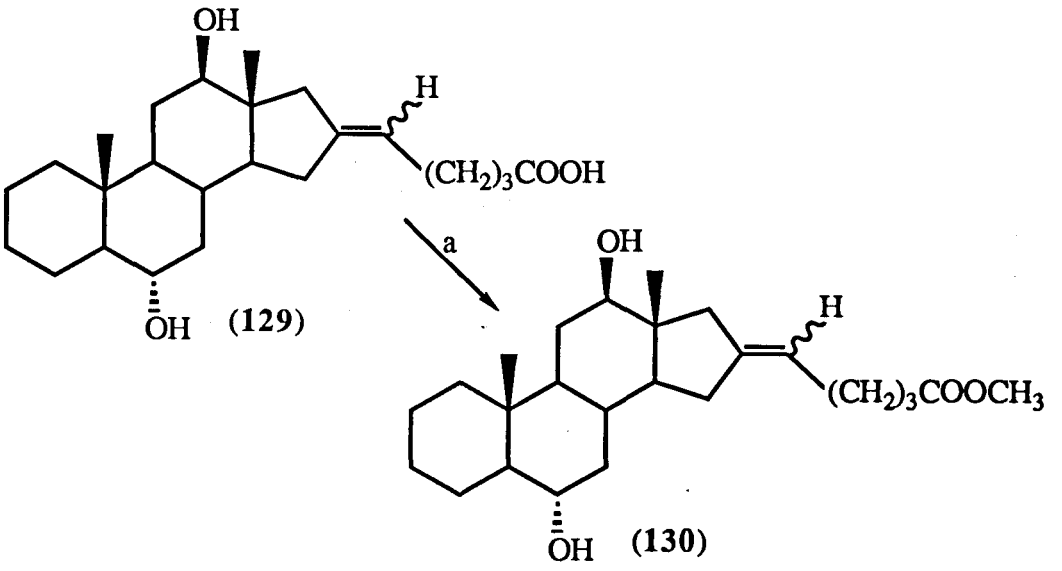
Scheme 25. Reagents: *a*, NaH, DMSO, $(Ph)_3P{=}CHC_5H_{11}$, 55 °C.

Similarly reaction of (**54**) with the ylid derived from (4-carboxybutyl) triphenylphosphonium bromide afforded (**129**) as a mixture of E/Z-isomers, the Z-isomer of which is the type III prostacyclin steroidal analogue (**53**) [Scheme 26].



Scheme 26. Reagents: *a*, NaH, DMSO, $(Ph)_3P{=}CH(CH_2)_3COONa$, 55 °C.

The E/Z mixture (**129**) was derivatised to the corresponding E/Z methyl esters by reaction with ethereal diazomethane (Scheme 27).

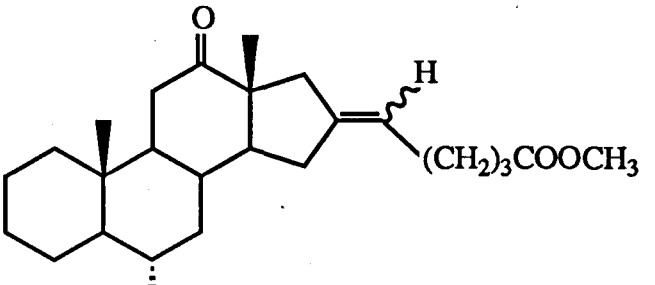


Scheme 27. Reagents: *a*, ethereal diazomethane (CH_2N_2).

The isomeric mixture of E/Z-isomers (**130**) resisted resolution by p.l.c. on silver nitrate (1-3%) impregnated silica gel and multiple elutions with EtOAc:Toluene (1 : 5).

A fraction of (**130**) was subjected to alkaline hydrolysis to give (**129**) after acidification of the reaction mixture with dilute hydrochloric acid. Column chromatography of the resulting oil afforded an off-white solid, mp 74-75 °C, for which satisfactory elemental analysis was obtained. However ^1H NMR analysis showed that even after column chromatography, (**129**) was still a mixture of geometric isomers [containing (**53**)], in which form it was submitted for biological screening as an inhibitor of platelet aggregation and a vascular smooth muscle relaxant.

At this stage it was decided that the time that was left would be most profitably spent in attempting to establish the conditions for the introduction of the ω mimetic side chain into the 12-position of **(130)**. This involved converting **(130)** into the corresponding 6-ol-12-one **(131)** and finally the introduction of the ω mimetic side chain at the 12-position by way of a Wittig or Grignard reaction.



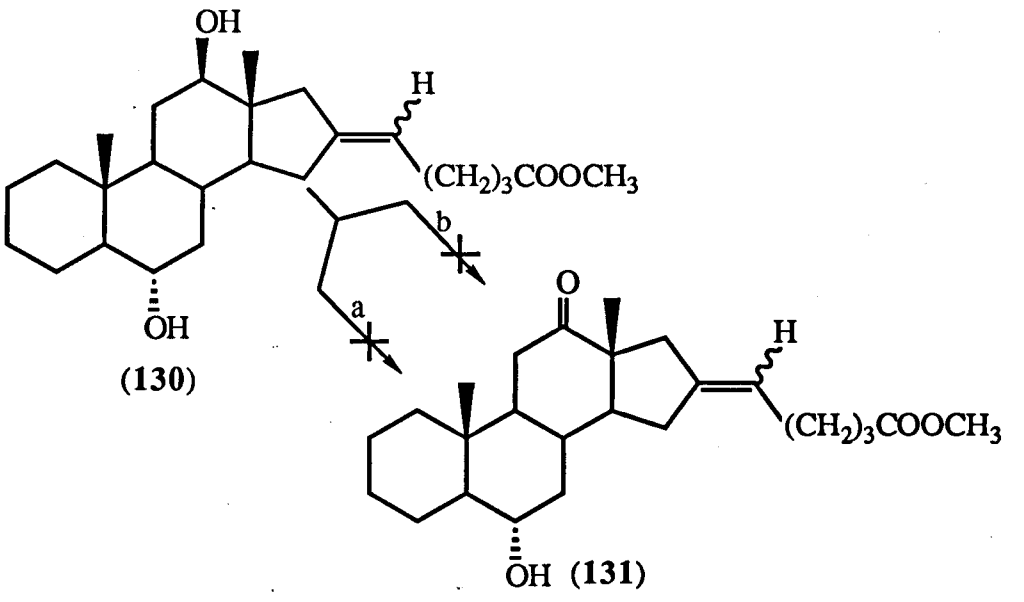
(131)

Initial attempts at this conversion [**(130)** to **(131)**] hoped to exploit the reported differences in the rates of oxidation of the C-6 and C-12 hydroxy groups.

Studies conducted by Schreiber, Enchenmoser, and Grimmer established that the 12β-hydroxy group is three times more reactive than the 6α-hydroxy group towards chromate oxidation [156,157].

The difference in reactivity of these two equatorial alcohols is considered to be due to the extent of their non-bonded interactions. It would appear that the 12β-hydroxy group experiences a greater degree of non-bonded interactions and its oxidation is sterically accelerated.

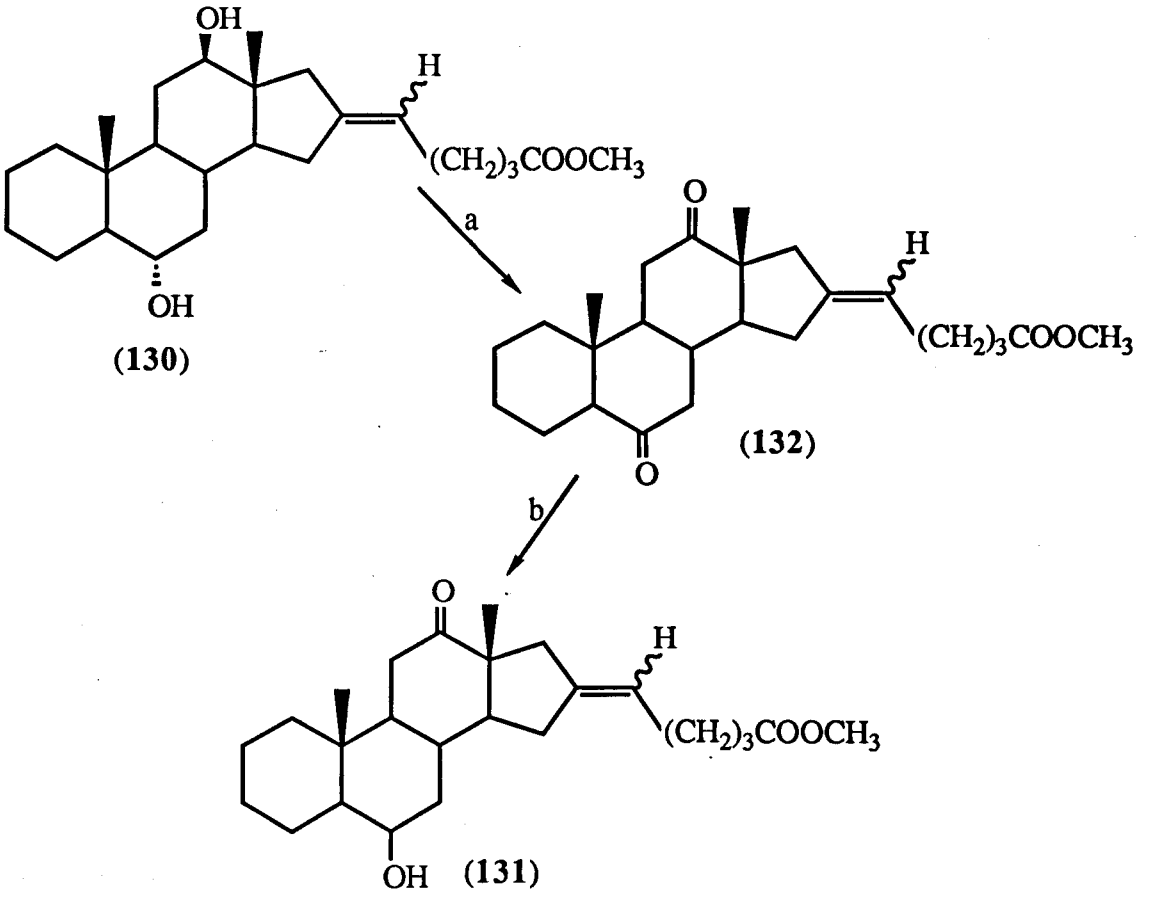
Despite the above, attempts at converting **(130)** to **(131)** [Scheme 28] using stoichiometric amounts of pyridinium dichromate failed to achieve reaction. The starting material **(130)** was recovered unchanged.



Scheme 28. Reagents: *a*, pyridinium dichromate - dry CH_2Cl_2 / DMF; *b*, pyridinium dichromate - dry DMF.

The use of dry DMF and dry CH_2Cl_2 as solvents for this reaction follows reports of the usefulness of pyridinium dichromate as an oxidant for organic synthesis in aprotic media and the general idea that anhydrous conditions are more conducive to complexation of the substrate with Cr ^{VI} species and therefore to mild oxidation [158,159]. Additionally the ready solubility of steroids in these solvents is advantageous.

Failure to prepare **(131)** by the selective oxidation of the C-12 hydroxy group of **(130)** led to the trial of a longer synthetic approach to **(131)**. This alternative two-stage sequence involved the conversion of **(130)** into the corresponding 6,12-dione **(132)** and then selectively reducing **(132)** to give the 6-ol-12-one **(131)** [Scheme 29, proposed].



Scheme 29. Reagents: *a*, Pyridinium dichromate-CH_2Cl_2, r.t.; *b*, $NaBH_4$-isopropanol, 25 °C.

The oxidation of (**130**) to the corresponding 6, 12-dione (**132**) proceeded readily in dry CH_2Cl_2 with an excess of pyridinium dichromate as oxidant; (**132**) was obtained in yields of 88%.

For the reduction of the 6-keto function in the presence of the 12-keto group of (**137**) use was made of the selectivity of sodium borohydride in isopropanol.

Sodium borohydride has been used as a selective reducing agent in steroid chemistry and preparative reductions have been reported of a 3-keto group, in the presence of 11-, 12-, 17-, and 20-keto groups[160-164], of a 3-keto group in the presence of both 11- and 20-keto groups[160], and of a 20-keto group in preference to

an 11-keto group[165] and a Δ^4-3-keto group [166].

Although the selective reduction of a 6-keto group in the presence of a 12-keto group does not appear to have been reported, kinetic studies have been performed with steroid monoketones involving positions in the steroid molecule which have different steric environments. These studies showed that the steroidal 6-keto group is six times more reactive than the steroidal 12-keto group towards $NaBH_4$-isopropanol reduction at 25 °C [167,168].

The principle effect decreasing the rate of reduction of the 12-keto group, relative to the 6-keto group, is thought to be due to the eclipsing effect of the α-hydrogen on C-17; a similar interaction is encountered between the 7-keto group and the α-hydrogen on C-15 and accounts for its lower reactivity than the 6-keto group [169].

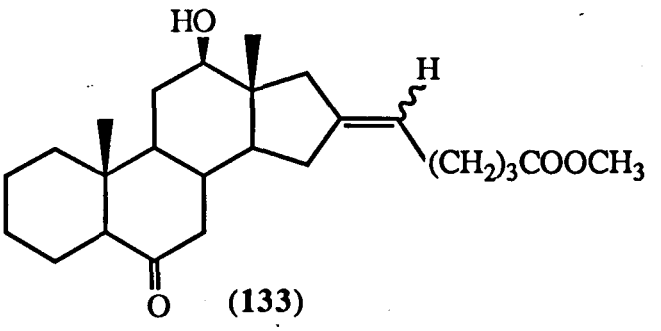
The suitability of isopropanol for these reductions is due to the stability of borohydride in this solvent, the solubility of the steroids, and the reasonably slow rates of reduction[170,171], the latter which allows for greater selectivity.

Following the reaction of the 6,12-dione (**132**) with $NaBH_4$, the crude product was isolated and purified by p.l.c. Thin layer chromatography of the purified product indicated the absence of the starting material (**132**).

The appearance of a strong absorption band at 3450 cm^{-1} in the infrared spectrum of the product inferred the presence of an OH-group. In addition, mass spectral data confirmed the accurate molecular mass and formula of the product to be 402.2766 and $C_{25}H_{35}O_4$. This data is consistent with a product in which one of the two keto groups present in the 6,12-dione (**132**) has been reduced.

^1H NMR studies on the product confirmed it to be a two-component mixture; duplication of the angular methyl and methoxy groups' resonances were observed. Additionally, the carbinolic protons resonated as slightly overlapping double doublets centred at δ 3.55 (J 10.82, 4.70) and 3.57 (J 10.88, 4.52). Such

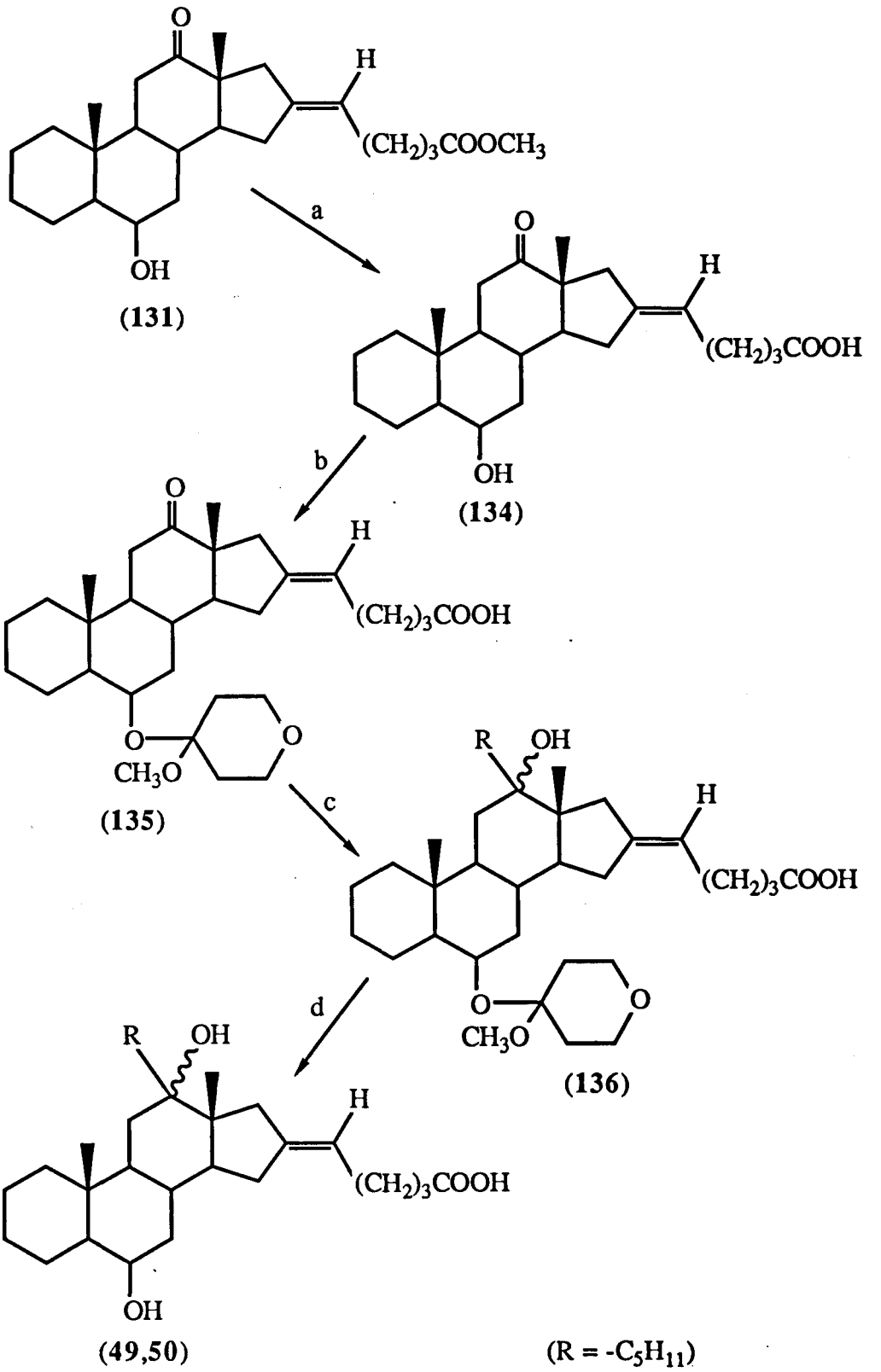
signal multiplicity, taken in conjunction with the coupling constant, is consistent with a product in which the 12-keto function of the 6,12-dione (**132**) has been reduced to an equatorial 12β-hydroxy group [*see* (**133**)].



(**133**)

In (**133**) the axial 12α-H is coupled to the axial 11β-H (J_{aa}) and to the equatorial 11α-H (J_{ae}). The *cis*-coupling constants (J_{ae} 4.70, 4.52) are consistent with an equatorial 12β-OH group [172]. Also, the presence of only one diaxial coupling (J_{aa} 10.88, 10.82) involving the carbinolic proton provides additional strong evidence that the product of selective reduction of the 6,12-dione (**132**) was (**133**). Unequivocal proof of structure would have required further NMR experimentation. For example, HMBC studies would have established connectivities between C-12 and 18-H, and C-18 and 12-H. However, constraints of time prevented the completion of this study, or any other synthetic operation from being carried out.

The remainder of this section will be restricted to a brief hypothetical discussion of the intended termination steps (Scheme 30, proposed) leading to analogues (**49**) and (**50**).

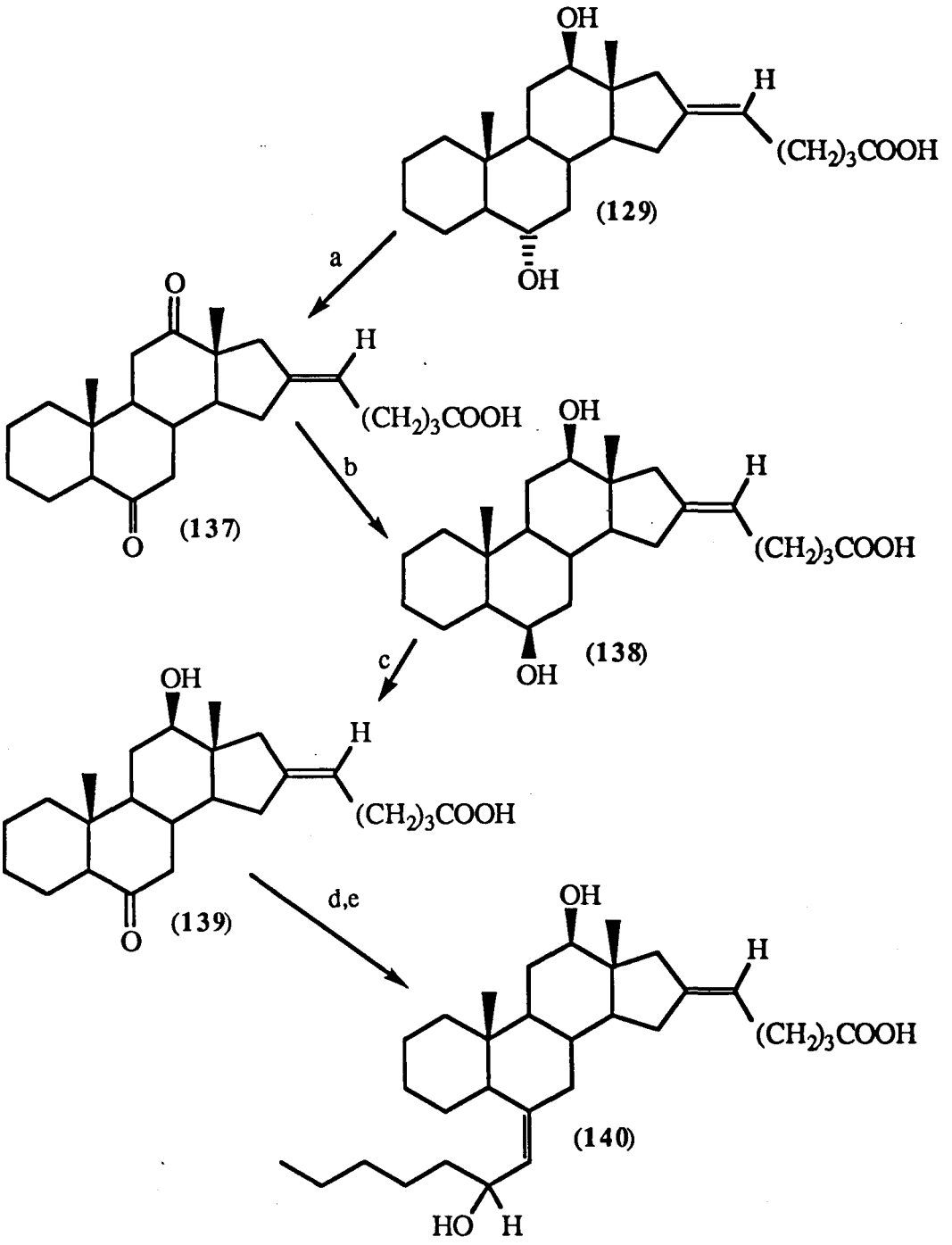


Scheme 30. Reagents : *a*, NaOH /MeOH; *b*, 4-methoxy-5,6-dihydro-2H-pyran / TsOH / dioxan[173]; *c*, $C_5H_{11}MgBr$ / dioxan[174,175]; *d*, 0.1 M HCl / EtOH (1:100).

Base catalysed hydrolysis of **(131)** will afford **(134)** as a mixture of E/Z isomers which, in the form of their sodium salts, would be separated by preparative h.p.l.c. (high pressure liquid chromatography). Protection of the E-isomer from **(134)** as the 6-O-methoxypyranyl derivative **(135)** will be followed by its **(135)** reaction with two equivalents of pentyl magnesium bromide in anhydrous dioxan to give **(136)** as a mixture of epimeric alcohols. Removal of the protecting groups from **(136)** will afford steroidal analogues **(49)** and **(50)** as a mixture.

Although the synthetic objectives of this project were only partially realised valuable ground work has been established to support future synthetic approaches to steroidal analogues of prostacyclin.

With the benefit of hindsight and experience gained the author truly believes that all synthetic objectives of this project are achievable and presents an approach (Scheme 31) which outlines a synthetic route to steroidal prostacyclin analogues in which the PGI_2-mimetic side chains are nuclear substituents at C-6 and C-16.



Scheme 31. Reagents; *a*, PDC/CH_2Cl_2; *b*, LS-Selectride® [176]; *c*, PDC/CH_2Cl_2[156,157]; *d*, dimethyl 2-oxo-*n*-pentyl phosphonate[177], *e*, $NaBH_4$-MeOH.

Reaction of (**129**) with pyridinium dichromate will afford the corresponding dione (**137**) which will be stereoselectively reduced with LS-Selectride® (lithium trisiamylborohydride) to give 6β,12β-dihydroxy compound (**138**). Selective chromate oxidation of (**138**) will generate (**139**) which will undergo the modified Wittig condensation followed by $NaBH_4$ reduction to give (**140**).

It is also being strongly suggested that in order to avoid the considerable number of repetition of synthetic steps, an undesirable and time consuming feature of the present work, future research should be carried out in collaboration with laboratories specialised in large scale fermentation technology and with facilities for large scale ozonolysis.

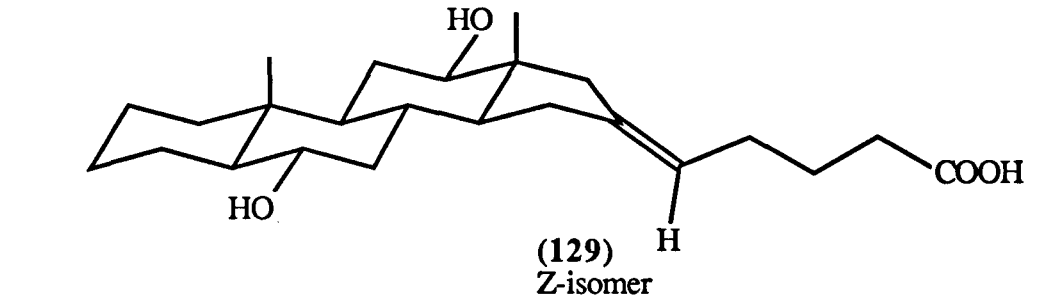
2.7 Pharmacological evaluation of prostacyclin analogue (129).

Preliminary pharmacological screening of analogue (**129**) showed that, in standard *in vitro* tests, using diluted human and pig whole blood, it was similar in potency to prostacyclin as an inhibitor of collagen-induced platelet aggregation.

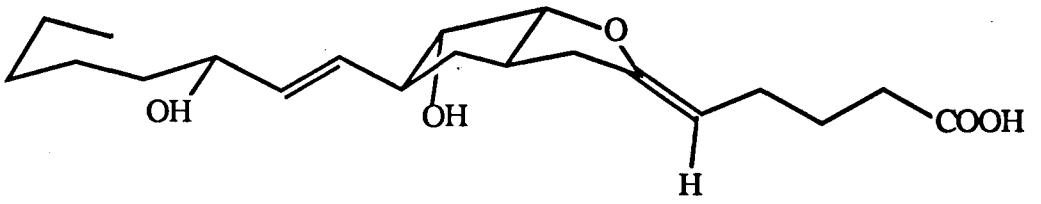
The details of the pharmacological testing are to be found in the Appendix.

2.8 Future development work.

As the biological potency of (**129**) has been shown to be comparable to that of prostacyclin, this analogue represents a biologically active development of type III steroidal prostacyclin analogues. This important lead compound (**129**) is undoubtedly a mixture of geometric isomers (E/Z) and this dictates that work in the immediate future involves the development of preparative isolation procedures (h.p.l.c.) to resolve this mixture. This will allow the biological evaluation of both isomers and the exciting possibility that the active isomers (E and/or Z) may be more potent than prostacyclin itself. Illustrated in figure 17, overleaf, is a conformer of prostacyclin which mimics the Z-isomer of (**129**).



(129)
Z-isomer

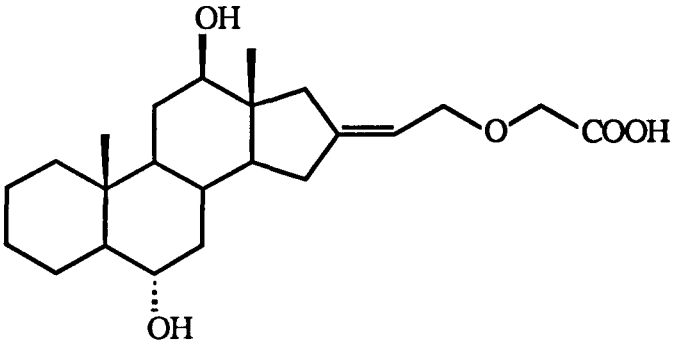


Prostacyclin Conformer

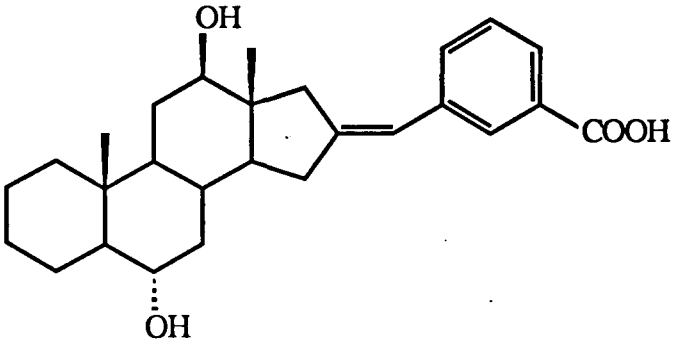
Figure 17.

In the type-III steroidal (**129** Z-isomer) the ω mimetic side chain is incorporated into the steroid nucleus (C-7 to C-1) and is of known configuration. On the other hand, the carboxyl side chain, like that of PGI_2, emanates from a cyclopentane ring and retains a degree of conformational mobility.

Analogue (**129** Z-isomer) is essentially a hydroxy fatty acid and is therefore a potential substrate for the non-specific enzymes that catalyse β-oxidation of fatty acids. In anticipation of this mode of metabolism, future development work on (**129**) may proceed on two fronts. Firstly, the synthesis and biological evaluation of potential dinor and tetranor metabolites resulting from β-oxidation of (**129**). And secondly, the synthesis and pharmacological screening of analogues of (**129**), such as (**141**) and (**142**), that incorporate structural features that are known to retard β-oxidation of the carboxyl side chain (*see* figure 18).



(141)



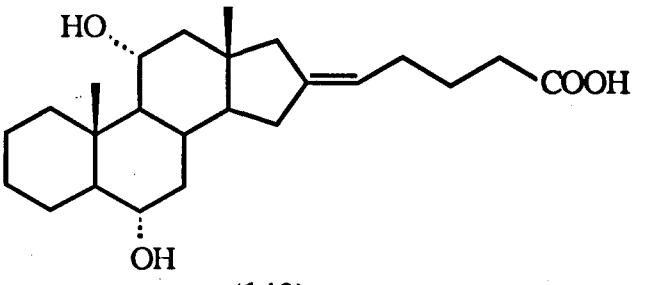
(142)

Figure 18

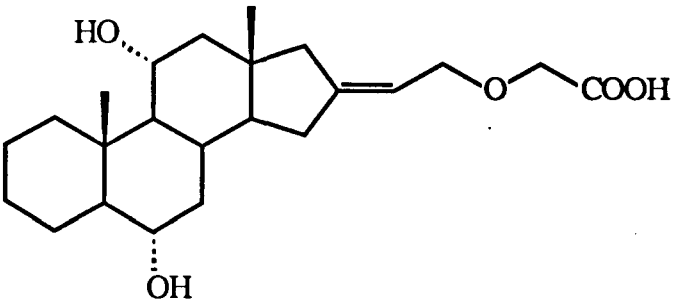
The susceptibility of the PGI_2-mimetic hydroxy groups of analogue **(129)** to 15-hydroxyprostaglandin dehydrogenase is not thought to be of major concern as it is unlikely to be a substrate for this enzyme. However, analogue **(129)** may be a substrate for oxidoreductases and the synthesis of analogues in which the OH-groups are protected against metabolizing enzymes is a projection for future developmental work. For example, the introduction of methyl or halogen groups at or near the site of oxidation may engage future synthetic efforts for improving metabolic stability. Also the synthesis of mono- and di-oxo analogues may allow the pharmacological evaluation of the potential metabolites of oxidoreductases.

Finally it would be of interest to determine the effects, on the biological activity of **(129)**, of a variation in its oxygenation pattern. For example, the corresponding 6α,11α-dihydroxy series of type III steroidal analogues [*see* **(143-**

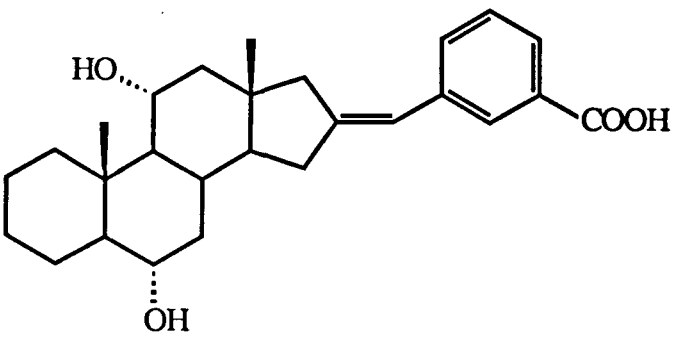
145)] may be readily obtained from 6α,11α-dihydroxy-5α-androstan-16-one. This precursor may be prepared by the 6α,11α-dihydroxylation of 5α-androstan-16-one by *Calonectria decora.*



(143)



(144)



(145)

3.0 Experimental

3.1 General experimental procedures.

The usual work-up procedure referred to in the text of the Experimental procedures (section 3.5) involved drying the organic solution over anhydrous sodium sulphate (a.Na_2SO_4), filtration of the hydrated sodium sulphate and finally removal of the organic solvent under reduced pressure.

Melting points (mp) were determined on a Reichart hot-stage apparatus and are uncorrected.

Infrared spectra (IR) were recorded on a Perkin-Elmer 781 spectrophotometer. Crystalline samples were prepared as KBr discs, and gums and oils as solvent-free thin films. The abbreviations used to describe the bands were as follows: str, stretching; def, deformation; s, strong; m, medium; w, weak; br, broad.

Ultraviolet spectra (UV) were recorded on a Perkin-Elmer 550 UV/visible spectrophotometer. Samples were prepared as solutions in ethanol.

Nuclear magnetic resonance (NMR). ^1H NMR spectra were recorded at 250 MHz, unless otherwise stated, on a Bruker WH 250 instrument and and chemical shifts are given in δ against $SiMe_4$ as internal standard.

^{13}C and 2D-NMR (COSY-45, NOESY, HMBC, and HMQC) spectra were recorded on a Bruker AMX 400 instrument.

All NMR spectra were recorded as solutions in deuterochloroform unless otherwise stated

Abbreviations used to describe signal multiplicity were as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; ddd, double double doublet; dt, double triplet; dq, double quartet; br, broad.

Mass (MS) spectra and accurate mass measurements were recorded on a MSS Ltd. AEI-MS 902 double focussing mass spectrometer using the direct insertion probe technique. An inlet temperature of 50-260 °C and an electron beam of 70 eV were used.

Elemental analyses were performed on a Carlo Erba elemental analyser.

Gas chromatography-mass spectrometry (GC-MS) were performed and recorded using a Hewlett-Packard GC-MS HP 5890-5988A instrument interfaced with a HP RTE-6/VM data system.

Thin layer chromatography (t.l.c.) was performed on pre-coated plastic plates (0.2 mm, E.Merck, silica gel 60 F_{254}). Visualisation was by viewing the developed plate under UV light (254 nm) and by means of spraying it with a 10% solution of ceric sulphate in 2N sulphuric acid and heating the sprayed plate at 120 °C for at least 15 minutes.

Preparative layer chromatography (p.l.c.) was performed on pre-coated glass plates (20 cm x 20 cm) [0.5 mm, Camlab, silica gel 60 F_{254}]. Visualisation : as for t.l.c., but only a fraction of the plate was sprayed and this fraction was heated with an industrial hot air blower. Removal of the adsorbent and extraction of the adsorbate with chloroform gave the pure compound.

Column chromatography purification procedures were carried out using the following adsorbents: silica gel 60 (E.Merck), Florisil 60-100 mesh (Aldrich Chem. Co).[activated chromatographic magnesium silicate] and neutral aluminium oxide (Brockmann grade 1, deactivated with 5% water).

Solvent systems used for chromatographic procedures are indicated in the text of the Experimental procedures (section 3.5).

Unless otherwise stated the method of chromatographic purification used after obtaining the crude product [as referred to in the text of the of the Experimental procedures (section 3.5)] was column chromatography.

Ozonisations were carried out using a Tack Laboratory ozoniser model-L5 and a Penwalt Wallace and Tiernan Ozoniser, Type number: BA 423012.

3.2 Purification of solvents.

Benzene: Thiophene-free benzene was distilled at 80-81 °C/760mm and stored over sodium wire (5 g/L).

Chloroform ($CHCl_3$) was slowly distilled form phosphorous pentoxide and the distillate at 61 °C/760mm collected and stored over 4A molecular sieves.

Dichloromethane (CH_2Cl_2): as for chloroform; distilled at 41 °C/ 760mm and stored over 4A molecular sieves.

N,N-Dimethylformamide (DMF) was allowed to stand over potassium hydroxide pellets overnight and then distilled from calcium oxide. The distillate at 153 °C/760mm was collected and stored over 4A molecular sieves.

Hexane was distilled at 69 °C/760mm and the distillate passed *via* a column of activated aluminium oxide. The filtrate was again slowly distilled at 69 °C.

Methanol (MeOH): magnesium turnings (5 g) and sublimed iodine were added to methanol (50 mL) and refluxed until the iodine disappeared and hydrogen evolution occurred. After methoxide formation, methanol (1 L) was added and refluxed for about 1/2 hour, followed by careful distillation (bp 65 °C). The distillate was collected and stored over 4A molecular sieves.

Propan-2-ol: as for methanol; distilled at 82 °C/760mm and stored over 4A molecular sieves.

Pyridine was dried by standing over KOH pellets for 20 hours, followed by distillation from barium oxide and the distillate at 115 °C/760mm was collected and stored over KOH pellets.

Petroleum ether 60-80 °C: as for hexane; distilled at 60-80 °C.

Tertiary butanol was refluxed over calcium oxide for 20 hours and distilled from the same at 83 °C/760mm and stored over 4A molecular sieves.

Tetrahydrofuran (THF) was stored over KOH pellets and distilled from lithium aluminium hydride prior to use.(bp 67 °C/760mm).

3.3 Reagent purification and preparation.

Benzaldehyde was freshly distilled before use (bp 178-179 °C).

***n*-Butanal** was freshly distilled before use (bp 74.8-75 °C).

3-Chloroperoxybenzoic acid (*m*-CPBA): wet solutions in CH_2Cl_2 were dried by standing over anhydrous sodium sulphate prior to use.

Ethylene glycol was stored over KOH pellets overnight and distilled from the same at 196-198 °C/760mm. The distillate was stored over 4A molecular sieves.

Isoamyl nitrite was freshly distilled prior to use (bp 99 °C/760mm).

Triethyl orthoformate was freshly distilled before use (bp 146 °C/ 760mm).

Zinc (Zn): commercial zinc powder (400 g) was stirred with 10% HCl (150 mL) for 2 minutes , filtered, and washed with water (300 mL), followed acetone (100 mL) and oven-dried.

Jones reagent in acetone: chromium trioxide (26.72 g) was dissolved in concentrated sulphuric acid (23 mL) and diluted with water to 100 mL.

Ethereal periodic acid: a solution containing about 16 mg/mL was prepared by stirring an excess of the powdered peracid with sodium-dried ether, under nitrogen, for 1 hour, allowing the solid to settle and decanting the supernatant.

Ethereal diazomethane: this reagent was prepared using the Mini-Diazald Apparatus (Aldrich Chem. Co.); ethanol (95%, 10 mL) was added to a solution of KOH (5 g) in water (8 mL) in the reaction vessel. The mixture was heated to 65 °C and then a solution of Diazald (N-methyl-N-nitroso-*p*-toluenesulphonamide, 5 g, 23 mmol) in ether (45 mL) was added dropwise over 20 minutes. The rate of distillation should approximate the rate of addition. The ethereal co-distillate is condensed by the acetone-dry ice (-78 °C) cold finger into the receiving vessel and will contain about 700 mg (16.6 mmol) of diazomethane.

Sodiomethylsulphinyl carbanion (dimsyl sodium).

Method I: 0.4 g of a 60% sodium hydride (NaH) dispersion in mineral oil (equiv. to 0.24 g NaH, 10 mmol) under nitrogen, in a dry, stoppered 3-neck round bottom flask was washed free of mineral oil using sodium-dried ether. Anhydrous DMSO (10 mL) was added to the dry NaH and the mixture stirred at 70 °C for 1 hour to give the base, dimsyl sodium (methyl sulphinyl methide, 10 mmol) as a tarry solution.

Method II: 0.4 g of a 60% NaH dispersion in mineral oil (equiv. to 0.24 g NaH, 10 mmol), under nitrogen, in a dry, stoppered 3-neck round bottom flask was washed free of mineral oil using sodium-dried toluene. Anhydrous DMSO (10 mL) was added to the dry NaH and the mixture stirred at 55 °C for 2 hours to give the base, dimsyl sodium (methyl sulphinyl methide, 10 mmol) as a green translucent solution.

These methods have been summarised in the text of the Experimental procedures (section 3.5) to, for example: To a stirred 1M solution of sodiomethyl-sulphinyl carbanion (10 mL, 10 mmol) at 25 °C, under nitrogen, was added a solution of, say, (4-carboxybutyl)triphenylphosphonium bromide.....

3.4 Microbiological hydroxylation.

Cultures of the micro-organism, *Calonectria decora* (*Cd.*) were obtained from the Centraalbureau Voor Schimmelcultures, Baarn, Netherlands. Broth cultures were made from Saboraud Liquid Medium [(Oxoid) 30 g/L] in distilled water, mixed well, distributed into final containers and sterilised by autoclaving at 121 °C for 15 minutes. The steam sterilised media were inoculated with the organism (*Cd*) from the master slope and developed for 5-7 days at 25 °C. (a continuous supply of broth culture was maintained by preparing new ones from existing cultures one week after the appearance of a healthy development). The broth cultures were used as a source of spore suspension for inoculating the fermentation media.

The medium used was prepared from malt extract (2 g), beef extract (2 g), yeast [(Difco) 2 g], corn steep liquor (2 mL), glucose (10 g) and distilled water (1 L). The pH of the medium was adjusted to 5.5 with 2N HCl and sucrose (2 g) was added. The medium was distributed into 1 L conical flasks (300 mL/flask), plugged with non-absorbent cotton wool and sterilised by autoclaving at 121 °C for 15 minutes.

The flasks were then inoculated with spore suspension (5 mL/flask), under sterile conditions, plugged with non-absorbent cotton wool, and swirled (150 rev. min^{-1}) in a Gallenkamp orbital incubator at 25 °C for 2-3 days. A solution of the steroid substrate in an organic solvent was added under sterile conditions and the flasks were swirled for 4 days at 25 °C. Different extraction procedures (I and II) were used.

Extraction I. The contents of the flasks were combined and filtered through a thick layer of NaCl. The mycelium was extracted by repeated soakings in boiling acetone and decantation. After evaporation of the acetone, the residue was partitioned between $CHCl_3$ (or EtOAc) and water. The organic layer afforded the mycelial extract. The culture fluid (i.e. the filtrate) was saturated with NaCl and

extracted by shaking with CH_2Cl_2 or continuously by Soxhlet extraction. The CH_2Cl_2 layer was dried (a.Na_2SO_4) and evaporated to give the broth extract. These extract were combined to give the combined extracts.

Extraction II. This differed from extraction I in the following respects: the contents of the flasks were filtered via a plug of glass wool and the mycelial extract was resuspended in a minimum volume of distilled water and added to filtrate and the whole distributed into 1 L thick-walled, round bottom flasks (100 mL/flask) and freeze-dried using an Edward high vacuum Modulyo Freeze Dryer. The freeze-dried residues were resuspended in a minimum volume of distilled water, combined, diluted with acetone and extracted with CH_2Cl_2, dried and evaporated to give the combined extracts.

The following abbreviated description for microbiological hydroxylation will be adopted in the text of the Experimental procedures (section 3.5). For example, Incubation with *Cd*: 0.8 g in DMSO (150 mL), 10 flasks (300 mL medium/flask), extraction I or II gave 1.0 g combined extracts, which were refluxed in acetone (250 mL)-H_2O (20 mL) with TsOH.H_2O (150 mg) for 4h. Chromat. on Al_2O_3 (neutral, deactivated with 5% H_2O, 60 g) indicates: A solution of the steroid (0.8 g) in DMSO (150 mL) was incubated for 4 days with the micro-organism grown in 10 flasks containing the nutrient medium. Extraction I or II gave the combined extracts which were hydrolysed and chromatographed.

3.5 Experimental procedures.

(i) 5α-Androstan-17β-ol (58):

To a solution of the steroid ketone (**57**) [5 g, 17.24 mmol] and KOH (40 g) in ethylene glycol (400 mL) was added 85% hydrazine hydrate (15 mL). The mixture was refluxed for 1.5 hours, then excess hydrazine hydrate and water were distilled off until the internal temperature of the reaction mixture rose to 195-200 °C. The reaction mixture was refluxed at this temperature for a further 5 hours, cooled to room temperature and diluted with water before acidifying with 6N HCl. Extraction with ether followed by the usual work-up procedure gave **the product (58)** as a white solid; yield: 4.52 g (95%); mp 168-169 °C [needles from petroleum ether (40:60°)/ether], lit., [97a,118] mp 164-167 °C; $\upsilon_{max.}$ 3300 cm^{-1} (br s, O-H str); δ_H 0.79 (s, 3H, 19-H), 0.73 (s, 3H, 18-H), 1.74-1.82 (m, 1H, 16-H), 1.97-2.10 (m, 1H, 16-H), 3.62 (t, 1H, J 8.39 Hz, 17-H); *m/z* 276 (M^+) (100%), 261 $(M-CH_3)^+$ (27.9), 258 $(M-H_2O)^+$ (9.4) [M^+, 276.2453. $C_{19}H_{32}O$ requires M, 276.2453].

(ii) 5α-Androstan-17-one (59):

To a stirred, ice-cold solution of the sterol (**58**) [5 g, 10.09 mmol] in acetone (250 mL) was added Jones reagent (10 mL) in a dropwise manner. The reaction mixture was stirred for a further 3 hours, after which the precipitated chromic salts were filtered off. After the addition of aqueous sodium metabisulphite (5%, 200 mL) to the filtrate it was extracted with ether. The organic extract was washed successively with aqueous NaOH (5%, 200 mL), water (200 mL) and brine (200 mL). After drying the ethereal extract (a.Na_2SO_4), the solvent was removed under reduced pressure to give the crude product which was purified by column chromatography using silica gel as adsorbent and $CHCl_3$ as eluant to give **the product (59)** as a clear crystalline solid; yield: 4.48 g (90%); mp 122-123 °C (platelets from acetone), lit., [97b] mp 121-122 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str);

δ_H 0.81 (s, 3H, 19-H), 0.86 (s, 3H, 18-H), 2.08 (m, 1H, 16α-H), 2.43 (dd, 1H, J 18.5, 9.3 Hz, 16β-H); *m/z* 273 (M^+) (100%), 246 $(M-CO)^+$ (0.7), 246 $(M-C_2H_4)^+$ (1.0), 230 $(M-CH_3CHO)^+$ (42.8), 218 $(M-C_3H_4O)^+$ (34.4), 203 $(M-C_4H_7O)^+$ (20.3) [M^+, 274.2292. $C_{19}H_{30}O$ requires M, 274.2297].

(iii) 16(E)-Benzylidene-5α-androstan-17-one (60):

A solution of freshly distilled benzaldehyde (4.2 g, 39.58 mmol) and 5α-androstan-17-one (**59**) [4.2 g,16.03 mmol] in absolute alcohol (115 mL) containing KOH (0.64 g) was kept in the dark, at room temperature for 16 hours during which the product precipitated. Filtration of the reaction mixture gave the crude product as a cream crystalline solid which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the product (60)** as a white crystalline solid; yield: 5.16 g (88.81%); mp 159-160 °C (platelets form aqueous EtOH), lit., [97c] 157-159 °C; $\upsilon_{max.}$ 1715 (s, C=O str), 1630 cm^{-1} (s, arom. conj. C=C str); $\lambda_{max.}$ 293 nm; δ_H (400 MHz) 0.86 (s, 3H, 19-H), 0.96 (s, 3H, 18-H), 2.41 (m, 1H, 15β-H), 2.88 (ddd, 1H, J 16.00, 6.60, 1.87 Hz, 15α-H), 7.38-7.56 (m, 6H, C_6H_5CH=); *m/z* 362 (M^+) (100%), 347 $(M-CH_3)^+$ (9.5), 271 $(M-C_7H_7)^+$ (36.4), 144 ($C_{10}H_8O^+$) (27.0), 116 ($C_9H_8^+$) (96.4); [M^+, 362.2629. $C_{26}H_{34}O$ requires M, 362.2610].

(iv) 16(E)-Benzylidene-5α-androstane (61):

To a stirred solution of aluminium chloride (1.2 g) and lithium aluminium hydride (0.2 g) in sodium-dried ether (25 mL) was added, in a dropwise manner over 20 minutes, a solution of of the benzylidene ketone (**60**) [1 g, 3.0 mmol] in sodium-dried ether (50 mL). The reaction mixture was boiled under reflux for 2.5 hours, cooled to room temperature and carefully added to ice-water (200 mL) containing 2N HCl (12 mL). Extraction with ether was followed by the usual work-up procedure to give the crude product as a cream solid which was chromatographed on silica gel.

Elution with hexane afforded **the product (61)** as a clear crystalline solid; yield: 0.72 g (75%), mp 124-125 °C (needles from aqueous acetone), lit., [97c] mp 123.5-124 °C. Further elution with ethyl acetate afforded **16(E)-benzylidene-5α-androstan-17β-ol (61a)**; yield: 0.28 g (23%),mp 199-200 °C (needles from aqueous acetone), lit., [97c] 198-199 °C.

16(E)-Benzylidene-5α-androstane (61):

$\upsilon_{max.}$ 1650 (s, conj. C=C str), 1590, 1570, 1490 cm^{-1} (arom. C=C str); $\lambda_{max.}$ 256 nm; δ_H (400 MHz) 0.82 (s, 3H, 18-H), 0.85 (s, 3H, 19-H), 2.14-2.19 (br m, 1H, 15β-H), 2.28 (br q, 2H, J 14.8 Hz, 17-H), 2.68 (br dd, 1H, J 16.4, 6.0 Hz, 15α-H), 6.37 (br s, 1H, C_6H_5CH=), 7.16-7.20 (m, 1H, arom. H), 7.31-7.36 (m, 4H, arom. H's); *m/z* 348 (M^+) (87.1%), 91 ($C_7H_7^+$) (100), 77 ($C_6H_5^+$) (21.8); [M^+, 348.2822. $C_{26}H_{36}$ requires M, 348.2817].

16(E)-Benzylidene-5α-androstan-17β-ol (61a):

$\upsilon_{max.}$ 3520 cm^{-1} (br s, O-H str); δ_H (400 MHz) 0.70 (s, 3H, 18-H), 0.83 (s, 3H, 19-H), 2.12-2.22 (m, 1H, 15β-H), 2.66 (br dd, 1H, J 16.86, 5.78 Hz, 15α-H), 4.06 (br s, 1H, 17α-H), 6.52 (br d, 1H, J 2.36 Hz, C_6H_5CH=), 7.18-7.22 (m, 1H, arom. H), 7.31-7.40 (m, 4H, arom. H's); δ_C (100.61 MHz) 11.37 (C-18), 12.49 (C-19), 85.34 (C-17), 123.03, 126.52, 128.41, 128.54 (aromatic C-H's), 138.15, 146.68 (aromatic *ipso* C's); *m/z* 364 (M^+) (73.1%), 247 ($M-C_9H_9$)$^+$ (100), 91 ($C_7H_7^+$) (59.6); [M^+, 364.2770. $C_{26}H_{36}O$ requires M, 364.2766].

(v) 5α-androstan-16-one (56):

A solution of the benzylidene steroid **(61)** [1 g, 2.90 mmol] in MeOH (250 mL) - EtOAc (100 mL) was ozonised (ozoniser model-L5) at -78 °C for 4 hours. Nitrogen was then passed through the solution for 10 minutes. After the addition of

glacial AcOH (50 mL), the mixture was warmed to 30 °C and was maintained between 30-35 °C by external cooling while Zn dust (20 g) was added in small portions over a period of 10-15 minutes. The excess Zn was filtered off and the filter-cake was washed with fresh EtOAc. The combined filtrate and washings were concentrated under reduced pressure, diluted with CH_2Cl_2 and washed with water. The usual work-up procedure gave the crude product as a gum which was chromatographed on neutral alumina (deactivated with 5% H_2O). Elution with 5% ether:petroleum ether 60-80 °C afforded **the product (56)** as a crystalline solid; yield: 163 mg (20.71%), mp 107-108 °C (needles from MeOH), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H 0.82 (s, 3H, 19-H), 0.87 (s, 3H, 18-H); *m/z* 274 (M^+) (100%).

(vi) 16-Benzoyl-5α-androst-16-ene (65):

A solution of the benzylidene steroid (**61**) [4.75 g, 13.63 mmol] in MeOH (350 mL) - EtOAc (150 mL) was ozonised (ozoniser model-L5) at -78 °C for 72 hours during which a true solution resulted. Nitrogen was then passed through the solution for 10 minutes. After the addition of glacial AcOH (250 mL), the mixture was warmed to 30 °C and maintained between 30-35 °C by external cooling while Zn dust (120 g) was added in small portions over a period of 60 minutes. The excess Zn was filtered off and the filter-cake washed with fresh EtOAc. The combined filtrate and washings were concentrated under reduced pressure, diluted with CH_2Cl_2, and washed with water. The usual work-up procedure gave a crude gum which was chromatographed on silica gel. Elution with 5% ether:petroleum ether 60-80 °C afforded **the product (65)** as a crystalline solid; yield: 3.05 g (61.74%), mp 125-126 °C (rods from MeOH) (Found: C, 86.54; H, 9.87. $C_{26}H_{34}O$ requires C, 86.13; H, 9.48); $\upsilon_{max.}$ 1667 (s, C=O str), 695, 670 cm^{-1} (s, arom. H def); $\lambda_{max.}$ 261 nm; δ_H (400 MHz) 0.81 (s, 3H, 19-H), 1.33 (s, 3H, 18-H), 2.27 (dd, 1H, J 17.60, 14.41 Hz, 15β-H), 2.71 (dd, 1H, J 17.6, 4.42 Hz, 15α-H) 6.79 (s, 1H, olefinic

17-H), 7.28-7.37 (m, 5H, arom. H's); *m/z* 362 (M^+) (100%), 347 $(M-CH_3)^+$ (7.0), 145 ($C_{10}H_9O^+$) (65.9) [M^+, 362.2607. $C_{26}H_{34}O$ requires M, 362.2610].

(vii) 5α-Androstan-16-one (56):

To a stirred solution of potassium permanganate (16 mg, 0.10 mmol) and potassium metaperiodate (552 mg, 2.40 mmol) in water (20 mL) was added, in a dropwise manner, a solution of the benzylidene steroid (**61**) [102.51 mg, 0.30 mmol] in pyridine (100 mL). The mixture was stirred vigorously for 8 hours at room temperature, diluted with with brine, extracted with ether, followed by the usual work-up procedure to give the crude product as a gum,which was purified by p.l.c. [1 large plate, 2 x $CHCl_3$]. 75.2 mg of the starting material (**61**) was recovered unchanged and **the product (56)** was obtained as a clear crystalline solid; yield: 12 mg (14.9%), mp 108-109 °C (needles from acetone), lit.,[114] mp 108 °C; $\upsilon_{max.}$1745 cm^{-1} (s, C=O str); δ_H 0.80 (s, 3H, 19-H), 0.86 (s, 3H, 18-H).

(viii) 16-Hydroxyimino-5α-androstan-17-one (84):

To a stirred solution of potassium-*t*-butoxide (10.6 g, 94.46 mmol) and the steroid ketone (**59**) [11.2 g, 40.81 mmol] in dry *t*-butanol (340 mL) was added, in a slow dropwise manner, freshly distilled isoamyl nitrite (11.2 g, 95.60 mmol). The resulting viscous pink suspension was stirred for 16 hours at 31 °C, then diluted with water and acidified with dilute acetic acid. Extraction with ether, followed by the usual work-up procedure procedure gave **the product (84)** as a crystalline solid; yield: 9.5 g (76.74%), mp 260-261 °C (dec.) [platelets from EtOH], lit.,[114] mp 260 °C (dec.); $\upsilon_{max.}$ 3310 (br s, H-bonded OH str, -CO-C=N-OH), 1740 (s, C=O str), 1630 (s, conj. C=N str), 945 cm^{-1} (s, N-O str); δ_H 0.83 (s, 3H, 19-H), 0.95 (s, 3H, 18-H), 2.14 (dd, 1H, J 17.65, 13.14 Hz, 15β-H), 2.93 (dd, 1H, J 17.65, 6.76 Hz, 15α-H), 9.45 (br s, 1H, =N-OH); *m/z* 303 (M^+) (36.1%) [M^+, 303.2210.

$C_{19}H_{29}NO_2$ requires M, 303.2198].

(ix) 17β-Hydroxy-5α-androstan-16-one (85):

To a stirred solution of the keto-oxime (**84**) [4.4 g, 14.17 mmol] in a mixture of glacial AcOH (80 mL) and water (6.3 mL) was added in small portions, 12.9 g of Zn powder . After the addition of Zn, a further volume of water (47 mL) was added and the mixture vigorously boiled under reflux for 1 hour. The reaction mixture was allowed to cool to room temperature and the precipitated zinc acetate and unreacted Zn filtered off. The filter-cake was washed with glacial AcOH and the combined filtrate and washings diluted with water. The precipitated solid was filtered off and dried to give **the product (85)** as a cream powder; yield: 3.8 g (92.68%), mp 143-144 °C (needles from acetone), lit.,[114] mp 144 °C; $\upsilon_{max.}$ 3390 (br s, OH str), 1755 cm^{-1} (s, C=O str); δ_H 0.71 (s, 3H, 19-H), 0.82 (s, 3H, 18-H), 1.81 (dd, 1H, J 18.86, 11.94 Hz, 15β-H), 2.32 (ddd, 1H, J 18.86, 7.58, 1.18 Hz, 15-αH), 3.75 (br s, 1H, 17-H); *m/z* 290 (M^+) (71.6%), 272 $(M-H_2O)^+$ (0.8), 217 $(M-C_3H_5O_2)^+$ (100) [M^+, 290.2551. $C_{19}H_{30}O_2$ requires M, 290.2246].

(x) 17β-Tosyloxy-5α-androstan-16-one (86):

To a stirred solution of the α-ketol (**85**) [0.5 g, 1.72 mmol] in anhydrous pyridine (8.7 mL) was added anhydrous *p*-toluenesulphonyl chloride (0.375 g, 1.96 mmol). After the sulphonyl chloride dissolved, the reaction was maintained under a static atmosphere of nitrogen and kept in the refrigerator for 16 hours, during which needles of pyridinium chloride separated. The reaction mixture was poured into a vigorously stirred ice-water mixture and the precipitated tosylate was filtered off to give **the product (86)** as a brownish solid; yield: 0.70 g (91%), mp 142-143 °C (platelets from benzene-petroleum ether 40:60 °C), lit.,[114] mp 144 °C; $\upsilon_{max.}$ 1755 (s, C=O str), 1600 (s, S=O str), 1170 cm^{-1} (s, S=O str); δ_H 0.80 (s, 3H, 19-H), 0.83

(s, 3H, 18-H), 2.24 (br dd, 1H, J 11.70, 4.45 Hz, 15-H), 2.45 (s, 3H, -C_6H_4-CH_3), 4.59 (s, 1H, 17-H), 7.34 (d, 2H, J 5.1 Hz, arom. H_m), 7.86 (d, 2H, J 5.1 Hz, arom. H_o); *m/z* 444 (M^+) (37.7%), 429 ($M-CH_3$)$^+$ (1.9), 289 ($M-C_7H_7O_2S$)$^+$ (100), 273 ($M-C_7H_7O_3S$)$^+$ (3.0), 91 ($C_7H_7^+$) (89.9) [M^+, 444.2330. $C_{26}H_{36}O_4S$ requires M, 444.2334].

(xi) 17β-Tosyloxy-5α-androstan-16β-ol (87):

To a stirred suspension of the keto-tosylate (**86**) [5.6 g, 12.60 mmol] in a mixture of anhydrous methanol (69 mL) and anhydrous pyridine (21 mL) was added sodium borohydride (1.4 g, 36.99 mmol) in small fractions at a time. The suspension soon dissolved and this was followed by a copious precipitate. The reaction mixture was cooled in an ice-bath and the precipitate filtered off. The filter-cake was suspended in cold dilute HCl and stirred at 0 °C for 2 hours, after which the suspension was extracted with ether. The ethereal extract was washed thoroughly with water, and followed by the usual work-up procedure to give **the product (87)** as a white solid; yield: 4.02 g (71.40%), mp 167-168 °C (platelets from acetone-ether), lit.,[114] mp 167 °C; $\upsilon_{max.}$ 3520 (s, O-H str), 1360 (s, S=O str), 1170 cm^{-1} (s, S=O str); δ_H 0.77 (s, 3H, 19-H), 1.0 (s, 3H, 18-H), 2.45 (s, 3H, -C_6H_5-CH_3), 3.9 (d, 1H, J 6.83 Hz, 17α-H), 4.24 (m, 1H, 16-H), 7.33 (d,2H, J 7.98 Hz, arom. H_m), 7.80 (d, 2H, J 8.3 Hz, arom. H_o); *m/z* 446 (M^+) (0.6%), 291 ($M-C_7H_7O_2S$)$^+$ (38.1), 276 ($M-C_7H_6O_3S$)$^+$ (56.6), 274 ($M-C_7H_8O_3S$)$^+$ (79.7), 217 ($M-C_{10}H_{13}O_4S$)$^+$ (100), 91 ($C_7H_7^+$) (38.0) [M^+, 446.2499. $C_{26}H_{38}O_4S$ requires M, 446.2491].

(xii) 5α-Androstan-16-one (56):

To a stirred solution of the hydroxy tosylate (**87**) [2.52 g, 5.71 mmol] in hot methoxy-methanol (100 mL) was added 2N sodium hydroxide (34 mL) in a dropwise manner. The reaction mixture was heated (160-170 °C) at reflux for 3 hours. On cooling to room temperature, a little water was added and the resulting precipitate filtered off to give **the product (56)** as a white solid; yield: 1.04 g (67.10%), mp 107-108 °C (needles from acetone), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H 0.81 (s, 3H, 19-H), 0.86 (s, 3H, 18-H).

(xiii) 5α-Androstan-17-one-tosylhydrazone (91):

A solution of the keto steroid (**59**) [2.74 g, 10.0 mmol] and *p*-toluenesulphonylhydrazide (2.23 g, 12.0 mmol) in absolute alcohol (100 mL) was refluxed for 10.5 hours. The reaction mixture was concentrated to about 20 mL at 60 °C under reduced pressure. On cooling to room temperature a white precipitate occurred, which was filtered off and washed with ice-cold ethanol to afford **the product (91)**; yield: 2.07 g (46.73%), mp 200-202 °C (dec.) [platelets from EtOH], lit.,[118] mp 200-202 °C (dec.); $\upsilon_{max.}$ 3200 (s, N-H str), 1665 (m, C=N str), 1600 (m, arom. C=C str), 1350 (s, S=O str), 1170 cm^{-1} (s, S=O str); δ_H 0.77 (s, 3H, 19-H), 0.78 (s, 3H, 18-H), 2.26 (dd, 1H, J 18.06, 8.12 Hz, 16-H), 2.43 (s, 3H, $-C_6H_4-CH_3$), 7.14 (br s, 1H, -N-H], 7.30 (d, 2H, J 8.20 Hz, arom. H_m), 7.83 (d, 2H, J 8.20 Hz, arom. H_o); *m/z* 442 (M^+) (3.8%), 287 $(M-C_7H_7O_2S)^+$ (100), 272 $(M-C_7H_8NO_2S)^+$ (6.5), 258 $(M-C_7H_8N_2O_2S)^+$ (39.0), 257 $(M-C_7H_9N_2O_2S)^+$ (58), 243 $(M-C_8H_{11}N_2O_2S)^+$ (15.9), 91 ($C_7H_7^+$) (39.2) [M^+, 442.2649. $C_{26}H_{38}N_2O_2S$ requires M, 442.2654].

(xiv) 5α-androst-16-ene (92):

A fine suspension of the tosylhydrazone (**91**) [1.0 g, 2.26 mmol] in sodium-dried ether was maintained at 0 °C and under a slight positive pressure of nitrogen. To this was added a 1.4 M ethereal solution of methyl lithium (5 mL, 7.0 mmol) in a slow dropwise manner. The suspension dissolved immediately, producing an orange-coloured solution. Stirring was continued at 0 °C for 1.5 hours and then at room temperature for 3.5 hours. The reaction mixture was then refluxed for 3 hours during which it acquired a red colour. After cooling to room temperature, water (10 mL) was cautiously added and the mixture stirred for 1 hour. Extraction with ether, followed by the usual work-up procedure gave the crude product, which was chromatographed on silica gel. Elution with hexane afforded **the product (92)** as a white solid; yield: 0.45 g (77.60%), mp 81 °C (platelets from MeOH), lit.,[118] mp 78-79 °C; $\upsilon_{max.}$ 3040 (m, =C-H str), 1590 (w,C=C str), 715 cm^{-1} (s, =C-H def); δ_H 0.75 (s, 3H, 18-H), 0.82 (s, 3H, 19-H), 1.88 (m, 1H, 15-H), 2.09 (m, 1H, 15-H), 5.67 (ddd, 1H, J 5.89, 2.50, 1.07 Hz, 17-H), 5.83 (ddd, J 5.89, 2.90, 1.43 Hz, 16-H); *m/z* 258 (M^+) (99.2%), 257 $(M-H)^+$ (7.7), 243 $(M-CH_3)^+$ (100) [M^+, 258.2342. $C_{19}H_{30}$ requires M, 258.2347].

(xv) 5α-Androstan-16-one (56):

A stirred solution of the steroid alkene (**92**) [181 mg, 0.71 mmol] in dry tetrahydrofuran (4 mL, freshly distilled from $LiAlH_4$) was maintained under nitrogen and cooled to 0 °C. To this was added 1.0 M borane in THF (2.5 mL, 2.5 mmol.) in a slow dropwise manner. Stirring was continued for 48 hours at room temperature then 6N NaOH (3 mL) and 30% H_2O_2 were carefully added. The reaction mixture was heated at reflux (60 °C) for 2 hours, cooled to room temperature, diluted with 5% $NaHCO_3$ and extracted with CH_2Cl_2. The usual work-up procedure gave 185 mg of the crude intermediate, which was taken up in acetone (10 mL), cooled to ice-bath

temperature, stirred and treated with Jones reagent (1.0 mL). After filtration of the green chromic salts, the filtrate was treated with 5% $NaHSO_3$ and extracted with CH_2Cl_2. The organic extract was washed successively with 5% $NaHCO_3$ and water to give the crude product which was chromatographed on silica gel. Elution with 5% ether-petroleum ether 60-80 °C afforded the product as a crystalline solid of isomeric composition (5α-androstan-16-one and 5α-androstan-17-one); yield: 31 mg (16.13%). The isomer ratio of the 16-ketone:17-ketone was determined by ^1H NMR integration studies to be 1.6:1. Melting range 102-111 °C, lit.,[114, 97b] mp 108 °C and 121-123 °C for the 16-ketone and 17-ketone respectively; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H 0.80 (s, 3H, 19-H), 0.82 (s, 3H, 19-H), 0.86 (s, 3H, 18-H), 0.87 (s, 3H, 18-H), 2.21 (dd, characteristic of pure 16-ketone), 2.43 (dd, characteristic of pure 17-ketone).

(xvi) 5α-Androstan-16-one (56):

A solution of the steroid alkene (**92**) [103 mg, 0.40 mmol] in dry tetrahydrofuran (2 mL, freshly distilled from $LiAlH_4$) was maintained under nitrogen and cooled to 0 °C. To this solution was added 0.5 M 9-borabicyclo[3.3.1]nonane (9-BBN) in THF (2.0 mL, 1.0 mmol) in a slow dropwise manner. Stirring was continued for 48 hours at room temperature, then 6N NaOH (2 mL) and 30% H_2O_2 (1 mL) were carefully added. The reaction mixture was heated (60 °C) at reflux for 2 hours, cooled to room temperature, diluted with 5% $NaHCO_3$ and extracted with CH_2Cl_2. The usual work-up procedure gave 118 mg of the crude intermediate, which was taken up in acetone (10 mL), cooled to ice-bath temperature, stirred and treated with Jones reagent (0.5 mL). After filtration of the green chromic salts, the filtrate was treated with 5% $NaHSO_3$ and extracted with CH_2Cl_2. The organic extract was washed successively with 5% $NaHCO_3$ and water, followed by the usual work-up procedure to give **the product (56)** as a crystalline solid; yield: 11 mg (10.06%),

mp 106-107 °C (needles from methanol), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H 0.82 (s, 3H, 19-H), 0.87 (s, 3H, 18-H).

(xvii) 16-Hydroxyphenylmethyl-5α-androstane (94):

A stirred solution of the benzylidene steroid (**61**) [508 mg, 1.46 mmol] in dry THF (3 mL, freshly distilled from $LiAlH_4$) was maintained under nitrogen and cooled to 0 °C. To this solution was added 1.0 M borane in THF (4.5 mL, 4.5 mmol) in a dropwise manner. Stirring was continued for 48 hours at room temperature, then 6N NaOH (8 mL) and 30% H_2O_2 (4 mL) were carefully added. The reaction mixture was heated (60 °C) at reflux for 2 hours, cooled to room temperature, treated with aqueous K_2CO_3 and then extracted with CH_2Cl_2. The usual work-up procedure gave the crude product which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the product (94)** as a white solid; yield: 374 mg (70.0%), mp 150-151 °C. (needles from acetone) (Found: C, 83.85; H, 9.76. $C_{26}H_{38}O.1/2\ H_2O$ requires C, 83.14; H,10.20); $\upsilon_{max.}$ 3370 cm^{-1} (br s, O-H str); δ_H 0.70 (s, 3H, 19-H), 0.77 (s, 3H, 18-H), 2.12 (s, 1H, benzylic OH), 2.31 (m, 1H, 16-H), 4.36 (d, 1H, J 9.66 Hz, benzylic H), 7.21-7.29 (m, 5H, arom. H's); *m/z* 366 (M^+) (9.0%), 348 $(M-H_2O)^+$ (15.6), 259 $(M-C_7H_7O)^+$ (12.5), 107 ($C_7H_7O^+$) (100) [M^+, 366.2924. $C_{26}H_{38}O$ requires M, 366.2922].

(xviii) 16-Benzoyl-5α-androstane (95):

A solution of the hydroxy benzyl steroid (**94**) [100 mg, 0.27 mmol] in acetone (10 mL) was cooled in an ice-bath. To this stirred solution was added Jones reagent (0.5 mL) in a slow dropwise manner. After 1 hour the precipitated green chromic salts were filtered off and the filtrate treated with 5% $NaHSO_3$ and extracted with CH_2Cl_2. The organic extract was washed successively with 5% $NaHCO_3$ and water, followed by the usual work-up procedure to give the crude product which was

chromatographed on Florisil. Elution with 5% ether-petroleum ether 60-80 °C afforded **the product (95)** as a white solid; yield: 91 mg (91.50%), mp 158-159 °C (needles from acetone) (Found: C, 85.64; H, 9.70. $C_{26}H_{36}O$ requires C, 85.66; H, 9.95); $\upsilon_{max.}$ 1680 cm^{-1} (s, C=O str); δ_H 0.72 (s, 3H, 18-H), 0.79 (s, 3H, 19-H), 3.71 (m, 1H, 16-H), 7.48 (m, 3H, arom. $H_{m,p}$), 7.59 (dd, 2H, J 8.26, 1.36 Hz, arom. H_o); *m/z* 364 (M^+) (100%), 259 $(M-C_7H_5O)^+$ (2.6), 105 ($C_7H_5O^+$) (68.6), 77 ($C_6H_5^+$) (13.5) [M^+, 364.2752. $C_{26}H_{36}O$ requires M, 364.2766].

(xix) 16-Benzoyl-5α-androstane (95):

A stirred solution of the benzylidene steroid **(61)** [514 mg, 1.47 mmol] in dry tetrahydrofuran (4 mL, freshly distilled from $LiAlH_4$.) was maintained under nitrogen and cooled to 0 °C. To this solution was added 0.5 M 9-BBN in THF (9 mL, 4.5 mmol) in a slow dropwise manner. Stirring was continued for 48 hours at room temperature, then 6N NaOH (8 mL) and 30% H_2O_2 (4 mL) were carefully added. The reaction mixture was heated (60 °C) at reflux for 2 hours, cooled to room temperature, treated with saturated aqueous K_2CO_3 and then extracted with CH_2Cl_2. The usual work-up procedure gave the crude product which was taken up in acetone (20 mL), cooled to ice-bath temperature, stirred and treated dropwise with Jones reagent (2 mL). After 1 hour the precipitated green chromic salts were filtered off and the filtrate treated with 5% $NaHSO_3$ and extracted with CH_2Cl_2. The organic extract was washed successively with 5% $NaHCO_3$ and water, followed by the usual work-up procedure to give the crude product which was chromatographed on silica gel. Elution with 5% ether-petroleum ether 68-80 °C afforded **the product (95)** as a white solid; yield 367 mg (68.27%), mp 158-159 °C (needles from acetone); $\upsilon_{max.}$ 1680 cm^{-1} (s, C=O str).

(xx) 5α-Androstan-16yl-benzoate (96):

To a stirred solution of the benzoyl steroid (**95**) [100 mg, 0.27 mmol] in dry CH_2Cl_2 (5 mL) was added in portions (2 mL) over 5 minutes, a solution of 50% w/w *m*-CPBA (120 mg, 0.35 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 72 hours, diluted with CH_2Cl_2, washed successively with an excess 10% $NaHSO_3$, 10% $NaHCO_3$, and water. The usual work-up procedure gave the crude product which was purified by p.l.c. on silica gel. Elution with petroleum ether 60-80 °C afforded **the product (96)** as an oil; yield: 20 mg (19.16%); $\upsilon_{max.}$ 1715 (s, C=O str), 1600, 1500, (m, arom. C=C str), 1270 cm^{-1} (s, C-O str); δ_H 0.80 (s, 3H, 19-H), 0.97 (s, 3H, 18-H), 2.38 (m, 1H), 5.39 (m, 1H, 16-H), 7.52 (m, 3H, arom. $H_{m,p}$), 8.03 (m, 2H, arom. H_o); *m/z* 380 (M^+) (3.5%), 365 $(M-CH_3)^+$ (1.0), 275 $(M-C_7H_5O)^+$ (1.9), 258 $(M-C_7H_6O_2)^+$ (100), 243 $(M-CH_3-C_7H_6O_2)^+$(75.5), 105 ($C_7H_5O^+$) (67.6), 77 ($C_6H_5^+$) (49.1) [M^+, 380.2717. $C_{26}H_{36}O_2$ requires M, 380.2715].

(xxi) 5α-Androstan-16-one (56):

A solution of the steroid ester (**96**) [20 mg, 0.05 mmol] in MeOH (10 mL) containing NaOH (100 mg) and water (1 mL) was refluxed for 4 hours, cooled to room temperature, diluted with water and extracted with CH_2Cl_2. The organic extract was washed several times with water, followed by the usual work-up procedure to give the crude product which was taken up in acetone (5 mL), cooled to ice-bath temperature, stirred and treated with Jones reagent (0.5 mL) in a dropwise manner. After 1 hour the precipitated green chromic salts were removed by filtration and the filtrate treated with 5% $NaHSO_3$ and extracted with CH_2Cl_2. The organic extract was washed successively with 5% $NaHCO_3$ and water, followed by the usual work-up procedure to give the crude product which was purified by p.l.c. on silica gel. Elution with $CHCl_3$ afforded **the product (56)**; yield: 6.93 mg (48.06%), mp 106-107 °C

(needles from MeOH), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str).

(xxii) 3'-Phenyl-5α-androstan-16-spiro-2'-oxirane (97):

To a stirred solution of the benzylidene steroid (**61**) [1.1 g, 3.16 mmol] in dry CH_2Cl_2 (100 mL) was added, in a dropwise manner, a dry solution of 50% w/w *m*-CPBA (1.2 g, 2.5 mmol) in CH_2Cl_2 (75 mL). The reaction mixture was stirred at room temperature for 18 hours under nitrogen and then treated successively with an excess 10% $Na_2S_2O_3$, 5% $NaHCO_3$, and water. The usual work-up procedure gave the product as a yellow gum which was chromatographed on neutral alumina (deactivated with 5% H_2O). Elution with 5% ether-petroleum ether 60-80 °C afforded **the product (97)** as a colourless oil of α- and β-epoxides; yield: 1.14 g (99%). The isomer ratio of epoxides was determined, by integration of the epoxide-methine signals in the ^1H NMR spectrum to be 55:45. $\upsilon_{max.}$ 1600, 1580 (m, arom. C=C str), 1500 (s, arom. C=C str), no O-H abs., no 1650 cm^{-1} band [C=C str in the benzylidene steroid (**61**)]; δ_H 0.72 (s, CH_3), 0.77 (s, CH_3), 0.79 (s, CH_3), 1.06 (s, CH_3), 3.86 (s, epoxide-methine-H), 3.95 (s, epoxide-methine-H); *m/z* 364 (M^+) (100%), 249 ($M-CH_3$)$^+$(5.9), 248 ($M-CH_3-H$)$^+$ (2.6), 336 ($M-CO$)$^+$ (1.9), 335 ($M-CHO$)$^+$(4.6), 105 ($C_8H_9^+$) (14.9), 105 ($C_7H_5O^+$) (9.0) [M^+, 364.2766. $C_{26}H_{36}O$ requires M, 364.2766].

(xxiii) Reaction of 3'-Phenyl-5α-androstan-16-spiro-2'-oxirane with alkaline DMSO:

The steroid epoxide (**97**) [176 mg, 0.48 mmol] was dissolved in a solution of 0.3N NaOH in 85% DMSO (20 mL) and heated at reflux for 6 hours. After cooling to room temperature, the reaction mixture was diluted with water and extracted with ether. The ethereal extract was washed thoroughly with water and followed by the usual work-up procedure to give the unchanged starting material as a

yellow gum which was chromatographed on neutral alumina (deactivated with 5% H_2O). Elution with 5% ether-petroleum ether 60-80 °C afforded **the starting (97)** material as a colourless oil (145.50 mg); $\upsilon_{max.}$ 1600, 1580 (m, arom. C=C str), 1500 (s, arom. C=C str), no O-H and C=O str, no 1650 cm^{-1} band [C=C str in the benzylidene steroid (**61**)]; δ_H 0.74 (s, CH_3), 0.78 (s, CH_3), 0.81 (s, CH_3), 1.05 (s, CH_3), 3.88 (s, epoxide-methine H), 3.97 (s, epoxide-methine H); *m/z* 364 (M^+) (100%), 349 $(M-CH_3)^+$ (8.4), 348 $(M-CH_3-H)^+$ (3.5), 336 $(M-CO)^+$ (1.5), 335 $(M-CHO)^+$ (3.0), 105 ($C_8H_9^+$) (16.1), 105 ($C_7H_5O^+$) (9.6), 77 ($C_6H_5^+$) (14.2) [M^+, 364.2766. $C_{26}H_{36}O$ requires M, 364.2766].

(xxiv) Reaction of 3'-Phenyl-5α-androstan-16-spiro-2'-oxirane with aqueous periodic acid in acetone:

To a stirred solution of the steroidal epoxide (**97**) [200 mg, 0.55 mmol] in acetone (15 mL) was added a solution of periodic acid (138 mg, 0.61 mmol) in water (5 mL). The reaction mixture was refluxed for 3 hours, cooled to room temperature, diluted with water and extracted with ether. The ethereal extract was washed thoroughly with water and followed by the usual work-up procedure to give a crude yellow gum which was chromatographed on neutral alumina (deactivated with 5% H_2O). Elution with 5% ether petroleum ether 60-80 °C afforded **5α-androstan-16-one (56)** as a crystalline solid; yield 45.5 mg, (28.82%), mp 107-108 °C, lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H 0.82 (s, 3H, 19-H), 0.87 (s, 3H,18-H); *m/z* 274 (M^+) (100%), 259 $(M-CH_3)^+$ (49.8), 256 $(M-H_2O)^+$ (3.8), 241 $(M-CH_3-H_2O)^+$ (7.7), 231 $(M-CH_3-CO)^+$ (3.8), 217 $(M-C_3H_5O)^+$ (19.2), 216 $(M-C_3H_6O)^+$ (4.6).

(xxv) 5α-Androstan-16-one (56):

To a solution of the steroidal epoxide (**97**) [201 mg, 0.55 mmol] in sodium-dried ether (10 mL) was added a solution of periodic acid (160 mg, 0.71 mmol) in sodium-dried ether (10 mL). The reaction mixture was stirred for 7 hours at room temperature, diluted with water and extracted with ether. The organic extract was washed thoroughly with water and followed by the usual work-up procedure to give the product as a crude yellow gum which was chromatographed on neutral alumina (deactivated with 5% H_2O). Elution with 5% ether-petroleum ether 60-80 °C afforded **the product (56)** as a crystalline solid; yield: 62.8 mg (40%), mp 107-108 °C (needles from MeOH), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H 0.82 (s, 3H, 19-H), 0.88 (s, 3H, 18-H); *m/z* 274 (M^+) (100%), 259 ($M-CH_3)^+$ (33), 256 ($M-H_2O)^+$ (1.6), 241 ($M-CH_3-H_2O)^+$ (5.1), 231 ($M-CH_3-CO)^+$ (2.1), 217 ($M-C_3H_5O)^+$ (11.6), 216 ($M-C_3H_6O)^+$ (25.7) [M^+, 274.2291. $C_{19}H_{30}O$ requires M, 274.2297].

(xxvi) 5α-Androstan-16-one (56):

A fine crystalline suspension of the benzylidene steroid (**61**) [5.6 g, 16.1 mmol] in a mixture of MeOH (350 mL) and EtOAc (150 mL) was ozonised at -78 °C, using the Penwalt Wallace and Tiernan Ozoniser, Type number: BA 423012, until all the crystals dissolved and the solution acquired a blue colour which persisted. Nitrogen was then passed through the solution for 10 minutes. After the addition of glacial AcOH (280 mL), the mixture was warmed to 30 °C and maintained between 30-35 °C by external cooling while Zn dust (160 g) was added in small portions over 60 minutes. The excess Zn was filtered off and the filter-cake was washed with fresh EtOAc. The combined filtrate and washings were concentrated under reduced pressure, diluted with CH_2Cl_2 and washed with water. The usual work-up procedure gave the crude product which was chromatographed on neutral alumina (deactivated

with 5% H_2O). Elution with 5% ether-petroleum ether 60-80 °C afforded **the product (56)** as a white solid; yield: 4.07 g (92%), mp 107-108 °C (needles from MeOH), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str); δ_H (400 MHz) 0.81 (s, 3H, 19-H), 0.86 (s, 3H, 18-H), 1.89 (br dd, 1H, J 18.14, 13.24 Hz, 15β-H), 1.94 (br d, 1H, J 16.92 Hz, 17α-H), 2.09 (br d, 1H, J 16.92 Hz, 17β-H), 2.18 (br dd, 1H, J 18.14, 6.88 Hz, 15α-H); δ_C (100.61 MHz) 12.21 (C-19), 18.10 (C-18), 34.94 (C-8), 36.43 (C-10), 38.28,38.38 (C-1, C-12), 39.16 (C-13), 39.32 (C-15), 51.79 (C-14), 55.93 (C-17), 219.10 (CO); *m/z* 274 (M^+) (100%), 259 $(M-CH_3)^+$ (33.8), 256 $(M-H_2O)^+$ (2.4), 241 $(M-CH_3-H_2O)^+$ (5.5), 216 $(M-C_3H_6O)^+$ (33) [M^+, 274.2281. $C_{19}H_{30}O$ requires M, 274.2297].

(xxvii) 16,16-Ethylenedioxy-5α-androstane (55):

To a solution of the steroid ketone **(56)** [5.5 g, 20.04 mmol] in sodium-dried benzene (100 mL) were added freshly distilled ethylene glycol (11 mL) and triethyl orthoformate (22 mL), and *p*-toluenesulphonic acid (0.6 g). The mixture was refluxed for 20 hours, cooled to room temperature, washed with saturated $NaHCO_3$ and extracted with benzene. The usual work-up procedure gave **the product (55)** as a white solid; yield: 5.4 g (84.64%), mp 110-111 °C [platelets from methanol/ pyridine (trace)], lit.,[96] mp 111.5 - 112.5 °C; $\upsilon_{max.}$ 1090 (s, C-O str), no C=O str at 1745 cm^{-1}; δ_H 0.79 (s, CH_3, 19-H), 0.89 (s, CH_3, 18-H), 1.79 (d, 1H, J 13.24 Hz, 17-H), 1.89 (dd, 1H, J 13.10, 6.73 Hz, 15-H), 3.84 (m, 4H, -O-CH_2-CH_2-O-); *m/z* 318 (M^+) (100%), 247 $(M-C_5H_{11})^+$ (25.5), 139 $(M-C_{13}H_{33})^+$ (74.4%, 114 $(M-C_{15}H_{24})^+$ (74.8), 99 $(M-C_{16}H_{27})^+$ (84.1) [M^+, 318.2555. $C_{21}H_{34}O_2$ requires M, 318.2559].

(xxviii) 6α,12β-Dihydroxy-5α-androstan-16-one (54):

Incubation with *Cd*: 16,16-ethylenedioxy-5α-androstane (**55**) [0.88 g, 2.76 mmol] in DMSO (132 mL), 11 flasks, 6d, medium B (160 mL/flask), extraction I gave 0.514 g combined extracts, which were refluxed in acetone (100 mL)-water (8 mL) with $TsOH.H_2O$ (60 mg) for 4 hours. Residual DMSO was removed by short path distillation, under reduced pressure, on a steam-bath. Chromatography of the residue on neutral alumina (deactivated with 5% H_2O) and elution with Et_2O-MeOH (10:1) gave **the product (54)** as a semi-crystalline gum; yield: 161 mg (19.10%). T.l.c. [silica gel/Et_2O-MeOH (10:1)] indicated that product had the same R_f as an authentic sample of 6α,12β-dihyroxy-5α-androstan-16-one obtained from Professor D.N. Kirk, Honorary Curator of the Steroid Reference Collection, Chemistry Department, Queen Mary College, University of London. The analytical sample was prepared by sublimation at 180 °C and under reduced pressure; mp 218-219 °C (rods from MeOH), lit.,[93] mp 214-216 °C; $\upsilon_{max.}$ 3420 (br s, O-H str), 1725 cm^{-1} (s, C=O str); δ_H 0.84 (s, CH_3, 19-H), 0.88 (s, CH_3, 18-H), 3.43 (ddd, 1H, J 10.64, 10.64, 4.46 Hz, 6β-H), 3.63 (dd, 1H, J 10.99, 4.54 Hz, 12α-H); *m/z* 306 (M^+) (24.9%), 291 $(M-CH_3)^+$ (3.1), 288 $(M-H_2O)^+$ (24.4), 270 $(M-2H_2O)^+$ (22.0), 96 $(M-C_{12}H_{18}O_2)^+$ (100) [M^+, 306.2196. $C_{19}H_{30}O_3$ requires M, 306.2195].

(xxix) Attempted synthesis of 6α,12β-dihydroxy-5α-androstan-16-one (54):

Incubation with *Cd*: 16,16-ethylenedioxy-5α-androstane (**55**) [0.084 g, 0.26 mmol] in EtOH (12 mL), 2 flasks, 6d, medium B (160 mL/flask), extraction I gave 123 mg combined extracts, which were refluxed in acetone (30 mL) containing H_2O (2 mL) and $TsOH.H_2O$ (15 mg) for 4 hours. T.l.c. [silica gel/Et_2O:MeOH (10:1)] indicated that the product spot was less polar than the authentic 6α,12β-dihydroxy-5α-androstan-16-one and identical in R_f to that of 5α-androstan-16-one (**56**). Chromatography of the residue on neutral alumina (deactivated with 5% H_2O) and elution with 5% ether-petroleum 40-60 °C gave **5α-androstan-16-one (56)**; yield: 23 mg, mp 106-107 °C (needles from MeOH), lit.,[114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str).

(xxx) 6α,12β-dihydroxy-5α-androstan-16-one (54):

Incubation with *Cd*: 16,16-ethylenedioxy-5α-androstane (**55**) [1.29 g, 4.06 mmol] in DMSO (195 mL), 10 flasks, 6d, medium B (260 mL/flask), extraction II gave 1.2 g combined extracts, which were refluxed in acetone (250 mL) containing H_2O (20 mL) and $TsOH.H_2O$ (150 mg) for 4 hours. T.l.c. [silica gel/Et_2O:MeOH (10:1)] indicated the product was indentical in R_f to that of an authentic sample of 6α,12β-dihydroxy-5α-androstan-16-one (**54**). Chromatography of the residue on neutral alumina (deactivated with 5% H_2O) and elution with Et_2O:MeOH (10:1) gave **the product (54)** as a semi-crystalline gum; yield: 476 mg (38%), mp 212-213 °C (rods from MeOH), lit.,[93] mp 214-216 °C; $\upsilon_{max.}$ 3420 (br s, O-H str), 1725 cm^{-1} (s, C=O str); *m/z* 306 (M^+) (60.1%) [M^+, 306.2196. $C_{19}H_{30}O_3$ requires M, 306.2195].

(xxxi) Attempted synthesis of 16-hexylidene-5α-androstane (102):

To a stirred solution of *n*-hexyltriphenylphosphonium bromide (0.31 g, 0.73 mmol) in dry THF (10 mL, freshly distilled from $LiAlH_4$) under nitrogen was added 1.6M BuLi in hexanes (1.2 mL, 1.92 mmol) at room temperature in a slow dropwise manner. Stirring was continued for 1 hour during which an orange solution was formed. To this ylid solution was added a solution of 5α-androstan-16-one (**56**) [0.1 g, 0.36 mmol] in dry THF (5 mL), in a slow dropwise manner. Stirring was continued for 46 hours at room temperature, then the reaction mixture was refluxed for 22 hours. After the addition of brine, the reaction mixture was extracted thoroughly with ether, followed by the usual work-up procedure to give a crude gum, t.l.c. [silica gel/$CHCl_3$:MeOH (9:1)] of which indicated the presence of one component identical in R_f to that of the starting material. The crude gum was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the starting material (56)** as a crystalline solid; mp 106-107 °C (needles from MeOH), lit., [114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str).

(xxxii) 5α-androstan-16β-ol (113):

To a stirred solution of 5α-androstan-16-one (**56**) [0.43 g, 1.57 mmol] in propan-2-ol (50 mL) was added in small portions at a time, $NaBH_4$ (0.5 g, 13.22 mmol). Stirring was continued at room temperature for 36 hours. The excess $NaBH_4$ was decomposed with 2N HCl and the precipitated steroid was filtered off, washed thoroughly with 2N NaOH and water, then dissolved in CH_2Cl_2. The usual work-up procedure afforded **the product (113)** as a white solid; yield: 422 mg (97.46%), mp 107-109 °C (needles from MeOH), lit., [114] mp 110 °C; $\upsilon_{max.}$ 3470 (br s, O-H str), 1380 cm^{-1} (m, CH_3 def); δ_H 0.80 (s, 3H, 19-H), 0.94 (s, 3H, 18-H), 4.40 (m, 1H, 16-H); *m/z* 276 (M^+) (44.9%), 261 $(M-CH_3)^+$ (11.2%), 258 $(M-H_2O)^+$ (35.3%), 243 $(M-CH_3-H_2O)^+$ (51.8%), 218 $(M-H_2O-C_3H_4)^+$ (31.5), 217 (M-H_2O-

C_3H_4-H)$^+$ (48.9%) [M$^+$, 276.2454. $C_{19}H_{30}O$ requires M, 276.2453].

(xxxiii) Attempted synthesis of 16-androstanyltriphenylphosphonium bromide (108):

To a stirred solution of the sterol (**113**) [265 mg, 0.96 mmol] in dry $CHCl_3$ (10 mL) was added triphenylphosphine hydrobromide (567 mg, 1.65 mmol) and the mixture boiled under reflux for 12.5 hours. After t.l.c. indicated no change in the starting material the reaction mixture was diluted with $CHCl_3$ and followed by the usual work-up procedure to give a crude gum which was chromatographed on silica gel. Elution with toluene: $CHCl_3$:EtOH (5:5:3) gave the unchanged **starting material (113)**; $\upsilon_{max.}$ 3470 (br s, O-H str), 1380 cm^{-1} (m, CH_3 def).

(xxxiv) Attempted synthesis of 16-bromo-5α-androstane (114):

To a stirred solution of the sterol (**113**) [0.089 g, 0.32 mmol] in dry DMF (12 mL) was added triphenylphosphine dibromide (360 mg, 0.55 mmol) in portions over 5 minutes. The resulting solution was refluxed under nitrogen for 16 hours. The solvent (DMF) was removed under reduced pressure. T.l.c. [silica gel/$CHCl_3$:toluene (1:1)] of the residue indicated complete disappearance of the starting material and one spot less polar than (**113**). The residue was chromatographed on silica gel. Elution with $CHCl_3$:toluene (1:1) afforded a clear oil which was identical in R_f to that of 5α-androst-16-ene (**93**) [silica gel/hexane]; yield: 18.4 mg (22.12%). GC-MS analysis showed the oil to be a mixture of 2 isomers, each of an *a.m.u.* of 258 (isomer ratio 56:44); $\upsilon_{max.}$ 3030 (m, =C-H str), 1590 cm^{-1} (w, C=C str); δ_H 5.67-5.82 (br m, olefinic H's) [M$^+$, 258.2352. $C_{19}H_{30}$ requires M, 258.2347].

(xxxv) Cholest-4-en-3yltriphenylphosphonium bromide (116):

To a stirred solution of cholesterol (**115**) [0.4 g, 1.04 mmol] in dry $CHCl_3$ (3 mL) was added triphenylphosphine hydrobromide (0.35 g, 1.03 mmol) and the resulting solution refluxed for 16 hours. T.l.c. [silica gel/$CHCl_3$-EtOH (9:4)] indicated complete disappearance of the starting material and the formation of a polar product. Dilution of the reaction mixture with $CHCl_3$ was followed by the usual work-up procedure to give **the product (116)** as an oil; yield 605 mg (82%). Crystallisation from acetone-petroleum ether 60-80 °C (4:1) gave needles; mp 230-232 °C (crystals darken at 210-212 °C) [Found: C, 74.31; H, 8.05. $C_{45}H_{60}BrP.H_2O$ requires C, 74.05; H, 8.29]; $\upsilon_{max.}$ 1435 (s), 1110 (s), 1000 (m) [quaternary $P^+(Ph)_3$], 750, 690 cm^{-1} (s, arom. H def); δ_H (400 MHz) 0.60 (s, 3H, 18-H), 0.88 (m, 9H, 21-, 26-, 27-H's), 0.97 (s, 3H, 19-H), 5.26 (br s, 1H, 4-H), 6.09 (br m, 3-H); 7.65-7.76 (m, 9H, arom. $H_{m,p}$), 7.99-8.07 (m, 6H, arom. H_o).

(xxxvi) 3-Butylidene-cholest-4-ene (117):

To a stirred solution of the steroidal phosphonium bromide (**116**) [100 mg, 0.14 mmol] in dry THF (4 mL, freshly distilled from $LiAlH_4$) at 25 °C, under N_2, was added, dropwise, 2N BuLi in hexane (0.2 mL, 0.4 mmol) to yield the deep red solution of the ylid. To this ylid solution was added, dropwise, an excess of freshly distilled *n*-butanal (1 mL, 13.9 mmol) in THF (4 mL). Decolourisation ensued almost immediately and after stirring at room temperature for 3 hours, the reaction mixture was diluted with water and extracted with hexane. The organic extract was washed thoroughly with water and brine, followed by the usual work-up procedure to give a crude oil which was purified by p.l.c. [silica gel/hexane] to give **the product (117)** as a clear oil; yield; 28 mg (47%). GC-MS analysis showed the oil to be an isomeric mixture (isomer ratio 65:35) of compounds, each of an *a.m.u.* of 424.70; isomer t_r 16.01 min. 35%, *m/z* 424 (M^+) (38.7%); isomer t_r 16.38 min. 65%, *m/z* 424

(M^+) (42.2%).

(xxxvii) Attempted synthesis of 16(4'-carboxybutylidene)-5α-androstane (124):

To a stirred 1M solution of sodiomethylsulphinyl carbanion (10 mL, 10 mmol, **Method I)** in anhydrous DMSO at 25 °C under nitrogen, was added a solution of (4-carboxybutyl)triphenylphosphonium bromide (2.32 g, 5.23 mmol) in anhydrous DMSO (10 mL) in a dropwise manner to yield the orange solution of the ylid. To this ylid solution was added dropwise, a solution of 5α-androstan-16-one (**56**) [152 mg, 0.55 mmol] in anhydrous DMSO and the reaction mixture was stirred at 35 °C for 24 hours under nitrogen, cooled, basified with 1N NaOH and extracted thoroughly with ether to give the E1 extract. Acidification of the aqueous phase with 2N HCl was followed by extraction with ether to give the E2 extract. Both extracts were thoroughly washed with brine and water and followed by the usual work-up procedure of E1 and E2 to give a yellow oil and a yellow semi-crystalline solid respectively as residues. T.l.c. [silica gel/EtOAc- Me_2CO (5:1)] of residues E1 and E2 revealed the presence of unchanged starting material in the E1 residue only; no chromatographic evidence of any steroid component in the E2 residue was detected. Chromatography [silica gel/$CHCl_3$] of the E1 residue afforded **the starting material (56)** as a crystalline solid; mp 107-108 °C (needles form MeOH), lit., [114] mp 108 °C; $\upsilon_{max.}$ 1745 cm^{-1} (s, C=O str).

(xxxviii) 16-Hexylidene-5α-androstane (102):

To a stirred 1M solution of sodiomethylsulphinyl carbanion (5 mL, 5 mmol, **Method I**) in dry DMSO at 25 °C under nitrogen was added a solution of *n*-hexyltriphenylphosphonium bromide (2.25 g, 5.26 mmol) in anhydrous DMSO (5 mL) in a slow dropwise manner to yield the orange solution of the ylid. To this ylid solution was added dropwise, a solution of 5α-androstan-16-one **(56)** [154 mg, 0.56 mmol] in anhydrous DMSO (5 mL) and the reaction mixture was stirred at 35 °C for 24 hours, cooled, diluted with water and extracted thoroughly with petroleum ether 60-80 °C. The organic extract was washed thoroughly with brine and water, followed by the usual work-up procedure to give a crude yellow oil, which was chromatographed on neutral alumina (deactivated with 5% H_2O). Elution with hexane-ether (9:1) afforded a pale yellow oil which was further purified by p.l.c (silica gel/hexane) to give **the product (102)** as a clear oil; yield: 10 mg, (5.2%). GC-MS analysis showed the oil to be an isomeric mixture (isomer ratio 65:35.) of compounds, each of an *a.m.u.* of 342; $\upsilon_{max.}$ 3000 (m, =C-H str), 1650 (w, C=C str), 1675 (w, C=C str), 1375 (m, CH_3 def), no C=O str at 1745 cm^{-1}; δ_H 5.22 (br m, olefinic H's); isomer, t_r 10.48 min., 65%; *m/z* 342 (M^+) (16%), 327 $(M-CH_3)^+$ (27), 286 $(M-C_4H_8)^+$ (1.2), 285 $(M-C_4H_9)^+$ (3.1), 271 $(M-C_5H_{11})^+$ (5.2), 217 $(M-C_9H_{17})^+$ (100); isomer, t_r 10.56 min., 35%; *m/z* 342 (M^+) (14.6%), 327 $(M-CH_3)^+$ (25.7), 285 $(M-C_4H_9)^+$ (1.8), 271 $(M-C_5H_{11})^+$ (6.9), 217 $(M-C_9H_{17})^+$ (100).

(xxxix) 1-Heptenyl benzene (126):

To a stirred 1M solution of sodiomethylsulphinyl carbanion (10 mL, 10 mmol, **Method II**) in anhydrous DMSO at 25 °C under nitrogen, was added dropwise, a solution of *n*-hexyltriphenylphosphonium bromide (4.4 g, 10 mmol) in anhydrous DMSO (10 mL) to yield the intense orange-red solution of the ylid. To this ylid solution was added an excess of freshly distilled benzaldehyde (5 mL) in

anhydrous DMSO (5 mL). Decolourisation ensued almost immediately. The reaction was stirred at 35 °C under nitrogen for 1 hour, cooled, diluted with ice-water and extracted with petroleum ether 60-80 °C. The organic extract was washed thoroughly with brine and water, and followed by the usual work-up procedure to give the crude product as a yellow oil, which was chromatographed on silica gel. Elution with hexane:toluene (2:1) afforded **the product (126)** as a clear oil; yield: 470 mg (27%). GC-MS analysis showed the oil to be an isomeric mixture (isomer ratio 60: 40) of compounds, each of an *a.m.u.* of 174; $\upsilon_{max.}$ 3080, 3060, 3020, 3010 (m, =C-H str), 1650 (w, C=C str), 1650 (w, C=C str), 1600, 1575, 1490 (m, arom C=C str), no aldehydic C=O str at 1700 cm^{-1}; δ_H 1.05, 1.07 (2 CH_3, overlapping triplets), 2.35 (dq, 2H, J 7.34, 0.95 Hz, C_6H_5CH=CH-CH_2-), 2.49 (dq, 2H, J 7.33, 1.78 Hz, C_6H_5CH=CH-CH_2-), 5.82 (dt, 1H, J 11.65, 7.25 Hz, C_6H_5CH=CH-CH_2-), 6.37 (dt, 1H, J 15.78, 6.65 Hz, C_6H_5CH=CH-CH_2-), 6.53 (d, 1H, J 15.83 Hz, C_6H_5-CH=CH-), 6.57 (d, 1H, J 11.68 Hz, C_6H_5-CH=CH-), 7.27-7.51 (m, 10H, arom. H's); isomer, t_r 2.78 min., 60%; 174 (M^+) (28.5%), 159 $(M-CH_3)^+$ (0.2), 145 $(M-C_2H_5)^+$ (1.1), 131 $(M-C_3H_7)^+$ (4.5), 118 ($C_9H_{10}^+$) (12.4), 117 $(M-C_4H_9)^+$ (100), 104 ($C_8H_8^+$) (88.2), 91 ($C_7H_7^+$) (34.1), 77 ($C_6H_5^+$) (6.2), 65 $(C_7H_7-C_2H_2)^+$ (5.7), 57 ($C_4H_9^+$) (0.6), 51 $(C_6H_5-C_2H_2)^+$ (5.4); isomer, t_r 3.23 min., 40%; *m/z* 174 (M^+) (28.2%), 159 $(M-CH_3)^+$ (0.1), 145 $(M-C_2H_5)^+$ (1.1), 131 $(M-C_3H_7)^+$ (4.5), 118 ($C_9H_{10}^+$) (11.9), 117 $(M-C_4H_9)^+$ (100), 104 ($C_8H_8^+$) (84.4), 91 ($C_7H_7^+$) (41.1), 77 ($C_6H_5^+$) (7.8), 65 $(C_7H_7-C_2H_2)^+$ (6.8), 57 ($C_4H_9^+$) (0.7), 51 $(C_6H_5-C_2H_5)^+$ (6.1).

(xl) 16-Hexylidene-5α-androstane (102):

To a stirred 1.25M solution of sodiomethylsulphinyl carbanion (20 mL, 25 mmol, **Method II**) in anhydrous DMSO at 25 °C under nitrogen, was added dropwise, a solution of *n*-hexyltriphenylphosphonium bromide (9.0 g, 21 mmol) in anhydrous DMSO (20 mL) to the yield the intense orange-red solution of the ylid. To this ylid solution, was added dropwise, a solution of 5α-androstan-16-one (**56**) [540 mg, 1.97 mmol] in anhydrous DMSO (15 mL). The reaction mixture was stirred at 55 °C for 20 hours, cooled, diluted with ice-water and extracted thoroughly with petroleum ether 60-80 °C. The organic extract was thoroughly washed with brine and water and followed by the usual work-up procedure to give a crude oil which was chromatographed on silica gel. Elution with hexane afforded **the product (102)** as an oil; yield: 490 mg (71.23%). GC-MS analysis showed the oil to be an isomeric mixture of compounds, each of an *a.m.u.* of 342; $\upsilon_{max.}$ 3000 (m, =C-H str), 1650, 1675 (w, C=C str), 1375 (m, CH_3 def); no C=O str at 1745 cm^{-1}; δ_H 0.72 (s, CH_3), 0.73 (s, CH_3), 0.80 (s, CH_3's), 0.90 (CH_3's, overlapping triplets), 5.25-5.31 (br m, olefinic H), 5.0 (t, J 4.58 Hz, olefinic H); *m/z* 342 (M^+) (77.4%), 327 $(M-CH_3)^+$ (58.8), 299 $(M-C_3H_7)^+$ (0.7), 286 $(M-C_4H_8)^+$ (2.2), 285 $(M-C_4H_9)^+$ (6.6), 271 $(M-C_5H_{11})^+$ (9.0), 217 $(M-C_9H_{17})^+$ (100).

(xli) 16(4'-Methoxycarbonylbutylidene)-5α-androstane (127):

To a stirred 1.2M solution of sodiomethylsulphinyl carbanion (10 mL, 12 mmol, **Method II**) in anhydrous DMSO at 25 °C under nitrogen was added, dropwise, a solution of (4-carboxybutyl)triphenylphosphonium bromide (2.3 g, 5 mmol) in anhydrous DMSO (10 mL) to yield the intense orange-red solution of the ylid. To this ylid solution was added dropwise, a solution of 5α-androstan-16-one (**56**) [274 mg, 1 mmol] in anhydrous DMSO (10 mL). The reaction mixture was stirred at 55 °C for 16 hours, cooled, diluted with water, basified with 1N NaOH and

extracted thoroughly with ether. Acidification of the aqueous phase with 2N HCl was followed by extraction with ether. The acidic extract was thoroughly washed with brine and water , and followed by the usual work-up procedure to give the crude product as an oil which was dissolved up in ether and reacted with an ethereal solution of diazomethane (equiv. 40 mmol CH_2N_2). The ether was allowed to evaporate slowly at room temperature and the residue was chromatographed on silica gel. Elution with ether-petroleum ether 40-60 °C (1:2) afforded **the product (127)** as an oil; yield 175 mg (47.04%). GC-MS analysis showed the oil to be an isomeric mixture (isomer ratio 68:32) of compounds, each of an *a.m.u.* of 372; $\upsilon_{max.}$ 1740 (s, C=O str), 1160 cm^{-1} (s, C-O str); δ_H 0.70 (s, CH_3), 0.71 (s, CH_3), 0.78 (s, CH_3's), 3.65 (s, OCH_3's), 5.15-5.25 (br m, olefinic H's); *m/z* 372 (M^+) (22.1%), 357 $(M-CH_3)^+$ (65.4), 341 $(M-OCH_3)^+$ (1.1), 257 $(M-C_6H_{11}O_2)^+$ (100), 74 ($C_3H_6O_2^+$) (2.6) [M^+, 372.3024. $C_{25}H_{40}O_2$ requires M, 372.3028].

(xlii) 16-Hexylidene-5α-androstan-6α,12β-diol (128):

To a stirred 2.5M solution of sodiomethylsulphinyl carbanion (10 mL, 25 mmol, **Method II**) in anhydrous DMSO at 25 °C under nitrogen, was added dropwise, a solution of *n*-hexyltriphenylphosphonium bromide (9.0 g, 20 mmol) in anhydrous DMSO (15 mL) to yield the intense orange-red solution of the ylid. To this ylid solution was added dropwise a solution of 6α,12β-dihydroxy-5α-androstan-16-one (**54**) [250 mg, 0.82 mmol] in anhydrous DMSO (10 mL). The reaction mixture was stirred at 55 °C for 18 hours, cooled to room temperature, diluted with water and extracted thoroughly with CH_2Cl_2. The organic extract was washed thoroughly with brine and water and followed by the usual work-up procedure to give a crude yellow oil, which was chromatographed on silica gel. Elution with $CHCl_3$ afforded the **product (128)** as an oil; yield: 23 mg (7.3%) ^1H NMR studies showed this oil to be a mixture; $\upsilon_{max.}$ 3350 (br s, O-H str); δ_H (400 MHz) 0.72 (s, CH_3), 0.73 (s, CH_3),

0.82 (s, CH_3's), 3.35-3.55 (br m, carbinolic H's), 5.25-5.40 (br m, olefinic H's); *m/z* 374 (M^+) (100%), 359 ($M-CH_3$)$^+$ (40.5), 356 ($M-H_2O$)$^+$ (31.4) 341 ($M-CH_3-H_2O$)$^+$ (10.5), 338 ($M-2H_2O$)$^+$ (0.8), 323 ($M-CH_3-2H_2O$)$^+$ (3.9), 317 ($M-C_4H_9$)$^+$ (3.6), 290 ($M-C_6H_{12}$)$^+$ (39.7) [M^+, 374.3187. $C_{25}H_{42}O_2$ requires M, 374.3185].

(xliii) 16(4'-carboxybutylidene)-5α-androstan-6α,12β-diol (129):

To a stirred 1.25M solution of sodiomethylsulphinyl carbanion (40 mL, 50 mmol, **Method II**) in anhydrous DMSO at 25 °C under nitrogen, was added dropwise, a solution of (4'-carboxybutyl)triphenylphosphonium bromide (8.87 g, 20 mmol) in anhydrous DMSO (30 mL) to yield the intense orange-red solution of the ylid. To this ylid solution was added dropwise, a solution of the diol-one (**54**) [940 mg, 3.07 mmol] in anhydrous DMSO (15 mL). The reaction mixture was stirred at 55 °C for 20 hours, cooled, basified with 1N NaOH and extracted thoroughly with ether. Acidification of the aqueous phase with 2N HCl was followed by extraction with CH_2Cl_2. The acidic extract was washed thoroughly with brine and water and followed by the usual work-up procedure to give a crude oil, which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the product (129)** as an oil; yield: 522 mg (43.57%); $\upsilon_{max.}$ 3560 (br s, combined acid and alcohol O-H str), 1700 cm^{-1} (s, C=O str); δ_H [(400 MHz), $CDCl_3$ + 3 drops CD_3OD] 0.65 (s, CH_3), 0.66 (s, CH_3), 0.80 (s, 2 CH_3), 3.29-3.40 (br m, carbinolic H's), 5.21 (br m, olefinic H's); *m/z* 390 (M^+) (17.8%), 375 ($M-CH_3$)$^+$ (22.3), 373 ($M-OH$)$^+$ (22.7), 372 ($M-H_2O$)$^+$ (96.3), 358 ($M-CH_3-OH$)$^+$ (13.3), 357 ($M-CH_3-H_2O$)$^+$ (58.8), 354 ($M-2H_2O$)$^+$ (28.6), 339 ($M-CH_3-2H_2O$)$^+$ (17.0), 249 ($M-C_8H_{13}O_2$)$^+$ (100) [M^+, 390.2775. $C_{24}H_{38}O_4$ requires M, 390.2770].

(xliv) 16(4'-Methoxycarbonylbutylidene)-5α-androstan-6α,12β-diol (130):

To a solution of the dihydroxy unsaturated steroid acid **(129)** [522 mg, 1.34 mmol] in dry MeOH (15 mL) was added an ethereal solution of diazomethane (equiv. 10 mmol CH_2N_2). The reaction mixture was left to stand for 20 hours, during which the yellow colour of the diazomethane was discharged. The methanolic solution of product was subjected to the usual work-up procedure to give crude **(130)** as an oil, which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the product (130)** as a clear oil; yield: 522 mg (96.49%); $\upsilon_{max.}$ 3350 (br s, O-H str), 1725 (s, C=O str), 1200 cm^{-1} (s, C-O str); *m/z* 404 (M^+) (4.4%), 389 $(M-CH_3)^+$ (3.4), 386 $(M-H_2O)^+$ (63.5), 373 $(M-OCH_3)^+$ (3.6), 371 $(M-CH_3-H_2O)^+$ (16.8), 368 $(M-2H_2O)^+$ (14.8), 74 ($C_3H_6O_2^+$) (3.8) [M^+, 404.2919. $C_{25}H_{40}O_4$ requires M, 404.2927].

(xlv) 16(4'-carboxybutylidene)-5α-androstan-6α,12β-diol (129):

To a stirred solution of the dihydroxy unsaturated ester **(130)** [102 mg, 0.26 mmol] in MeOH (5 mL) was added dropwise, a solution of NaOH (200 mg, 5 mmol) in a mixture of water (2 mL) and MeOH (18 mL). The reaction mixture was stirred at 60 °C under reflux for 4 hours, cooled to room temperature, acidified with 2N HCl, extracted with CH_2Cl_2, and followed by the usual work-up procedure to give crude **(129)** as an oil, which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the product (130)** as a solid; yield: 33.2 mg (33.72%), mp 74-75 °C (Found: C, 72.74; H, 10.13. $C_{24}H_{38}O_4.1/5\ H_2O$ requires C, 73.13; H, 9.72); $\upsilon_{max.}$ 3560-2500 (br s, combined acid and alcohol O-H str), 1700 cm^{-1} (s, C=O str); δ_H (400 MHz) 0.68 (s, CH_3), 0.78 (s, CH_3), 1.04 (s, 2 CH_3's), 3.46 (br m, carbinolic H), 3.81 (br m, carbinolic H), 5.28 (br m, olefinic H); *m/z* 390 (M^+) (21.5%), 375 $(M-CH_3)^+$ (15.8), 373 $(M-OH)^+$ (22.4), 372 $(M-H_2O)^+$ (83.8), 358

$(M-CH_3-OH)^+$ (13.3), 357 $(M-CH_3-H_2O)^+$ (40.9), 354 $(M-2H_2O)^+$ (76.1), 339 $(M-CH_3-2H_2O)^+$ (62.5), 249 $(M-C_8H_{13}O_2)^+$ (100) [M^+, 390.2765. $C_{24}H_{38}O_4$ requires M, 390.2770].

(xlvi) Attempted synthesis of 6α-Hydroxy-16(4'-methoxy-carbonylbutylidene)-5α-androstan-12-one (131):

To a stirred suspension of powdered 4A molecular sieves (2 g) in dry CH_2Cl_2 (10 mL) under nitrogen was added, a solution of the dihydroxy unsaturated steroid ester (**130**) [64 mg, 0.16 mmol] in dry CH_2Cl_2 (10 mL). To this mixture was added dropwise over 10 minutes, a solution of pyridinium dichromate (14.6 mg, 0.04 mmol) in a mixture of dry CH_2Cl_2 (5 mL) and dry DMF (5 mL). The reaction mixture was stirred at room temperature for 20 hours, then diluted with water and extracted with ether. The ethereal extract was filtered *via* a bed of silica gel and the filtrate washed thoroughly with water. The usual work-up procedure gave **the unchanged starting material (130)** as an oil; $\upsilon_{max.}$ 3390 (br s, O-H str), 1730 (br s, C=O str), 1200 cm^{-1} (s, C-O str).

(xlvii) Attempted synthesis of 6α-Hydroxy-16(4'-methoxy-carbonylbutylidene)-5α-androstan-12-one (131):

To a stirred suspension of powdered 4A molecular sieves (2 g) in dry DMF (5 mL) under nitrogen, was added a solution of the dihydroxy unsaturated steroid ester (**130**) [60 mg, 0.07 mmol] in dry DMF (3 mL). To this mixture was added, dropwise over 10 minutes, a solution of pyridinium dichromate (26 mg, 0.07 mmol) in dry DMF (5 mL). The reaction mixture was stirred at room temperature for 20 hours, then diluted with water and extracted with ether. The ethereal extract was filtered *via* a bed of silica gel and the filtrate was thoroughly washed with water. The usual work-up procedure gave **the unchanged starting material (130)** as an

oil; $\upsilon_{max.}$ 3390 (br s, O-H str), 1730 (br s, C=O str), 1200 cm^{-1} (s, C-O str).

(xlviii) 16(4'-Methoxycarbonylbutylidene)-5α-androstan-6,12-dione (132):

To a stirred solution of the dihydroxy unsaturated ester **(130)** [450 mg, 1.11 mmol] in dry CH_2Cl_2 (40 mL) were added powdered 4A molecular sieves (5 g) and pyridinium dichromate (5 g, 14.35 mmol). The reaction mixture was stirred at room temperature, under nitrogen, for 16 hours, diluted with ether and filtered The filtrate was subjected to the usual work-up procedure to give the crude product as an oil, which was chromatographed on silica gel. Elution with $CHCl_3$ afforded **the product (132)** as a clear oil; yield: 390 mg (87.6%); $\upsilon_{max.}$ 1705 (s, C=O str), 1735 (s, C=O str), 1200 cm^{-1} (s, C-O str); δ_H (400 MHz) 0.84 (s, 6H, 2 CH_3's, 19-H's), 1.03 (d, 3H, J 0.84 Hz, 18-H), 1.05 (d, 3H, J 0.80 Hz, 18-H): *long range 18-H - 17α-H coupling* [178], 3.67 (s, 6H, 2 OCH_3's), 5.33 (br m, 2H, olefinic H's); *m/z* 400 (M^+) (73.8%), 382 $(M-H_2O)^+$ (14.4), 369 $(M-OCH_3)^+$ (9.7), 357 $(M-C_2H_3O)^+$ (100), 74 ($C_3H_6O_2^+$) (10.8) [M^+, 400.2612. $C_{25}H_{36}O_4$ requires M, 400.2614].

(xlix) Reaction of (132) with sodium borohydride in isopropanol.

To the dioxo-unsaturated steroid **(132)** [68 mg, 0.17 mmol] in dry propan-2-ol (2 mL), under nitrogen, was added a 0.053N solution of $NaBH_4$ in dry propan-2-ol (3.5 mL, 0.05 mmol). The reaction mixture was stirred at 25 °C for 20 hours, diluted with water and extracted with CH_2Cl_2. The organic extract was washed with water and followed by the usual work-up procedure to give an oil, which was purified by p.l.c. on silica gel. Elution with EtOAc:Me_2CO (5:1) afforded **the product (133)** as an oil. ^1H NMR showed this product to contain 2 steroidal components; $\upsilon_{max.}$ 3450 (br s, O-H str), 1720 (br s, C=O str), 1200 cm^{-1} (s, C-O str); δ_H (400 MHz) 0.73 (s, CH_3), 0.75 (s, CH_3), 0.76 (s, CH_3's), 3.55 (dd, J

10.82, 4.70 Hz, carbinolic H), 3.57 (dd, J 10.88, 4.52 Hz, carbinolic H), 3.66 (s, OCH_3), 3.67 (s, OCH_3), 5.30 (br m, olefinic H's); *m/z* 402 (M^+) (16.4%), 284 ($M-H_2O)^+$ (100), 371 ($M-OCH_3)^+$ (9.8) [M^+, 402.2766. $C_{25}H_{38}O_4$ requires M, 402.2770].

4.0 References

4.1 References.

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Appendix

This appendix contains reports on the preliminary biological screening of the type III steroidal prostacyclin analogue (**129**), as an inhibitor of collagen-induced platelet (derived from human and porcine blood) aggregation.

The pharmacological study was carried out by Mohanjit Panesar under the supervision of Dr C. L. Wainwright, Department of Physiology and Pharmacology, University of Strathclyde.

Effect of prostacyclin analogue on platelet aggregation in porcine blood

Introduction

The formed elements in blood are known to be able to undergo aggregation reactions both in physiological and pathological situations. These aggregation reactions are important in host defence mechanisms and maintaining haemostasis. Numerous studies have shown that prostacyclin inhibits platelet aggregation. The purpose of this study is to assess any anti-platelet aggregatory properties of a novel prostacyclin analogue.

Materials and methods

Freshly withdrawn, diluted, pig whole blood (0.5 mL)was added to a cuvette containing 0.5 mL saline in the warming chamber (37°C) of the aggregometer. Platelet aggregation was induced in the cuvette by the addition of collagen with a final blood concentration range of 1-8 mg/mL. The electrode assembly of the aggregometer measures any change in impedance which gives an index of platelet aggregation. Details of the principle and method of operation of the aggregometer are as described by Smith, N.C.R. *et al*, 1981. For experiments determining the anti-aggregatory properties, the anti-aggregatory agents (prostacyclin and prostacyclin analogue) were left to incubate in the blood containing cuvettes for 5 minutes prior to the addition of collagen.

The control dose-response curves were obtained in the absence of inhibitor. The anti-aggregatory effects were assessed by obtaining dose-response curves in presence of varying concentrations of the test anti-aggregatory agent ranging from 1-30 ng/mL.

Results

The anti-aggregatory agents used in this study (prostacyclin and prostacyclin analogue) produced an inhibition of the collagen induced aggregation.

These initial results show that prostacyclin and the analogue at low concentrations of 1, 3 and 10 ng/mL, do not have any effect on collagen induced aggregation as shown in Figures 1-3. Although these figures do not conclusively show any inhibition by the anti-aggregatory agents, closer examination of Figure 3 suggests a tendency of a decrease in percentage aggregation in presence of both anti-aggregatory agents (10 ng/mL), particularly at higher collagen concentrations. With increased sample numbers the results should show an inhibition. Figure 4 shows that prostacyclin and the analogue 30 ng/mL, inhibit platelet aggregation. The maximum aggregation appears to be reduced by 20 percent. There was little difference between the inhibition produced by prostacyclin and it's analogue 30 ng/mL, in fact, the results indicate that the novel prostacyclin analogue may be more potent than prostacyclin at 30 ng/mL.

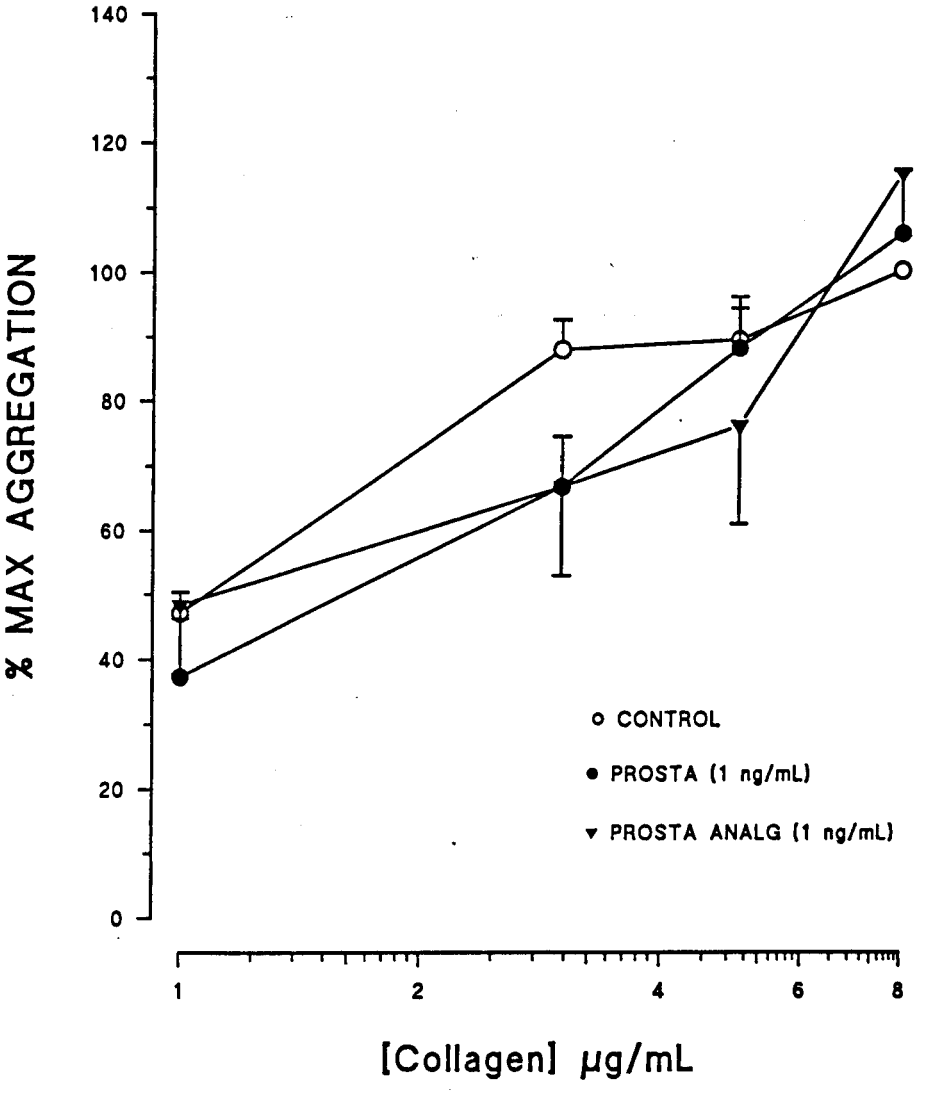

Figure 1 : Effect of prostacyclin and a prostacyclin analogue on platelet aggregation in diluted porcine whole blood induced by collagen. Each point represents the mean of a minimum of three observations; vertical bars show s.e.mean.

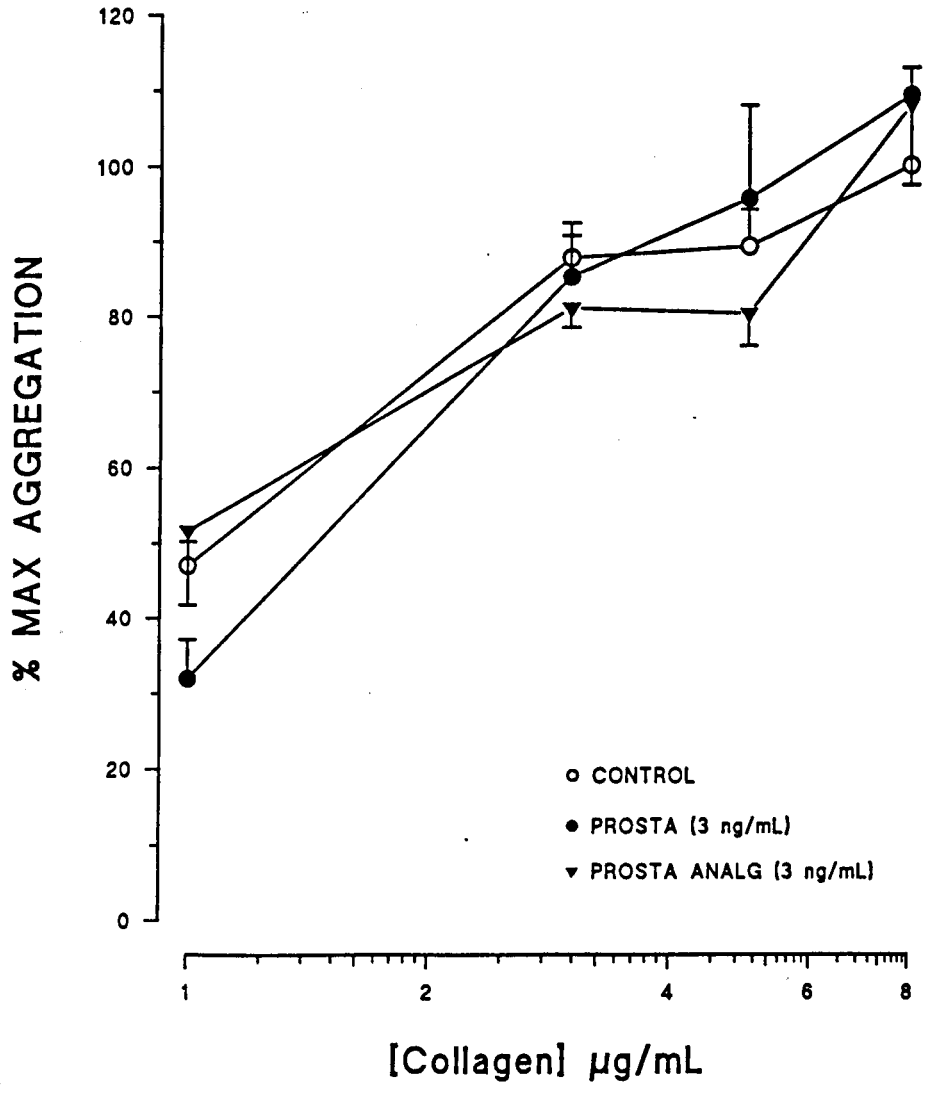

Figure 2 : Effect of prostacyclin and a prostacyclin analogue on platelet aggregation in diluted porcine whole blood induced by collagen. Each point represents the mean of a minimum of three observations; vertical bars show s.e.mean.

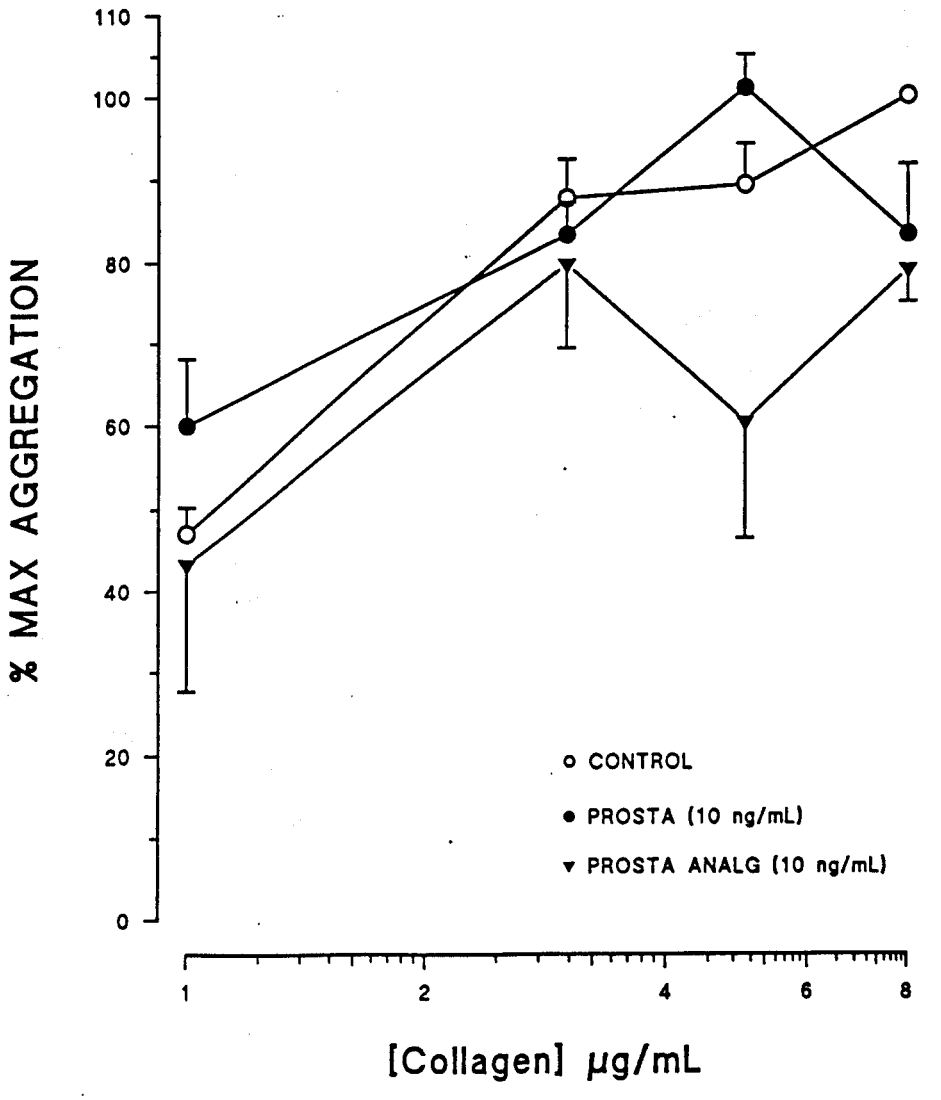

Figure 3 : Effect of prostacyclin and a prostacyclin analogue on platelet aggregation in diluted porcine whole blood induced by collagen. Each point represents the mean of a minimum of three observations; vertical bars show s.e.mean.

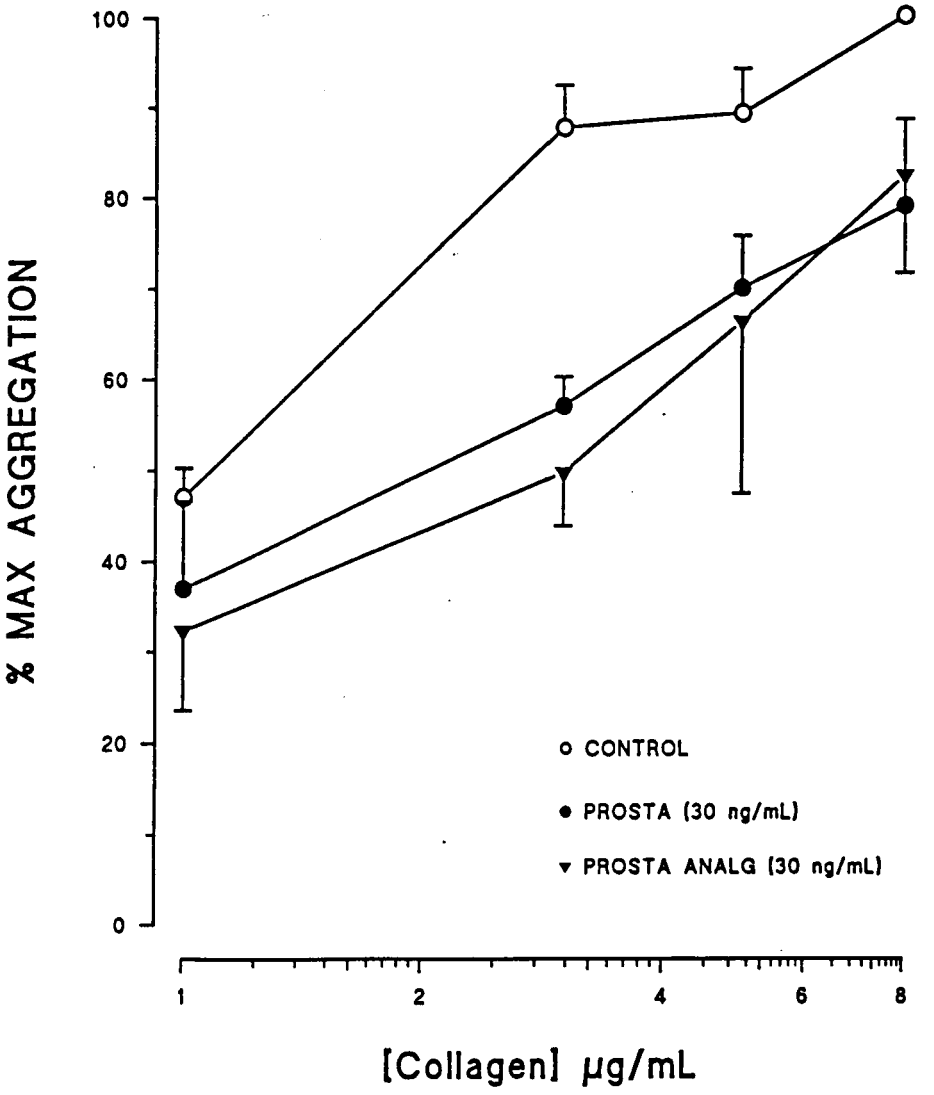

Figure 4 : Effect of prostacyclin and a prostacyclin analogue on platelet aggregation in diluted porcine whole blood induced by collagen. Each point represents the mean of a minimum of three observations; vertical bars show s.e.mean.

Conclusions

1 The effect of prostacyclin analogue on platelet aggregation in pig whole blood is inhibitory at higher concentrations (>10 ng/mL). The results suggest that the analogue is slightly more potent than prostacyclin.

2 An increase in the sample numbers should show that prostacyclin and it's analogue produce a concentration dependent inhibition.

Reference

Smith, N. C. R., *et al* (1981). Measuring platelet and leucocyte aggregation / adhesion responses in very small volumes of whole blood. *Journal of Pharmacological Methods*. **6** : 315-333.

Effect of prostacyclin analogue on platelet aggregation in Human blood

Introduction

Prostacyclin is a compound with a well established action of being a potent inhibitor of platelet aggregation (Moncada, S. *et al*, 1976). Prostacyclin inhibits platelet aggregation by stimulating adenylcyclase. There exist chemically stable analogues which have retained the capability of prostacyclin to inhibit platelet aggregation. These novel analogues are biologically tested to identify those with any prostacyclin-like abilities of inhibiting platelet aggregation and/or reducing blood pressure via vasodilation. The objective of this study is to assess anti-platelet aggregatory activity of a novel prostacyclin analogue in human blood.

Materials and methods

The general methodology for the experiment was similar to that described in progress report 1.

0.5 mL of freshly withdrawn, citrated, human whole blood was added to a cuvette containing 0.5 mL saline in the warming chamber (37°C) of the aggregometer. Platelet aggregation was stimulated in the cuvette by the addition of collagen with a final blood concentration range of 0.1-8 µg/mL. Prostacyclin and it's analogue were allocated a 5 minute incubation time prior to the addition of collagen while testing their anti-aggregatory property. Two concentrations (30 and 100 ng/ml) of the anti-aggregatory agents were used. The lower concentration (30 ng/ml) was chosen as it

appeared to be the minimum dose causing a decrease in platelet aggregation in pig whole blood. It was ensured that the volunteers who gave blood had not taken any aspirin at least one week prior to the experiment.

The control dose-response curves were obtained in the absence of inhibitor. The anti-aggregatory effects were assessed by obtaining dose-response curves in presence of the mentioned concentrations of the test anti-aggregatory agent.

Results

Prostacyclin and it's analogue produced a dose dependent inhibition of the platelet aggregation in human blood.

At 30 ng/ml, both the anti-aggregatory agents showed a tendency of inhibiting the maximum by 35% and 18% respectively (Fig. 1).The inhibitors appear to affect only the maximum of the control but not the rising phase. Fig. 1 suggests that prostacyclin is slightly more potent as an anti-aggregatory agent.

Fig. 2 shows a marked inhibition of the control maximum by both prostacyclin and it's novel analogue. Prostacyclin appears to have inhibited the maximum by 55% while the analogue produced a 45% inhibition. The prominent inhibitory activity is evident at every point in the entire control sigmoidal curve. In fact, judging by the S.E.M. bars, prostacyclin is only slightly, if at all, more potent than the analogue as an inhibitory agent.

Although the study could only be conducted on blood from three volunteers the results have clearly shown that the analogue at both 30 and 100 ng/ml concentrations

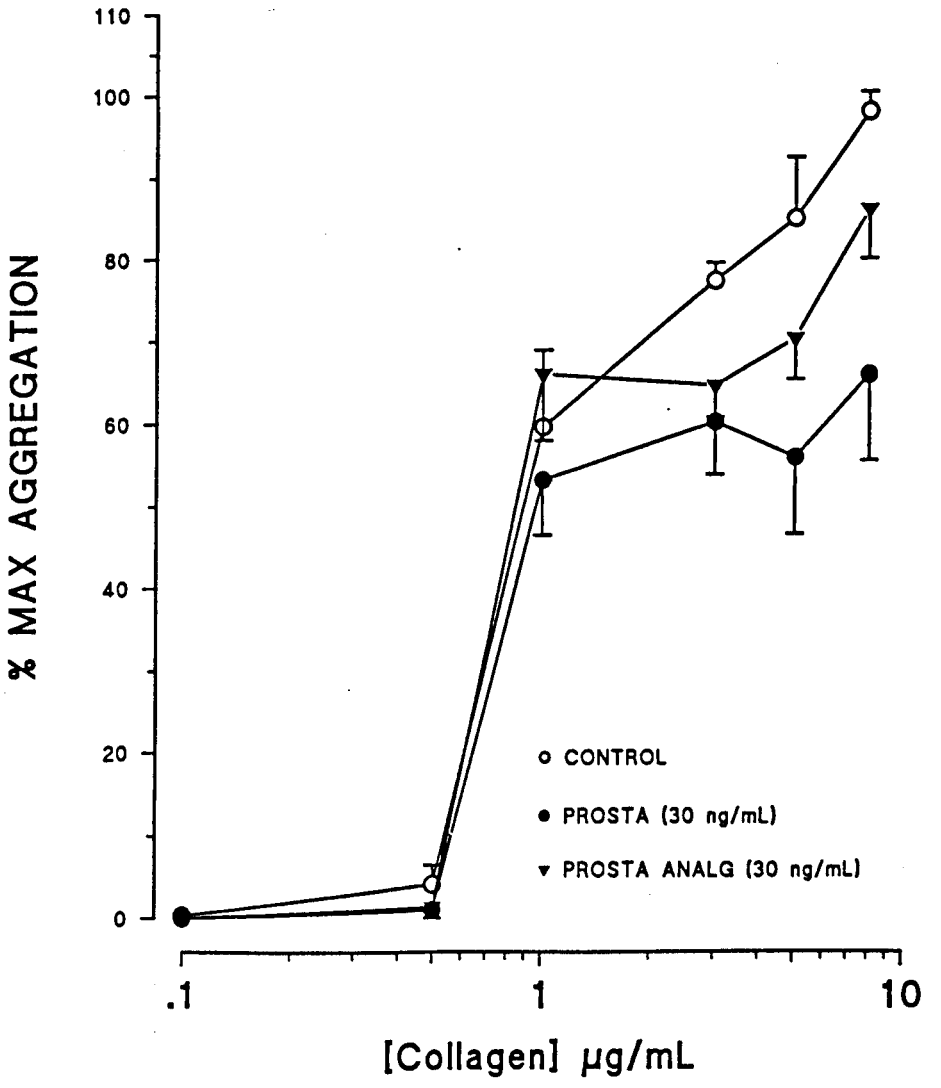

Figure 1 : Effect of prostacyclin and a prostacyclin analogue on platelet aggregation in diluted human whole blood induced by collagen. Each point represents the mean of a minimum of three observations; vertical bars show s.e.mean.

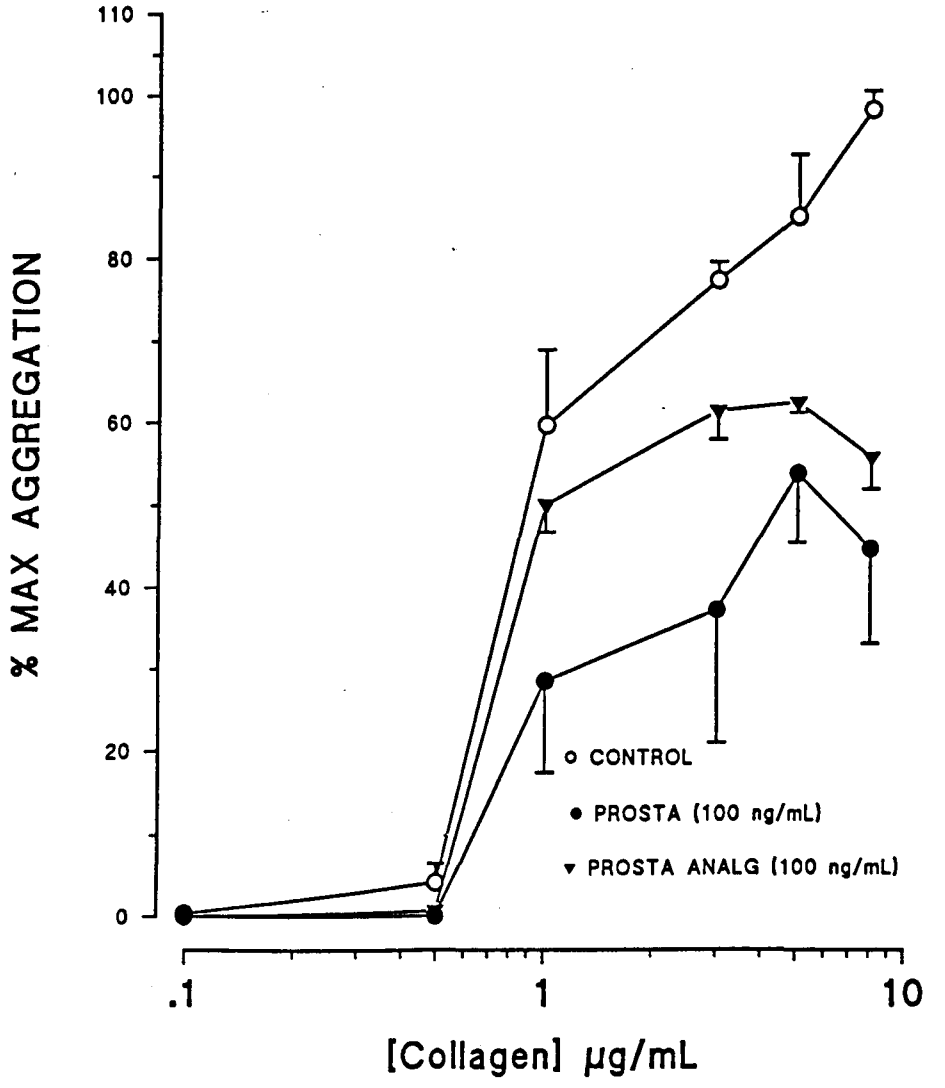

Figure 2 : Effect of prostacyclin and a prostacyclin analogue on platelet aggregation in diluted human whole blood induced by collagen. Each point represents the mean of a minimum of three observations; vertical bars show s.e.mean.

produced an inhibition which may be similar to prostacyclin at the higher concentration. An increase in the sample numbers would determine it's exact potency as compared to prostacyclin.

Conclusions

1 As shown previously with pig whole blood, the novel prostacyclin analogue has a similar inhibitory effect on platelet aggregation in human whole blood. The results suggest that the analogue may have a similar potency to prostacyclin at 100 ng/ml in human blood.

2 The inhibition produced by the analogue is concentration dependent.

3 Larger sample numbers would enlighten it's effectiveness as an inhibitor of platelet aggregation and a broader study could determine if it has another prostacyclin-like property of being a potential vasodilator.

Reference

Moncada, S., *et al* (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*. **263** : 663-665.