STEREOSELECTIVE SYNTHESIS OF PERHYDROBENZO[4.5.6]CHOLESTANES

by

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Aan mijn ouders, door wie ik ben.

Aan Astrid, voor wie ik ben.

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Summary

An intramolecular Michael-aldol reaction sequence has been developed for the stereocontrolled synthesis of pentacyclic steroids, with the new six-membered ring attached to the C(4) and C(6) positions.

Cholesterol was converted into 3ß-hydroxycholest-4-en-6-one by standard methods, and the corresponding 3α-isomer was obtained through Mitsunobu inversion. Acetoacetylation of the 3-alcohols afforded the corresponding 3ß- and 3α-acetoacetoxycholest-4-en-6-ones, which served as substrates for an investigation of intramolecular condensation routes to the target ring systems.

Base treatment of the 3ß-ester resulted in an efficient and stereocontrolled intramolecular Michael addition to give (2*R*)-2-(3ß-hydroxy-6-oxo-5ß-cholestan-4ß-yl)-3-oxobutanoic acid 1,3'-lactone, and reaction conditions were developed to achieve sequential lactone cleavage, decarboxylation, and aldol closure, leading to 3ß,6-dihydroxy-4α,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one. Although this product resisted base-mediated ß-elimination, acid treatment resulted in dehydration to give the corresponding Δ^6-compound, which underwent double bond isomerisation and 5-epimerisation, to give 3ß-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one.

A similar series of reactions was performed on the 3α-acetoacetate, leading finally to formation of 3α-hydroxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one.

Modification of foregoing reaction conditions, resulted in the design of a tandem Michael-aldol sequence, in which the 3-acetoacetates could be converted directly into the corresponding pentacyclic enones.

These products were interrelated *via* base-mediated equilibration of their respective 3,6-diketones, leading to the thermodynamically

favoured 4ß,5α-isomer. Preliminary investigations into the stereoselective reduction of the olefinic bond in the 4α,5α-isomer, resulted in the formation of a new class of hexahydrobenzo[4.5.6]cholestane derivatives.

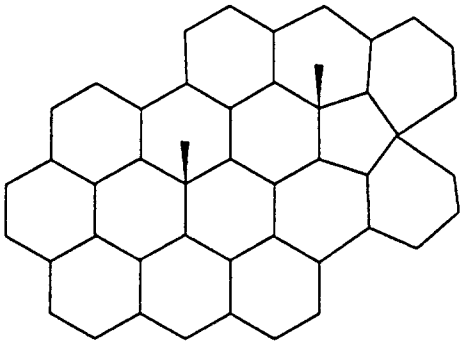
The structural and conformational properties of the condensation products were studied with the aid of ^{1}H NMR, ^{13}C NMR, and IR spectroscopy.

Introduction

The addition of carbon bridges to the tetracyclic steroid nucleus results in pentacyclic structures and represents a method for the introduction of alkyl residues and conformational deformations which are associated with changes in the biological activity of the steroid. A variety of pentacyclic steroids have been synthesised to evaluate the effect of such factors on the hormonal activity.

If one considers constructing pentacyclic structures, formed by carbon bridges which result in the creation of an additional six-membered ring, 11 possibilities exist (Figure 1). More effort, however has been done on the construction of additional three-membered and/or five-membered rings on the steroid nucleus than on the construction of an additional six-membered ring, to what this discussion is restricted to.

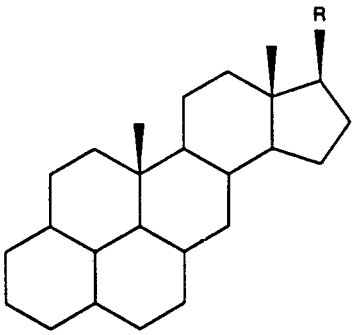
Figure 1



Possibilities for additional six-membered rings on the steroid nucleus

Pitt and co-workers[1,2,3] have synthesised pentacyclic steroids of the type (**1**) and (**2**), in order to investigate the effect of a bridge on the hormonal activity. Formation of a bridge serves to eliminate nonbonded

interactions (e.g. between C(1) and C(11)-substituents, and between C(11ß) and C(13ß)-substituents), which are responsible for reducing hormonal activity. The C(1),C(11)-bridge did not have any significant effect on the hormonal activity, unlike the C(11ß),C(13ß)-bridge, which greatly enhanced the hormonal activity.



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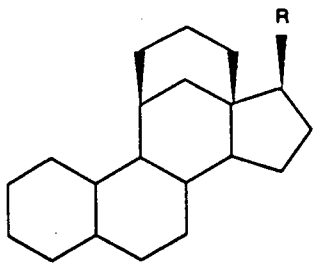

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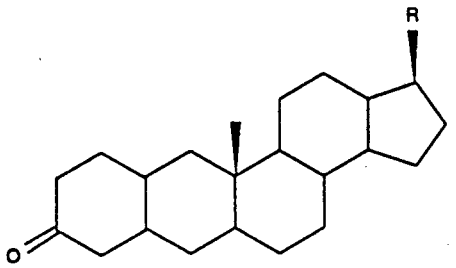

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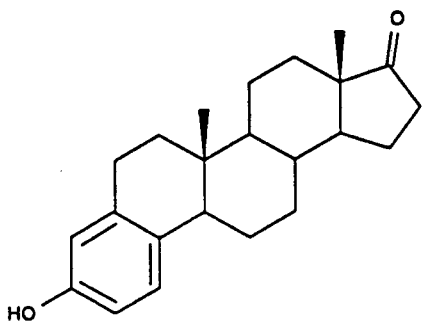
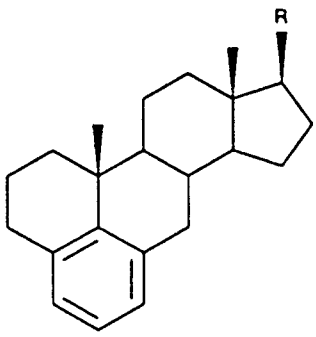


(4)



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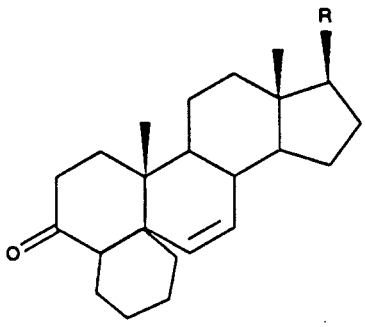


(5)

Pentacyclic steroids, with the new ring bonded at (2) and C(3) like type (**3**), have been synthesised by Cooley and co-workers[4] and were found to have no significant effect on the biological activity.

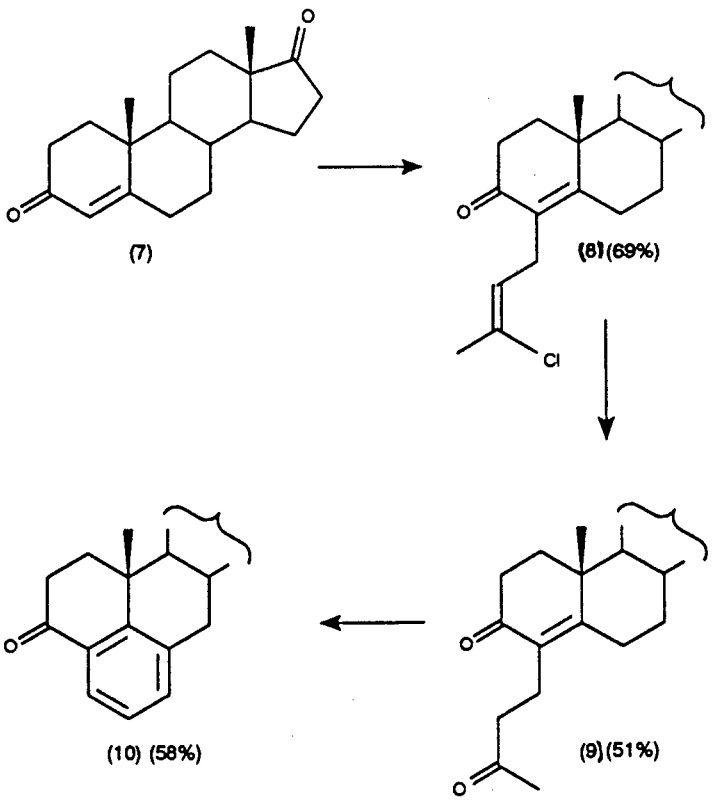
Graves and Ringold,[5] in their study on structure-activity relationships, have constructed steroids of the androstane series with the additional ring bonded at positions C(3) and C(4).

Lenz,[6] in his study of photo-Diels-Alder reactions on steroidal 3-oxo-4,6-dienes, has synthesised pentacyclic steroids of type (**5**). Finally, the benzo[4.5.6]steroid (**6**), which has a propano-bridge bonded to C(4) and C(6) has received much attention[5,7-11].

A variety of methods, including the well known Robinson annelation procedure[12] have been used to append new rings to the steroid nucleus. Some of these methods are exemplified in the synthesis of benzo[4.5.6]steroids.

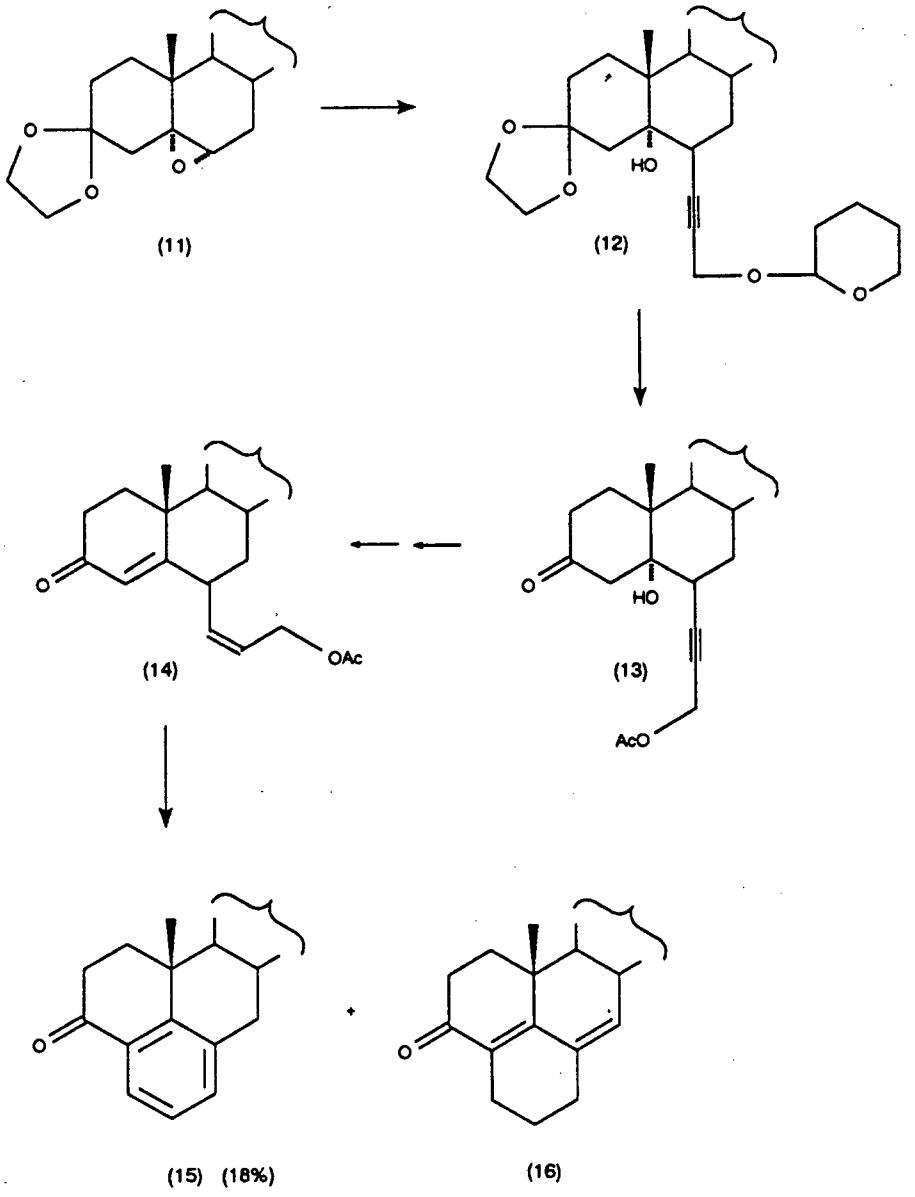
Graves and Ringold[5] used the following procedure (Scheme 1). The starting material (**7**) was alkylated with 1.5 equivalents of 1,3-dichlorobutene. Hydrolysis with sulphuric acid afforded the triketone (**9**). The base catalysed cyclisation was not straightforward and led to difficulties. Depending on the reaction conditions, different cyclisation products could be obtained. The benzo[4.5.6]steroid (**10**) was obtained by refluxing (**9**) in 2% methanolic sodium hydroxide for 3 h.

Scheme 1



Komeno and co-workers[7] have synthesised benzo[4.5.6]steroids by an intramolecular condensation of a 6-(3-acetoxy-1-propenyl)-4-en-3-one steroid (**14**) (Scheme 2). This compound (**14**) was synthesised by treatment of a 5α,6α-epoxide (**11**) with 3-tetrahydropyranyloxyprop-1-yn-magnesiumbromide. Dehydration with thionyl chloride in pyridine afforded the required product (**14**). The benzo[4.5.6]steroid (**15**) was formed (18%) by treatment of (**14**) with sodium hydride in refluxing xylene for 18 h. A small amount of the dienone (**16**) was also isolated.

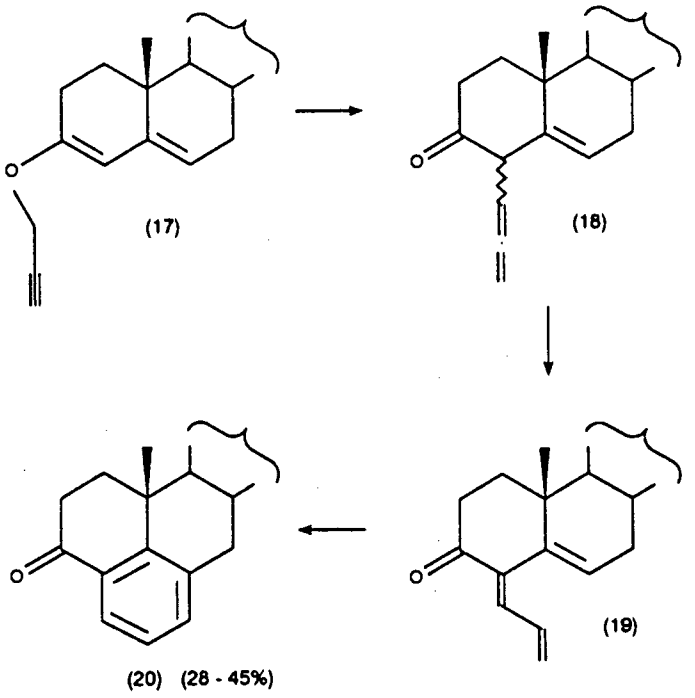
Scheme 2



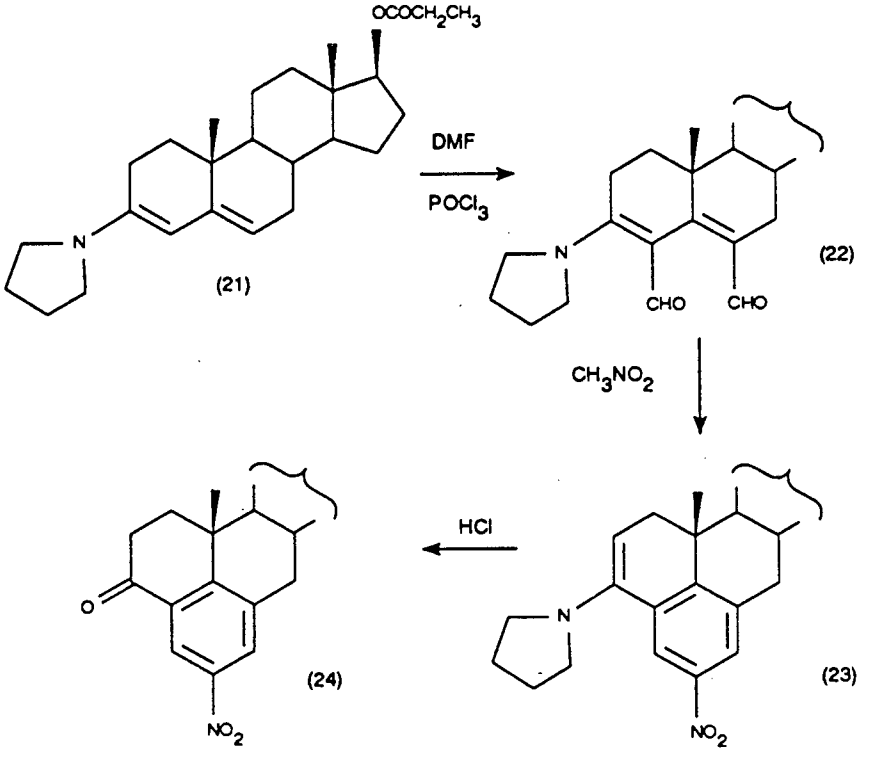
Vitali and co-workers[8] have examined the Claisen rearrangement of propargyl ethers as a route to benzo[4.5.6]steroids. The propargyl enol ether (**17**) (Scheme 3) was refluxed in toluene for 5 h, resulting in rearrangement to the allene (**18**). On passage through alumina, migration of the double bond occurred to form the conjugated ketone (**19**). However, by refluxing the enol ether (**17**) for 3 h in pyridine, the benzo[4.5.6]steroid (**20**) (27%) was isolated. The yield increased to 45% by working in the presence of palladium charcoal. The authors have proposed the following sequence (Scheme 3). Since the isomerisation of the allene (**18**) is fast under the reaction conditions, benzosteroid (**20**) arises from the 4-allylideneketone (**19**) by cyclodehydrogenation. The nature of the reaction is yet unknown and autooxidative processes are not involved, since the reaction occurred also in the presence of antioxidants. Addition of a hydrogen acceptor did not affect the yield. The authors claim that some of their results of research suggest that cyclisation and aromatisation occur partially by hydrogen disproportionation, the 4-allylideneketone acting as both hydrogen donor and acceptor.

Sciaky and Pallini[9] used the Vilsmeier-Haack reagent (DMF-$POCl_3$) for the diformylation of the dienamine (**21**) (Scheme 4) at C(4) and C(6) in their approach to benzo[4.5.6]steroids. The diformyl steroid derivative (**22**) was cyclised via an aldol-type condensation using nitromethane and sodium ethoxide in ethanol at room temperature to obtain the nitrobenzene derivative (**23**). Hydrolysis with aqueous hydrochloric acid gave the 3-oxo-nitrobenzo[4.5.6]steroid (**24**).

Scheme 3

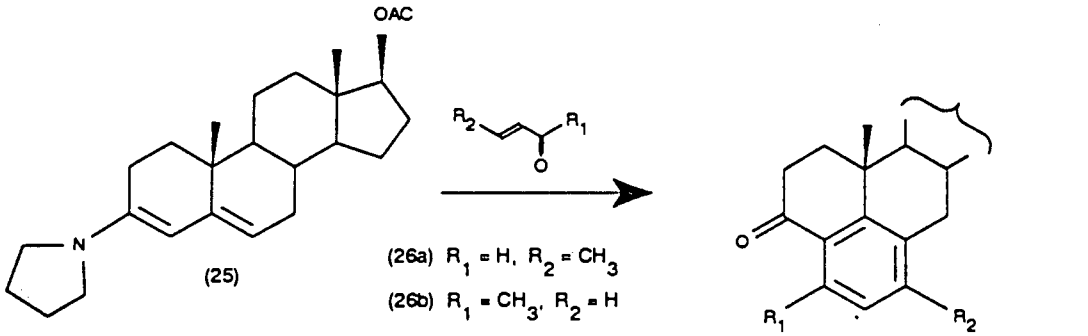


Scheme 4



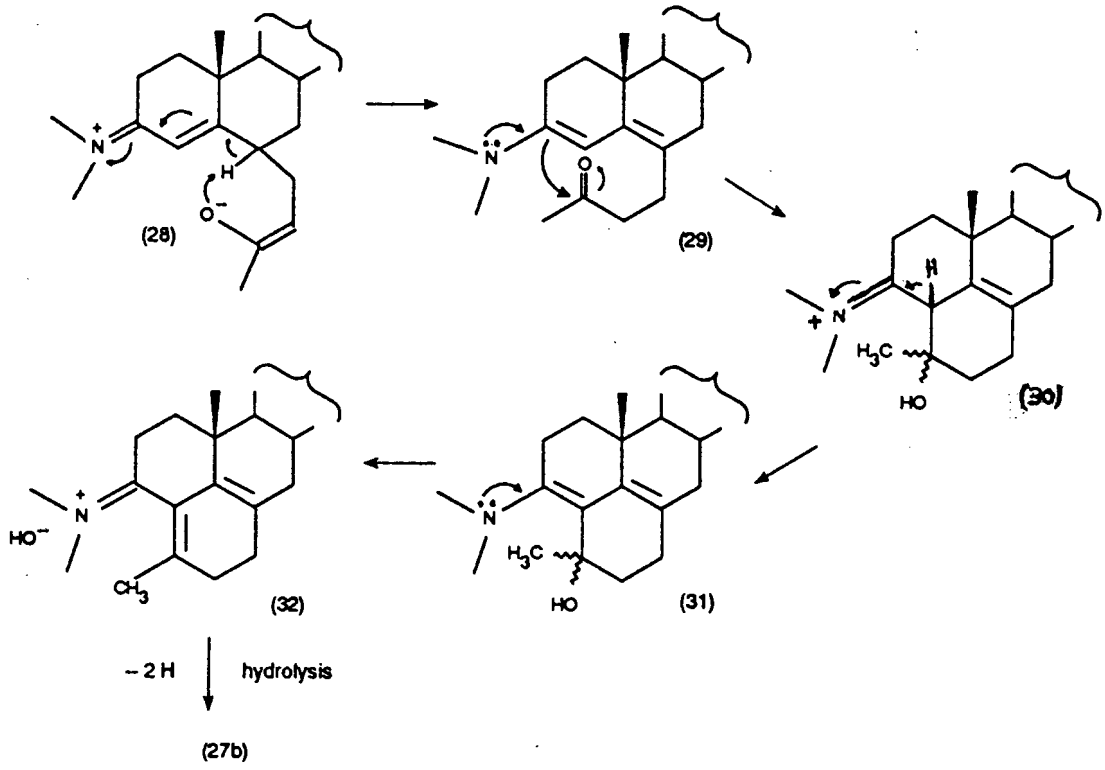
Pandit and co-workers[10,11] developed a convenient, single step procedure (Scheme 5) for the synthesis of benzo[4.5.6]steroids (**27a**) and (**27b**), which involves the annelation of the steroidal enamine (**25**), using crotonaldehyde (**26a**) or a methyl vinyl ketone (**26b**) respectively.The initial step is a Michael-type addition at C(6), resulting in the formation of a dipolar intermediate (**28**) (Scheme 6), which neutralises to (**29**) by proton transfer. An intramolecular attack of the enamine function on the carbonyl group occurs, to yield the intermediates (**30**) and (**31**). Transformation of (**31**) to (**27b**) proceeds *via* (**32**), followed by dehydrogenation and hydrolysis. The aromatisation of the dihydrobenzene system in (**32**) or its hydrolysed equivalent, might either involve an air oxidation during work-up or a disproportionation process.

Scheme 5

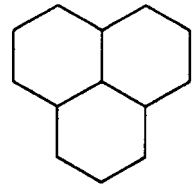


(27a) R_1 = H, R_2 = CH_3 (11%)
(27b) R_1 = CH_3, R_2 = H (15%)

Scheme 6



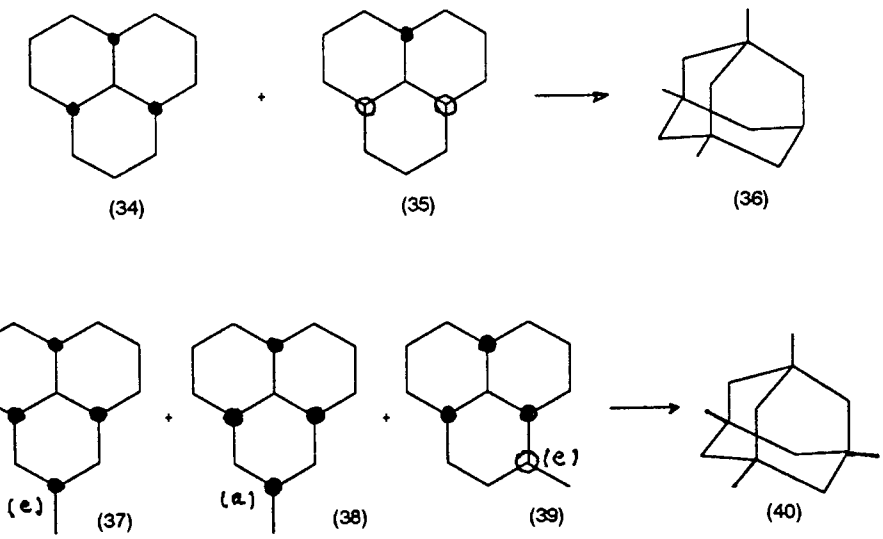
Little work has been conducted towards the synthesis of the hexahydrobenzo[4.5.6]steroids, or the dodecahydrophenalene system (**33**), which resembles rings A, B, and E of the hexahydrobenzo[4.5.6]steroids.



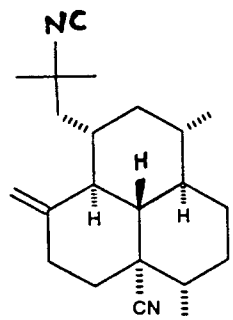
(33)

Schneider, Warren, and Janoski[13,14] have found that dodecahydrophenalenes (**34**) and (**35**) (Scheme 7), and their methyl derivatives (**37**), (**38**), and (**39**) undergo aluminium halide catalysed isomerisations to adamantane derivatives (**36**) and (**40**) respectively, which are strainless molecules.

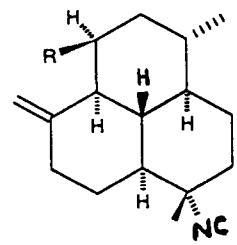
Scheme 7



The dodecahydrophenalene nucleus (**33**), unlike the hexahydrobenzo[4.5.6]steroids, occurs in nature. Wells and co-workers[15] have isolated the tricyclic isocyanide (**41**) from the sponge *Hymeniacidon amphilecta* and three isocyanides of the type (**42**) from a species of the sponge genus *Adocia*.



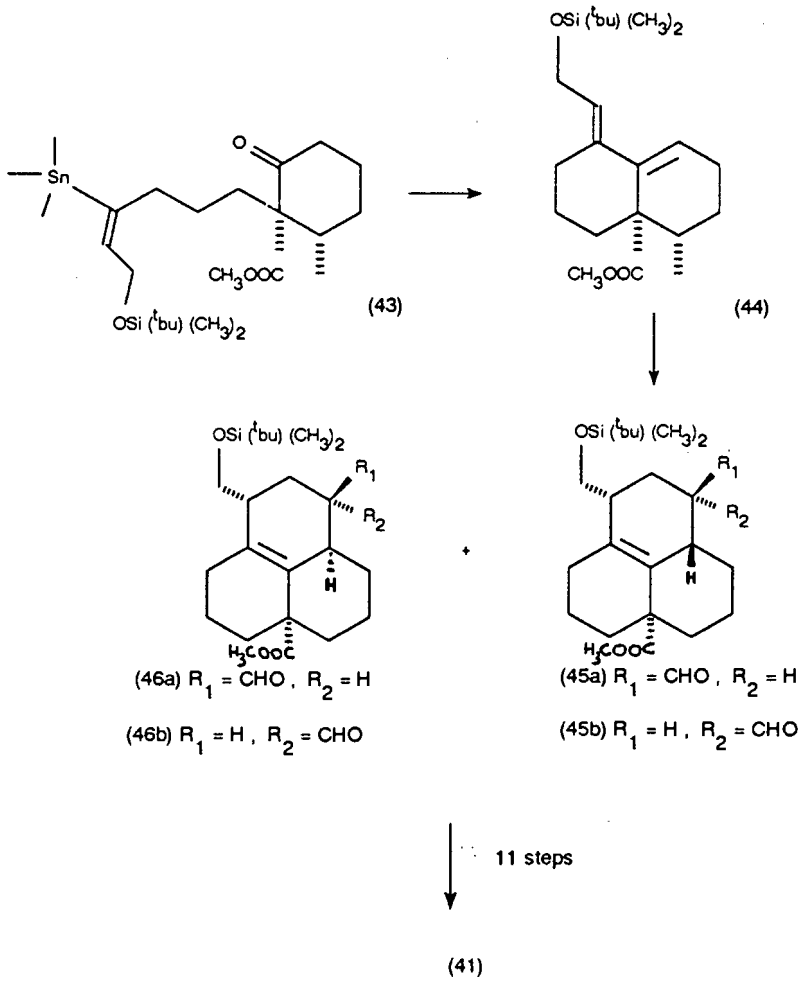
(41)



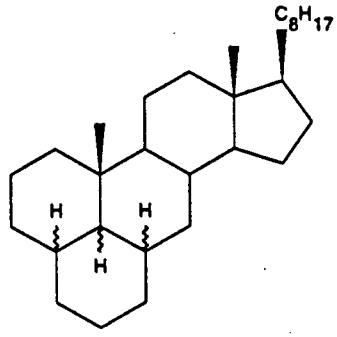
(42)

Piers and Llinas-Brunet[16] have developed a stereocontrolled total synthesis of (**41**) in 20 steps. The key step of the synthesis was the construction of the tetrahydrophenalene systems (**45a**),(**45b**),(**46a**),(**46b**) (Scheme 8). A Diels-Alder reaction of the diene (**44**) with propenal, followed by equilibration with sodium methoxide in methanol of the resultant mixture of four adducts, provided a mixture of the two aldehydes (**45a**) and (**46b**) in a ratio of 3:7 respectively. Separation on silica gel afforded the clean adducts (**45a**) and (**46b**) in 29 and 58% respectively.

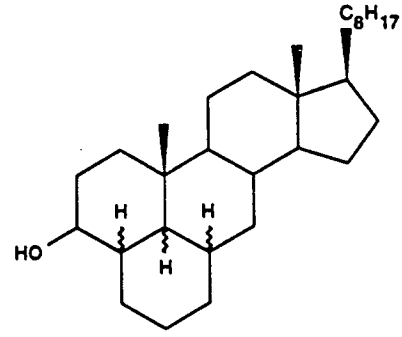
Scheme 8



There are four chiral centres in hexahydrophenalene, three of which are equivalent, thus four stereoisomers are possible. However, in the steroid nucleus, the nonequivalence of the four chiral centres leads to sixteen possible stereoisomers, which reduces to eight if C(10) is fixed as in the natural steroid skeleton. Some of the isomers may be difficult to synthesise because of the strain created in the molecule by the new ring. The presence of rings C and D renders the steroid molecule less flexible than the hexahydrophenalene system. The most likely conformations of the possible isomers of the hexahydrobenzo[4.5.6]steroids are illustrated in Scheme 9.



(47)



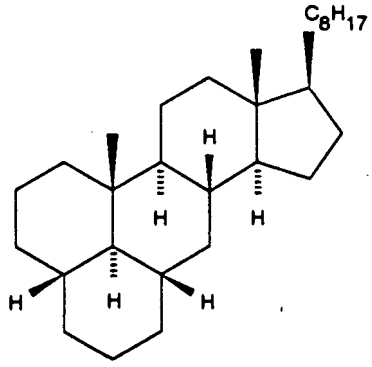
(55)

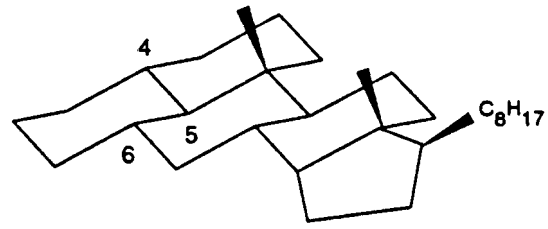
Concerning the synthesis of steroids with a partially or fully saturated ring E, Bull and co-workers[17,18,32] reported a practical route to pentacyclic steroids of the type (**47**). Treatment of (**48**) (Scheme 10) with the homocuprate derived from the reaction of lithiated acetone-N,N-dimethylhydrazone with copper(I) iodide in the presence of diisopropyl sulphide afforded the product (**49**). Hydrolysis with aqueous hydrochloric acid gave a mixture of (**50**) and (**51**). The authors further demonstrated that base treatment of the mixture of (**50**) and (**51**) gave the aldol condensation product (**52**) and that (**50**) epimerised to (**51**)

prior to cyclisation. Prolonged base treatment of (**52**) afforded the enone (**53**) in 64%. The enone (**53**) was reduced by a variety of methods, followed by a Wolff-Kishner reduction, to give in each case the parent hydrocarbon (**54**), having the 6α(H)-configuration.

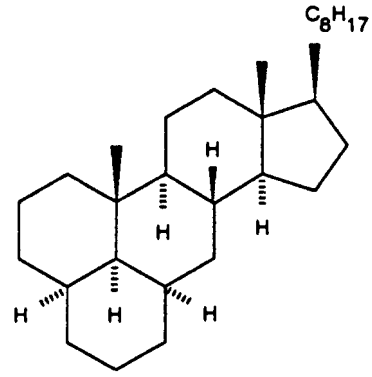
In view of the paucity of literature on the synthesis of hexahydrobenzo[4.5.6]steroids, and the analogy of the work of Bull and co-workers,[17,18,32] the investigations conducted were directed towards the construction of steroidal analogues with the basic skeleton (**55**).

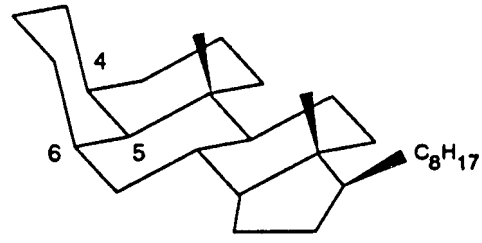
Scheme 9



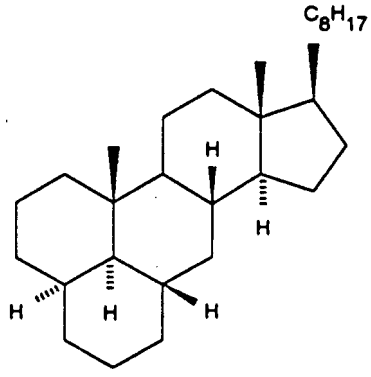


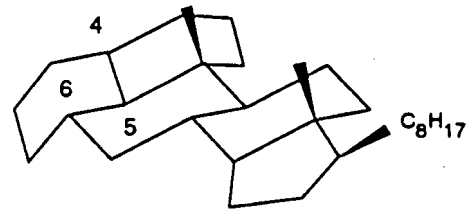
43.4 kcal mol^{-1}



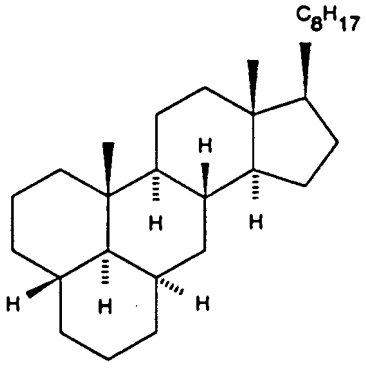


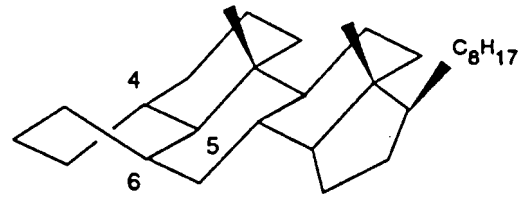
53.2 kcal mol^{-1}



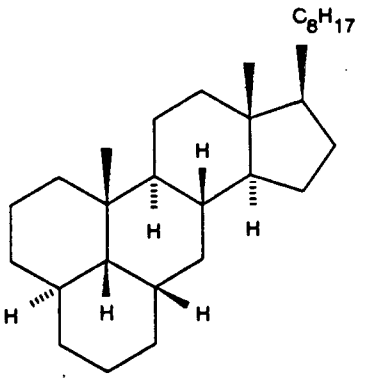


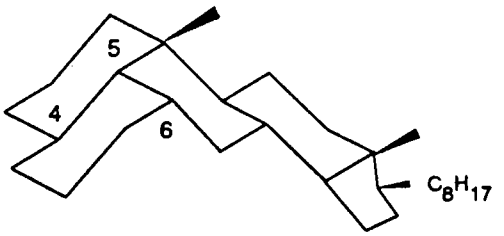
54.2 kcal mol^{-1}



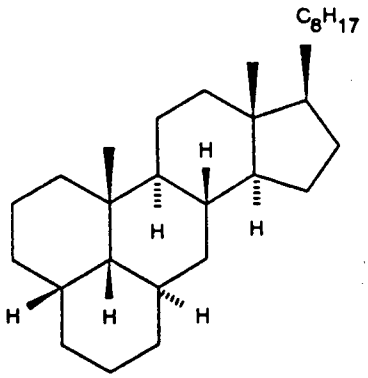


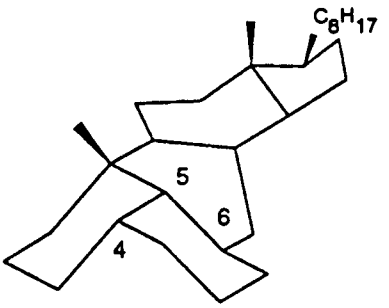
55.7 kcal mol^{-1}



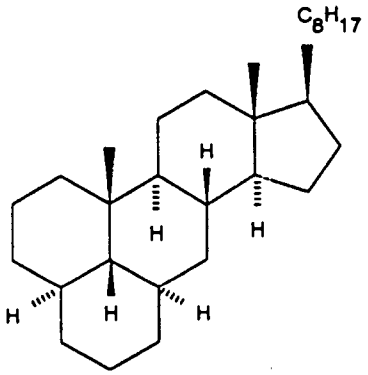


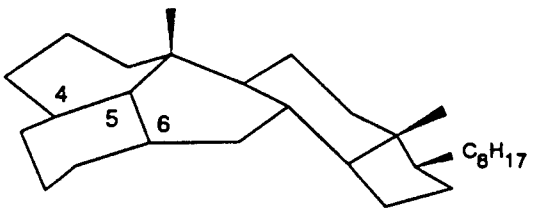
44.9 kcal mol^{-1}



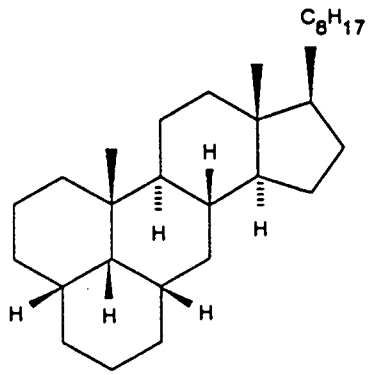


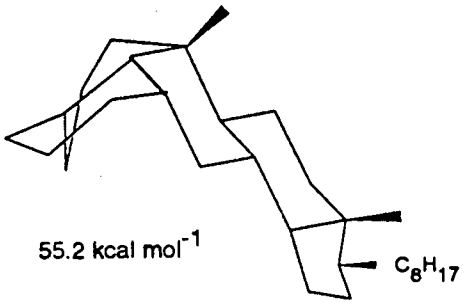
50.4 kcal mol^{-1}





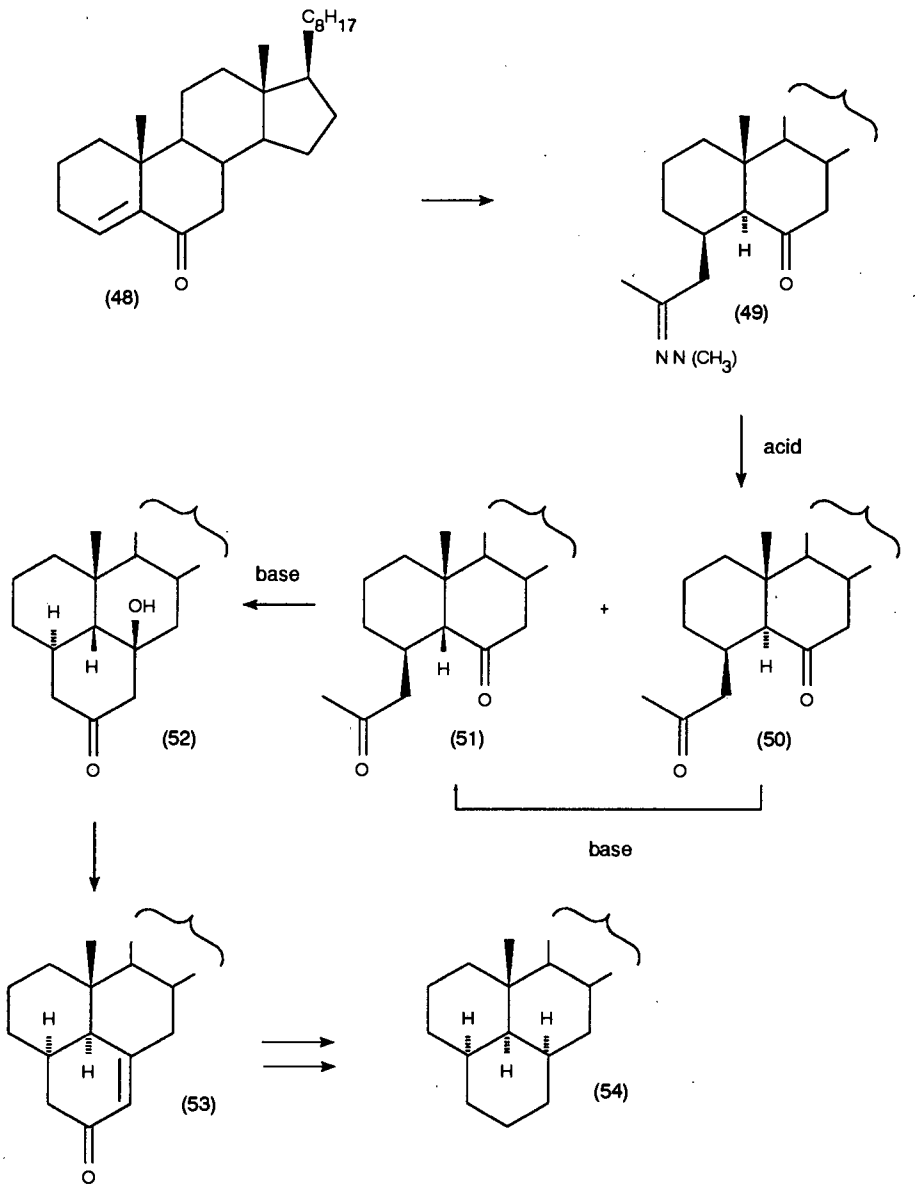
52.4 kcal mol^{-1}





55.2 kcal mol^{-1}

Scheme 10


C_8H_{17}
(48)
O
H
O
(49)
N N (CH_3)
acid
base
H
OH
H
O
(52)
H
O
(51)
+
H
O
(50)
O
base
H
H
O
(53)
H
H
H
(54)

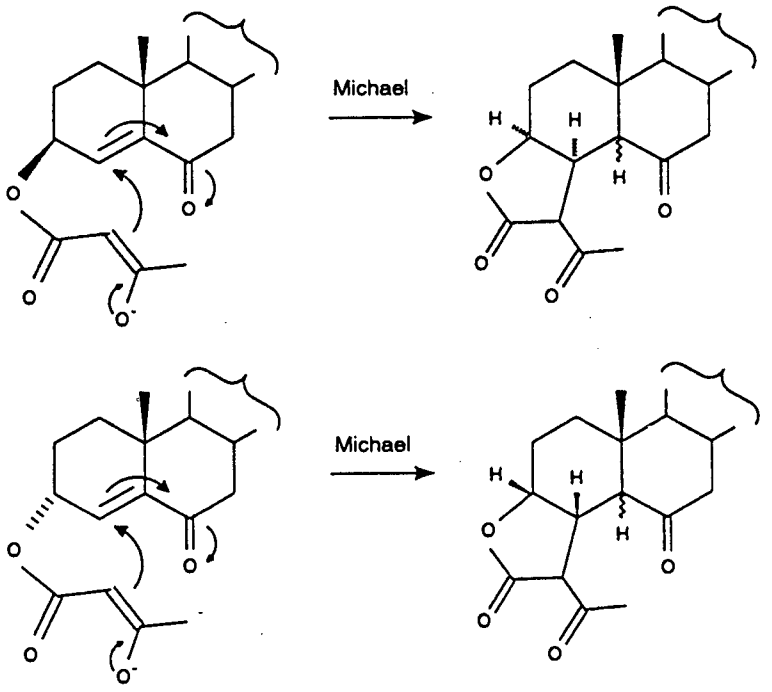
The hydroxyl group at C(3) was part of the strategy to construct pentacyclic compounds of the type (**55**). Furthermore, after oxidation of the hydroxyl group to the ketone, a means of equilibrating the ring at the C(4) junction *via* enolisation, is created, giving access to the entirely novel 4ß(H)-series. The work was carried out on the cholesterol skeleton, in order to establish stereochemical control at C(4) and C(6). The synthetic routes here developed, it is thought, can be extrapolated to any steroid series, provided one or more protective goups are used throughout the synthesis. Such systems would serve as models for 4,6-dialkyl hormones, the alkyl substituents thus being constrained into various orientations by the formation of a bridge. The conformations of rings A and B are also affected by the presence of ring E. In this investigation, aspects of the synthesis and properties of representative 4,6-propanocholestanes are discussed.

Objectives and approach

In this investigation, the overall objective was to apply a tandem Michael-aldol strategy for the stereocontrolled synthesis of pentacyclic steroids, in which the additional six-membered ring E, joined at C(4) and C(6) of the steroid nucleus, had either a 4ß(H), or a 4α(H) configuration.

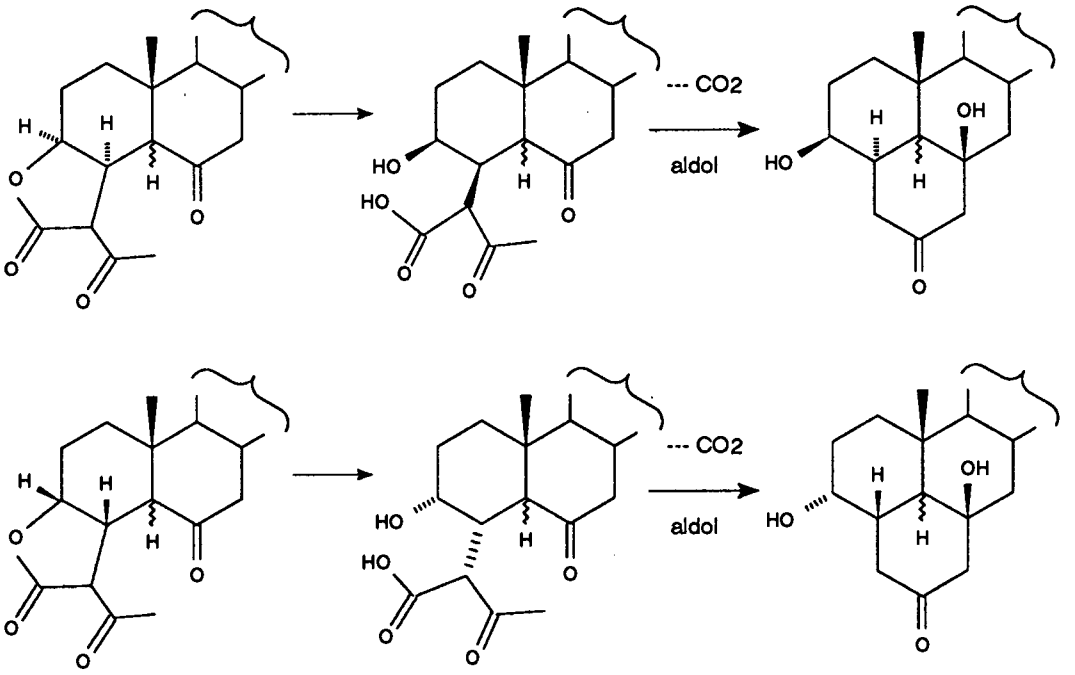
In the first phase of the work, it was planned to prepare two isomeric precursors to pentacyclic steroids. The first was 3ß-acetoacetoxycholest-4-en-6-one, upon which an intramolecular Michael reaction was expected to lead stereoselectively to a 3ß,4ß-fused γ-lactone (Scheme 11). The isomeric 3α-acetoacetoxycholest-4-en-6-one, in turn, was expected to lead to the 3α,4α-fused γ-lactone. The preferred 5-configuration of these lactones was uncertain at this stage, but it was expected that this might influence subsequent intramolecular reactions.

Scheme 11



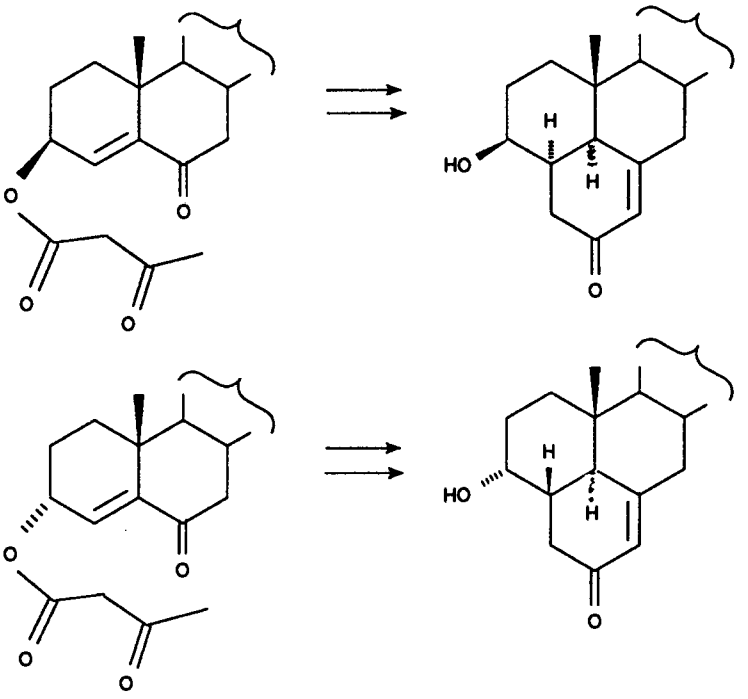
With the respective stereodefined γ-lactones in hand, it was planned to study the sequential lactone opening, decarboxylation, and intramolecular aldol condensation, leading to the corresponding 3ß,6-dihydroxy-4α,5ξ,6ß- and 3α,6-dihydroxy-4ß,5ξ,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-ones (Scheme 12).

Scheme 12



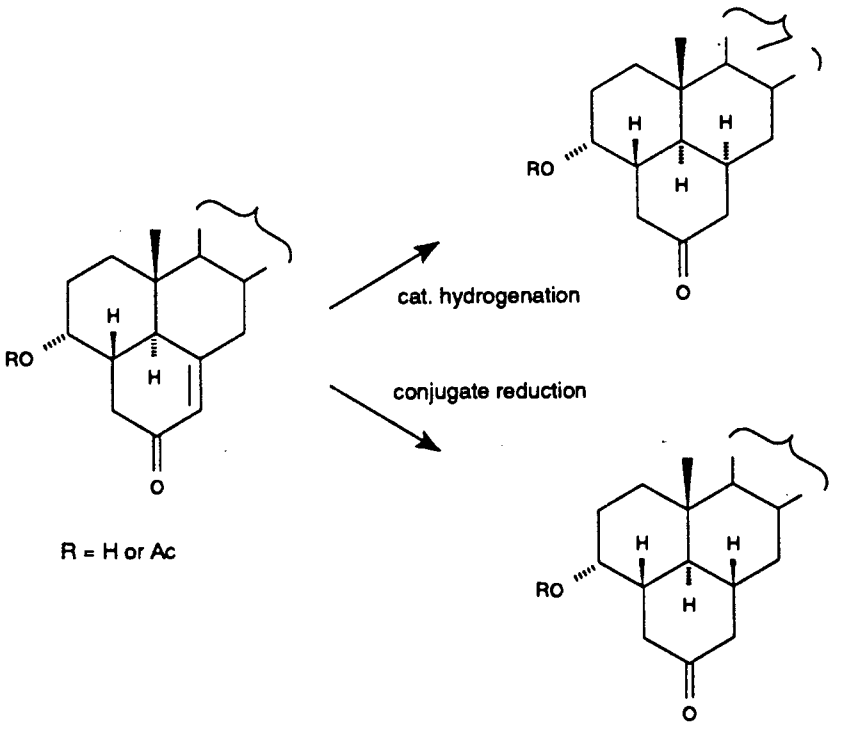
It was further intended to optimize the reaction conditions of the stepwise transformations of the lactones to the pentacyclic structures in order to eventually develop a tandem Michael-aldol strategy for direct conversion of the acetoacetates into 3ß-hydroxy-4α,5α- and 3α -hydroxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-ones, without isolation of any intermediates of the five consecutive steps of intramolecular Michael addition, lactone cleavage, decarboxylation, aldol condensation, and ß-elimination (Scheme 13).

Scheme 13



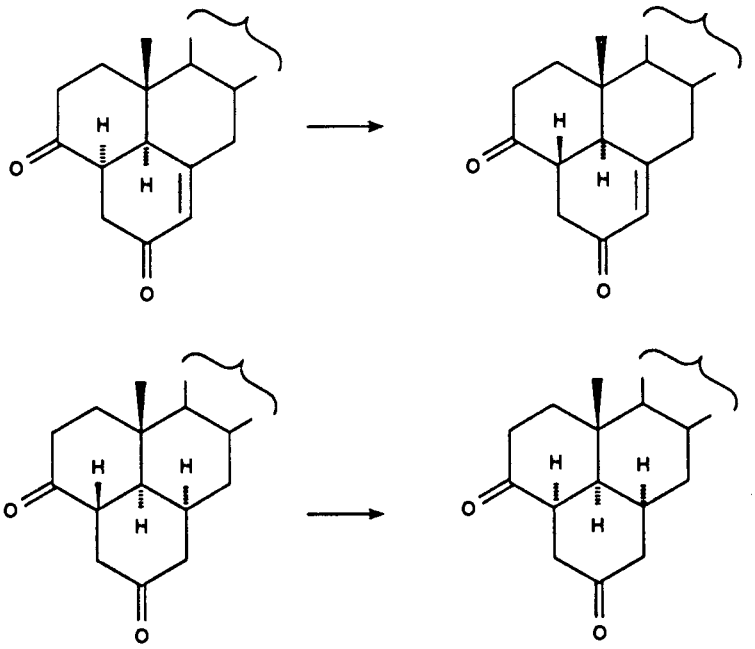
The planned third phase of the project entailed a study of the stereoselective reduction of the olefinic bond in the pentacyclic enones. The 4ß,5α-series was of particular interest, since it was hoped that catalytic hydrogenation and dissolving metal reduction might follow different stereoselective courses, leading to the 4ß,5α,6α- and 4ß,5α,6ß-series respectively (Scheme 14). The former ring system was expected to display interesting conformational properties, since the saturated ring E was expected to adopt an enforced twist conformation.

Scheme 14



The availability of different 4,5,6-isomers in the pentacyclic compounds, was also expected to provide scope for studying interconversions *via* equilibrations of derived 3-ketones (Scheme 15).

Scheme 15



Discussion

1. Synthesis of starting materials

1.1. Synthesis of 3ß-hydroxycholest-4-en-6-one

The preparation of 3ß-hydroxycholest-4-en-6-one (**61**), the key intermediate for the investigation of the proposed stereoselective routes to the hexahydrobenzo[4.5.6]cholestanes, was carried out using known procedures[19] (Scheme 16).

Cholesterol (**56**) was converted into the corresponding 3ß,5α,6ß-triol (**57**) by sequential treatment with performic acid (generated *in situ* by treatment of the substrate with performic acid and aqueous 30% hydrogen peroxide), followed by alkaline hydrolysis. The reaction proceeded efficiently and without isolation of intermediates, *via* 3-formylation, 5α,6α-epoxidation, *trans*-opening by formate, and hydrolysis of the presumed 5α-hydroxy-3ß,6ß-diformate.

The triol (**57**) underwent efficient selective oxidation with *N*-bromosuccinimide in aqueous dioxane, to give the 3ß,5α-dihydroxy-6-ketone (**58**) (95%). The selectivity of this reaction[20] relies upon the relative ease of access of the equatorial 6α-proton to the reagent during the rate-determining step, which entails the oxidation of the C-H bond.

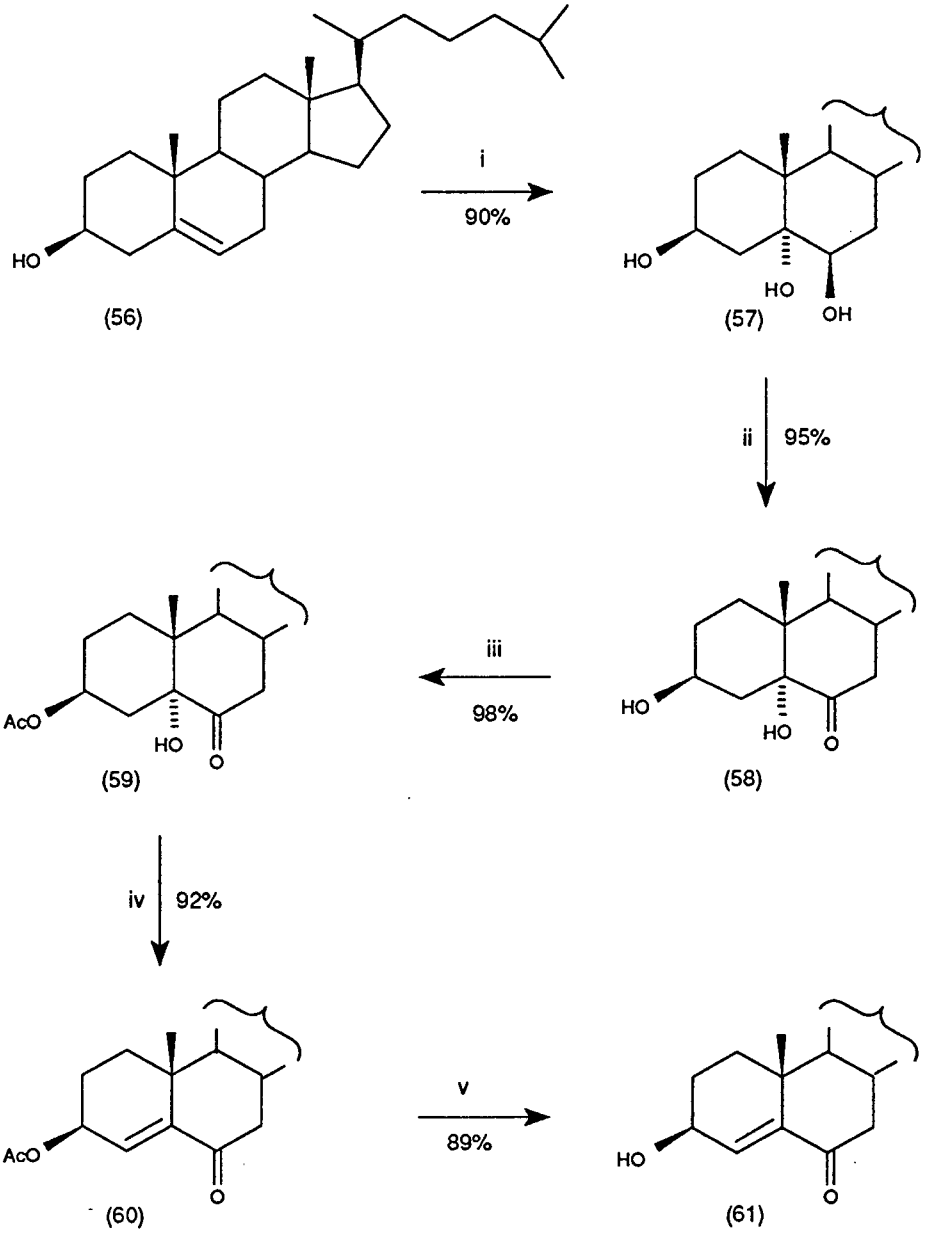
The 3ß,5α-dihydroxy-6-ketone (**58**) was acetylated with acetic anhydride in pyridine at 20°C to give 3ß-acetoxy-5-hydroxy-5α-cholestan-6-one (**59**) (98%).

Dehydration[21] of (**59**) by treatment with 5 equivalents of thionyl chloride in pyridine at 0°C afforded 3ß-acetoxycholest-4-en-6-one (**60**)

(92%). Finally, alkaline hydrolysis provided the desired 3ß-hydroxycholest-4-en-6-one (**61**) (89%).

The properties of the intermediates, and the final product (**61**) in this reaction sequence corresponded with those reported in the literature, and the conversion of (**56**) into (**61**) proceeded in an overall yield of *ca.* 68%. The procedure was amenable to large-scale preparation of (**61**), since the intermediates and the product were formed cleanly, and chromatography was not required.

Scheme 16



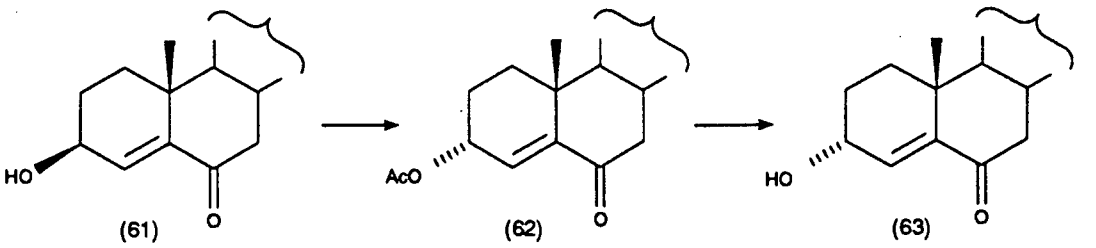
Reagents and conditions: i, HCOOH, H_2O_2, then NaOH; ii, NBS, aq.dioxane; iii, Ac_2O, py; iv, $SOCl_2$, py;v, KOH, EtOH

In a recent communication,[22] it has been claimed that oxidation of cholesterol (**56**) with silver(I) chromate-iodine in pyridine-dichloromethane at 0°C leads directly and efficiently (75%) to 3ß-hydroxycholest-4-en-6-one (**61**). However, we have attempted repeatedly and unsuccessfully to reproduce this result. All the reaction variables were examined, and silver(I) chromate was prepared and purchased from commercial sources. The reagents and solvents were purified and distilled prior to use. Despite all of these precautions, we have consistently obtained yields of the desired product (**61**) in the range of 17-21%. Furthermore, the product (**61**) was always accompanied by 5,6α-epoxy-5α-cholestan-3ß-ol (**98b**) (up to 45%) as the major compound of the oxidation mixture (see appendix). This product was not mentioned in the publication, and it must therefore be questioned whether the reported results are reproducible, or whether an unspecified experimental factor is crucial to the success of this method of direct oxidation.

1.2. Synthesis of 3α-hydroxycholest-4-en-6-one

The proposed method for the synthesis of 3α-hydroxycholest-4-en-6-one (**63**) entails Mitsunobu inversion of the corresponding 3ß-hydroxycholest-4-en-6-one (**61**), in the presence of acetic acid, followed by alkaline hydrolysis of the expected 3α-acetoxycholest-4-en-6-one (**62**) (Scheme 17).

Scheme 17



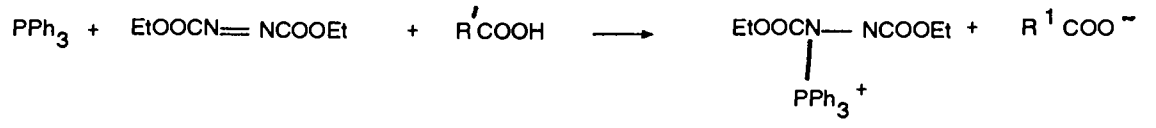
A literature search revealed that a similar reaction had been carried out.[23] The authors used benzoic acid as the nucleophile for the Mitsunobu reaction, and 3ß-hydroxycholest-4-ene as the substrate. They obtained 3α-benzoyloxycholest-4-ene in 81% yield. After studying the mechanistic details,[26] it was expected that the presence of a conjugated ketone would not significantly influence the reaction. A Mitsunobu reaction with γ-acyloxy α,ß-unsaturated ketones could however not been found in the literature, which is quite extensive (over 400 reviews and articles have been published on this subject).[26] Pusset and co-workers[24] have synthesised the 3-methoxy derivatives *via* a photochemical strategy, however, they did not prepare the corresponding alcohols. Fieser and co-workers[46] prepared the 3α-acetoxy and 3α-hydroxy-Δ^4-6-ketones (**62**) and (**63**) by fractional crystallisation of the mixture of α- and ß-acetoxycholest-4-en-6-ones, obtained by acetic acid mediated partial equilibration of 3ß-acetoxycholest-4-en-6-one (**60**). Their method was too complicated and was not amenable to efficient large scale synthesis of (**63**).

The Mitsunobu reaction[25] is firmly established as a favoured method for S_N2 inversion of secondary alcohols. The reaction is generally

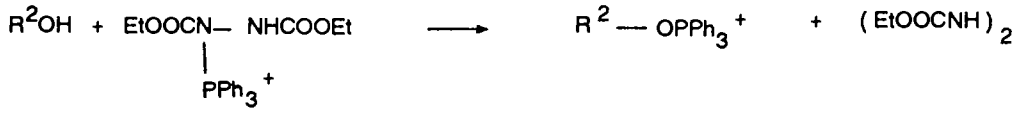
carried out by adding dialkylazodicarboxylate to a solution of the carboxylic acid, the alcohol, and triphenylphosphine. In this investigation, however, the alcohol was added last, and this did not adversely affect the reaction, since the product yields were excellent.

Scheme 18

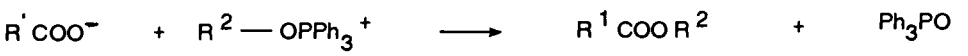
step 1: adduct formation



step 2: alcohol activation



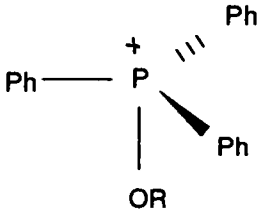
step 3: S_N2 reaction



The mechanism[26] involves three steps (Scheme 18). The formation of the adduct between diethyl azodicarboxylate (DEAD) and triphenylphosphine occurs within seconds at 20°C, as evidenced by the decolourisation of DEAD upon addition. In the presence of one equivalent of acetic acid, virtually all acetic acid anion will be hydrogen bonded in non-polar solvents such as benzene. This species is apparently orders of magnitude less reactive than the free acetic acid anion. When the amount of acetic acid is increased to two equivalents or more, the Mitsunobu adduct (the DEAD-PPh_3 adduct) is far more stable.

Under these conditions, clean displacement of the alcohol takes place with inversion of configuration.

The second step in the Mitsunobu reaction is transfer of the triphenylphosphine cation from the DEAD-PPh_3 adduct to the alcohol. In the presence of a carboxylic acid, an oxyphosphonium intermediate (**64**) is formed.



(64)

R = substrate (steroid nucleus)

The rate of transfer of the PPh_3^+ group from the DEAD-PPh_3 adduct to the alcohol is highly dependent on the basicity of the counterion. This indicates that the role of the counterion in this step is as a base to deprotonate the alcohol, which must occur before PPh_3^+ transfer takes place.

The final step is a S_N2 reaction of the carboxylate anion with the oxyphosphonium intermediate, resulting in the inversion of configuration. This is the rate-limiting step. If the attack of the nucleophilic carboxylate anion from the face of the molecule that would lead to inversion of configuration is a problem, because of hindered access, then a slow esterification will take place, with retention of

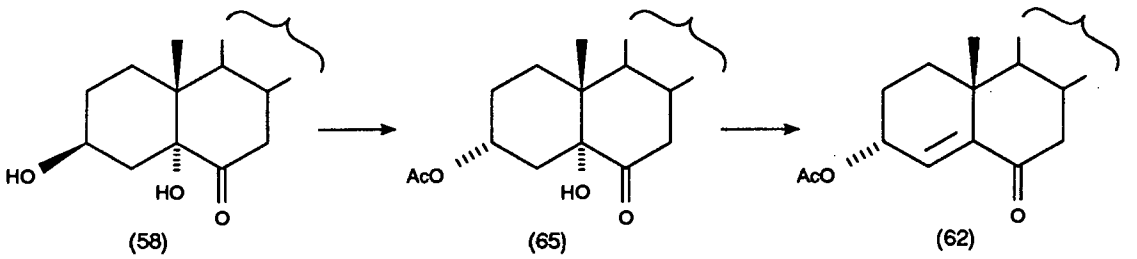
configuration. The S_N1 component however, if at all present, is very small.

3ß-hydroxycholest-4-en-6-one (**61**) was treated with triphenylphospine and diethyl azodicarboxylate (DEAD) in the presence of acetic acid in benzene at 20°C. The reaction proceeded rapidly to give 3α-acetoxycholest-4-en-6-one (**62**) in 90% yield. A possible explanation for the smoothness of the reaction is that the enone system tends to flatten ring A, thus creating ample accessibility for the nucleophilic acetic acid anion to attack from the α-face of ring A.

The structure of 3α-acetoxycholest-4-en-6-one (**62**) was proved, following from the spectroscopic data. The retention of the enone moiety was obvious from diagnostic NMR and IR data. The IR spectrum displayed the absorption for the enone system at υ_{max} 1680 and 1628 cm^{-1}, and an absorption at υ_{max} 1731 cm^{-1}, clearly indicated the presence of the acetoxy group. The NMR spectrum displayed the vinyl proton resonance at δ 6.14. Evidence for the 3α-orientation of the acetoxy group, and thus pseudo-axial substitution came from the 3ß-proton signal at δ 5.2, for which $W_{1/2}$ was distinctively smaller ($W_{1/2}$ 8 Hz) than in the comparable signal in the pseudo-equatorial 3ß-acetoxy compound (**60**) ($W_{1/2}$ 17 Hz).

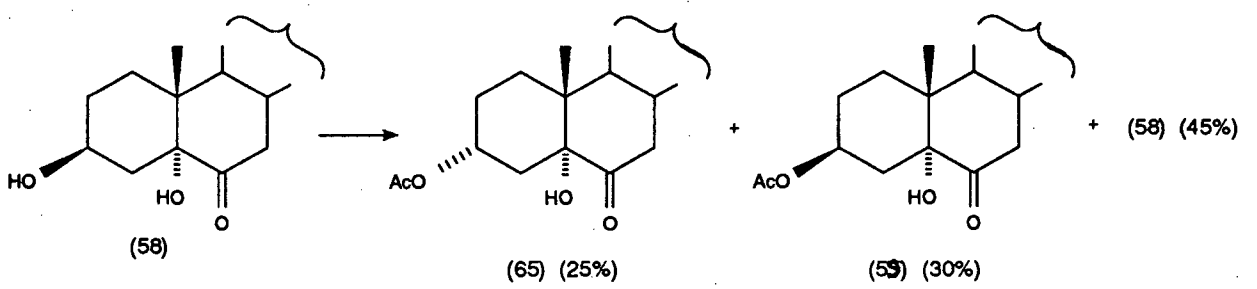
We envisaged a possible short-cut towards the synthesis of 3α-hydroxycholest-4-en-6-one. If it were possible to carry out a Mitsunobu reaction on 3ß,5-dihydroxy-5α-cholestan-6-one (**58**), it would then be possible to dehydrate the resulting 3α-acetoxy-5-hydroxy-5α-cholestan-6-one (**65**), which, after saponification, would afford the desired product (**63**) (Scheme 19)

Scheme 19



The attempted Mitsunobu inversion of (**58**) proceeded slowly and the reaction did not go to completion after 20 h in refluxing benzene. About **30**% of the product mixture comprised the acetylated product (**59**) as was indicated by the ^{1}H NMR spectrum, and direct comparison with authentic material (**59**).

scheme 20



Reagents and conditions: CH_3COOH, PPh_3, DEAD, benzene, reflux, 20 h.

The inverted product (**65**) was isolated in only 25% yield after 20 h reflux in benzene. This unreactivity towards Mitsunobu inversion can be explained by a 1,3-diaxial interaction between the 5α-hydroxyl group,

and the approaching nucleophile in an S_N2 process. Approach from the ß-face is much less hindered, and the S_N1 process, leading to (**57**) is able to compete.

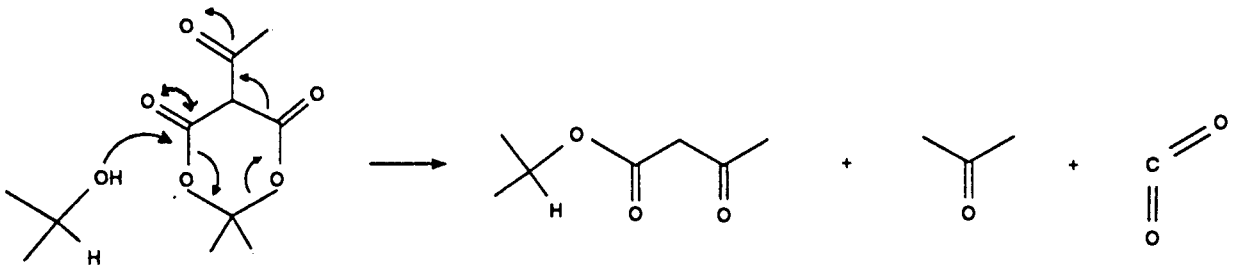
Evidence for the inversion in the 3α-acetoxy compound (**65**) came from the ^1H NMR spectrum, in which the signal for the 3-proton was an unresolved multiplet ($W_{1/2}$ 8 Hz), consistent with the absence of the antiperiplanar coupling with the 2ß-and 4ß-protons. This approach to the synthesis of 3α-hydroxycholest-4-en-6-one (**63**) was abandoned.

1.3. Acetoacetylation of the 3-alcohols

With the hydroxy-enones (**61**) and (**63**) in hand, it was decided to synthesise the respective 3-acetoacetoxy derivatives by treatment with 5-acetyl-Meldrum's acid (**69**). The method described by Yonemitsu and co-workers[27] was used for this purpose. They found that 5-acyl derivatives of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) react with alcohols to afford the corresponding ß-ketoesters. This method is referred to as an alcoholysis process, in which the ß-ketoester is obtained by refluxing the appropriate 5-acyl-2,2-dimethyl-1,3-dioxane-4,6-dione in the alcohol, or in the case of solid alcohols, by reflux in benzene, toluene, or xylene.

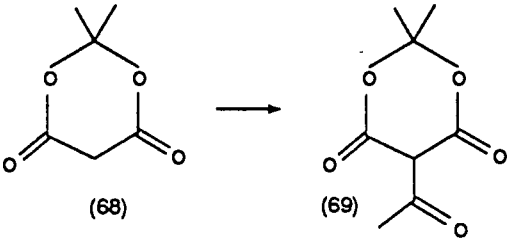
The mechanism of the esterification[27] involves the nucleophilic attack of the hydroxyl group onto the carbonyl carbon of 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione. A subsequent concerted loss of acetone and carbon dioxide produces the desired ß-ketoester (Scheme 21).

Scheme 21



Meldrum's acid (**68**) was treated with acetyl chloride and pyridine in dichloromethane to afford the solid 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**69**) (93%) (Scheme 22).

Scheme 22



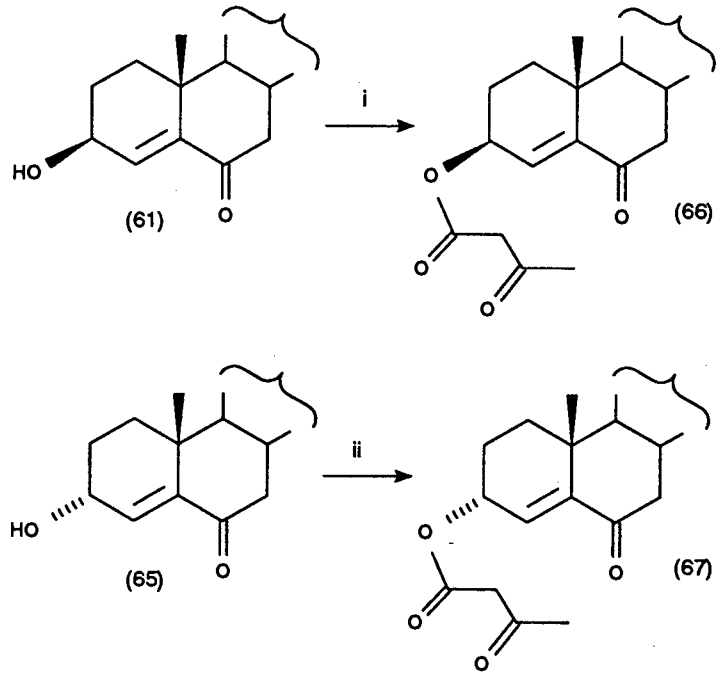
(68) (69)

Reagents and conditions: CH_3COCl, py, CH_2Cl_2, 0°C, 1h, then 20°C, 1h.

Treatment of 3ß-hydroxycholest-4-en-6-one (**61**) with 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**69**) in refluxing benzene for 3 h, afforded 3ß-acetoacetoxycholest-4-en-6-one (**66**) (96%). Similar treatment of 3α-hydroxycholest-4-en-6-one (**63**) at 65°C afforded 3α-acetoacetoxycholest-4-en-6-one (**67**) (94%) (Scheme 23).

Preliminary investigations on this method indicated that in the 3α-case (**63**), yields were better at lower reaction temperatures. The optimal reaction temperature was found to be 65°C, as a compromise between the yield of the acetoacetoxy esters and the reaction time. This was thought to be due to the relative instability of the product (**67**). The pure compound failed to crystallise, even at freezer temperatures. When the oily product was stored at 20°C, yellowing occurred after a few days, and t.l.c. showed the gradual accumulation of baseline spots. However, the ester (**67**) was stable at freezer temperature, under nitrogen.

Scheme 23



Reagents and conditions: i, 5-acetyl-Meldrum's acid, benzene, reflux, 3 h; ii, 5-acetyl-Meldrum's acid, benzene, 65°C, 5 h.

Proof of the structure of (**66**) and (**67**) comes from the IR and the NMR (Tables 1 and 2).

Table 1: comparative ^{13}C NMR and IR data of the acetoacetoxy esters

compound	^{13}C NMR (δ)	IR (cm^{-1})	assignment
(**66**)	166.7	1741	1'-C=O
	202.4	1714	3'-C=O
		1688	6-C=O
(**67**)	166.3	1738	1'-C=O
	203.0	1715	3'-C=O
		1686	6-C=O

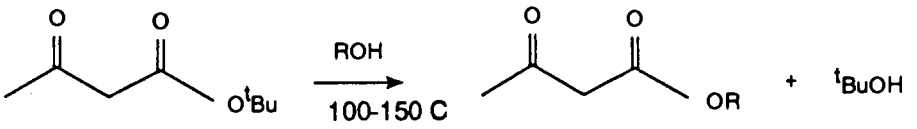
Table 2: comparative ^{1}H NMR data (ppm) of the acetoacetoxy esters

δ(**66**)	δ(**67**)	integral,mult.	assignment
2.25	2.24	3H, s	4'-H_3
3.44	3.42	2H, s	2'-H_2
5.38		1H, ddd, *J* 13.8,6.3,2.0 Hz	3α-H
	5.32	1H, m, $W_{1/2}$ 12 Hz	3ß-H
6.03	6.19	1H, s	4-H

A recent publication[28] decribes the synthesis of a large variety of acetoacetoxy esters from alcohols, with t-butyl acetoacetate. The reaction is carried out by simply refluxing the acetoacetate and the

appropriate alcohol in toluene or xylene, in the absence of a catalyst (Scheme 24).

Scheme 24



The authors found that the more hindered *t*-butyl acetoacetate is *ca* 15-20 fold more reactive than the methyl or ethyl analogues. They report yields of 72-90% for the formation of the acetoacetoxy esters from the corresponding alcohols (primary, secondary, and tertiary). This publication appeared after the completion of this phase of our work and, because of the excellent yields we achieved, this reaction variation was not tried.

2. Intramolecular reactions of the 3-acetoacetoxycholest-4-en-6-ones

With the 3-acetoacetoxycholest-4-en-6-ones (**66**) and (**67**) in hand, the stage was set for an investigation of intramolecular reaction sequences leading to the target pentacyclic steroids. The eventual goal was to elaborate a tandem Michael-aldol strategy, but it was recognised that the success of such an approach would depend crucially upon an efficient and stereoselective intramolecular Michael reaction, and upon favourable juxtaposition of the carbonyl groups in the derived

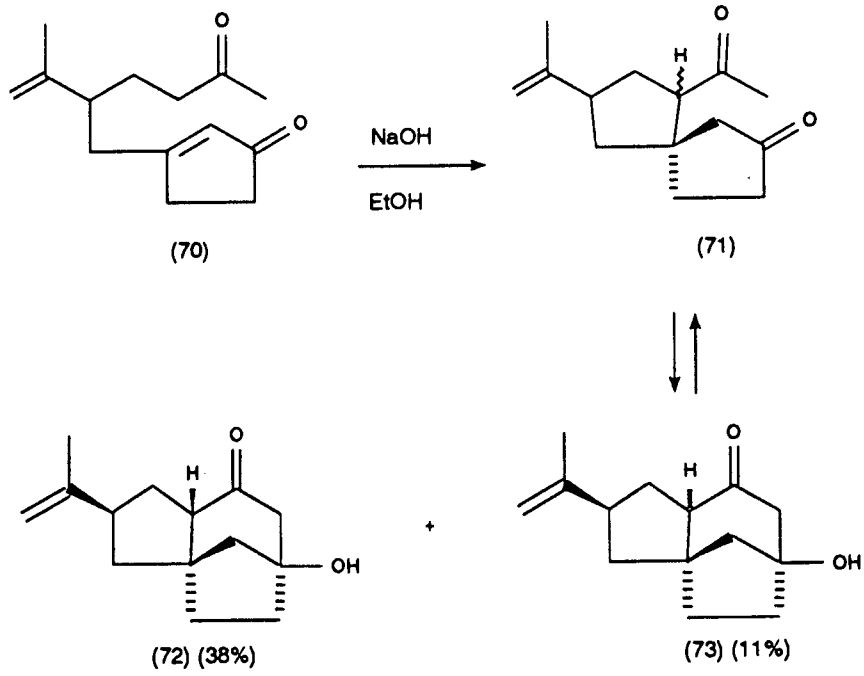
intermediates, for intramolecular aldol closure before or after decarboxylation. Accordingly, attention was first given to studying and optimising the intramolecular Michael reaction of the 3-acetoacetoxycholest-4-en-6-ones (**66**) and (**67**).

2. 1. Michael reaction of 3ß-acetoacetoxycholest-4-en-6-one

Intramolecular Michael reaction of the type envisaged in this work have been described.[29-31] An interesting reaction sequence was achieved by Alexakis and co-workers[29] (Scheme 25). When they exposed the cyclopentenone (**70**) to 3 equivalents of sodium hydroxide in aqueous ethanol for 2 h at 25°C, they obtained a mixture of (**72**) and (**73**) in 38 and 11% respectively. The mother liquor from the repeated fractional crystallisation of (**72**) and (**73**) had spectral properties consistent with the spirocyclopentenone (**71**). Treatment of the mother liquor with sodium hydroxide in aqueous ethanol produced the same mixture of (**71**), (**72**), and (**73**) as was formed from the monocyclic cyclopentenone (**70**). Likewise, ketols (**72**) and (**73**), when submitted to these reaction conditions, produced the same product distribution.

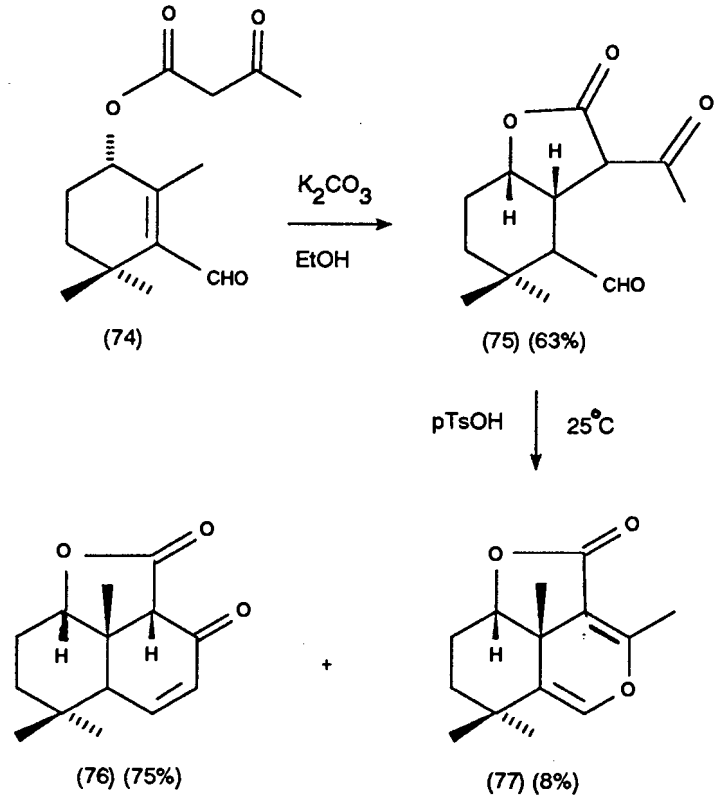
The authors concluded that the initial Michael reaction was irreversible for this system under the above conditions, whereas the subsequent aldol condensation seemed to be reversible. Their approach represents an analogy to our investigations into the elaboration of an intramolecular tandem Michael-aldol strategy. Their system however differs in the absence of an ester precursor and the more rigid steroid nucleus which, as discussed further, requires additional manipulations to assemble the desired pentacyclic steroid skeleton.

Scheme 25



Ruveda and co-workers[30] have investigated a closely analogous strategy for a potential forskolin precursor (Scheme 26). After several unsuccessful attempts to conduct an intramolecular Michael reaction on (**74**), they found that treatment of (**74**) with potassium carbonate in ethanol at 25°C afforded the lactone (**75**) in 63% yield. Further manipulation of (**75**) under basic conditions failed to produce the aldol product (**76**). However, treatment of (**75**) with toluene-*p*-sulphonic acid in anhydrous benzene for 5 h at 25°C produced the desired tricyclic α,ß-unsaturated ketone (**76**) in 75% yield, along with 8% of the enol ether (**77**).

Scheme 26



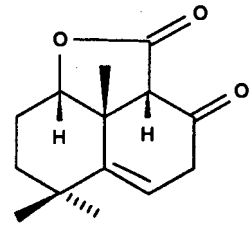
(74)

(75) (63%)

(76) (75%)

(77) (8%)

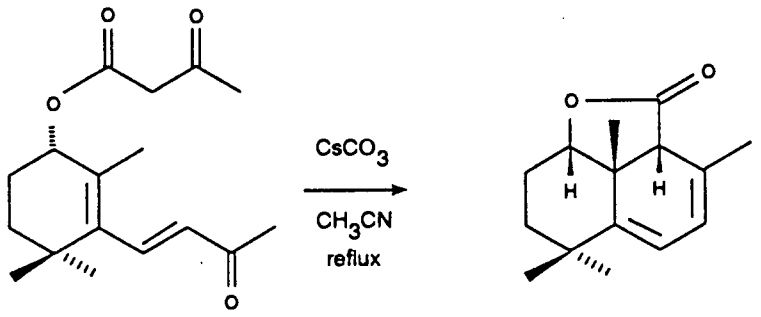
Prolonged exposure of (**75**) to the above reaction conditions (48 h) yielded the ß,γ-unsaturated ketone (**78**) as the main product.



(78)

Koft and co-workers[31] used an analogous Michael-aldol strategy towards advanced forskolin intermediates (Scheme 27). Exposure of (**79**) to 0.2 equivalents of $CsCO_3$ in refluxing acetonitrile for 6 h led directly to (**80**). They concluded that, in a single operation, intramolecular Michael addition, aldol condensation, deacylation, and olefin isomerisation had occurred.

Scheme 27



(79) (80)

Our initial experiments were carried out using the mildly basic reaction conditions described by Ruveda and co-workers.[30] This proved to be gratifyingly successful, since treatment of the 3ß-acetoacetate (**66**) with potassium carbonate in aqueous ethanol at 60°C led rapidly and efficiently to a single product (95%), which was formulated as (2*R*)-2-(3ß-hydroxy-6-oxo-5ß-cholestan-4ß-yl)-3-oxobutanoic acid-1,3'-lactone (**81**) (Scheme 28).

The structure of the product (**81**) was confirmed by spectroscopic examination. Thus, an IR absorption band at 1774 cm^{-1} was diagnostic for the γ-lactone group, and those at 1715 and 1698 cm^{-1} could be assigned to the carbonyl groups at C(6) and C(3') respectively. The ^1H

NMR spectrum of (**81**) revealed the absence of signals for the olefinic 4'-proton, and the acetoacetoxy methylene group of the starting material (**66**). Instead, a pattern of signals assigned to protons attached at C(3),C(4),C(5), and C(2') uniquely defined the nature and configuration of the substituents on ring A and B (Table 3).

Table 3: diagnostic ^{1}H NMR data of (**81**)

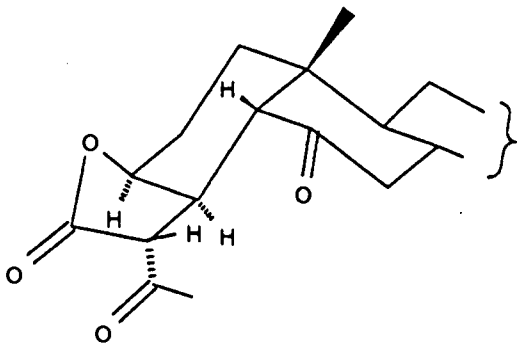
δ	multiplicity	assignment
1.82 (1H)	d, *J* 12.2 Hz	5ß-H
2.31 (3H)	s	4'-H_3
3.06 (1H)	dd , *J* 12.2 and 4 Hz	4α-H
3.57 (1H)	s	2'-H
4.63 (1H)	br.m , $W_{1/2}$ 11 Hz	3α-H

The assignments were confirmed with the aid of a COSY plot, which clearly revealed correlations between the signals at δ 1.82, 3.06, 3.57, and 4.63. Furthermore, a weak *trans*-carbonyl coupling between the 4'-CH_3 and 2'-proton of the lactone moiety was recognised.

A molecular model of (**81**) confirmed that the observed couplings are appropriate and are uniquely associated with a 5ß-configuration. Thus, the 4α-proton must have an antiperiplanar neighbour at C(5) to accommodate the 12 Hz splitting. Similarly, the signal for the 3α-proton is clearly diagnostic for axial attachment of the lactone ring. Furthermore, the lack of coupling between the 2'-and 4α-protons demonstrated their near-orthogonal alignment, and hence 2'*R*-configuration.

The structure and conformation of (**81**) was thus established as depicted in figure 2.

Fig.2: structure and conformation of (**81**)

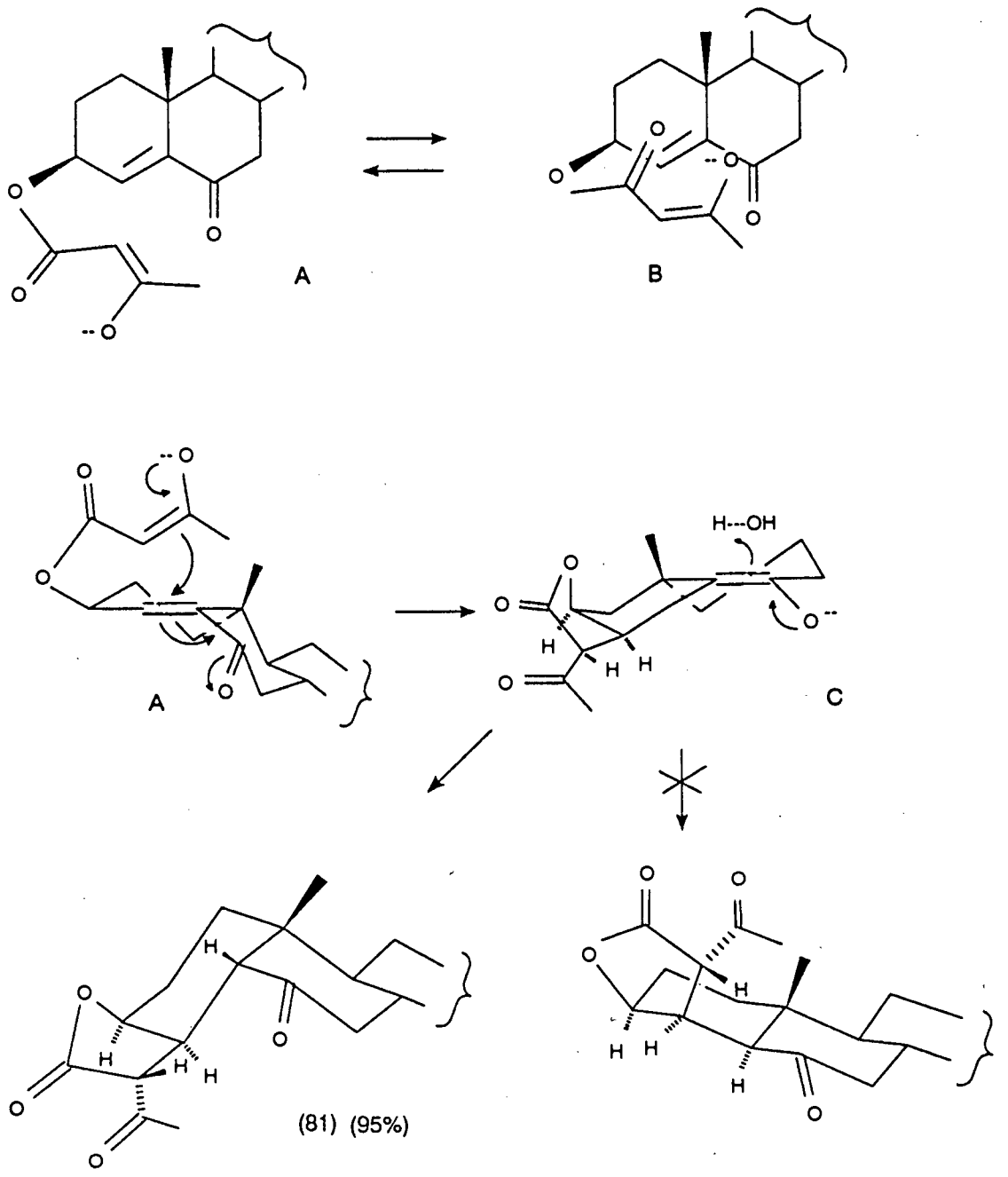


In the light of the foregoing structural assignment, it was possible to interpret the mechanism of the reaction in terms of stereoelectronic and steric constraints (Scheme 28). Thus, deprotonation of the acetoacetoxy moiety under the mildly basic conditions was assumed to lead to the reactive species A, in which the enolic component adopts the favoured s-*cis* conformation. The orientation of this component in species A enables *syn* bond formation to take place between the *re*-face and the ß-face at C(4). *Anti*-addition is obviously forbidden by steric constraints.

The alternative orientation of the nucleophile, in which the *si*-face is presented to the Michael acceptor (species B), is stereoelectronically disfavoured, since models reveal that orthogonality of the interacting centres cannot be achieved. Intramolecular closure of A leads to species C, in which the configuration at C(2') is thus fixed, and 5α-protonation would result in severe steric crowding between the C(4)-C(2') bond and

the 10ß-methyl group in the product; accordingly, 5ß-protonation predominates to give the thermodynamically-favoured lactone (**81**).

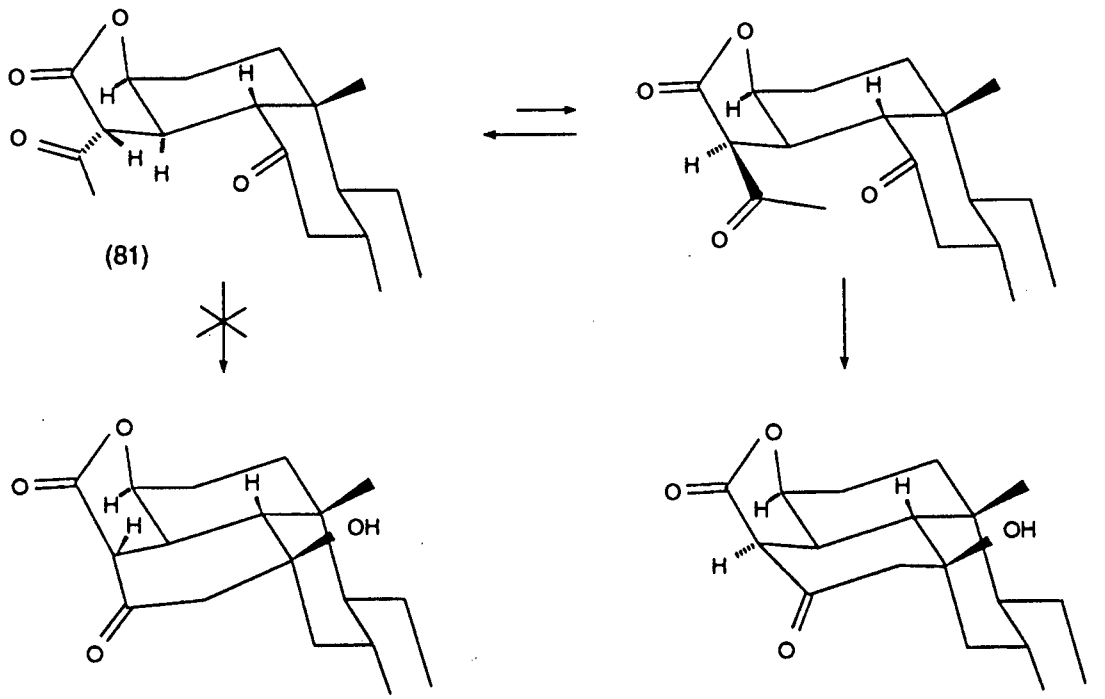
Scheme 28



The foregoing interpretation thus provides an explanation for the remarkable stereoselectivity achieved at three centres in the final product (**81**).

The orientation of the side chain in the lactone ring in (**81**) follows also from the failure to detect at least a small amount of the further aldol closed product in the presence of an intact lactone ring. From the model of (**81**), it is obvious that this aldol closure cannot take place with the side-chain in its initial orientation. The isomeric orientation however should lead to a certain amount, if not all, of the aldol closed product (Scheme 29). Attempts to obtain this product have failed, even upon prolonged exposure to the above reaction conditions (up to 48 h). Isomerisation of the acetyl moiety on the lactone ring in (**81**) can thus not be achieved under these reaction conditions. An attempt to achieve this under acid conditions, as proposed in an analogous reaction sequence carried out by Ruveda and co-workers[30] (toluene-*p*-sulphonic acid in ethanol) also failed. No further attempts were carried out to obtain this aldol product.

Scheme 29



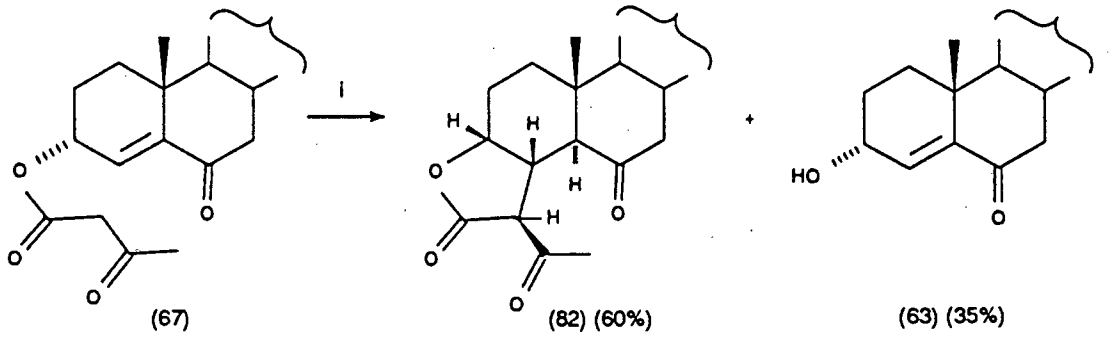
2.2. Michael reaction of 3α-acetoacetoxycholest-4-en-6-one

Examination of the models of the various options available revealed that the 3α-acetoacetoxy compound (**67**) should close at C(4) exclusively from the α-face of the steroid nucleus. This Michael reaction, which would lead to the irreversible formation of the C(4)-C(2') bond, was expected to proceed efficiently and stereoselectively, because of the proximity of the two reaction centres C(4) and C(2'), and the absence of any group causing steric hindrance for the nucleophilic attack upon the Michael acceptor from the α-face of ring A. Stereoelectronic considerations obviously exclude any Michael attack on the ß-face, which is *anti* to the 3α-O bond.

It seemed logical to assume that identical conditions as applied for the Michael reaction of the 3ß-compound (**66**) would lead to a smooth

conversion of 3α-acetoacetoxycholest-4-en-6-one (**67**) to the lactone derivative. Accordingly, the first experiment carried out, was the treatment of (**67**) with aqueous potassium carbonate in ethanol at 60°C (Scheme 30). Two products were observed on t.l.c. and the isolation and characterisation of the two readily separable compounds revealed that a major product, obtained in 60% yield was a lactone, and the minor product, obtained in *ca* 35% yield was the hydrolysed product, 3α-hydroxycholest-4-en-6-one (**63**). The lactone compound was formulated as (2*S*)-2'-(3α-hydroxy-6-oxo-5α-cholestan-4α-yl)-3-oxobutanoic acid 1,3'-lactone (**82**).

Scheme 30



Reagents and conditions: i, Aq.K_2CO_3, EtOH, 60°C, 4 h.

The structure of the product (**82**) was confirmed by spectroscopic examination. Thus, an IR absorption band at υ_{max} 1771 cm^{-1} was diagnostic for the γ-lactone group, and those at υ_{max} 1718 and 1704 cm^{-1} were assigned to the carbonyl groups at C(6) and C(3') respectively. The ^1H NMR spectrum of (**82**) revealed the absence of signals for the olefinic 4-proton, and the acetoacetoxy methylene group of the starting material (**67**). Again, a pattern of signals assigned to protons attached at C(3),

C(4), C(5), and C(2') uniquely defined the nature and configuration of compound (**82**) (Table 4).

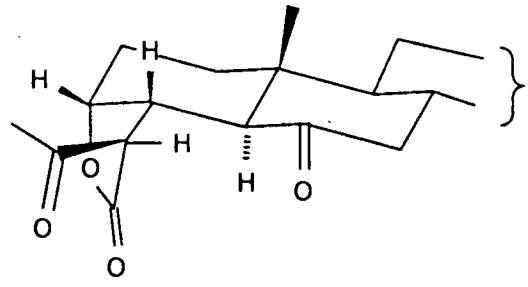
Table 4: diagnostic ^1H NMR data of product (82)

δ	multiplicity	assignment
2.17 (1H)	d (*J* 11.7 Hz)	5α-H
2.37 (3H)	s	4'-H_3
3.02 (1H)	dd (*J* 11.7 and 4.6 Hz)	4ß-H
3.22 (1H)	s	2'-H
4.72 (1H)	m ($W_{1/2}$=7 Hz)	3ß-H

The assignments were confirmed with the aid of a COSY plot, which clearly displayed correlations between the signals at δ 2.17, 3.02, 3.22, and 4.72. Again, a weak *trans*-carbonyl coupling between the 4'-CH_3 and 2'-proton of the lactone ring was observed.

A molecular model confirmed the observed couplings in the case of the proposed formulation of (**82**). In this case, 5α-configuration is obligatory, since the couplings between the 4- and 5-protons (12 Hz), can only be accommodated by an antiperiplanar arrangement. Notable features included the evidence of an axial substituent at C(3), since the signal for the 3-proton was displayed as an unresolved multiplet with $W_{1/2}$ 7 Hz. Furthermore, the lack of coupling between the 2'-and 4α-protons demonstrated in this case also their near-orthogonal alignment and hence 2'S-cofiguration. The structure and configuration was thus established as depicted in Figure 3.

Fig 3: structure and conformation of (**82**)

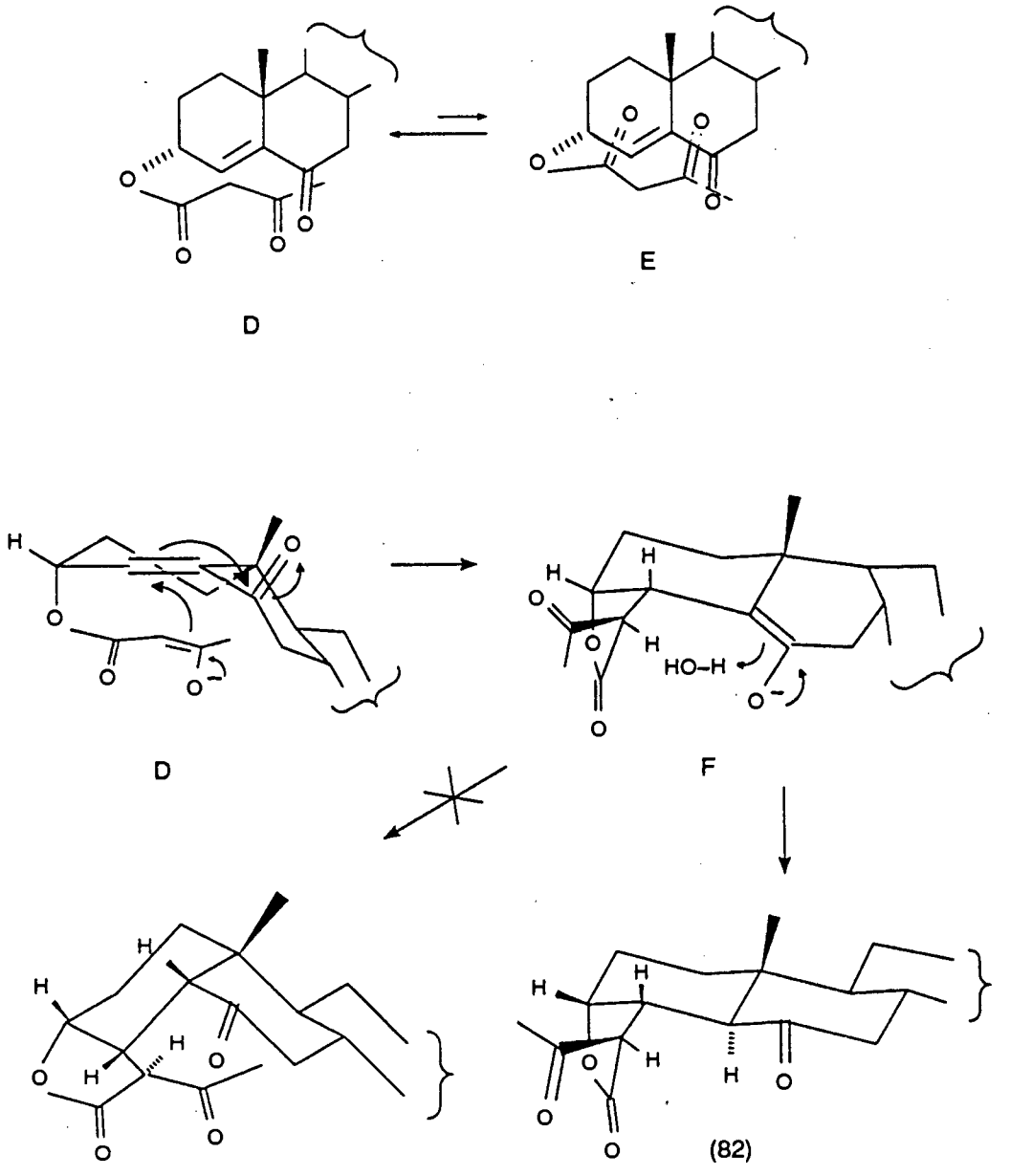


The foregoing structural assignment made it possible to interpret the mechanism of the reaction in terms of stereoelectronic and steric constraints (Scheme 31). Again, deprotonation of the acetoacetoxy group under the mildly basic conditions employed was assumed to lead to the reactive species D, in which the enolic component adopts a s-*cis* conformation. The orientation of this component in D enables bond formation to take place between the *si*-face and the α-face at C(4).

The alternative orientation, in which the *re*-face of the nucleophile is presented to the Michael acceptor (species E), is stereoelectronically disfavoured. Intramolecular closure of D leads to a species F, in which the configuration at C(2') is thus fixed, and 5α-protonation leads to the observed product (**82**). 5ß-Protonation would lead to a severe crowding between the C(4)-C(2') bond and ring B.

The foregoing interpretation thus provides an explanation for the remarkable stereoselectivity achieved at three centres in the final product (**82**).

Scheme 31

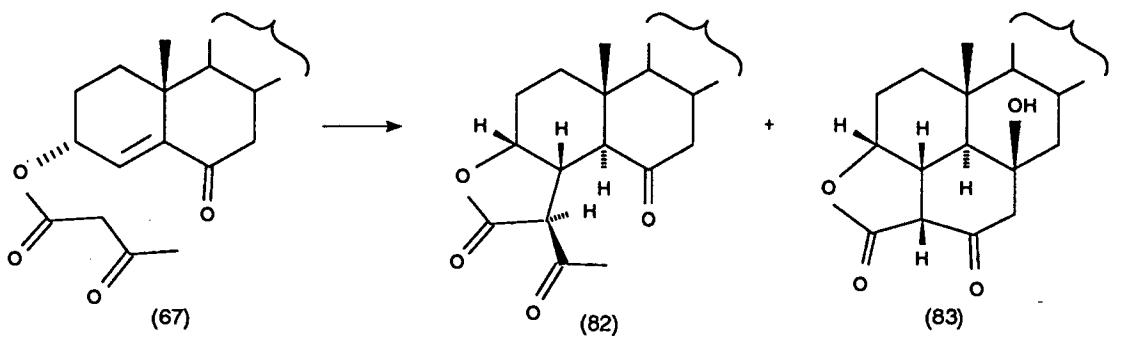


With the structure and conformation of (**82**) being fully established, attempts were made to optimise the conversion of (**67**) into (**82**). The obvious criterion for the improved reaction was the exclusion of hydrolytic conditions, since hydrolysis of the ester moiety in the starting material (**67**) appeared to be the only competing reaction.

The same reaction conditions, however at 0°C, still produced a significant amount of the hydrolysis product (**63**), and no further attempts in this direction were made, since the reaction was much slower at lower temperatures.

With the knowledge that the 3α-acetoacetoxy ester hydrolysed very easily, the use of a non-nucleophilic base as the catalyst for the Michael reaction was considered. Accordingly, when the starting material (**67**) , dissolved in a small amount of tetrahydrofuran, was added to a solution of potassium t-butoxide (1 eq. generated *in situ* by the addition of potassium metal to dry t-butyl alcohol) in t-butyl alcohol and tetrahydrofuran at 40°C, a rapid and clean conversion of (**67**) into the lactone (**82**) proceeded in a yield of *ca* 80%, together with *ca* 20% of the lactone (**83**) (Scheme 32). The latter was clearly the result of a base-catalysed intramolecular reaction of lactone (**82**), after epimerisation of the acetyl moiety on the lactone ring in (**82**).

Scheme 32



Reagents and conditions: t-BuOK, t-BuOH, THF, 40°C, 2.5 h

The product (**83**) was identified as 6-hydroxy-5'(6'*H*)-oxo-4ß,4',5α,6ß-tetrahydrobenzo[4.5.6]cholestane-6'α,3α-carbolactone .

Evidence for the proposed structure of (**83**) follows from the spectral interpretations. From the IR spectrum of (**83**), it was clear that the lactone ring was still in place, as judged from the carbonyl absorption band at υ_{max} 1775 cm^{-1}. The other carbonyl absorption band at υ_{max} 1712 cm^{-1} was assigned to the 5'-carbonyl group. An absorption band at υ_{max} 3596 cm^{-1} indicated the presence of a hydroxyl group. From theoretical considerations and the IR data, the only obvious structure was this one for the further aldol closed product. The ^1H NMR revealed a complete set of data for the positions of the protons attached to C(3), C(4), C(5), and C(6'). A COSY plot confirmed these data. The diagnostic resonances are listed in table 5.

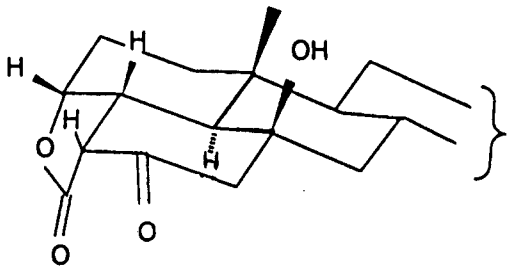
table 5: diagnostic ^1H NMR data of (**83**)

δ	multiplicity	assignment
1.47 (1H)	d, *J* 12.3 Hz	5α-H
2.35 (1H)	d, *J ca* 15 Hz	4' -H
2.44 (1H)	d, *J ca* 15 Hz	4' -H
3.18 (1H)	ddd, *J* 12.3, 5.7, and 3.5 Hz	4ß-H
3.52 (1H)	d, *J* 5.7 Hz	6'ß-H
4.55 (1H)	m, $W_{1/2}$ 10 Hz	3ß-H

The data indicated the absence of the acetyl methyl resonance of the starting material, and the ^{13}C NMR spectrum displayed an additional quaternary carbon at δ 73.3, assigned to C(6). Furthermore, the carbonyl group at C(6) was absent. The ^1H NMR displayed the 4'-methylene protons as two degenerate doublets, overlapping each other, at δ 2.35 and 2.44. The 5α-configuration had not changed, and again, the antiperiplanar arrangement with the 4-proton was reflected in a

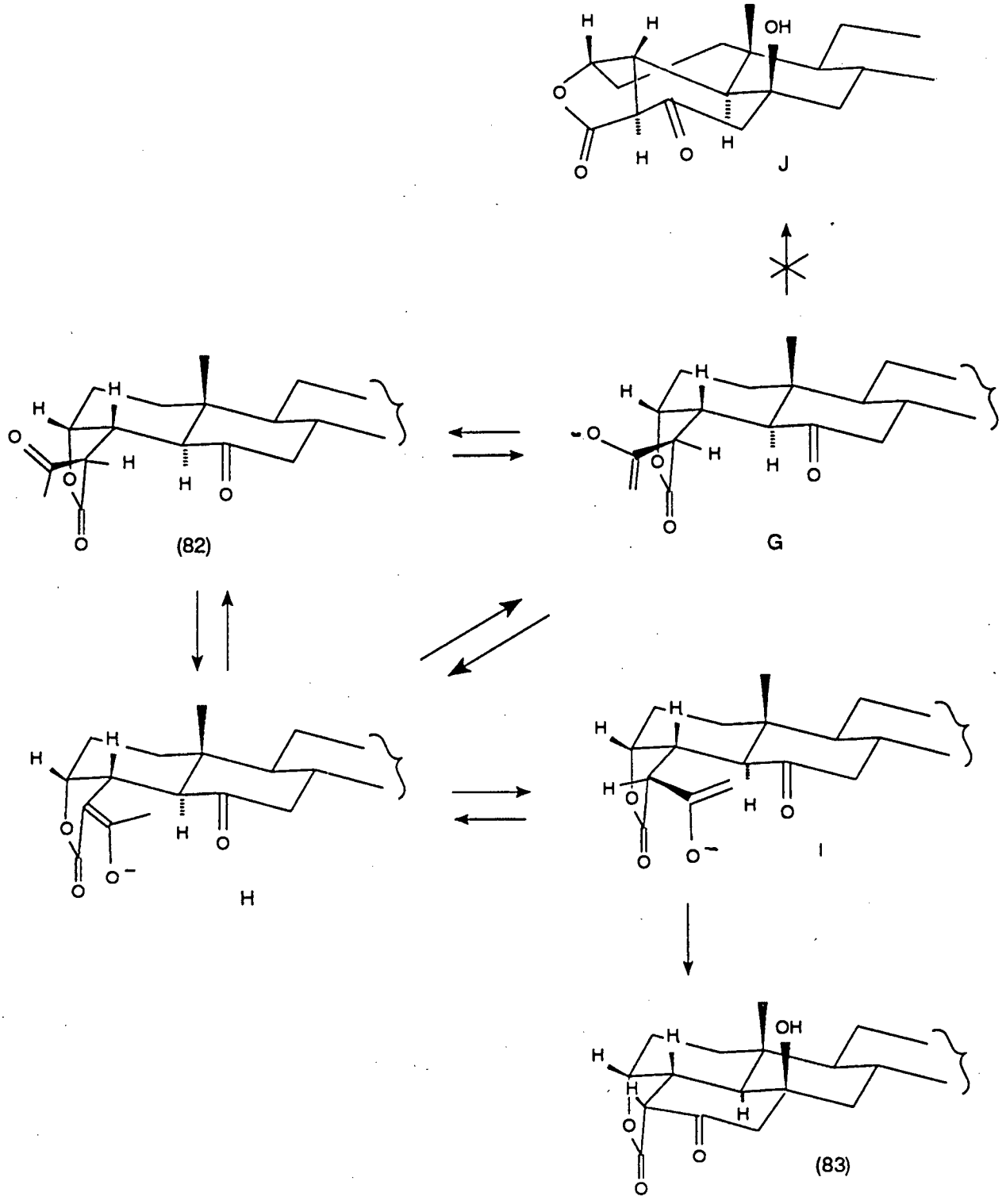
coupling constant of *ca* 12 Hz. It was further observed that the 6'-proton occupied a synclinal position with the 4ß-proton, as was reflected in a coupling constant of 5.7 Hz. Thus, it was concluded that isomerisation at C(2') of the precursor (**82**) had taken place, prior to aldol closure. This isomerisation was not observed for the analogous lactone (**81**), or at least, it did not lead to an analogous aldol closure at C(6). The structure and conformation of (**83**) was thus established as depicted in figure 4.

Fig. 4: structure and conformation of (**83**)



With this information in hand, it was possible to formulate the mechanism of the reaction (Scheme 33). Base treatment under the above conditions converted the lactone (**82**) into a series of enolised species at the acetyl moiety on the lactone ring. Species G cannot aldol-close because this closure would result in a highly strained species J, in which ring A would have to adopt a highly energetic twist conformation. It was very difficult to build a model according to the representation J. If however, a species H was formed, isomerisation can take place, and *via* keto-enol tautomerism, this species is transformed into species I, which can form the aldol product (**83**). The final product (**83**) adopts a stable all-chair conformation.

Scheme 33



For practical reasons, attempts were made to minimize the formation of lactone (**83**). Variation of the reaction temperatures (25, 50,

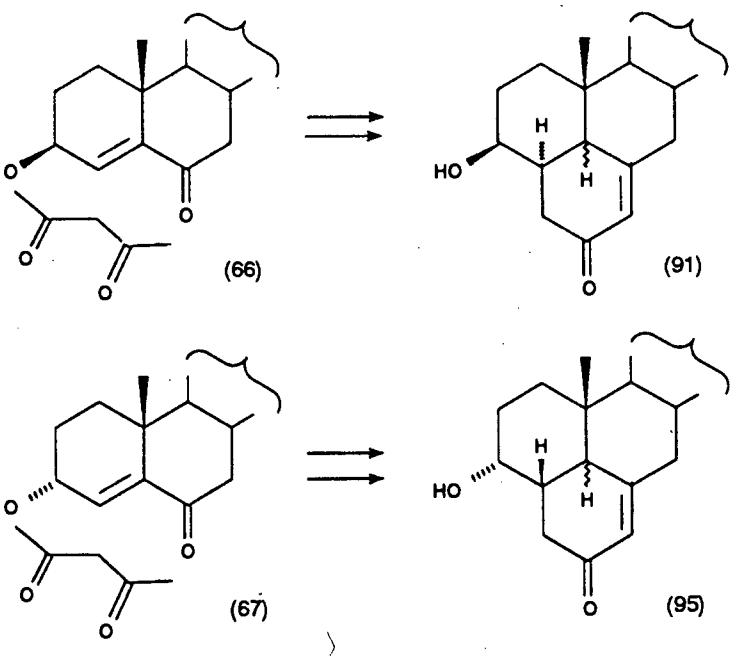
and 80°C) did not significantly affect the formation rate and amount of lactone (**83**). However, when the reaction was conducted at 40°C with 0.5 equivalents of potassium t-butoxide, it was possible to reduce the formation of (**83**) to a negligible amount (less than 10%). It appeared to be impossible to totally exclude the formation of (**83**), and the optimised yield for the formation of (**82**) was 93%. In view of the planned subsequent transformations (lactone cleavage, decarboxylation, aldol closure, and ß-elimination), the formation of (**83**) was not of any influence on the yields of the final products, since the same transformations, though possibly in an other order, would be attainable under the same conditions.

2. 3. Aldol condensation and tandem Michael-aldol reactions of intermediates

With the optimised reaction conditions for the intramolecular Michael additions of both 3ß- and 3α-acetoacetoxycholest-4-en-6-one in hand, it was now planned to study the various options to form the desired pentacyclic steroids.

Mechanistic considerations, especially drew attention to the pathway in which a series of successive transformations, i.e. lactone cleavage, decarboxylation, aldol closure and eventually ß-elimination would lead to the pentacyclic enones (**91**) and (**95**). It was realised that lactone cleavage, at least in the case of (**82**), did not necessarily need to take place to form the aldol closed derivative. If the pentacyclic enones (**91**) and (**95**) could be synthesised according to these preliminary considerations, it was reasonable to assume that it would then be possible to obtain these compounds in a 'one-pot preparation' from their respective acetoacetoxy precursors (Scheme 34).

Scheme 34



2.3.1. Reactions of the 3ß,4ß-lactone (81)

Preliminary investigations on this strategy were carried out on the 3ß,4ß-lactone. It was more attractive to start the investigations in the ß-series, as it was expected that some of the intermediates would be the 3-substituted (hydroxy or acetoxy) analogues of the series of products, prepared by Steer.[18,32] However, the emphasis would then be on the 3α,4α-lactone and precursors, since this represented an entirely novel series, and it was hoped that the observations made in the ß-series could then be extrapolated into this α-series.

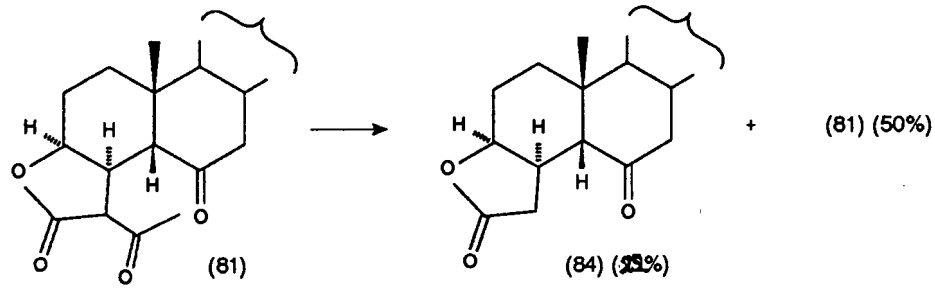
In a first series of experiments, it was decided to examine the problem of stereoselective assembly of the pentacyclic compounds stepwise. This would facilitate the design of a 'one-pot' reaction protocol

for the conversion of the 3ξ-acetoacetoxy-Δ^4-6-ketones, directly into the 3ξ-hydroxy-4ξ,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-ones.

Ruveda and co-workers[30] were able to induce an acid catalysed aldol reaction of their lactone intermediate (Scheme 26). Treatment of lactone (**81**) with a catalytic amount of toluene-*p*-sulphonic acid in anhydrous benzene at various reaction temperatures did not lead to the desired products, but led after prolonged reaction times to complex polar mixtures, which were not characterised. It was thus concluded that an acid catalysed aldol closure was not applicable to this system, probably because under these reaction conditions, the lactone ring could not be cleaved, and aldol closure at C(6) was thus not observed.

The stage was now set for investigations into various methods for lactone cleavage. The most straightforward way to achieve this goal was cleavage with a nucleophilic base. Treatment of lactone (**81**) with excess (10 equivalents) of aqueous potassium hydroxide in refluxing ethanol gave mainly starting material, though, when the reaction was prolonged to 42 h, t.l.c. displayed an incomplete reaction with one new product (49%) (Scheme 35). The spectral data of this product confirmed that again the γ-lactone ring was present, but that the acetyl group was absent. The product was thus formulated as the deacylated starting material 2(3ß-hydroxy-6-oxo-5ß-cholestan-4ß-yl)-acetic acid-1,3'-lactone (**84**).

Scheme 35



Reagents and conditions: Aq. KOH, EtOH, reflux, 42 h.

The structural evidence for this unexpected product was obvious from analytical and spectroscopic data. Thus, microanalytical and mass spectral data confirmed the loss of an acetyl group, whilst IR absorption at υ_{max} 1781 cm^{-1} revealed that the γ-lactone ring was still present. The loss of the acetyl group was corroborated by the absence of the appropriate methyl resonance (δ *ca* 2.07 as for (**81**)) in the ^{1}H NMR spectrum and corresponding resonances in the ^{13}C NMR spectrum. In addition, the similarity of the C(5), C(9), and C(19) resonances in (**81**) and (**84**) supported the retention of 5ß-configuration.

An analogous deacylation of a γ-lactone has been described by Koft and co-workers[31].

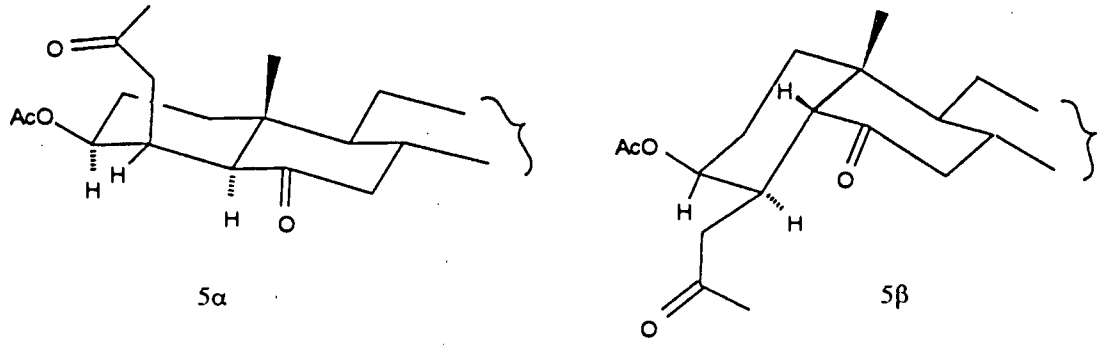
It was now clear that the lactone ring in (**81**) is very resistant to cleavage, and that, in order to prevent the competing deacylation, higher reaction temperatures were required to achieve a fast lactone cleavage. The medium chosen for that purpose was dioxane, and treatment of (**81**) with 10 equivalents of aqueous potassium hydroxide in refluxing dioxane led to the complete disappearance of the starting material (**81**) after 5 h. Furthermore, no trace of the lactone (**84**) was observed on t.l.c. Instead, two more polar, readily separable products had formed. The respective

products were isolated in yields of 35 and 34%, and were characterised as 3ß-hydroxy-4ß(2-oxopropyl)-5ß-cholestan-6-one (**85**) and 3ß,6-dihydroxy-4α,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**86**) in order of increasing polarity (Scheme 36).

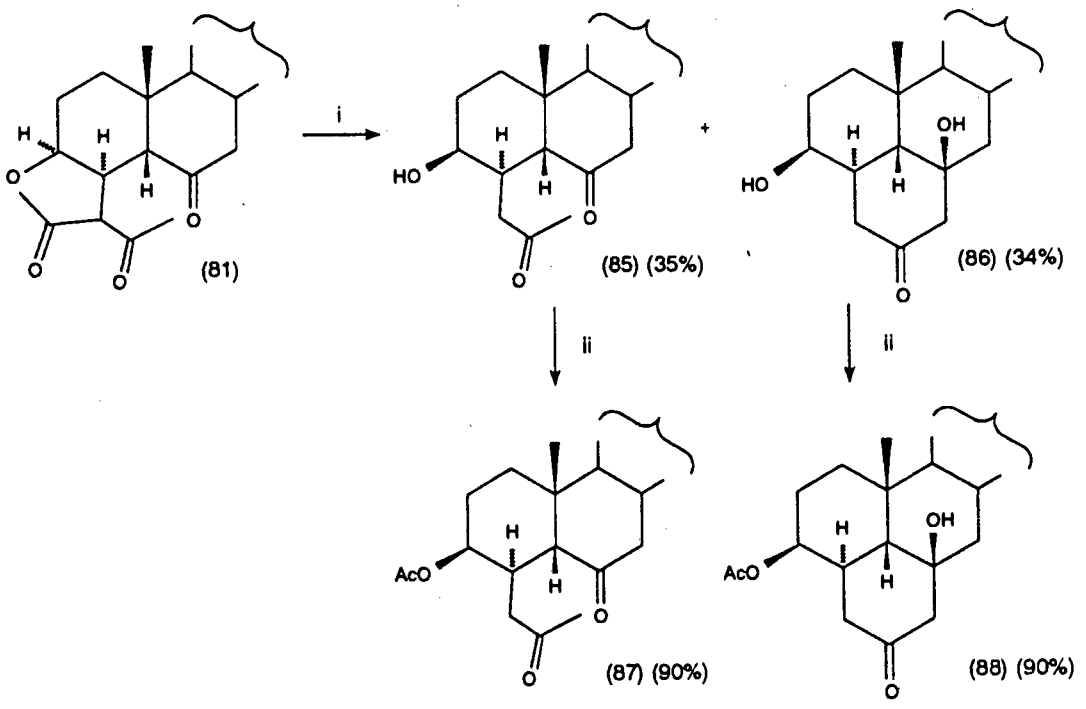
Product (**85**) was difficult to crystallise, but acetylation with acetic anhydride in pyridine proceeded smoothly to afford the 3-acetate (**87**) (89%). This derivative (**87**) was easily crystallisable and was fully characterised. Product (**86**) was acetylated under similar conditions to give (**88**) (90%), which was also fully characterised.

The IR spectrum of (**87**) displayed carbonyl absorption bands at υ_{max} 1725 (AcO), 1715 (6-C=O), and 1691 cm^{-1} (2'-C=O). The ^1H NMR signals for the terminal 3'-methyl group appeared at δ 2.06. The configuration at C(5) was confirmed by ^{13}C NMR and by comparison of the models of both possible configurations at C(5) (Figure 5). It was not possible to recognise the signal for the 5-proton in the ^1H NMR spectrum, however the ^{13}C NMR spectrum confirmed the ß stereochemistry (by comparison).[32]

Fig. 5: comparative representation of the possible C(5) isomers of (**87**)



Scheme 36

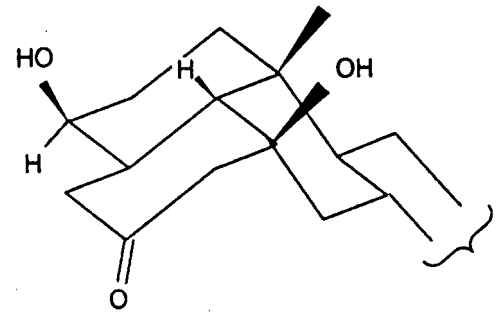


Reagents and conditions: i, Aq. KOH, dioxane, reflux, 5 h; ii, Ac_2O, py, 20°C, 4 h.

The complex multiplet at δ 2.80 in the ^1H NMR spectrum of (**87**) was confidently assigned to the 4α-proton on the basis of the extensive couplings with the neighbouring protons. The assignments of the other protons were not obvious, except for the 3α-proton, which indicated an axial acetoxy group, and hence again 5ß-configuration. The ^{13}C NMR spectrum of (**87**) displayed the C(19) signal at δ 23.6, while for the 5α-configuration, C(19) would be subject to two more γ-gauche interactions, which are known[33,34,35] to cause a shielding of *ca* 12 ppm. In the ß-case, this shielding is absent. Furthermore, in the 5ß-configuration, C(9) experiences two less γ-gauche interactions, and is therefore relatively deshielded by *ca* 12-15 ppm. The observed values for C(9) and C(19) were thus in accordance with the proposed 5ß-stereochemistry. From

these data, it was clear that (**85**) is a direct intermediate for the formation of (**86**), since (**86**) was recognised as the aldol closed product at C(6). Only one carbonyl absorption band was observed in the IR spectrum of (**86**), which appeared at υ_{max} 1708 cm^{-1}. ^1H NMR revealed the absence of the acetyl methyl peak at δ 2.07 of the precursors, while in the ^{13}C NMR spectrum, a new quaternary carbon signal was observed at δ 76.3, which was assigned to the hydroxy-substituted C(6). The configuration at C(5) had remained ß, since the ^{13}C NMR signal for C(19) was now strongly shifted downfield to δ 27.1, owing to the 1,3 diaxial interaction with the 6-hydroxyl group. The signal for C(9) was recognised at δ 40.55, a chemical shift which is in accordance with the above rules. The conformation of (**86**) is represented in Figure 6.

Fig. 6: conformation of (86)



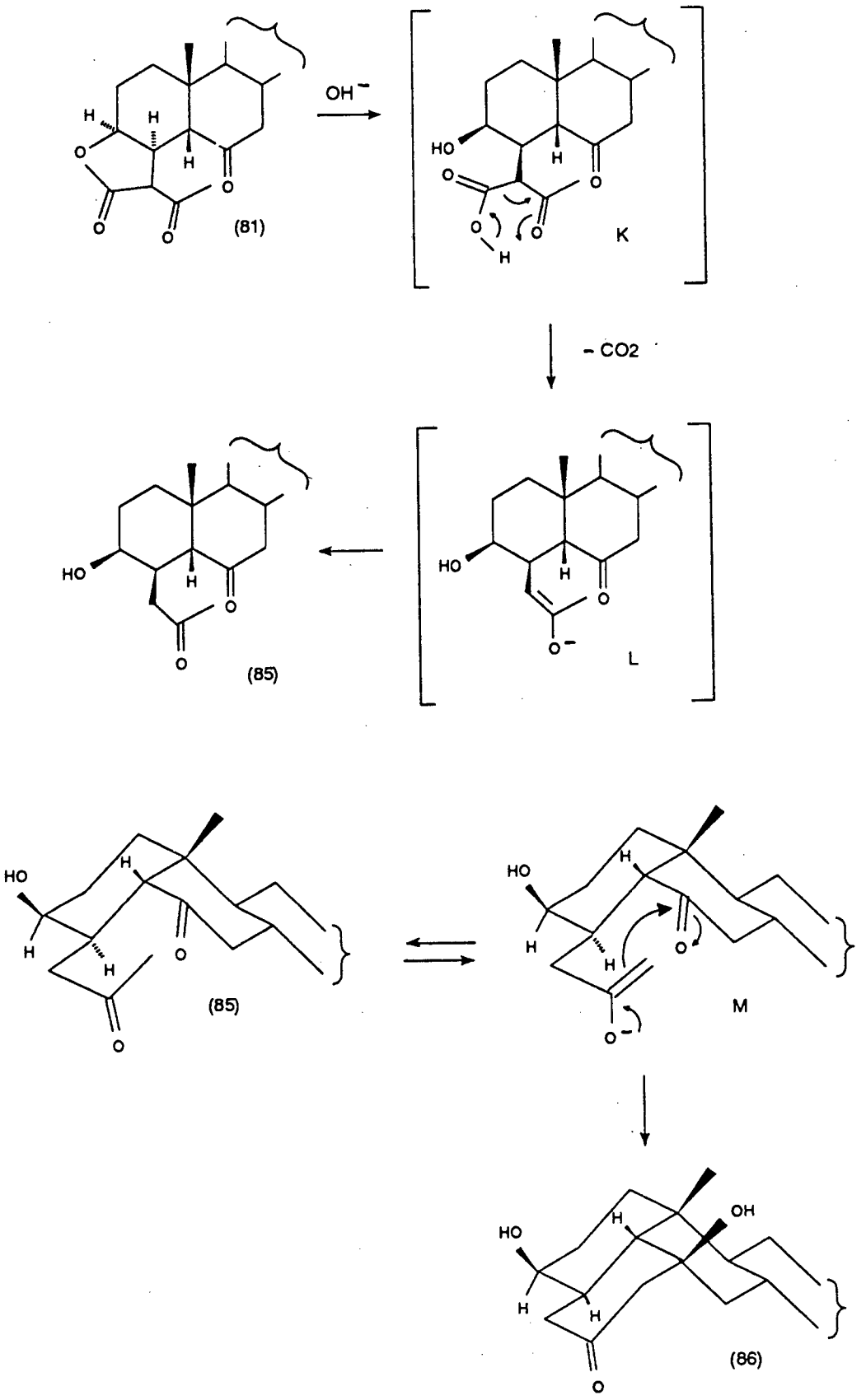
With these clear data, it was now possible to uncover the reaction mechanism for the formation of (**86**) (Scheme 37). A first step in the sequence involves the nucleophilic cleavage of the lactone ring, producing a short-lived (not detected on t.l.c.) carboxylic acid intermediate K. It was thus assumed that fast decarboxylation led to the enolate species L, which tautomerised to the ketone (**85**). The decarboxylation reaction is widely known to proceed mainly at elevated

temperatures, especially in aqueous media, since protonation must occur to achieve the six-membered cyclic transition state. The subsequent aldol closure seemed to be a problem under the above conditions, since no significant change in the ratio of (**85**) and (**86**) was observed upon prolonged reaction times. It was our goal, in view of the planned 'one-pot reaction', to obtain the aldol product (**86**) as the sole product of the reaction.

Accordingly, the aldol closure was examined on the isolated compound (**85**). When the intermediate (**85**) was treated with six equivalents of aqueous potassium hydroxide in ethanol at 0°C, the starting material was consumed after 4 h, and was completely converted into (**86**).

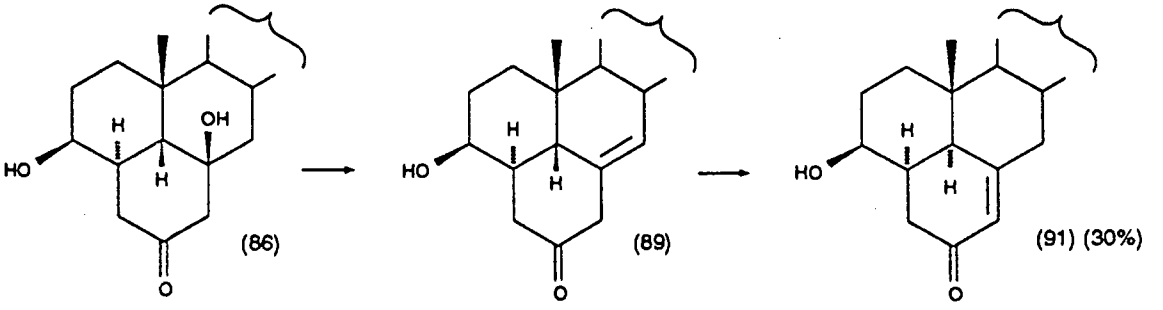
This result suggests that the rate-limiting step in the aldol closure is formation of a species M, the kinetic enolate. Evidently, the lower reaction temperature promotes formation of the kinetic enolate M, at the expense of the competing thermodynamic enolate. It was thus possible to convert the lactone (**81**) into (**86**) as the sole reaction product, by cooling the reaction mixture to *ca* 0°C, once the lactone cleavage had been achieved.

Scheme 37



A further step towards achieving a 'one-pot' conversion of the 3ß-acetoacetoxy enone (**66**) into (**91**) would require ß-elimination in (**86**). However, prolonged treatment of (**86**) with aqueous potassium hydroxide in ethanol, as described[32] for the analogous 3-deoxy compound, failed to produce the desired enone (**91**). Various solvent systems and reaction temperatures were tried, but prolonged treatment always led to gradual decomposition of the starting material (**86**). However, when (**86**) was treated with toluene-*p*-sulphonic acid in refluxing benzene, the enone (**91**) could eventually be isolated in *ca* 30% yield (Scheme 38).

Scheme 38



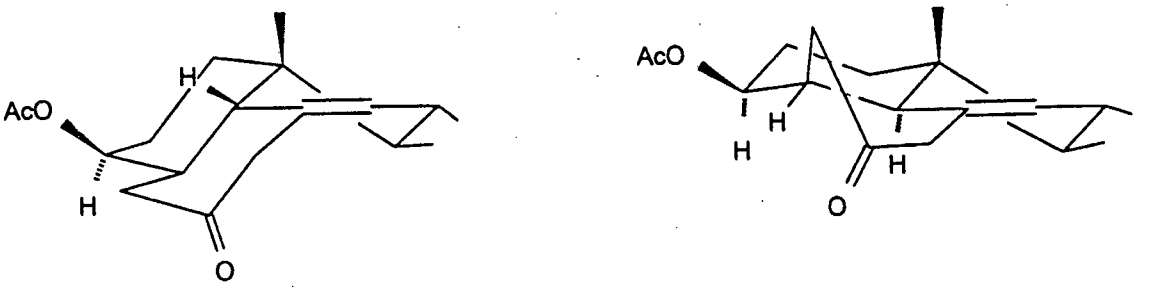
Reagents and conditions: *p*TsOH, benzene, reflux, 12 h.

The reaction proceeded with formation of two products during reaction times shorter than 12 h, and the ratio of the two gradually changed in favour of the formation of (**91**). The less polar intermediate for the formation of the enone (**91**) was found to be 3ß-hydroxy-4α,4',5ß,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'*H*)-one (**89**).

A product of that nature had also been encountered in analogous work.[17,32] The structure and conformation could be assigned by comparison of the observed with those reported for the corresponding 3-

deoxy compound.[32] The mass spectrum of (**89**) displayed a molecular ion at *M* 440, and the IR spectrum displayed carbonyl absorption at υ_{max} 1710 cm^{-1} for a saturated cyclohexanone. Further evidence was obtained from the derived 3-acetate (**90**), which showed the presence of a saturated carbonyl group at υ_{max} 1708 cm^{-1}, an olefinic proton signal in the ^1H NMR spectrum at δ 5.25, and evidence of 5ß configuration derived from the axial substitution of the 3-acetoxy group. The 3α-proton signal had $W_{1/2}$ 7 Hz, which is diagnostic for equatorial orientation, since antiperiplanar relationship of the 3α- and 2ß-proton in the alternative orientation would lead to a broader signal (Figure 7).

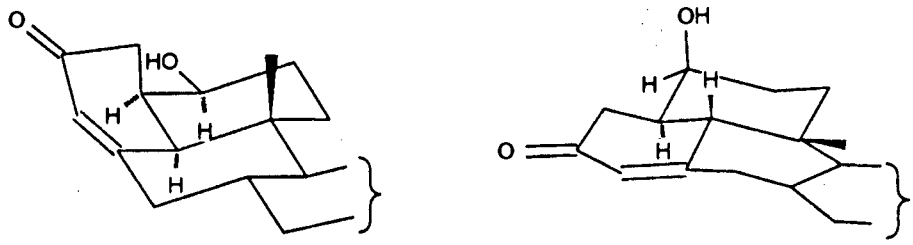
Fig.7: comparative representation of the two isomeric compounds at C(5) of the acetylated compound (**90**)



The more polar product was formulated as 3ß-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**91**). The IR spectrum displayed the enone absorption at υ_{max} 1652 (C=O) and 1625 (C=C) cm^{-1}. The ^1H NMR spectrum displayed a singlet at δ 5.90, which is characteristic for the 4'-olefinic proton. The ^{13}C NMR signals at δ 124.3, 165.7, and 201.5 were assigned to C(4'), C(6), and C(5') respectively. In this case, the NMR data supported the assignment of 5α-configuration. Thus, the 3ß-acetoxy group is equatorial, since the 3α-proton appeared as a partially resolved multiplet ($W_{1/2}$ 18 Hz), consistent with axial

orientation. In addition, a COSY plot made possible the assignment of a doublet at δ 2.03 to the 5α-proton, of which the coupling to the 4α-proton (*J* 5.2 Hz) is consistent with a synclinal relationship (Figure 8).

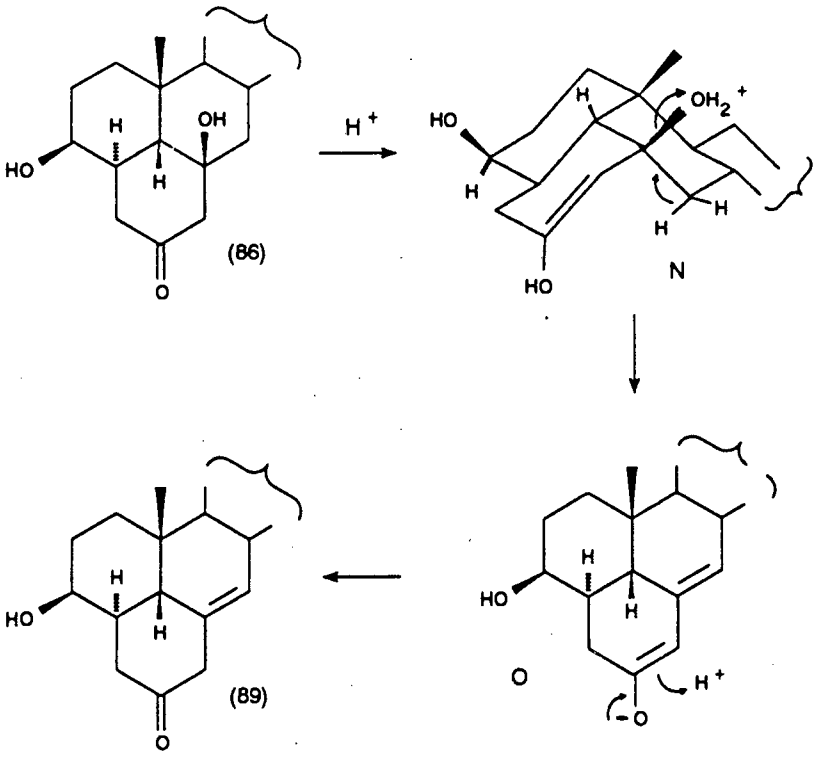
Fig. 8: comparative representation of the C(5) isomers of enone (**91**)



5α 5β

With the reaction products fully characterised, it was possible to propose the likely sequence of events during the reaction of (**89**) (Scheme 39 and 41). Protonation of the tertiary hydroxy group, with possible enolisation of the 5'-carbonyl group (N), would facilitate kinetically controlled dehydration, followed by reprotonation of the dienol at C(4'), presumably also kinetically controlled. The subsequent transformation of (**89**) into the conjugate enone (**91**) is slow and inefficient, and it is assumed that formation of the homoannular dienol (Q) by isomerisation of (P) is slow, and competes poorly with protonation of (O). However, it is assumed that thermodynamic control results in 5α-protonation of (O), leading eventually to the conjugate enone (**91**).

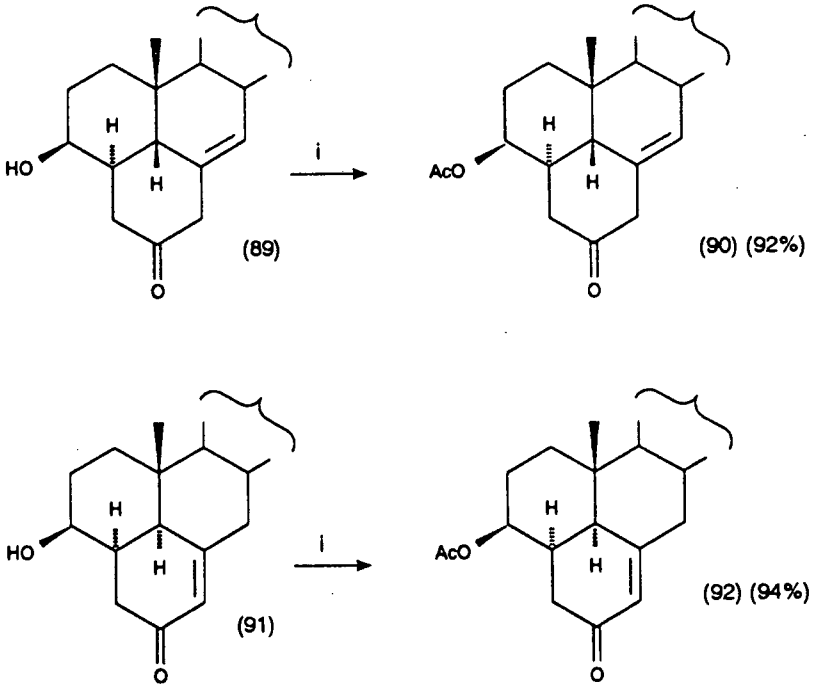
Scheme 39



When the derivatised 3-acetate (**87**) was treated under the same conditions, acetoxy hydrolysis was faster than the formation of the conjugated enone (**91**). It was in that way not possible to study the effect of the 3-hydroxyl group on the reaction mechanism, and no further attempts were done to achieve the 3-acetoxy-enone (**92**) from (**87**).

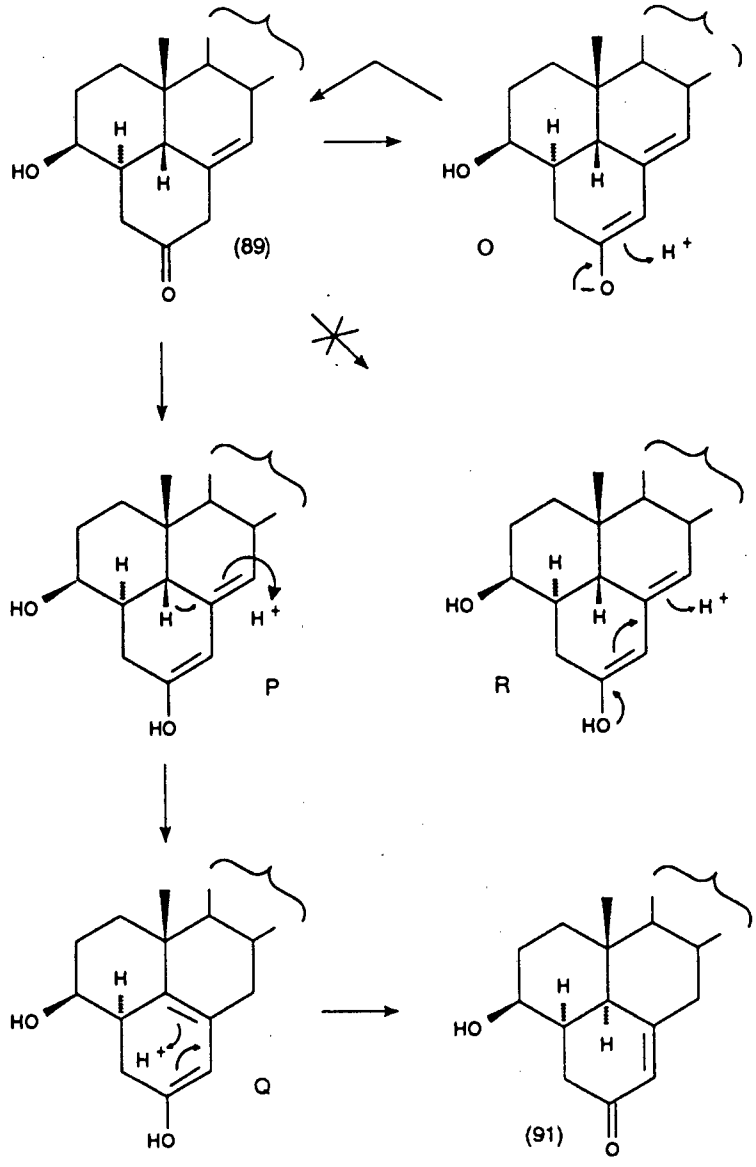
The respective 3-acetoxy derivatives were obtained by treating (**89**) and (**91**) with acetic anhydride in pyridine to yield (**90**) and (**92**) in 92 and 94% respectively (Scheme 40).

Scheme 40



Reagents and conditions: i, Ac_2O, py, 40°C, 10 h.

Scheme 41



The foregoing information upon the discrete steps in the conversion of the 3ß-acetoacetoxy-Δ^4-6-ketone (**66**) into the pentacyclic enone (**91**) enabled a reaction protocol to be designed in order to achieve a 'one-pot' reaction. Thus, the starting material (**66**) was dissolved in dioxane, aqueous potassium carbonate was added, and the reaction temperature was maintained at *ca* 40°C until all starting material was consumed (t.l.c.). Thereupon, aqueous potassium hydroxide was added

(*ca* 8 equivalents), and the reaction mixture was heated to reflux temperature, until lactone cleavage was complete. The reaction mixture was then cooled and maintained at 0°C until a single product was observed (t.l.c.). The reaction mixture was then acidified with toluene-*p*-sulphonic acid, and refluxed untill the enone (**91**) was present in the reaction mixture (t.l.c.). In this way, an overall conversion of (**66**) into (**91**) of *ca* 20% was achieved without isolation of intermediates.

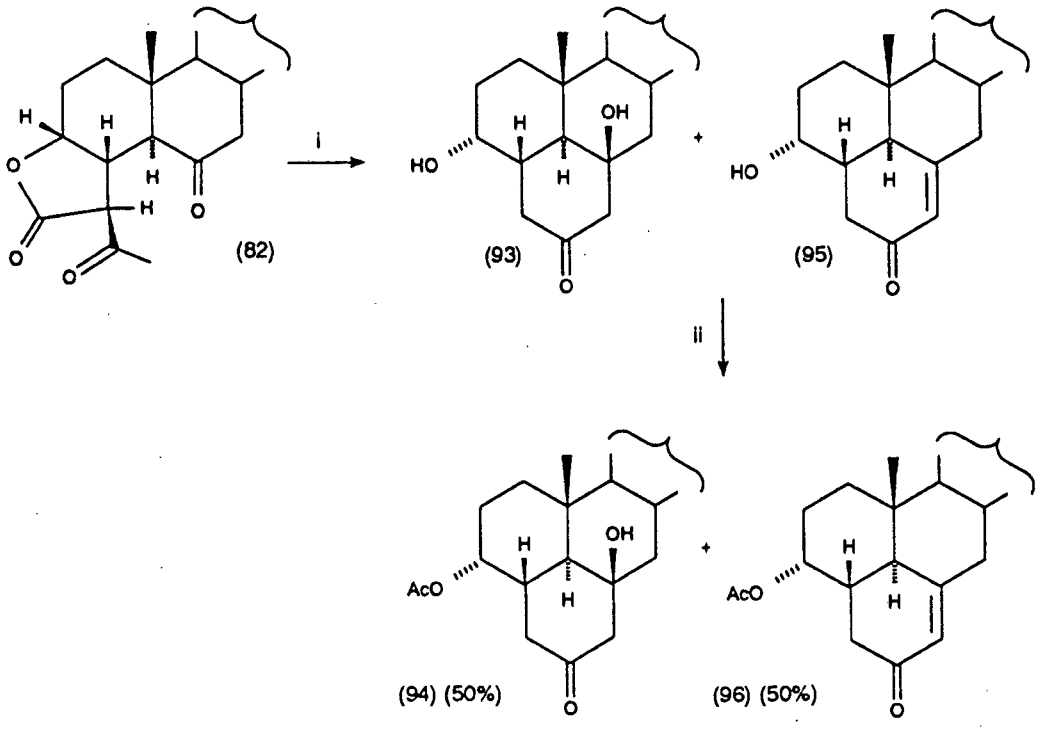
2.3.2. Reactions of the 3α,4α-lactone (82)

Attention was now given to conduct a similar series of reactions in the α-series. When lactone (**82**) was treated with toluene-*p*-sulphonic acid in ethanol at either 20°C or at reflux temperature, no reaction was observed. The lactone (**82**) also proved to be resistant to treatment with toluene-*p*-sulphonic acid. Accordingly, it was concluded that steric constraints similar to those encountered with the isomeric lactone (**81**) militated against acid-mediated aldol condensation prior to lactone opening and decarboxylation.

Here too, a strategy based upon sequential lactone cleavage, decarboxylation, aldol closure, and eventual ß-elimination had to be envisaged. When the lactone (**82**) was treated with aqueous potassium hydroxide in ethanol or dimethylformamide at reflux, the starting material (**82**) was rapidly consumed. The reaction in ethanol was preferred above dimethylformamide as the medium, since the reaction was cleaner, though slower, and the work-up procedure more straightforward. The reaction mixture comprised two major products of very similar polarity (t.l.c.). However, acetylation of the mixture with acetic anhydride-pyridine, followed by chromatography, afforded readily separable products, formulated as 3α-acetoxy-6-hydroxy-4ß,4',5α,6ß-

tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**94**) (50%), and 3α-acetoxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**96**) (50%) (Scheme 42). The structure of (**94**) followed from the interpretation of the spectroscopic and analytical data.

Scheme 42

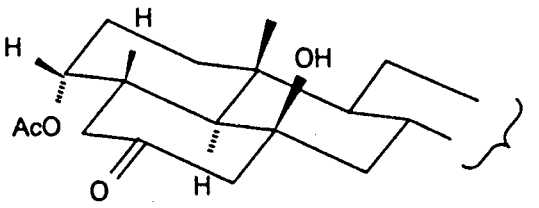


Reagents and conditions: i, Aq. KOH, DMF or EtOH, reflux, 1h; ii, Ac_2O, py, 40°C, 10 h.

Thus, IR absorption at υ_{max} 1723 and 1712 cm^{-1} was assigned to the 3-acetoxy and 5'-carbonyl groups respectively. The ^1H NMR spectrum displayed diagnostic signals for the 5α-configuration; thus the axial orientation of the 3α-acetoxy group was evident from the signal for the 3ß-proton ($W_{1/2}$ 8 Hz), and a sharp doublet at δ 1.66 (*J* 12.1 Hz), assigned to the 5α-proton showed the expected antiperiplanar relationship with the 4ß-proton. The configuration at C(6) was

confidently assigned as a 6ß-configuration, as judged from the deshielding influence of 6ß-hydroxy upon the signal for the C(10) methyl group (δ 1.03). The conformation of (**94**) is depicted in Figure 9.

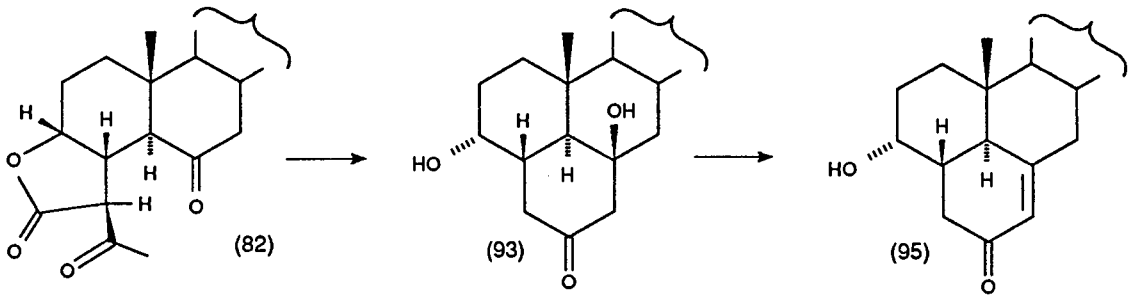
Fig.9: structure and conformation of (94)



The other product was clearly an enone, since the IR spectrum exhibited the typical enone absorption bands at υ_{max} 1659 and 1614 (C=O and C=C respectively) cm^{-1}. The ^1H NMR spectrum displayed an olefinic proton signal at δ 5.82.

The reaction pattern was thus found to be different from the one observed in the ß-series. Analogous intermediates, such as the cleaved and decarboxylated lactone were not observed, and the reaction proceeded smoothly to give, after prolonged reaction times essentially one product, the desired 3α-hydroxy-enone (**95**). The reaction sequence is outlined in Scheme 43.

Scheme 43

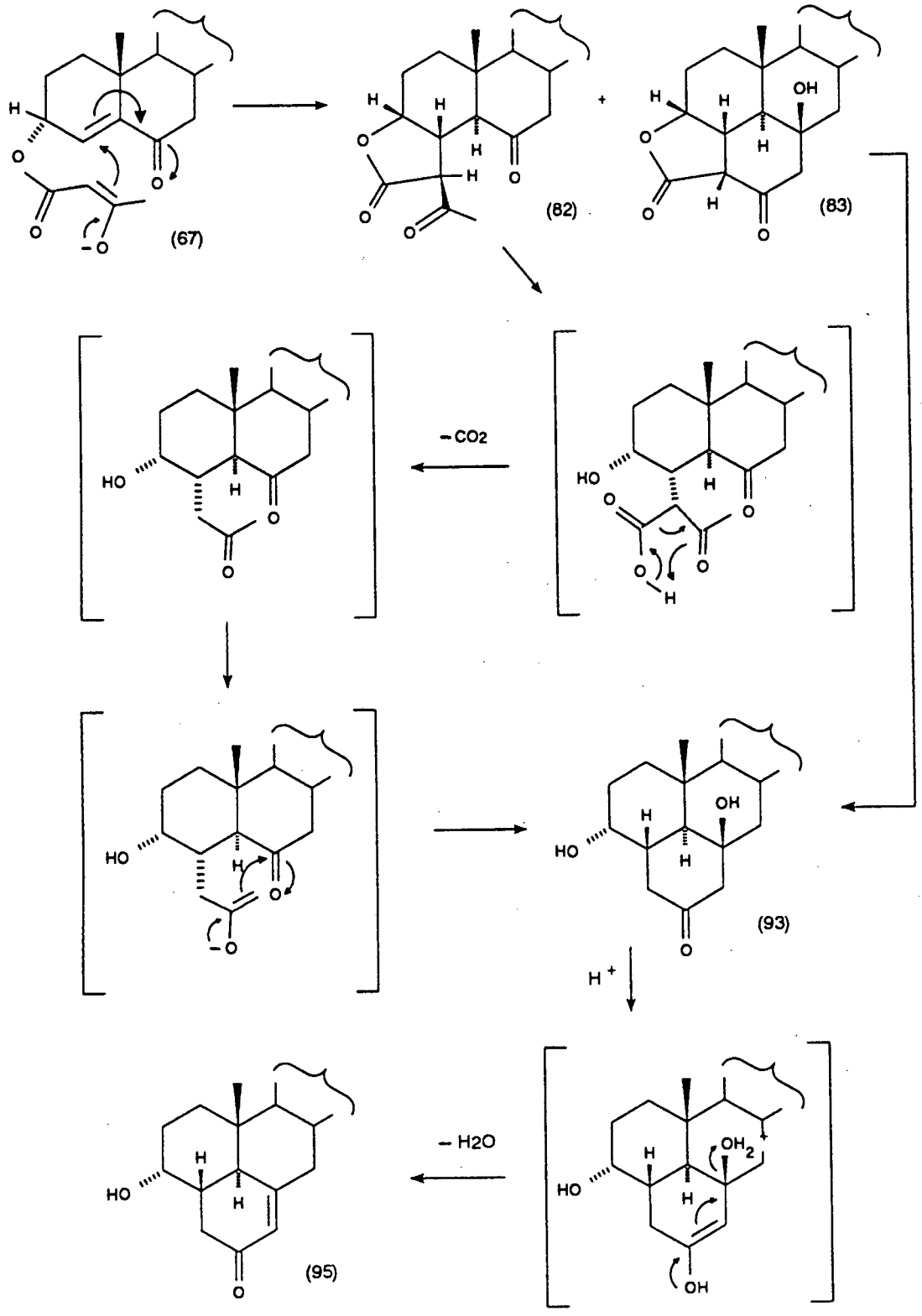


The final step in the reaction appeared to be rate-limiting, i.e. ß-elimination of the oxo-diol (**93**), was involved to give the enone (**95**). Prolonged alkaline treatment of (**93**) led ultimately to (**95**). However, treatment of (**93**) with *ca* one equivalent of toluene-*p*-sulphonic acid in refluxing ethanol, produced the enone (**95**) in a yield of 70%. The same reaction conditions could be applied on the 3-acetoxy derivative, to obtain (**96**) from (**94**) in comparable yields.

The foregoing observations made it possible to design a 'one-pot' procedure for conversion of the 3α-acetoacetoxy-Δ^4-6-ketone (**67**) into the enone (**95**). 3α-Acetoacetoxy-Δ^4-6-ketone (**67**) was dissolved in a small amount of tetrahydrofuran, and added dropwise to a solution of potassium butoxide in t-butyl alcohol and tetrahydrofuran (4:1). After completion of the Michael reaction (t.l.c.), a small amount of water (*ca* 5% of the total volume), and an additional amount of aqueous potassium hydroxide were added in order to adjust the base-level to *ca* 6 equivalents. The reaction mixture was heated to reflux temperature, and after one h under reflux, the lactone (82) had been consumed. Toluene-*p*-sulphonic acid was added to the reaction mixture, to ensure *ca* five equivalents of residual acid, and the reaction was allowed to proceed at 50°C for 12 hrs. The final product was isolated in a very satisfactory

overall yield of 72%. The result suggests that the five consecutive steps Michael addition, lactone cleavage, decarboxylation, tautomerisation and aldol closure, and ß-elimination each proceeded efficiently (each in more than 95% yield) under the above conditions. The complete set of events is outlined in Scheme 44.

Scheme 44

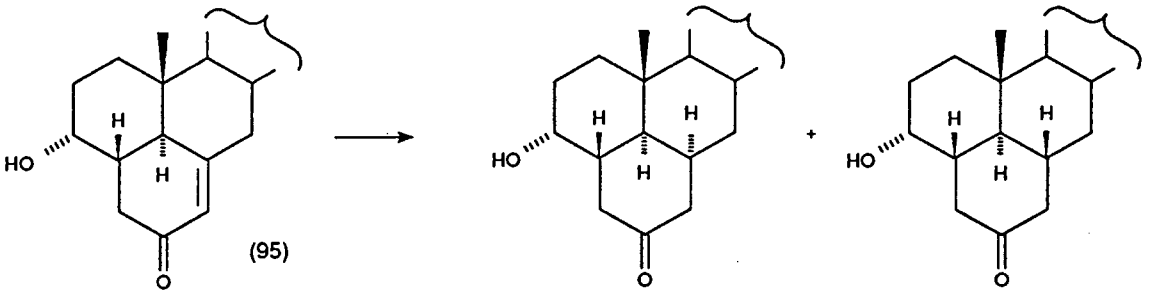


3. Reactions of the enones

3.1. Reductions of the 4ß(H)-enone

The 4ß(H)-enone (**95**) provided a possible entry to the 4ß(H),6α(H)- and 4ß(H),6ß(H)-isomers (Scheme 45) by stereoselective reduction of the double bond.

Scheme 45

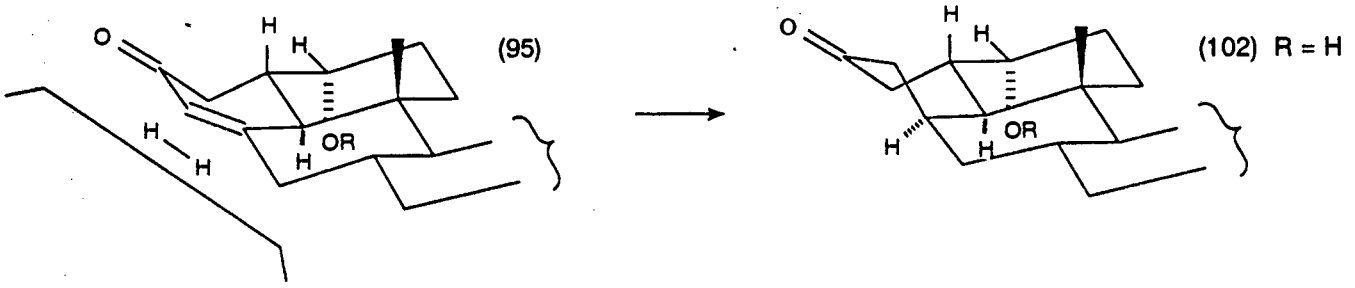


Catalytic hydrogenation is one of the most straightforward methods used for the reduction of a double bond in an enone system. By careful choice of the catalyst, it is possible to selectively reduce the double bond without reduction of the carbonyl function. Catalysts such as palladium on carbon, or the milder palladium on calcium carbonate can be utilised because of the higher reactivity of the conjugated double bond in comparison with an isolated double bond,[36] thus preventing possible reduction of the carbonyl group.

Hydrogenation takes place by adsorption of the compound to the catalyst surface, followed by *cis*-addition of two hydrogen atoms. Adsorption onto the catalyst surface is largely controlled by steric factors, resulting in hydrogenation from the less hindered side. In steroids, hydrogenation generally takes place from the α-face, because of the steric hindrance to the approach of the catalyst from the ß-face in

the presence of the C(10) and C(13) angular methyl groups.[37,38] Exceptions to this 'rule of α-attack' occur when the steric effect of the angular methyl groups, especially the C(10) methyl group is not dominant and there is abnormal steric hindrance on the α-face (e.g. ring A in 5ß-steroids).[38] It was thus expected that the approach to the catalyst on the ß-face of the 4ß(H),5α(H)-enone (**95**) would be inhibited by the presence of the C(10) angular methyl group (Scheme 46), and thus hydrogenation should occur preferentially from the α-face.

Scheme 46



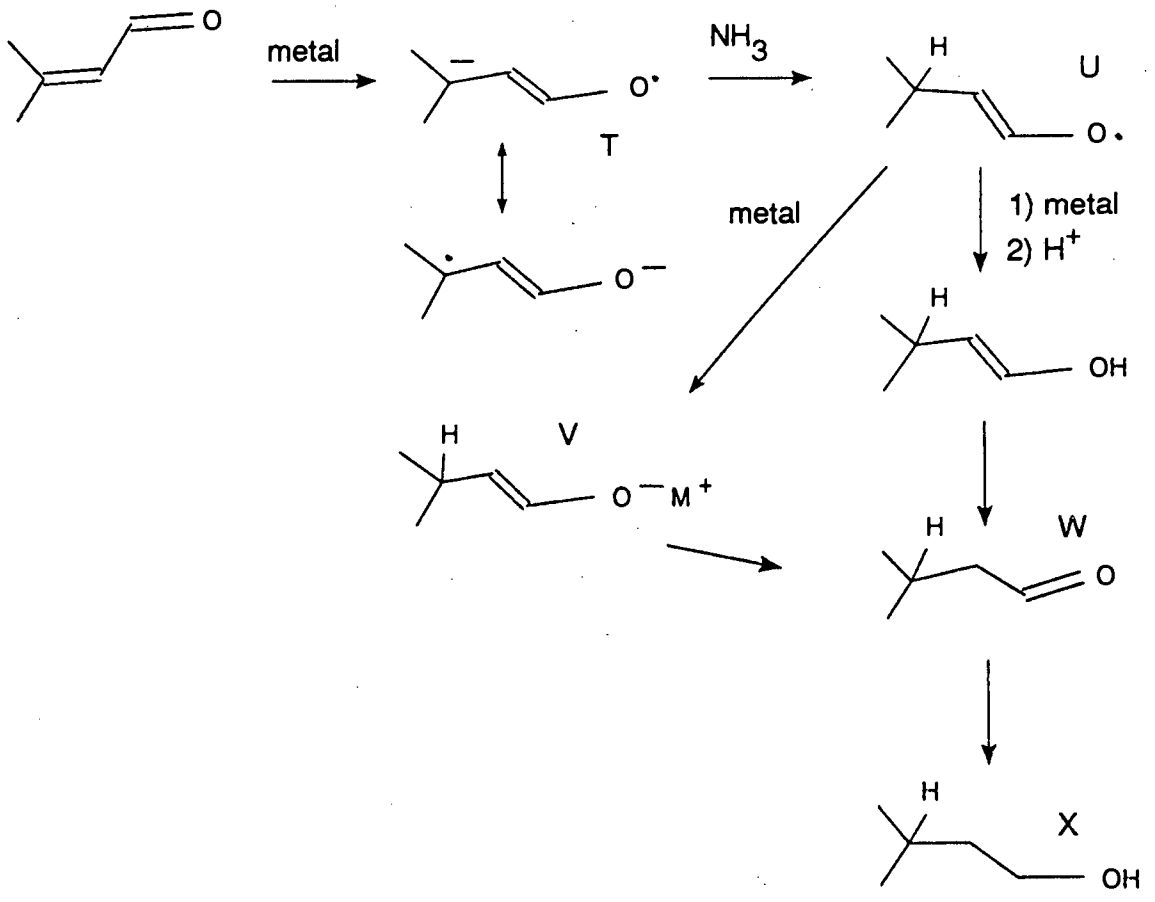
In this case however, the resultant α-hydrogenated ring E at C(6) would adopt an obligatory twist conformation and, since this conformation is energetically disfavoured, some doubt remained on whether the 4ß,5α,6α-isomer would be obtained as the sole product of the catalytic hydrogenation.

The stereochemistry of hydrogenation is also importantly affected by the presence of polar functional groups that can govern the mode of adsorption of the molecule to the catalyst surface. For instance, there are a number of examples [39,40] where the presence of a hydroxyl group results in hydrogen being introduced from the side of the molecule carrying the polar group. This implies that the molecule is adsorbed preferentially in such a way that the hydroxyl group can interact strongly

with the catalyst surface. Therefore, in the case of catalytic hydrogenation of (**95**), steric factors supported by the possible complexation of the 3α-hydroxy group, suggested that α-face attack might be favoured.

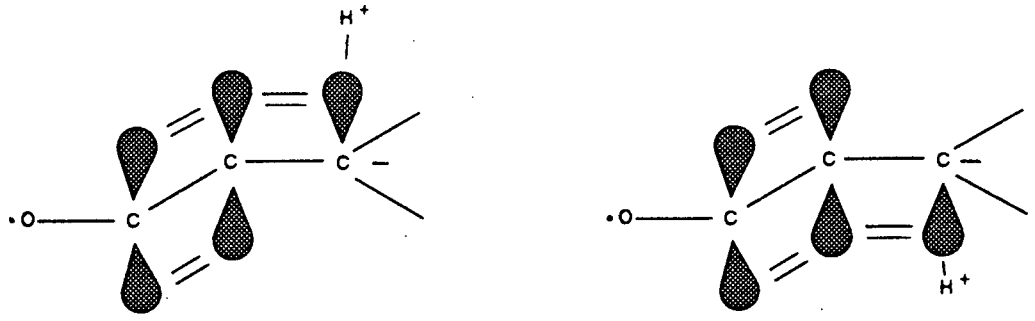
Dissolving metal reductions in liquid ammonia can also be used for the selective reduction of a double bond in an enone system. These reductions involve a more complex procedure than the catalytic hydrogenation and are carried out at the boiling point of ammonia (-33°C). Since the solubility of many compounds in liquid ammonia is low at this temperature, a co-solvent such as tetrahydrofuran is used to aid solubility. The reduction may be conducted in the presence or absence of a proton donor, e.g. t-butyl alcohol. The initial stage of the reduction is the addition of an electron to form a mesomeric radical anion T (Scheme 47), which is sufficiently basic to abstract a proton from ammonia. The stereochemistry of the final product is established at that stage. The enolate radical U accepts another electron to form the enolate anion V which is stabilised as a lithium salt. Acidification during work-up affords the saturated ketone W. In the presence of an excess of proton donor, the radical anion T will be rapidly protonated to form U. Transfer of another electron from the solution will result in an enolate anion which would undergo α-protonation in the presence of a proton donor to form the saturated ketone W. Further reduction in the metal dissolving liquid ammonia solution of W affords the saturated alcohol X.

Scheme 47



Generally, reductions of α,ß-unsaturated ketones in which two products epimeric at the ß-carbon are possible, afford the thermodynamically more stable isomer.[36] The radical anion T will adopt the conformation of lowest energy prior to protonation. This conformation must allow continuous overlap of the p-orbital on the ß-carbon atom with the orbital system of the enolate radical (Figure 10). Thus protonation of the ß-carbon is controlled by stereoelectronic factors, the ß-hydrogen being introduced perpendicular to the plane of the radical anion.

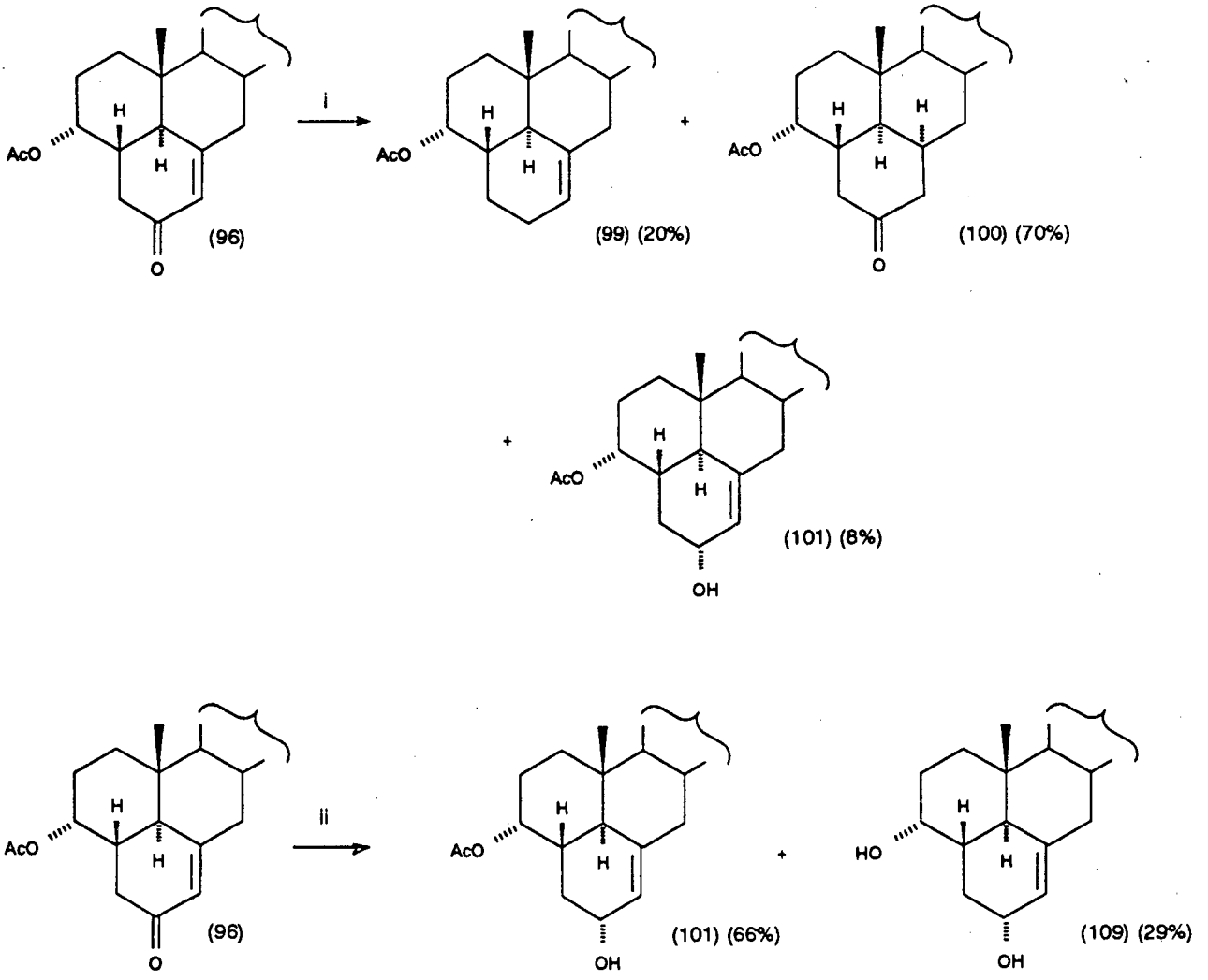
Fig. 10



It is possible, according to the above considerations, that the stereochemistry resulting from the metal dissolving liquid ammonia reduction will be different from that of the catalytic hydrogenation, since one would expect the more stable chair conformation for ring E. However, Stork and Darling[41] have shown that the saturated ketone afforded by a metal dissolving liquid ammonia reduction is not always the more stable of the two possible isomers. Continuous overlap of the orbitals during the protonation step is the most important criterion for the stereochemical outcome. Also steric hindrance to the close approach of the proton carrier to the anion can prevent protonation to the more stable isomer. Accordingly, the stereochemical outcome of the metal dissolving liquid ammonia reduction cannot be predicted with confidence.

Catalytic hydrogenation of the 3α-acetoxy-enone (**96**), using palladium on carbon (5%) and tetrahydrofuran as solvent, was conducted at 20°C under atmospheric pressure over 15 h. The major product of the reaction was the expected and desired 4ß,5α,6α-5'-ketone (**100**) (70%), together with the minor products (**99**) (20%), and (**101**) (8%) (Scheme 48).

Scheme 48



Reagents and conditions: i, 5% Pd on C, THF, 20°C, 10 h; ii, CuI, MeLi, HMPA, DIBAL-H, -78°C, 1h, then, 20°C, 2h.

The IR spectrum of the major product (**100**), exhibited the characteristic carbonyl absorption at υ_{max} 1700 cm^{-1}. The ^1H NMR spectrum displayed the unresolved multiplet ($W_{1/2}$ 6 Hz) for the 3ß-proton at δ 3.72. The only other isolated resonance was a triplet at δ 2.62, which could only be uniquely assigned to the 6'α-proton signal, since the geminal coupling with the 6'ß-proton and the *trans*-coupling

with the 4ß-proton was reflected in the coupling constant (2 x 13 Hz). A complex two-proton multiplet at δ 2.19 ($W_{1/2}$ 45 Hz) was assigned tentatively to the overlapping resonances of the 6'ß- and 4'ß-protons, since only the geminal and axial-equatorial coupling of the 6'ß-proton and the geminal and *trans*-coupling of the 4'ß-proton could match up to the observed width of the multiplet. Apart from the methyl signals, the rest of the ^1H NMR spectrum was too complex to be interpreted. Clear evidence for 6-configuration was not found in the ^1H NMR spectrum, since either configuration was expected to give similar proton splittings. The ^{13}C NMR evidence was also inconclusive, and comparative data for analogous ring systems could not be found in the literature. The C(8) signal was expected to display an upfield shift in comparison to the all-chair conformation, since one more γ-gauche interaction is created in the twist conformation. However since C(8) is tertiary, this shift was very small. Conclusive evidence for the configuration was obtained from from subsequent transformations (see later). Two minor products were also isolated from the hydrogenation experiment. The least polar product was assigned as 4ß,5α,5',6'-tetrahydrobenzo[4.5.6]cholestan-3α-yl acetate (**99**) (20%), and the 5'α-hydroxy-steroid (**101**) (8%). The IR spectrum of (**99**) showed only the acetoxy absorption band. The ^1H NMR spectrum confirmed the presence of the olefinic 4'-proton at δ 5.38 ($W_{1/2}$ 10 Hz). Unambiguous data for retention of the olefinic bond in (**99**) also came from the ^{13}C NMR spectrum, which displayed signals for a tertiary carbon at δ 120.7, and a quaternary carbon at δ 137.2, assignable to C(4') and C(6) respectively. The allylic alcohol (**101**), similarly showed spectroscopic properties which confirmed retention of the olefinic bond, but in addition IR absorption at υ_{max} 3597 cm^{-1}, and a ^1H NMR signal for the 5'-proton at δ 4.28 ($W_{1/2}$ 20 Hz). ^{13}C NMR also confirmed that the 5'-

carbonyl group had undergone reduction to a 5'-OH group, with the appearance of a doublet at δ 67.9.

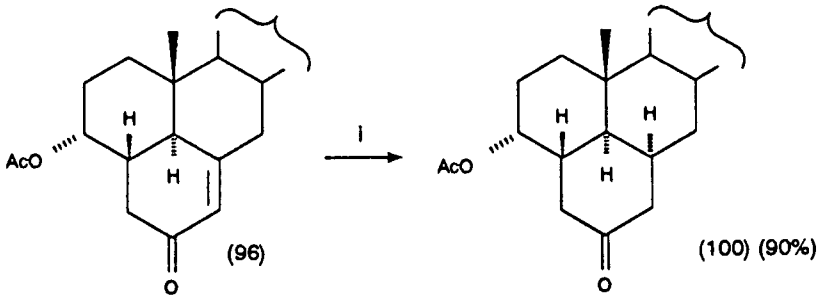
Formation of (**99**) and (**101**) was unexpected, since the usual course of reactions of enones is chemoselective reduction of the olefinic bond, with occasional over-reduction or hydrogenolysis of the carbonyl group. Expected by-products were either the product with a fully saturated ring E, or the saturated diol. Recrystallisation of the starting material (**96**) had no significant effect on the ratio of formation of the observed products. The only explanation for this reaction course was that competing reduction of the carbonyl group, was facilitated by steric hindrance to reduction of the olefinic bond.

The configuration at C(5') in (**101**) was assigned as the 5'α-H configuration, through direct comparison with the major product obtained *via* DIBAL-H reduction of (**96**); a mixed melting point was undepressed, and the ^{1}H and ^{13}C NMR data were identical. In an experiment, referred to as the attempted conjugate reduction with MeCu/DIBAL-HMPA in the experimental part, it was attempted to use a milder way for the conjugate reduction[43] of the 3α-acetoxy-enone (**96**) before the lithium liquid ammonia reduction would be used for the same purpose. However, this method proved unsuccessful, and when the reaction mixture was allowed to warm to 20°C, two products were observed. The major product was (**101**), isolated in 66% yield, while the minor product was the hydrolysed product (**109**), isolated in a yield of 29%. These products have thus arisen from the DIBAL-H reduction of the starting material (**96**), together with a partial hydrolysis of the acetoxy functionality at C(3) (Scheme 48).

When the catalytic hydrogenation of (**96**) was repeated in the presence of the milder palladium on calcium carbonate as the catalyst, the reaction pattern was significantly different. Hydrogen uptake was

significantly slower (24 h), and only the desired saturated ketone (**100**) was now isolated in a yield of 92% (Scheme 49). This method of choice for the preparation thus proved to be more efficient to obtain a high yield of the ketone (**100**).

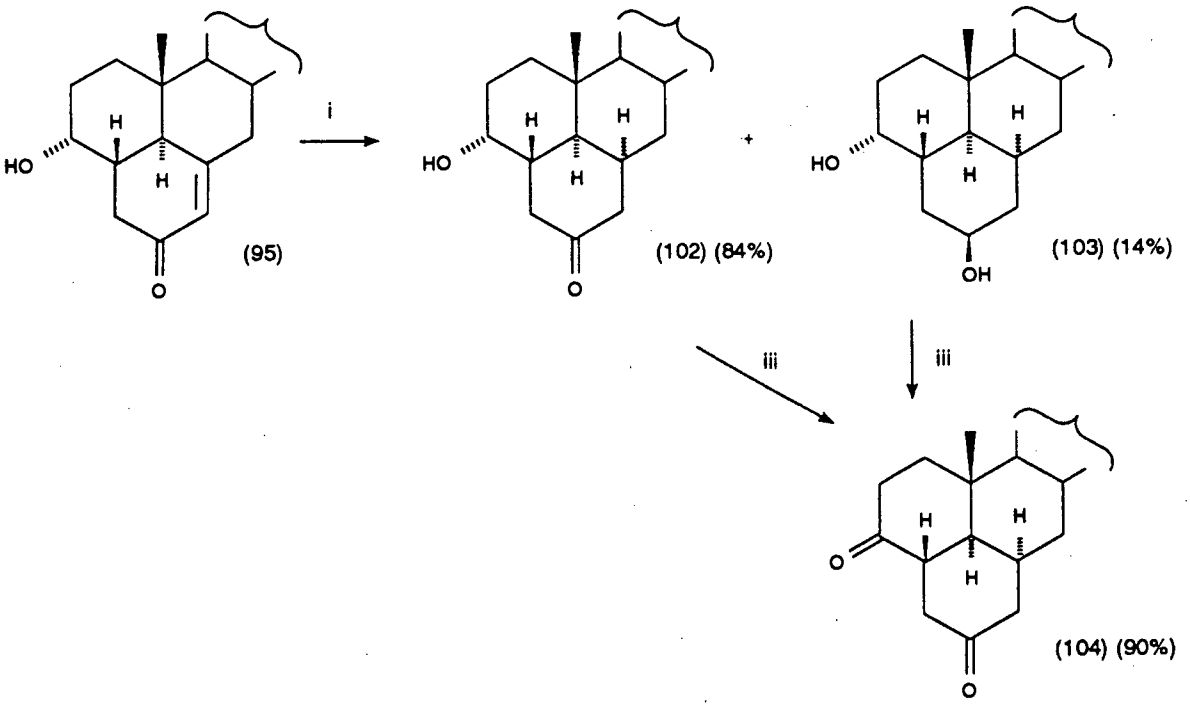
Scheme 49



Reagents and conditions: i, 5% Pd on $CaCO_3$, THF, 20°C, 24 h.

It was of interest to ascertain whether the nature of the substituent at C(3) would influence the course of the catalytic hydrogenation. Therefore, the reaction was repeated on the 3α-hydroxy-enone (**95**). After hydrogenation for 24 h at 20°C, two products were isolated (Scheme 50). The major product was the 3α-hydroxy-4ß,5α,6α-5'-ketone (**102**), identical to the product of the alkaline hydrolysis of (**100**), and a minor product, formulated as the saturated diol (**103**), which was isolated in a yield of 14%.

Scheme 50

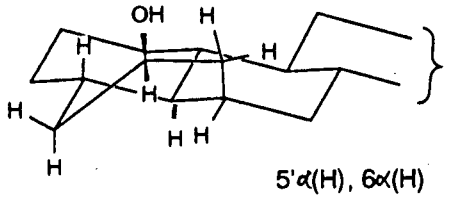


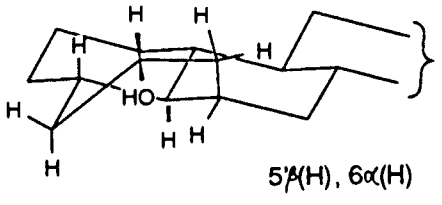
Reagents and conditions: i, 5% Pd on $CaCO_3$, THF, 20°C, 24 h.; ii, CrO_3, py, CH_2Cl_2, 20°C, 10 min.

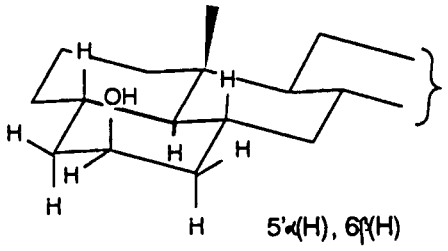
Product (**103**) was formulated as 4ß,4',5α,5'ß,6α,6'-hexahydrobenzo[4.5.6]cholestan-3ß,5'-diol. The IR spectrum of (**103**) showed no carbonyl absorption, but absorption at υ_{max} 3596 cm^{-1} was assigned to the hydroxyl groups. Evidence for the saturated nature of ring E came from the absence of olefinic signals in the NMR data. A ^1H multiplet at δ 3.72 ($W_{1/2}$ 8 Hz) was assigned to the equatorial 3ß-proton. A broad unresolved multiplet ($W_{1/2}$ 25 Hz) was thus assigned to the 5'-proton. The stereochemistry at C(5') could again not be unambiguously assigned, but since the hydroxyl group was assumed to have been bound tightly to the catalyst surface during the catalytic hydrogenation, and hydrogen delivery to the olefinic bond had proceeded from the α-face of

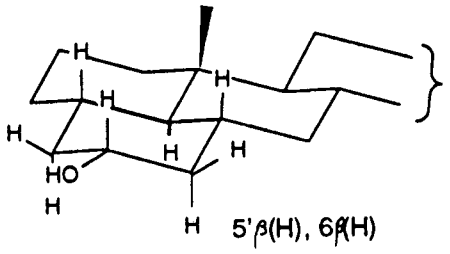
ring E, it was reasonable to expect that α-delivery of hydrogen to the 5'-keto group was also likely, resulting in a 5'ß-hydroxy group. Furthermore, in a closely analogous reaction conducted by Steer,[32] hydrogen delivery also proceeded from the α-face, and the overreduced product had the 5'-hydroxy configuration. The structure and conformational analysis of compound (**103**) was important, since analysis of the ^{1}H NMR spectrum would reveal the most reliable information on whether ring E had a twist conformation. A careful analysis of the four possible isomers at C(6) in combination with C(5') led to the conclusion that the formulations of the compounds (**100**), (**102**), and (**103**) were the correct ones (Figure 11). With the aid of the well-known Karplus-Conroy equation for vicinal coupling constants, the following calculations were made. In the all-chair conformation, the 5'α(H),6ß(H) conformation would result in a complex multiplet with $W_{1/2}$ *ca.* 15-20 Hz, in the 5'ß(H),6ß(H) conformation *ca.* 30-35 Hz, and in the 5'ß(H),6α(H) and 5'α(H),6α(H) conformations *ca.* 25 Hz. Since the observed $W_{1/2}$ was 25 Hz, it was concluded that the configuration at C(6) was the 6α(H)-configuration, and that ring E thus adopted a twist conformation.

Fig.11: Configuration of the 4 possible isomers of (**103**) at C(6) and C(5'). The C(19) methyl group is ommitted for a clearer representation of ring E and its protons in the 6α(H) conformations. The 3-substituent is ommitted for the same reason in the 6ß(H) configurations



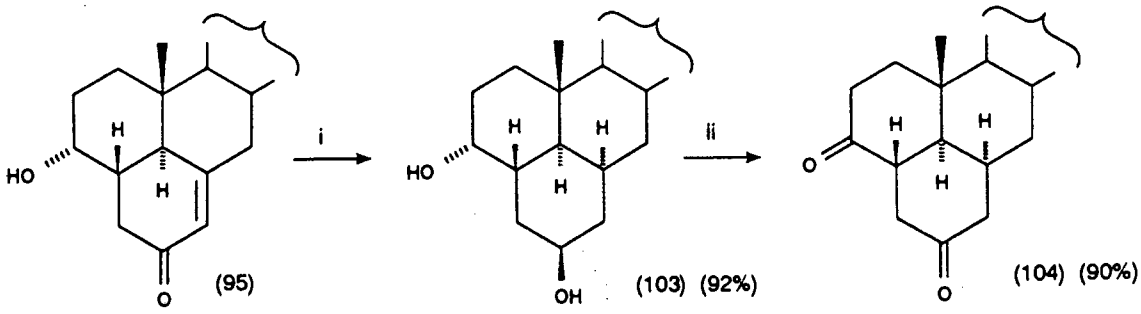






Reduction using lithium metal in liquid ammonia, was carried out on the 3α-hydroxy-enone (**95**). It was decided to use t-butyl alcohol as the proton donor, in order to avoid dimerisation to take place, as it was observed by Steer.[32] After quenching of the reaction with methanol, and standard work-up, one major product was isolated (Scheme 51), which was identical to the saturated diol (**103**) (92%). Oxidation of the diol (**103**) with the chromium trioxide-pyridine complex gave (**104**) (90%) (Scheme 51).

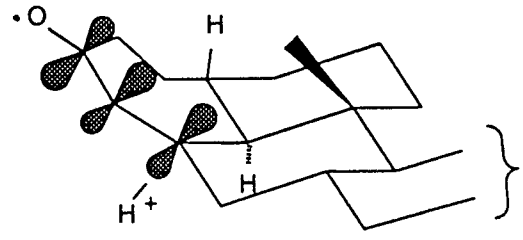
Scheme 51



Reagents and conditions: i, Li, liquid NH_3, -33°C, 30 min; ii, CrO_3, py, CH_2Cl_2, 20°C, 20 min.

Protonation had thus occurred from the α-face and not, as was hoped, from the ß-face. It is therefore possible that the proposal of Stork and Darling[41] applies here. In the radical anion (type T, Scheme 47), the approach of the proton carrier to C(6) is probably sterically less favoured from the ß-face, than from the α-face. Examination of a Buchi model (with real Van Der Waals spheres) of the transition state (Figure 12), revealed that the α-face was far less crowded than the ß-face. This result was surprising, since the chair conformation is energetically favoured.

Fig.12: α-face approach of the proton donor



No further work was carried out on the lithium-liquid ammonia reduction, since it has failed to afford the desired stereodifferentiation at C(6). Of the two procedures for the preparation of (**102**), catalytic hydrogenation is preferred, because of its simpler procedure.

Completion of this aspect of the work entailed the oxidation of the compounds (**102**) and (**103**) (Scheme 50) to obtain the 4ß,4',5α,6α-tetrahydrobenzo[4.5.6]cholestan-3,5'(6'*H*)-dione (**104**). If the oxidation of (**102**), obtained *via* catalytic hydrogenation and lithium-liquid ammonia reduction, and (**103**) were to give the same compound (**104**), this would confirm that the 6α-configuration was a common feature, and hence that all the foregoing configuration assignments are correct. The procedure for the oxidation was with the chromium trioxide-pyridine complex,[42] recognised as a reagent of choice in almost all situations calling for the oxidation of especially secondary alcohols. This method proved successful, since for all the oxidations carried out, a fast and clean conversion of the alcohols to the respective ketones proceeded in high yields. Chromatography was usually not necessary and crystallisation of the reaction residue, afforded the oxidation product of satisfactory purity. Thus, when compounds (**102**) and (**103**) were treated with the chromium trioxide-pyridine complex (generated *in situ*) in dichloromethane, the starting material was consumed in *ca* 10 min, affording one reaction product, isolated in a yield of *ca* 90%. The oxidation product of each starting material, as described above, was identical, as proved by the necessary characterisations, carried out independently. The oxidation product was formulated as 4ß,4',5α,6α-tetrahydrobenzo[4.5.6]cholestan-3,5'(6'*H*)-dione (**104**). The IR spectrum of (**104**) clearly exhibited intense carbonyl stretching at υ_{max} 1710 cm^{-1}, and the ^1H NMR spectrum revealed the absence of the 3- and 5'-proton

signals of the starting material (**103**), and ^{13}C NMR displayed two carbonyl carbon resonances at δ 209.7 and 210.3.

We do realise that more work has to be done on the products of the reduction. Circular dichroism, X-ray crystallography of the most suitable candidate (**103**), and isomerisation studies on the diketone (**104**) will provide unambiguous evidence for the 6-configuration.

4. Equilibration study

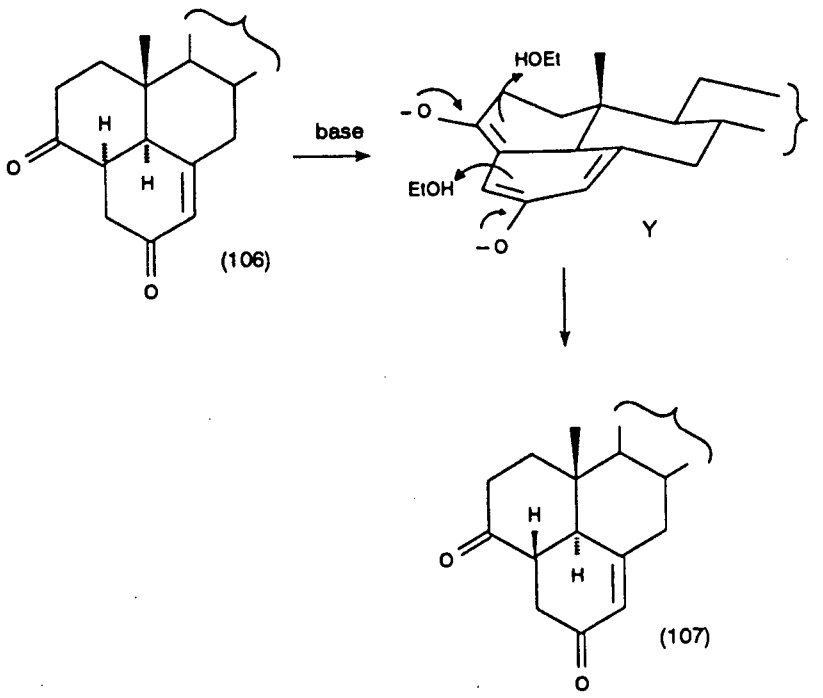
The final stage of the investigation entailed a preliminary study of isomerisations in this series of pentacyclic steroids. The relative steric energies of the various isomers in this series,[18] suggested that all-*trans* fusion around the perhydrophenalene moiety would be strongly favoured in the 5α-series, where possible, but that the relative steric energies of other configurational assemblies were similar for confident prediction of preferred isomers. Furthermore, it was expected that the presence of unsaturated bonds influences the steric relationships.

It was envisaged that equilibration could possibly be achieved at the 4ß,5α-dihydrobenzo stage of the route. The isomerisation at C(4), to produce the more stable compound, would in this way provide a by-pass in the development of pentacyclic steroids, which could then be applied to interconvert the series at this stage, and follow the most interesting route to either of the target pentacyclic compounds.

Accordingly, the first equilibration studies were done on the 3-oxo-4α(H),5α(H)-enone (**106**). It was believed that isomerisation possibility at C(4), created by the presence of the 3-keto functionality, could lead to the 3-oxo-4ß(H),5α(H)-enone (**107**). Thus alkaline treatment as a means of generating the enolate species Y (Scheme 52) would, after reprotonation result in the more stable 4ß(H) isomer (**107**), because of the relief of the

1,3-diaxial interaction of the C(4)-C(6') bond and the angular methyl group at C(10) in that conformation.

Scheme 52



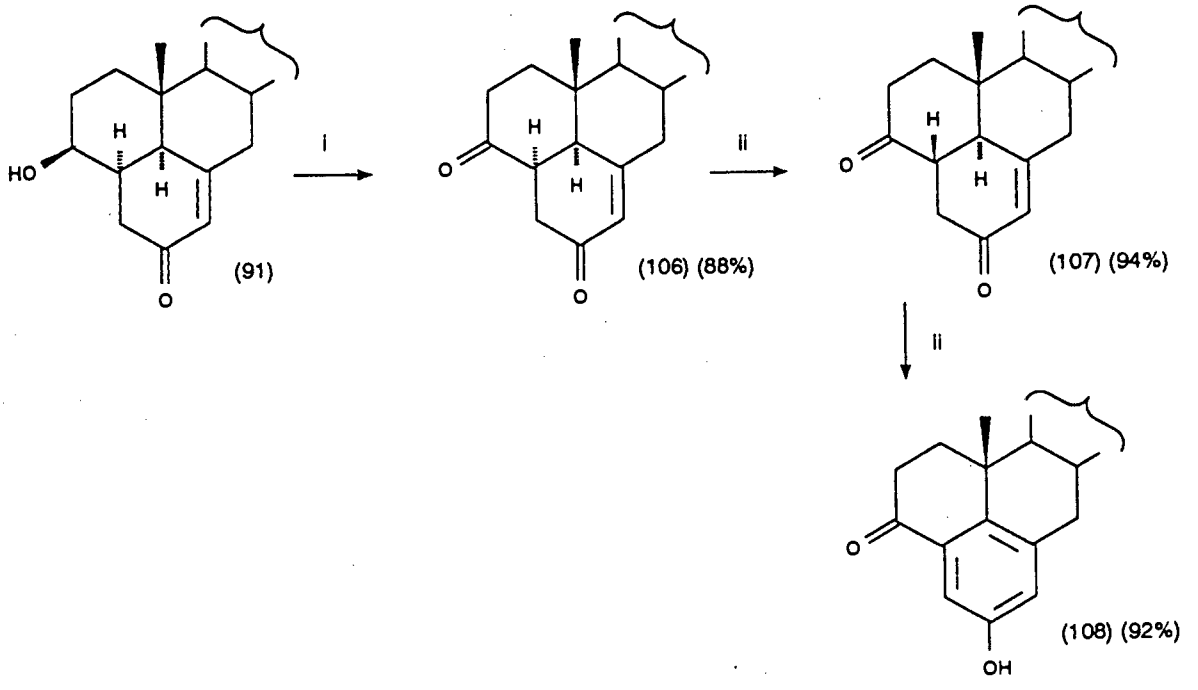
When the 3-oxo-4α(H),5α(H)-enone (**106**) was treated with aqueous potassium hydroxide in ethanol at 20°C, a fast consumption of the starting material (**106**) was observed (t.l.c.), in favour of the formation of one new product.

To have an unambiguous comparative basis, it was decided to oxidise the 3α-hydroxy-4ß(H),5α(H)-enone (**95**), and compare the thus obtained 3-oxo product (**107**) with the one formed by the alkaline treatment of (**106**). Isolation and characterisation of the new product formed by the alkaline treatment of (**106**), carried out independently of the characterisation of (**107**), revealed that the two products were

identical. The expected isomerisation at C(4) had thus taken place by alkaline treatment of (**106**) in a yield of 94%.

However, when the reaction mixture was left under the above conditions overnight, a completely new, more polar product had been formed as the only product, and starting material was no longer present (t.l.c.). The product was isolated in 92% yield. Characterisation of the product revealed that aromatisation of ring E had taken place. From the ^{1}H NMR spectrum, it was clear that two aromatic protons were present, which appeared as doublets at δ 6.77 and 7.33 (*J* 2.95 Hz). The presence of a hydroxyl proton signal at δ 5.45 was also confirmatory to formulate the product as 5'-hydroxybenzo[4.5.6]cholestan-3-one (**108**) (Scheme 53).

Scheme 53



Reagents and conditions: i, CrO_3, py, CH_2Cl_2, 20°C, 10 min; ii, Aq. KOH, EtOH, 20°C, 20 min or longer.

The formation of the aromatised product (**108**) proceeded at a comparable rate, even when precautions were taken to exclude oxygen, and it is therefore not evident what mechanism is involved. However, aromatisation proceeded much more slowly than isomerisation of (**106**), so it was possible to conduct the latter reaction efficiently without serious interference.

Experimental

General

Spectra were recorded as follows: infrared, Perkin-Elmer 983, chloroform solutions; ^{1}H and ^{13}C N.M.R., Varian VXR-200 (tetramethylsilane as internal standard) (200.1 and 50.3 MHz respectively), deuteriochloroform solutions; mass (electron impact) VG micromass 16F (recorded at 70 eV, with ion source temperature of 180-200°C). Optical rotations were determined in chloroform solutions at 20°C with a Perkin-Elmer 141 polarimeter. Microanalysis for C and H were carried out using a Heraues CHN-rapid combustion analyser.

Melting points were determined on a Reichert-Jung hot-stage microscope and are uncorrected.

Thin layer chromatography was performed on aluminium-backed silica gel 60 F_{254} plates in various solvents systems, applying the ascending technique. Upon development, the plates were sprayed with a solution of ammoniun ceric sulphate in 5*M*-sulphuric acid and heated to 200°C for 5 min. Silica gel for column chromatography refers to Merck Kieselgel 60: 70-230 mesh for gravity columns, and 230-400 mesh for flash chromatography.

Commonly used solvents were purified as described below. *Tetrahydrofuran, diethyl ether, benzene, ethanol, methanol, t-butyl alcohol*: Dried over sodium wire and distilled prior to use from sodium wire, using benzophenone as indicator. *Acetic anhydride*: fractionally distilled. *Pyridine*: dried over calcium hydride and fractionally distilled from calcium hydride prior to use.

5α-*Cholestane*-3ß,5,6ß-*triol* (57)

A suspension of cholesterol (**56**) (10.6 g, 27.5 mmol) in aqueous 88% formic acid (107 ml) was heated to 80°C with stirring. An oily layer separated and after 30 min, starting material was no longer present (t.l.c.). The solution was cooled to 25°C, whereupon the oil became semi-solid. 30% Hydrogen peroxide (16 ml) was added in one portion and the mixture was shaken periodically. An exothermic reaction commenced, with foaming of the medium. The temperature rose slowly and was maintained at 35-40°C by periodic external cooling. After 1 h, the mixture formed a homogeneous pale-blue solution and the exothermic reaction subsided. After 6 h at 25°C, boiling water (200 ml) was added and the mixture was allowed to cool to 25°C with occasional shaking, and was then cooled to 0°C in an ice bath for 30 min. A colourless precipitate separated, which was collected by filtration, washed repeatedly with water until free of formic acid, and dried at 60°C under vacuum for 12 h. The product was dissolved in boiling methanol (400 ml), cooled to 50°C, and aqueous 25% sodium hydroxide (10 ml) was added. After 15 min, the solution was heated to the boiling point and filtered. The filtrate was acidified with 3*M*-hydrochloric acid (5 ml) and water (200 ml) was added. The resultant precipitate was collected by filtration, washed with water, and dried at 60°C under vacuum . Crystallisation of the product (11 g) from methanol yielded the triol (**57**) (10.4 g, 90%), m.p. 169–172°C; $[\alpha]_D$ +15° (*c* 1.3) (lit.,[19] m.p. 168–173°C; $[\alpha]_D$ +13°).

3ß,5-*Dihydroxy*-5α-*cholestan*-6-*one* (58)

A solution of the triol (**57**) (10 g) in a mixture of diethyl ether (50 ml) and methanol (30 ml) was diluted with water (10 ml). The mixture was warmed on the steam–bath until clear, then cooled to 25°C. *N*-Bromo-

succinimide (4.5 g) was added to the solution, and the temperature was kept below 40°C with external cooling. The colour of the reaction mixture changed from yellow to dark yellow-orange and dark red in the course of 10 min. Finally, the colour became light orange and material started precipitating out. The organic phase was washed with aqueous 5% sodium disulphite, whereafter the bulk of the material precipitated out in the organic phase. The organic phase was then washed with aqueous 5% sodium hydroxide. The precipitate was collected by filtration and washed with methanol-water (1:1) until colourless. In addition, the filtrate was concentrated to give further precipitate, which was isolated, washed, and combined with the first precipitate to afford 3ß,5-dihydroxy-5α-cholestan-6-one (**58**) (9.2 g, 93%), m.p. 228–232°C (from methanol); $[\alpha]_D$ -30° (*c* 1.0) (lit.,[19] m.p. 229–231°C; $[\alpha]_D$ -32°).

3ß-*Acetoxy*-5-*hydroxy*-5α-*cholestan*-6-*one* **(59)**

3ß,5-Dihydroxy-5α-cholestan-6-one (**58**) (4 g, 9.56 mmol) was treated with acetic anhydride (10 ml) in pyridine (12 ml) at 20°C. After 4 h, the reaction mixture was poured into an acidified (HCl) water-ice mixture. After 30 min, the mixture was extracted with ethyl acetate (2 x 200 ml). The combined organic phase was washed with aqueous saturated sodium hydrogen carbonate, brine, and dried ($MgSO_4$). Evaporation under reduced pressure followed by co-evaporation with toluene to remove residual pyridine gave a crude product (4.8 g) which was crystallised from ethyl acetate-methanol to give 3ß-acetoxy-5-hydroxy-5α-cholestan-6-one (**59**) (4.3 g, 98%), m.p. 233–236°C (lit.,[19] m.p. 232–233°C); $[\alpha]_D$ –57° (c 1.0) ; υ_{max} 3590 (OH), 1738 (AcO), and 1717 (6-C=O) cm^{-1}; δ 0.64 (3H, s, 13ß-CH_3), 0.92 (3H, s, 10ß-CH_3), 2.01 (3H, s, 3ß-OAc), 2.67 (1H, s, exch. by D_2O, OH), 2.75 (1H, t, *J* 12.6 Hz, 7α-H), and

5.02 (1H, m, $W_{1/2}$ 15 Hz, 3α-H) (Found: C, 75.5; H, 10.8%; M^+, 460. $C_{29}H_{48}O_4$ requires C, 75.6; H, 10.5%; *M*, 460)

***Attempted Mitsunobu reaction of 3β,5-dihydroxy-5α-cholestan-6-one* (58).**

Glacial acetic acid (72 mg, 1.195 mmol), triphenylphosphine (157 mg, 0.6 mmol), and diethyl azodicarboxylate (105 mg, 0.61 mmol) were added successively to a stirred solution of 3ß,5-dihydroxy-5α-cholestan-6-one (**58**) (200 mg, 0.478 mmol) in benzene (4 ml) at 20°C. The reaction mixture was heated to reflux. After 20 h, the reaction mixture was allowed to cool to 20°C, filtered, and evaporated under reduced pressure to give a residue (930 mg), which was adsorbed on silica gel (93 g), and eluted with ethyl acetate-toluene (5:95), to afford 3α-acetoxy-5-hydroxy-5α-cholestan-6-one (**65**) (56 mg, 25%), m.p. 153–156°C (lit.,[45] m.p. 156–157°C); $[\alpha]_D$ −34° (c 1.0); υ_{max} 3590 (OH), 1740 (AcO), and 1716 (6-C=O) cm^{-1}; δ 0.63 (3H, s, 13ß-CH_3), 0.90 (3H, s, 10ß-CH_3), 2.06 (3H, s, 3α-OAc), 2.69 (1H, t, *J* 12.4 Hz, 7α-H), 3.35 (1H, s, exch. by D_2O, OH), and 5.27 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H) (Found: C, 75.8; H, 10.75%; M^+, 460. $C_{29}H_{48}0_4$ requires C, 75.6; H, 10.5%; *M*, 460), followed by 3ß-acetoxy-5-hydroxy-5α-cholestan-6-one (**57**) (65 mg, 30%), and starting material (**58**) (89 mg, 45%).

***3ß-Acetoxycholest-4-en-6-one* (60)**

Thionyl chloride (1.6 ml, 21.7 mmol) was added dropwise during 8 min to a stirred solution of 3ß-acetoxy-5-hydroxy-5α-cholestan-6-one (**59**) (2.15 g, 4.34 mmol) in pyridine (8.7 ml) at −5°C under nitrogen. The reaction temperature was maintained at −5°C for 45 min, was then allowed to warm to 20°C, and was stirred for an additional 30 min at 20°C. Pyridinium hydrochloride precipitated during the reaction. The reaction

mixture was poured into an acidified (HCl) water-ice mixture, and extracted into ethyl acetate (2 x 50 ml). The combined organic phase was washed with 3*M*-hydrochloric acid, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a residue (1.95 g), which was chromatographed on silica gel (150 g), with ethyl acetate-toluene (1:9) as the eluent, to give 3ß-acetoxycholest-4-ene-6-one (**60**) (1.80 g, 89%), m.p. 108–110°C (from methanol); $[\alpha]_D$ −47° (c 1.0) (lit.,[7] m.p. 110°C; $[\alpha]_D$ −48°); δ 0.69 (3H, s, 13ß-CH_3), 1.01 (3H, s, 10ß-CH_3), 2.23 (1H, s, 3ß-OAc), 5.1 (1H, br.m, $W_{1/2}$ 17 Hz, 3α-H), and 6.25 (1H, d, *J* 1.8 Hz, 4-H) (Found: C, 80.0; H, 10.3%; M^+, 442. $C_{29}H_{46}O_3$ requires C, 79.8, H, 10.5%; *M*, 442).

3ß-Hydroxycholest-4-en-6-one **(61)**

Aqueous 2*M*-potassium hydroxide (12 ml, 24 mmol) was added dropwise during 3 min to a stirred solution of 3ß-acetoxycholest-4-en-6-one (**60**) (4 g, 9.05 mmol) in ethanol (250 ml). The reaction mixture was stirred for 5 min at 25°C, then 3*M*-hydrochloric acid (5 ml) was added. The mixture was partly evaporated under reduced pressure, water was added, and the mixture was extracted with ethyl acetate (2 x 150 ml). The combined organic phase was washed with aqueous saturated sodium hydrogen carbonate, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give the crude 3ß-hydroxycholest-4-en-6-one (**60**) (3.63 g), which was crystallised from acetone (3.2 g, 89 %), m.p. 150–153°; $[\alpha]_D$ −13° (*c* 1.0) (lit.,[7] m.p. 152°C, $[\alpha]_D$ −12°); υ_{max} 3400 (OH), 1685 (C=O), and 1620 (C=C) cm^{-1}; δ 0.69 (3H, s, 13ß-CH_3), 1.00 (3H, s, 10ß-CH_3), 2.16 (1H, s, exch. by D_2O, OH), 4.22 (1H, m, $W_{1/2}$ 6 Hz, 3α-H), and 6.14 (1H, s, 4-H) (Found: C, 81.2; H, 11.2%; M^+, 400. $C_{27}H_{44}O_2$ requires C, 80.9; H, 11.1%; *M*, 400).

Attempted one step conversion of cholesterol to 3ß-hydroxycholest-4-en-6-one

To a stirred suspension of silver(I) chromate (1.09 g, 3.3 mmol) in dichloromethane (20 ml) at 0°C was added iodine (1.26 g, 5 mmol), followed by pyridine (316 mg, 4 mmol) in dichloromethane (1 ml), and the mixture was stirred for 10 min at 0°C under nitrogen. A solution of cholesterol (**56**) (773 mg, 2 mmol) in dichloromethane (8 ml) was added dropwise during 15 min, and the reaction mixture was stirred for an additional 20 min at 0°C. The ice-bath was removed and the reaction mixture was allowed to warm to 25°C. The temperature was maintained at 25°C for an additional 2 h, after which the mixture was filtered. The solvent was removed under reduced pressure to give a residue, which was redissolved in ethyl acetate (50 ml),and washed with aqueous 5% sodium thiosulphate (2 x 10 ml). The organic phase was washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure, to give a residue (805 mg), which was adsorbed on silica gel (70 g), and eluted with ethyl acetate-toluene (1:4) to give 3,6-diketo-cholest-4-ene (**98a**) (80 mg, 10%) m.p. 123-125°C (from methanol); $[\alpha]_D$ −40° (lit.,[10] m.p. 122–125°C, $[\alpha]_D$ −38°), followed by 3ß-hydroxycholest-4-en-6-one (**63**) (225 mg, 21%), and finally 5,6α-epoxy-5α-cholestan-3ß-ol (**98b**) (430 mg, 53%), m.p. 138–142°C (from methanol); $[\alpha]_D$ −45° (*c* 1.0) (lit.,[44] m.p. 141–143°C; $[\alpha]_D$ −47°).

3α-*Acetoxycholest-4-en-6-one* (62)

Glacial acetic acid (2.25 g, 37.5 mmol), triphenylphosphine (4.9 g, 18.75 mmol) and diethyl azodicarboxylate (3.26 g, 18.75 mmol) were added successively to a solution of 3ß-hydroxycholest-4-en-6-one (**61**) (3 g, 7.5 mmol) in dry benzene (120 ml) at 20°C. A precipitate of triphenylphosphine oxide appeared after 20 min. After 30 min, the

reaction mixture was partly evaporated to enhance the precipitation of triphenylphosphine oxide, and was filtered. The precipitate was washed with benzene. The filtrate was washed with aqueous saturated sodium hydrogen carbonate (10 ml), brine (2 x 30 ml), and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (6 g), which was chromatographed on silica gel (240 g), with ethyl acetate-toluene (3.5:96.5) as the eluent, to afford 3α-acetoxycholest-4-en-6-one (**62**) (3.80 g, 90%), m.p. 87–90°C (from methanol); $[\alpha]_D$ +92° (c 1.0) (lit.,[46] m.p. 90°C; $[\alpha]_D$ +94°); υ_{max} 1730 (AcO), 1680 (C=O), and 1628 (C=C) cm^{-1} ; δ 0.63 (3H, s, 13ß-CH_3), 0.88 (3H, s, 10ß-CH_3), 1.97 (3H, s, 3α-OAc), 5.2 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H), and 6.14 (1H, d, *J* 4.9 Hz, 4-H) (Found: C, 78.5; H, 10.4%; M^+, 442. $C_{29}H_{46}O_3$ requires C, 78.6; H, 10.4%; *M*, 442).

3α-*Hydroxycholest-4-en-6-one* **(63)**

Aqueous 2*M*-potassium hydroxide (10 ml, 20 mmol) was added to a solution of 3α-acetoxycholest-4-en-6-one (**62**) (3 g, 6.78 mmol) in ethanol (150 ml) and the reaction mixture was kept at 20°C for 1 h. The mixture was acidified with 3*M*-hydrochloric acid, partly evaporated under reduced pressure, and extracted with ethyl acetate (200 ml). The organic phase was washed with aqeuous saturated sodium hydrogen carbonate, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (3 g), which was crystallised from acetone to yield 3α-hydroxycholest-4-en-6-one (**63**) (2.47 g, 91%), m.p. 123–125°C; $[\alpha]_D$ +61° (c 1.0) (lit.,[46] m.p. 124–125°C; $[\alpha]_D$ +62°); υ_{max} 3601 (OH), 1681 (C=O), and 1624 (C=C) cm^{-1}; δ 0.68 (3H, s, 13ß-CH_3), 0.92 (3H, s, 10ß-CH_3), 1.58 (1H, s, OH), 4.22 (1H, m, $W_{1/2}$ 11 Hz, 3ß-H), and 6.26 (1H, d, *J* 4.7 Hz, 4-H) (Found: C, 81.2, H, 10.9%; M^+, 400. $C_{27}H_{44}O_2$ requires C, 80.9, H, 11.1%; *M*, 400).

3ß-*Acetoacetoxycholest-4-en-6-one* **(66)**

A mixture of 3ß-hydroxycholest-4-en-6-one (**61**) (3.88 g, 9.7 mmol) and 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**69**) (2.15 g, 11.6 mmol) was stirred in benzene (20 ml) at 75°C under nitrogen. After 1 h, the reaction was complete (t.l.c.). The reaction mixture was cooled to 20°C, washed with brine and water, dried ($MgSO_4$), and evaporated under reduced pressure to give a residue (6 g), which was chromatographed on silica gel (35 g), with ethyl acetate-toluene (7:93) as the eluent, to afford *3ß-acetoacetoxycholest-4-en-6-one* (**66**) (4.5 g, 96%), m.p. 119–122°C (from acetone-methanol); $[\alpha]_D$ −49.5° (c 1.0); υ_{max} 1741 (1'-C=O), 1714 (3'C=O), 1688 (6-C=O) and 1637 (C=C) cm^{-1} ; δ_H 0.69 (3H, s, 13ß-CH_3), 1.00 (3H, s, 10ß-CH_3), 2.25 (3H, s, 4'-CH_3), 3.44 (2H, s, 2'-CH_2), 5.38 (1H, ddd, *J* 13.8, 6.3 and 2.0 Hz, 3α-H), and 6.03 (1H, t, *J* 2.0 Hz, 4-H); δ_C 11.9 (q, C-18), 18.5 (q, C-21), 19.4 (q, C-19), 20.7 (t, C-11), 22.3 (t, C-2), 22.5 (q, C-26), 22.7 (q, C-27), 23.8 (t, C-23), 24.0 (t, C-15), 27.9 (d, C-25 and t, C-16), 30.1 (q, C-4'), 34.1 (d, C-8), 35.6 (d, C-20), 34.3 (t, C-1), 36.0 (t, C-22), 38.3 (s, C-10), 39.2 (t, C-24), 39.4 (t, C-12), 42.5 (s, C-13), 50.0 (t, C-2' and t, C-7), 51.1 (d, C-9), 55.9 (d, C-17), 56.5 (d, C-14), 70.4 (d, C-3), 127.6 (d, C-4), 148.5 (s, C-5), 166.7 (s, C-1'), and 202.4 (s, C-3'), not observed C(6) (Found: C, 76.8; H, 9.95; M^+, 484. $C_{31}H_{48}O_4$ requires C, 76.8; H, 9.7%; *M*, 484)

3α-*Acetoacetoxycholest-4-en-6-one* **(67)**

A mixture of 3α-hydroxycholest-4-en-6-one (**63**) (2.25 g, 5.63 mmol) and 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**69**) (1.26 g, 6.7 mmol) was stirred in dry benzene (20 ml) at 75°C under nitrogen. After 2h, the starting material had been consumed (t.l.c.). The reaction mixture was allowed to cool to 20°C, washed with water, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue,

which was chromatographed on silica gel (85 g) with ethyl acetate-toluene (6:94) as the eluent, to give 3α-*acetoacetoxycholest-4-en-6-one* (**67**) (3 g, 94%) as a colourless oil, $[\alpha]_D$ +131° (c 1.3); υ_{max} 1738 (1'-C=O), 1715 (3'-C=O), 1686 (6-C=O), and 1634 (C=C) cm^{-1}; δ_H 0.68 (3H, s, 13ß-CH_3), 0.94 (3H, s, 10ß-CH_3), 2.24 (3H, s, 4'-CH_3), 3.42 (2H, s, 2'-CH_2), 5.32 (1H, m, $W_{1/2}$ 12 Hz, 3ß-H), and 6.19 (1H, d, *J* 5.3 Hz, 4-H); δ_C 12.0 (q, C-18), 18.3 (q, C-19), 18.7 (q, C-21), 21.45 (t, C-11), 22.6 (q, C-26), 22.9 (q, C-27), 23.9 (t, C-23), 24.0 (t, C-2), 24.3 (t, C-15), 28.1 (t, C-16, and d, C-25), 30.2 (q, C-4'), 30.8 (t, C-1), 34.1 (d, C-8), 35.8 (d, C-20), 36.2 (t, C-22), 38.7 (s, C-10), 39.4 (t, C-12), 39.5 (t, C-24), 42.6 (s, C-13), 46.5 (t, C-7), 50.3 (t, C-2'), 51.0 (d, C-9), 56.1 (d, C-17), 66.9 (d, C-3), 125.1 (d, C-4), 151.5 (s, C-5), 166.3 (s, C-1'), and 203.2 (s, C-3'), not observed C(6) (*M*+, 484.353. $C_{31}H_{48}O_4$ requires *M*, 484.355).

5-*Acetyl*-2,2-*dimethyl*-1,3-*dioxane*-4,6-*dione* (**69**)

Acetyl chloride (3,45 g, 44 mmol) in dichloromethane (30 ml) was added dropwise during 15 min to a stirred solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (**68**) (5.76 g, 40 mmol) in dichloromethane (50 ml) and pyridine (6.3 ml, 80 mmol). During the reaction, pyridinium hydrochloride precipitated. The mixture was concentrated under reduced pressure, and benzene was added to complete the precipitation. The mixture was filtered, and the filtrate was concentrated to give a crystalline product, which was dissolved in toluene and filtered through Celite to remove oily material. The filtrate was co-evaporated with toluene several times to remove residual pyridine, and the product was crystallised from benzene-hexane to give 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**69**), m.p. 79–81°C (lit.,[9] m.p. 80–81°C).

(2R)-2-(3ß-*Hydroxy*-6-*oxo*-5ß-*cholestan*-4ß-*yl*)-3-*oxobutanoic acid* 1, 3'-*lactone* (81)

Aqueous 1*M*-potassium carbonate (14 ml) was added to a vigorously stirred solution of 3ß-acetoacetoxycholest-4-en-6-one (**66**) (3.7 g, 7.64 mmol) in ethanol (200 ml) at 60°C. After 2 h, the starting material had been consumed (t.l.c.) and the reaction mixture was filtered, partly evaporated under reduced pressure, and diluted with water. The mixture was extracted with ethyl acetate (2 x 200 ml). The combined organic phase was washed with 1*M*-hydrochloric acid (20 ml), water, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to give (2R)-2-(3ß-*hydroxy*-6-*oxo*-5ß-*cholestan*-4ß-*yl*)-3-*oxobutanoic acid* 1,3'-*lactone* (**81**) (3.51 g, 95%), m.p. 130–133°C (from acetone-methanol); $[\alpha]_D$ +1° (*c* 1.1); υ_{max} 1774 (1'-C=O), 1715 (6-C=O), and 1698 (3'-C=O) cm^{-1}; δ_H 0.65 (3H, s, 13ß-CH_3), 0.92 (3H, s, 10ß-CH_3), 1.82 (1H, d, *J* 12.2 Hz, 5ß-H), 2.31 (3H, s, 4'-CH_3), 3.06 (1H, dd, *J* 12.2 and 4.0 Hz, 4α-H), 3.57 (1H, s, 2'-H) and 4.63 (1H, m, $W_{1/2}$ 11 Hz, 3α-H); δ_C 11.9 (q, C-18), 18.6 (q, C-21), 20.8 (t, C-11), 21.6 (t, C-2), 22.5 (q, C-26), 22.7 (q, C-27), 23.15 (q, C-19), 23.8 (t, C-23, and t, C-15), 27.9 (d, C-25), 28.0 (t, C-16), 28.3 (t, C-1), 28.7 (q, C-4'), 35.6 (d, C-20), 36.0 (t, C-22), 36.5 (d, C-8), 36.7 (d, C-4), 37.4 (s, C-10), 39.4 (t, C-24, and t, C-12), 40.8 (d, C-9), 42.7 (t, C-7), 43.0 (s, C-13), 56.1 (d, C-14), 56.6 (d, C-17), 58.8 (d, C-5), 62.4 (d, C-2'), 77.3 (d, C-3), 170.9 (s, C-1'), 199.2 (s, C-3'), and 212.2 (s, C-6) (Found: C, 76.5; H, 9.6%; M^+, 484. $C_{31}H_{48}O_4$ requires C, 76.8; H, 9.65%; *M*, 484).

Attempted lactone cleavage-decarboxylation-aldol reaction of the lactone (81)

Aqueous 2*M*–potassium hydroxide (3 ml) was added dropwise during 1 min to a stirred solution of (2*R*)-2-(3ß-hydroxy-6-oxo-5ß-cholestan-4ß-yl)-3-oxobutanoic acid 1,3'-lactone (**81**) (300 mg, 0.62 mmol) at 40°C with stirring. After the addition, the reaction mixture was heated to reflux. After 42 h at reflux, the reaction mixture was cooled to 20°C, neutralised with aqueous 1*M*–hydrochloric acid and partly evaporated under reduced pressure. The mixture was diluted with water and extracted with ethyl acetate (2 x 50 ml). The combined organic phase was washed with aqueous saturated sodium hydrogen carbonate and brine. The washed organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure to give a residue (340 mg), which was adsorbed on silica gel (30 g), and eluted with ethyl acetate-toluene (35:65), to afford starting material (**81**) (78 mg), followed by 2-(3ß-*hydroxy*-6-*oxo*-5ß-*cholestan*-4ß-*yl*)-*acetic acid* 1,3'-*lactone* (**84**) (100 mg, **22**%), m.p. 143–144°C (from methanol); $[\alpha]_D$ –62° (*c* 1.0); υ_{max} 1781 (1'-C=O) and 1700 (6-C=O) cm^{-1}; δ_H 0.65 (3H, s, 13ß-CH_3), 0.91 (3H, s, 10ß-CH_3), 1.8 (1H, d, *J* 12.2 Hz, 5ß-H), 2.5 (1H, d, *J* 17.5 Hz, 2'ß-H), 2.6 (1H, br.m, $W_{1/2}$ 20 Hz, 4α-H), and 4.6 (1H, m, $W_{1/2}$ 8 Hz, 3α-H); δ_C 12.2 (q, C-18), 18.85 (q, C-21), 21.1 (t, C-11), 22.2 (t, C-2), 22.7 (q, C-26), 23.0 (q, C-27), 23.4 (q, C-19), 24.0 (t, C-23), 24.1 (t, C-15), 28.2 (d, C-25), 28.3 (t, C-16), 28.55 (t, C-1), 35.45 (d, C-20), 35.85 (d, C-4), 36.2 (t, C-22), 36.3 (t, C-12), 36.9 (d, C-8), 37.7 (s, C-10), 39.65 (t, C-24), 39.7 (t, C-7), 40.95 (d, C-9), 43.05 (t, C-2'), 43.3 (s, C-13), 56.4 (d, C-14), 57.1 (d, C-17), 60.4 (d, C-5), 77.5 (d, C-3), 176.0 (s, C-1'), and 212.0 (s, C-6) (Found: C, 78.6; H, 10.15%; M^+, 442. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.5%; *M*, 442).

(2S)-2-(3α-*Hydroxy*-6-*oxo*-5α-*cholestan*-4α-*yl*)-3-*oxobutanoic acid*-1,3'-*lactone* (82)

(a) Aqueous 1*M*-potassium carbonate (2 ml) was added to a vigorously stirred solution of 3α-acetoacetoxycholest-4-en-6-one (**67**) (390 mg, 0.806 mmol) in ethanol (30 ml) at 60°C. After 4 h, the starting material had been consumed (t.l.c.) and the reaction mixture was filtered, partly evaporated under reduced pressure, and diluted with water. The mixture was extracted with ethyl acetate (2 x 30 ml). The combined organic phase was washed with 1*M*-hydrochloric acid (5 ml), water, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a residue (450 mg), which was adsorbed on silica gel (30 g), and eluted with ethyl acetate-hexane (1:3), to give (2S)-2-(3α-*hydroxy*-6-*oxo*-5α-*cholestan*-4α-*yl*)-3-*oxobutanoic acid* 1,3'-*lactone* (**82**) (235 mg, 60%), m.p. 155–157°C (from acetone-methanol); $[\alpha]_D$ −11° (*c* 1.2); υ_{max} 1771 (1'-C=O), 1718 (6-C=O) and 1704 (3'-C=O) cm^{-1}; δ_H 0.63 (3H, s, 13ß-CH_3), 0.92 (3H, s, 10ß-CH_3), 2.17 (1H, d, *J* 11.7 Hz, 5α-H), 2.37 (3H, s, 4'-CH_3), 3.02 (1H, dd, *J* 11.7 and 4.6 Hz, 4ß-H), 3.22 (1H, s, 2'-H), and 4.72 (1H, m, $W_{1/2}$ 7 Hz, 3ß-H); δ_C 11.9 (q, C-18), 12.6 (q, C-19), 18.5 (q, C-21), 21.0 (t, C-11), 22.3 (t, C-2), 22.5 (q, C-26), 22.7 (q, C-27), 23.7 (t, C-15), 23.8 (t, C-23), 27.9 (d, C-25 and t, C-16), 29.4 (q, C-4'), 30.8 (t, C-1), 34.8 (d, C-4), 35.6 (d, C-20), 36.0 (t, C-22), 38.1 (d, C-8), 39.4 (t, C-24), 39.4 (t, C-12), 41.5 (s, C-10), 42.8 (s, C-13), 47.1 (t, C-7), 53.2 (d, C-9), 56.0 (d, C-14), 56.6 (d, C-17), 57.1 (d, C-5), 64.0 (d, C-2'), 77.9 (d, C-3), 172.4 (s, C-1'), 200.1 (s, C-3'), and 209.7 (s, C-6) (Found C, 76.4; H, 9.3%; M^+, 484. $C_{31}H_{48}O_4$ requires C, 76.4; H, 9.5%; *M*, 484), followed by the hydrolysis product, 3α-hydroxycholest-4-en-6-one (**63**) (35%).

(b) Potassium metal (0.2 g, 5.2 mmol) was added to *t*-butyl alcohol (freshly distilled from potassium metal) (25 ml) at 40°C under nitrogen. A solution of 3α-acetoacetoxycholest-4-en-6-one (**67**) (2.5 g, 5.2 mmol) in tetrahydrofuran (25 ml) was added dropwise during 20 min. After 2.5 h at 40°C, the starting material had been consumed (t.l.c.) and the reaction mixture was neutralised with 3*M*-hydrochloric acid. The neutralised mixture was diluted with water and extracted with ethyl actate (250 ml). The organic phase was washed with 1*M*-hydrochloric acid (10 ml), water and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a residue (2.6 g), which was adsorbed on silica gel (250 g) and eluted with ethyl acetate-toluene (3.5: 96.5), to give (2S)-2-(3α-*hydroxy*-6-*oxo*-5α-*cholestan*-4α-*yl*)-3-*oxobutanoic acid* 1,3'-*lactone* (**82**) (2 g, 80%). Further elution with ethyl acetate-toluene (15:85) afforded 6-*hydroxy*-5'(6'H)-*oxo*-4ß,4',5α,6ß-*tetrahydrobenzo*[4.5.6]*cholestane*-6'α,3α-*carbolactone* (**83**) (0.5 g, 20%), m.p. 210–212°C (from methanol); $[\alpha]_D$ −51° (*c* 0.8); υ_{max} 3596 (OH), 1775 (6'[1]-C=O) and 1712 (5'-C=O) cm^{-1}; δ_H 0.68 (3H, s, 13ß-CH_3), 1.00 (3H, s, 10ß-CH_3), 1.47 (1H, d, *J* 12.3 Hz, 5α-H), 2.35 and 2.44 (1H each, d, *J* *ca* 16 Hz, 4'-H_2), 3.18 (1H, ddd, *J* 12.3, 5.7 and 3.5 Hz, 4ß-H), 3.52 (1H, d, *J* 5.7 Hz, 6'ß-H), and 4.55 (1H, m, $W_{1/2}$ 10 Hz, 3ß-H); δ_C 11.9 (q, C-18), 13.5 (q, C-19), 18.6 (q, C-21), 20.5 (t, C-11), 22.5 (s, C-27), 22.7 (s, C-26), 23.0 (t, C-2), 23.7 (t, C-23), 24.2 (t, C-15), 27.9 (d, C-25), 28.0 (t, C-16), 30.5 (d, C-4), 34.6 (t, C-1), 35.2 (s, C-10), 35.7 (d, C-20), 36.0 (t, C-22), 39.2 (d, C-8), 39.4 (t, C-24), 39.5 (t, C-12), 42.4 (s, C-13), 46.2 (d, C-5), 46.25 (t, C-7), 53.2 (d, C-9), 55.6 (d, C-17), 56.0 (d, C-14), 56.2 (t, C-4'), 58.3 (d, C-6'), 73.3 (s, C-6), 78.7 (d, C-3), 172.0 (s, C-6'[1]), and 201.0 (s, C-5') (Found: C, 75.7; H, 9.4%; M^+, 484. $C_{31}H_{48}O_4$ requires C, 75.4; H, 9.5%; *M*, 484).

(c) Potassium metal (0.1 g, 2.6 mmol) was added to t-butyl alcohol (freshly distilled from potassium metal) (20 ml) under a nitrogen atmosphere at 40°C. A solution of 3α-acetoacetoxycholest-4-en-6-one (**67**) (2.5 g, 5.2 mmol) in tetrahydrofuran (25 ml) was added dropwise during 20 min. After 4 h at 40°C, the starting material had been consumed (t.l.c.) and the reaction mixture was neutralised with 3*M*-hydrochloric acid, and diluted with water. The mixture was extracted with ethyl acetate (250 ml). The organic phase was washed with 1*M*-hydrochloric acid (10 ml), water, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a residue (2.6 g), which was adsorbed on silica gel (70 g), and eluted with ethyl acetate-hexane (1:3), to afford (2*S*)-2-(3α-hydroxy-6-oxo-5α-cholestan-4α-yl)-3-oxobutanoic acid 1,3'-lactone (**82**) (2.32 g, 93%).

3ß,6-*Dihydroxy*-4α,4',5ß,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (86)

(a) Aqueous 2*M*−potassium hydroxide (27 ml) was added dropwise during 2 min to a stirred solution of (**81**) (2.6 g, 5.4 mmol) in dioxane (140 ml) and water (10 ml) at 50°C. After the addition, the reaction mixture was heated to reflux. After 5 h at reflux, the starting material had been consumed (t.l.c.), the reaction mixture was cooled and maintained at 0°C during 14 h. The reaction mixture was neutralised with aqueous 1*M*−hydrochloric acid and partly evaporated under reduced pressure. Water was added and the pH of the mixture was adjusted to 5 with aqueous 1*M*−hydrochloric acid. The acidified mixture was extracted with ethyl acetate (3 x 200 ml), and the combined organic phase was washed with aqueous saturated sodium hydrogen carbonate and brine. The organic phase was dried ($MgSO_4$), and the solvent was

evaporated under reduced pressure to afford a residue (3 g), which was adsorbed on silica gel (120 g), and eluted with ethyl acetate-toluene (65:35), to give 3ß,6-*dihydroxy*-4α,4',5ß,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**86**) (1.72 g, 70%), m.p. 180–183°C; $[\alpha]_D$ +3° (*c* 1.0); υ_{max} 3611 (3-and 6-OH) and 1708 (5'-C=O) cm^{-1}; δ_H 0.66 (3H, s, 13ß-CH_3), 1.21 (3H, s, 10ß-CH_3), 2.0 (1H, d, *J* 13.1 Hz, 4'ß-H), 2.55 (1H, d, *J* 13.1 Hz, 4'α-H), and 3.76 (1H, br.s, $W_{1/2}$ 8 Hz, 3α-H); δ_C 12.1 (q, C-18), 18.6 (q, C-21), 20.9 (t, C-11), 22.5 (q, C-26), 22.7 (q, C-27), 23.8 (t, C-23), 24.0 (t, C-15), 27.1 (q, C-19), 27.9 (t, C-2), 28.2 (t, C-16, and d, C-25), 30.9 (t, C-1), 32.35 (d, C-8), 35.7 (d, C-20), 36.05 (t, C-22), 36.65 (s, C-10), 38.25 (d, C-4), 39.1 (t, C-7), 39.4 (t, C-24), 40.0 (t, C-12), 40.55 (d, C-9), 42.8 (s, C-13), 45.2 (t, C-6'), 50.3 (d, C-5), 55.9 (d, C-17), 56.25 (d, C-14), 58.2 (t, C-4'), 69.6 (d, C-3), 76.3 (s, C-6), and 208.6 (s, C-5') (Found: C, 78.7; H, 10.8%; M^+, 458. $C_{30}H_{50}O_3$ requires C, 78.55; H, 11.0%; *M*, 458).

(b) Aqueous 2*M*–potassium hydroxide (3 ml) was added dropwise during 1 min to a stirred solution of (**81**) (307 mg, 0.63 mmol) in dioxane (12 ml) and water (1 ml) at 20°C. The reaction mixture was heated to reflux, and after 5 h, the starting material had been consumed (t.l.c.). The reaction mixture was cooled to 20°C, neutralised with aqueous 1*M*–hydrochloric acid, and partly evaporated under reduced pressure. The mixture was diluted with water and the pH was adjusted to 5 with aqueous 1*M*–hydrochloric acid. The acidified mixture was extracted with ethyl acetate (3 x 50 ml). The combined organic phase was washed with aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give a residue (320 mg), which was adsorbed on silica gel (16 g), and eluted with ethyl acetate-toluene (35:65), to afford 3ß-*hydroxy*-4ß(2-*oxopropyl*)-5ß-*cholestan*-6-*one* (**85**) (96 mg, 34%),

m.p. 124–127°C (from hexane); $[\alpha]_D$ –37° (*c* 0.8); υ_{max} 3589 (3-OH), 1700 (6-C=O), and 1693 (2'-C=O) cm^{-1}; δ_H 0.64 (3H, s, 13ß-CH_3), 0.90 (3H, s, 10ß-CH_3), 1.55 (1H, s, exch. by D_2O wash, OH), 2.08 (3H, s, 2'-CH_3), 2.40 (1H, br.m, $W_{1/2}$ 26 Hz, 4α-H), and 3.88 (1H, m, $W_{1/2}$ 10 Hz, 3α-H).(Found: C, 79.25;H, 10.7%; M^+, 458. $C_{30}H_{50}O_3$ requires C, 78.9; H, 10.6%; *M*, 458). Further elution with ethyl acetate-toluene (65:35) afforded 3ß,6-dihydroxy-4a,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**86**) (98 mg, 34%).

(c) Aqueous 2*M*–potassium hydroxide (3 ml) was added dropwise to a stirred solution of 3ß-hydroxy-4ß(2-oxopropyl)-5ß-cholestan-6-one (**85**) (480 mg, 1.0 mmol) in ethanol (25 ml) during 1 min at 0°C. After 4 h at 0°C, the reaction mixture was neutralised with aqueous 1*M*–hydrochloric acid. The neutralised reaction mixture was partly evaporated under reduced pressure and diluted with water. The pH was adjusted to 5 with aqueous 1*M*–hydrochloric acid. The acidified mixture was extracted with ethyl acetate (2 x 60 ml). The combined organic phase was washed with aqueous saturated sodium hydrogen carbonate, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 3ß,6-dihydroxy-4a,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**85**) (355 mg, 77%).

3ß-Acetoxy-4ß(2-oxopropyl)-5ß-cholestan-6-one **(87)**

3ß-Hydroxy-4ß(2-oxopropyl)-5ß-cholestan-6-one (**85**) (200 mg, 0.44 mmol) was treated with acetic anhydride (2 ml) in pyridine (4 ml) at 20°C. After 4 h at 20°C, the reaction mixture was poured into an acidified (HCl) water-ice mixture. The suspension was extracted into ethyl acetate (50 ml), and the aqueous layer was back-extracted with ethyl acetate (50 ml). The combined organic phase was washed with water (2 x 10 ml), and neutralised with aqueous saturated sodium hydrogen carbonate. The

neutralised organic phase was washed with brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give 3ß-*acetoxy*-4ß(2-*oxopropyl*)-5ß-*cholestan*-6-*one* (**87**) (193 mg, 89%), m.p. 94–97°C (from methanol); $[\alpha]_D$ −19° (*c* 0.7); υ_{max} 1725 (AcO), 1715 (6-C=O), and 1691 (2'-C=O) cm^{-1}; δ_H 0.64 (3H, s, 13ß-CH_3), 0.89 (3H, s, 10ß-CH_3), 2.04 (3H, s, AcO), 2.06 (3H, s, 2'-CH_3), 2.24 obsc(1H, m, $W_{1/2}$ *ca.*22 Hz, 1' -H), 2.80 (1H, br.m, $W_{1/2}$ 30 Hz, 4α-H), and 5.00 (1H, m, $W_{1/2}$ 9 Hz, 3α-H); δ_C 11.9 (q, C-18), 18.6 (q, C-21), 21.05 (q, AcO), 21.2 (t, C-11), 22.6 (q, C-26), 22.8 (q, C-27), 23.6 (q, C-19), 23.81 (t, C-15), 23.83 (t, C-23), 25.0 (t, C-2), 28.0 (d, C-25), 28.1 (t, C-16), 29.1 (t, C-1), 30.3 (q, C-3'), 33.25 (d, C-8), 35.7 (d, C-20), 36.1 (t, C-22), 37.3 (d, C-9), 39.2 (s, C-10), 39.5 (t, C-24), 39.65 (t, C-12), 40.2 (d, C-4), 43.05 (s, C-13), 43.55 (t, C-7), 43.7 (t, C-1'), 56.2 (d, C-17), 56.7 (d, C-14), 60.9 (d, C-5), 70.6 (d, C-3), 170.5 (s, AcO), 206.8 (s, C-2'), and 215.0 (s, C-6) (Found: C, 76.5; H, 10.3%; M^+, 500. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.5%; *M*, 500).

3ß-*Acetoxy*-6-*hydroxy*-4α,4',5ß,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (88)

3ß,6-Dihydroxy-4α,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**86**) (600 mg, 1.31 mmol) was treated with acetic anhydride (2 ml) in pyridine (4 ml) at 40°C for 7 h. The reaction mixture was allowed to cool to 20°C, and was poured into an acidified (HCl) water-ice mixture. The suspension was extracted into ethyl acetate (2 x 150 ml). The combined organic phase was washed with water (2 x 15 ml), aqueous saturated sodium hydrogen carbonate, and brine. The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to give 3ß-*acetoxy*-6-*hydroxy*-4α,4',5ß,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**88**) (583 mg, 89%), m.p. 95–97°C (from methanol); $[\alpha]_D$ +14° (*c* 0.85); υ_{max} 3591 (OH), 1731 (AcO), and 1709 (C=O) cm^{-1}; δ_H 0.66 (3H,

s, 13ß-CH_3), 1.23 (3H, s, 10ß-CH_3), 2.09 (3H, s, AcO), 2.34 (1H, dd, *J* 13.2 and *ca.*2 Hz, 4'α-H), 2.38 (1H, t, *J* 2 x 13.1 Hz, 6'ß-H), 2.58 (1H, d, *J* 13.2 Hz, 4'ß-H), and 4.85 (1H, m., $W_{1/2}$ 5 Hz, 3α-H); δ_C 12.5 (q, C-18), 19.0 (q, C-21), 21.3 (t, C-11), 21.5 (q, AcO), 22.9 (q, C-26), 23.1 (q, C-27), 24.2 (t, C-23), 24.4 (t, C-15), 25.2 (t, C-2), 27.5 (q, C-19), 28.3 (d, C-25), 28.6 (t, C-16), 32.05 (t, C-1), 32.8 (d, C-8), 36.1 (d, C-20), 36.5 (t, C-22), 36.95 (s, C-10), 37.2 (d, C-4), 39.6 (t, C-7), 39.8 (t, C-24), 40.3 (t, C-12), 41.1 (d, C-9), 43.2 (s, C-13), 45.0 (t, C-6'), 51.5 (d, C-5), 56.2 (d, C-17), 56.6 (d, C-14), 58.5 (t, C-4'), 72.9 (d, C-3), 76.6 (s, C-6), 170.7 (s, C-5'), and 209.8 (s, AcO) (Found: C, 76.7; H, 10.35%; M^+, 500. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.5%; *M*, 500).

***Forced acid-mediated elimination of* 3ß,6-*dihydroxy*-4α,4',5ß,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (86)**

(a) 3ß,6-Dihydroxy-4α,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one (**86**) (990 mg, 2.16 mmol) was treated with toluene-*p*-sulphonic acid (350 mg, 2.16 mmol) in ethanol (25 ml) at reflux. After 5 h at reflux, the starting material had been consumed (t.l.c.). The reaction mixture was diluted with water, allowed to cool to 20°C, and solid sodium hydrogen carbonate was added. The neutralised reaction mixture was filtered, and the filtrate was partly evaporated under reduced pressure. The mixture was extracted with ethyl acetate (2 x 100 ml). The combined organic phase was washed with brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (1 g), which was adsorbed on silica gel (60 g), and eluted with ethyl acetate-toluene (3:7), to afford 3ß-*hydroxy*-4α,4',5ß,6-*tetrahydrobenzo*[4.5.6]*cholest*-6-*en*-5'(6'H)-*one* (**89**) (518 mg, 60%), m.p. 69–72°C (from methanol); $[\alpha]_D$ +26° (*c* 1.0); υ_{max} 3615 (OH) and 1710 (C=O) cm^{-1}; δ_H (90 MHz) 0.67 (3H, s, 13ß-CH_3), 0.94 (3H, s, 10ß-CH_3), 2.96 (2H, m, $W_{1/2}$ 10 Hz, 4'-H_2), 3.75

(1H, m., $W_{1/2}$ 10 Hz, 3α-H), and 5.30 (1H, s, 7-H) (Found: C, 81.5; H, 10.9%; M^+, 440. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%; *M*, 440). Further elution with ethyl acetate-toluene (6:4) afforded 3ß-*hydroxy*-4α,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'*H*)-*one* (**91**) (114 mg, 12%), m.p. 169–172°C (from acetone); $[\alpha]_D$ +105° (*c* 0.9); υ_{max} 3605 (OH), 1652 (C=O), and 1625 (C=C) cm^{-1}; δ_H 0.62 (3H, s, 13ß-CH_3), 0.85 (3H, s, 10ß-CH_3), 2.03 (2H, d, *J* 5.2 Hz, 5α-H), 3.83 (1H, br.m, $W_{1/2}$ 18 Hz, 3α-H), and 5.90 (1H, s, 4'-H); δ_C 12.1 (q, C-18), 15.6 (q, C-19), 18.6 (q, C-21), 21.0 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 24.15 (t, C-15), 26.7 (t, C-2), 28.0 (d, C-25), 28.1 (t, C-16), 34.0 (t, C-7), 35.7 (d, C-20, and t, C-6, and t, C-1), 36.1 (t, C-22), 38.1 (d, C-4), 39.5 (t, C-24), 39.6 (d, C-8), 40.8 (s, C-10), 41.5 (t, C-12), 43.1 (s, C-13), 49.9 (d, C-5), 56.15 (d, C-17, and d, C-9), 56.6 (d, C-14), 71.35 (d, C-3), 124.3 (s, C-4'), 165.7 (d, C-6), and 201.5 (s, C-5') (Found: C, 81.7; H, 11.2%; M^+, 440. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%; *M*, 440).

(b) 3ß,6-Dihydroxy-4α,4',5α,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**86**) (1 g, 2.18 mmol) was treated with toluene-*p*-sulphonic acid (352 mg, 2.18 mmol) in benzene (40 ml) at reflux. After 14 h at reflux, the reaction mixture was allowed to cool to 20°C, and solid sodium hydrogen carbonate was added. The neutralised mixture was washed with water (5 ml), brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to afford a residue (1 g), which was adsorbed on silica gel (80 g), and eluted with ethyl acetate (1:1), to afford 3ß-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**91**) (268 mg, 28%).

(c) 3ß-Hydroxy-4α,4',5ß,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'*H*)-one (**89**) (220 mg, 0.5 mmol) was treated with toluene-*p*-sulphonic acid (80 mg, 0.5 mmol) in benzene at reflux. After 8 h at reflux, the starting material had been consumed (t.l.c.), and the reaction mixture was

allowed to cool to 20°C. Solid sodium hydrogen carbonate was added and the neutralised mixture was washed with water (2 ml) and brine. The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to give a residue (200 mg), which was adsorbed on silica gel (6 g), and eluted with ethyl acetate-toluene (1:1), to afford the enone (**91**) (110 mg, 50%).

(d) 3ß-Acetoxy-6-hydroxy-4α,4',5ß,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**88**) (1 g, 2 mmol) was treated with aqueous 10*M*–sulfuric acid (1 ml) in ethanol (20 ml) at reflux. After 40 h at reflux, the starting material had been consumed (t.l.c.), and the reaction mixture was allowed to cool to 20°C. Solid sodium hydrogen carbonate was added, and the neutralised mixture was partly evaporated under reduced pressure. The mixture was diluted with water, and extracted with ethyl acetate (2 x 100 ml). The combined organic phase was washed with brine, dried ($MgSO_4$), and the solvent was removed under reduced pressure to give a residue (1.1 g), which was adsorbed on silica gel (80 g), and eluted with ethyl acetate-toluene (3:7), to afford 3ß-hydroxy-4α,4',5ß,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'*H*)-one (**89**) (178 mg, 20%). Further elution with ethyl acetate-toluene (3:2), gave 3ß-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**91**) (160 mg, 18%).

(e) Aqueous 2*M*–potassium carbonate (2 ml) was added dropwise to a vigorously stirred solution of 3ß-acetoacetoxycholest-4-en-6-one (**66**) (484 mg, 1 mmol), in ethanol (30 ml) at 60°C. The reaction temperature was maintained at 60°C for 1 h, after which the starting material had been consumed (t.l.c.). Thereupon, aqueous 2*M*–potassium hydroxide (2 ml) was added dropwise during 1 min, and the reaction mixture was heated to reflux temperature. After 5 h at reflux temperature, the reaction mixture was cooled and maintained at

0°C for 12 h. Then, 2*M*-ethanolic toluene-*p*-sulphonic acid (10 ml) was added, and the reaction mixture was heated and maintained at reflux temperature for 30 h, after which the reaction mixture was cooled to 20°C, neutralised with solid carbon dioxide, filtered, and the filtrate was partly evaporated under reduced pressure. Water was added, and the suspension was extracted into ethyl acetate (200 ml). The organic phase was washed with brine, dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure, to afford a residue (105 mg), which was adsorbed on silica gel (5 g), and eluted with ethyl acetate-toluene (1:1), to afford the conjugate enone (**91**) (86 mg, 20%).

3ß-Acetoxy-4α,4',5ß,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'H)-one **(90)**

3ß-Hydroxy-4α,4',5ß,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'*H*)-one (**89**) (200 mg, 0.45 mmol) was treated with acetic anhydride (1 ml) in pyridine (2 ml) at 25°C for 3 h. The reaction mixture was poured into an acidified (HCl) water-ice mixture. The precipitate was extracted into ethyl acetate (2 x 100 ml). The combined organic phase was washed with water (2 x 10 ml), aqueous saturated sodium hydrogen carbonate, and brine. The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to give 3ß-*acetoxy*-4α,4',5ß,6-*tetrahydrobenzo*[4.5.6]*cholest*-6-*en*-5'(6'H)-*one* (**90**) (200 mg, 92%), m.p. 112–118°C (from methanol); $[\alpha]_D$ +47° (*c* 0.9); υ_{max} 1730 (AcO), and 1708 (C=O) cm^{-1} ; δ_H 0.68 (3H, s, 13ß-CH_3), 0.96 (3H, s, 10ß-CH_3), 2.09 (3H, s, AcO), 2.16 (2H, br.m, $W_{1/2}$ 20 Hz, 6'-CH_2), 2.94 (1H, d, *J* 14.7 Hz, 4' -H), 3.05 br.(1H, dt, *J* 14.7 and 2 x ca.2 Hz, 4' -H), 4.82 (1H, m, $W_{1/2}$ 8 Hz, 3α-H), and 5.29 (1H, s, 7-H); δ_C 11.9 (q, C-18), 18.6 (q, C-21), 20.7 (t, C-11), 21.1 (q, C-19), 22.5 (q, C-26), 22.7 (q, C-27), 23.3 (q, AcO), 23.8 (t, C-23, and t, C-15), 24.9 (t, C-2), 27.9 (d, C-25), 28.2 (t, C-16), 29.0 (t, C-

1), 33.95 (s, C-10), 35.7 (d, C-20), 36.05 (t, C-22), 37.05 (d, C-4), 39.4 (t, C-24), 39.9 (t, C-12), 40.1 (d, C-9), 41.0 (d, C-8), 43.1 (s, C-13), 44.7 (t, C-6'), 47.3 (d, C-5), 52.7 (t, C-4'), 54.5 (d, C-17), 56.0 (d, C-14), 71.2 (d, C-3), 124.6 (d, C-7), 133.8 (s, C-6), 170.3 (s, AcO), and 207.3 (s, C-5') (Found: C, 79.35; H, 10.35%, M^+, 482. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.4%; *M*, 482).

3α,6-*Dihydroxy*-4ß,4',5ß,6-tetrahydrobenzo[4.5.6]*cholestan*-5'(6'H)-*one* (93)

(a) Aqueous 2*M*−potassium hydroxide (2 ml) was added dropwise during 1 min to a stirred solution of (2*S*)-2-(3α-hydroxy-6-oxo-5α-cholestan-4α-yl)-3-oxobutanoic acid 1,3'-lactone (**82**) (175 mg, 0.36 mmol) in dimethylformamide (12 ml) and water (2 ml) at 50°C. After the addition, the reaction mixture was heated to reflux. After 1 h at reflux, the starting material had been consumed (t.l.c.). The reaction mixture was allowed to cool to 20°C and was diluted with water, acidified with aqueous 1*M*−hydrochloric acid, and extracted with diethyl ether (3 x 50 ml). The combined organic phase was washed with 1*M*−hydrochloric acid, water, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a residue (190 mg), which was redissolved in pyridine (4 ml), and acetic anhydride (2 ml) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred for 2 h, and poured into an acidified (HCl) water-ice mixture. The suspension was extracted into ethyl acetate (200 ml) and washed with aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was then washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (180 mg), which was adsorbed on silica gel (10 g), and eluted

with ethyl acetate-toluene (10:90), to afford 3α-*acetoxy*-4ß,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'*H*)-*one* (**96**) (50%), m.p. 180–182°C (from methanol); $[\alpha]_D$ −50° (*c* 0.9); υ_{max} 1731 (AcO), 1659 (C=O), and 1614 (C=C) cm^{-1}; δ_H 0.66 (1H, s, 13ß-CH_3), 0.87 (3H, s, 10ß-CH_3), 2.06 (3H, s, AcO), 2.45 (2H, br.m, $W_{1/2}$ 30 Hz, 6'-H_2), 4.95 (1H, m, $W_{1/2}$ 7 Hz, 3ß-H), and 5.82 (1H, s, 4'-H); δ_C 12.0 (q, C-18), 12.6 (q, C-19), 18.6 (q, C-21), 20.7 (t, C-11), 21.1 (q, AcO), 22.5 (q, C-26), 22.7 (q, C-27), 27.9 (d, C-25), 28.1 (t, C-2), 31.9 (t, C-1), 34.1 (d, C-4), 35.4 (d, C-20), 35.65 (d, C-8), 36.1 (t, C-22), 37.2 (s, C-10), 39.4 (t, C-12, and t, C-24), 39.6 (t, C-7), 40.1 (t, C-6'), 42.55 (s, C-13), 46.6 (d, C-5), 52.6 (d, C-9), 56.1 (d, C-17), 56.55 (d, C-14), 71.3 (d, C-3), 125.4 (d, C-4'), 164.0 (s, C-6), 170.3 (s, AcO), and 199.0 (s, C-5') (Found: C, 79.3; H, 10.25%; M^+, 482. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.5%; *M*, 482), followed by 3α-*acetoxy*-6-*hydroxy*-4ß,4',5α,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**94**) (50%), m.p. 167–170°C (from methanol); $[\alpha]_D$ −22° (*c* 1.0); υ_{max} 3591 (OH), 1723 (AcO), and 1712 (C=O) cm^{-1}; δ_H 0.68 (3H, s, 13ß-CH_3), 1.03 (3H, s, 10ß-CH_3), 1.66 (1H, d, *J* 12.1 Hz, 5α-H), 2.08 (3H, s, AcO), 2.46 (2H, br. m, $W_{1/2}$ 32 Hz, 4'-H_2), and 4.91 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H); δ_C 12.0 (q, C-18), 15.8 (q, C-19), 18.6 (q, C-21), 20.7 (t, C-11), 21.2 (q, AcO), 22.5 (q, C-26), 27.9 (d, C-25), 28.1 (t, C-1), 31.3 (d, C-8), 34.5 (t, C-2), 35.7 (d, C-20), 36.1 (t, C-22), 36.15 (d, C-4), 36.8 (s, C-10), 39.4 (t, C-24), 39.7 (t, C-12), 42.6 (s, C-13), 43.3 (t, C-7), 46.4 (t, C-6'), 48.1 (d, C-5), 54.2 (d, C-9), 55.8 (d, C-17), 56.2 (d, C-14), 56.8 (t, C-4'), 72.2 (d, C-3), 75.5 (s, C-6), 170.3 (s, AcO), and 209.1 (s, C-5') (Found: C, 76.5; H, 10.2%; M^+, 500. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.0%; *M*, 500).

Hydrolysis of (**94**) with aqueous potassium hydroxide in ethanol afforded 3α,6-*dihydroxy*-4ß,4',5α,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**93**) (89%), m.p. 199–202°C (from methanol); $[\alpha]_D$ −1.5° (*c* 1.0); υ_{max} 3615

(OH) and 1708 (C=O) cm^{-1}; δ_H 0.68 (3H, s, 13ß-CH_3), 1.02 (3H, s, 10ß-CH_3), 2.35 (1H, dd, *J* 13.1 and 2.3 Hz, 4'α-H), 2.57 (1H, d, *J* 13.1 Hz, 4'ß-H), 2.64 (1H, t, *J* 2 x 13.0 Hz, 6'ß-H), and 3.78 (1H, m, $W_{1/2}$ 7 Hz, 3ß-H); δ_C 12.0 (q, C-18), 15.7 (q, C-19), 18.6 (q, C-21), 20.7 (t, C-11), 22.5 (q, C-26), 22.7 (q, C-27), 23.8 (t, C-23), 24.1 (t, C-15), 27.9 (d, C-25), 28.1 (t, C-16), 29.45 (t, C-2), 31.3 (d, C-8), 33.9 (t, C-1), 35.7 (d, C-20), 36.1 (t, C-22), 36.95 (s, C-10), 37.8 (d, C-4), 39.4 (t, C-24), 39.8 (t, C-12), 42.6 (s, C-13), 43.7 (t, C-7), 46.4 (t, C-6'), 46.8 (d, C-5), 54.25 (d, C-9), 55.8 (d, C-17), 56.15 (d, C-14), 56.65 (t, C-4'), 68.8 (d, C-3), 76.0 (s, C-6), and 210.6 (s, C-5') (Found: C, 78.25; H, 11.0%; M^+, 458. $C_{30}H_{50}O_3$ requires C, 78.55; H, 11.0%; *M*, 458).

Hydrolysis of (**96**) with aqueous potassium hydroxide in ethanol afforded 3α-*hydroxy*-4ß,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**95**) (91%), m.p. 150–153°C (from hexane); $[\alpha]_D$ −28°C (*c* 1.0); υ_{max} 3617 (OH), 1657 (C=O), and 1625 (C=C) cm^{-1}; δ_H 0.65 (3H, s, 13ß-CH_3), 0.88 (H, s, 10ß-CH_3), 1.63 (1H, s, exch. by D_2O, OH), 2.45 (2H, br.m, $W_{1/2}$ 25 Hz, 6'-H_2), 3.84 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H), and 5.82 (1H, s, 4'-H); δ_C 12.0 (q, C-18), 12.5 (q, C-19), 18.6 (q, C-21), 20.7 (t, C-11), 22.4 (q, C-26), 22.7 (q, C-27), 23.7 (t, C-23), 23.9 (t, C-15), 27.9 (d, C-25), 28.1 (t, C-16), 29.3 (t, C-2), 31.4 (t, C-1), 34.1 (d, C-4), 35.6 (d, C-20), 36.1 (t, C-22), 36.9 (d, C-8), 37.4 (s, C-10), 39.4 (t, C-12, and t, C-24), 39.7 (t, C-7), 40.6 (t, C-6'), 42.5 (s, C-13), 45.45 (d, C-5), 52.7 (d, C-9), 56.0 (d, C-17), 56.7 (d, C-14), 68.5 (d, C-3), 125.2 (d, C-4'), 164.9 (s, C-6), and 200.2 (s, C-5') (Found: C, 81.95; H, 11.2%; M^+, 440. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%; *M*, 440).

(b) Aqueous 1*M*–potassium carbonate (2 ml) was added dropwise to a vigorously stirred solution of 3α-acetoacetoxycholest-4-en-6-one (**67**)

(390 mg, 0.8 mmol) in ethanol (20 ml) at 20°C. The reaction mixture was stirred for 3 h at 20°C, after which the reaction mixture was heated to reflux. After a further 4 h at reflux, the reaction mixture was allowed to cool to 20°C, and solid carbon dioxide was added. The neutralised reaction mixture was filtered, and the filtrate was partly evaporated under reduced pressure. The mixture was extracted with ethyl acetate (2 x 100 ml). The combined organic phase was washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (329 mg), which was adsorbed on silica gel (30 g), and eluted with ethyl acetate-toluene (45:55) to afford 3α-hydroxycholest-4-en-6-one (**63**) (96 mg, 30%), followed by 3α,6-dihydroxy-4ß,4',5α,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**93**) (64%).

3α-*Acetoxy*-6-*hydroxy*-4ß,4',5α,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (94)

3α,6-Dihydroxy-4ß,4',5α,6ß-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**93**) (700 mg, 15.3 mmol) was treated with acetic anhydride (2 ml) in pyridine (4 ml) at 40°C. After 6 h at 40°C, the reaction mixture was allowed to cool to 20°C, and was poured into an acidified (HCl) water-ice mixture. The suspension was extracted into ethyl acetate (2 x 150 ml). The organic phase was washed with water (2 x 15 ml), aqueous saturated sodium hydrogen carbonate, brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 3α-*acetoxy*-6-*hydroxy*-4ß,4',5α,6ß-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**94**) (688 mg, 90%).

3α-*Hydroxy*-4ß,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (95)

(a) Potassium metal (35 mg, 0.25 mmol) was added to t-butyl alcohol (freshly distilled from potassium metal) (20 ml) at 40°C under nitrogen.

After 20 min at 40°C, a solution of 3α-acetoacetoxycholest-4-en-6-one (**67**) (400 mg, 0.826 mmol) in dry tetrahydrofuran (5 ml) was added dropwise during 2 min. After 2h, the starting material had been consumed (t.l.c.), water (1.5 ml) and aqueous 1*M*−potassium hydroxide (1.5 ml) was added. The reaction mixture was heated to reflux. After 1 h at reflux temperature, the reaction mixture was allowed to cool to 30°C, and the pH was adjusted to 3 with an 1*M*−ethanolic toluene-*p*-sulphonic acid solution. The reaction mixture was heated to 50°C for 12 h, then allowed to cool to 30°C, and solid sodium hydrogen carbonate was added. The neutralised mixture was filtered, and the filtrate was extracted with diethyl ether (2 x 150 ml). The combined organic phase was washed with water (10 ml), brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (390 mg), which was adsorbed on silica gel (18 g), and eluted with ethyl acetate-toluene (45:55), to afford 3α-*hydroxy*-4ß,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**95**) (246 mg, 70%).

3α-*Acetoxy*-4ß,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (96)

3α-Hydroxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**95**) (230 mg, 0.5 mmol) was treated with acetic anhydride (2 ml) in pyridine (4 ml) at 50°C for 2 h. The reaction mixture was poured into an acidified (HCl) water-ice mixture, and the suspension was extracted into ethyl acetate (2 x 100 ml). The combined organic phase was washed with water (2 x 15 ml), aqueous saturated sodium hydrogen carbonate, and brine. The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to give 3α-*acetoxy*-4ß,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'*H*)-*one* (**96**) (236 mg, 94%).

***Catalytic hydrogenation of the 3α-acetoxy-enone* (96)**

(a) A solution of 3α-acetoxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**96**) (200 mg, 0.415 mmol) in tetrahydrofuran (10 ml), was hydrogenated with 5% palladium on carbon (50 mg). The suspension was stirred at 20°C for 10 h under hydrogen, after which the catalyst was removed by filtration through Celite. The filtrate was evaporated under reduced pressure to give a residue (200 mg), which was adsorbed on silica gel (10 g), and eluted with ethyl acetate-toluene (5:95 / 15:85 / 50:50), to afford 4ß,5α,5',6'-*tetrahydrobenzo*[4.5.6]*cholestan*-3α-*yl acetate* (**99**) (40 mg, 20%), m.p. 106–108°C (from ethyl acetate-methanol); $[\alpha]_D$ – 53° (*c* 0.6); υ_{max} 1722 (AcO) cm^{-1}; δ_H 0.65 (3H, s, 13ß-CH_3), 0.90 (3H, s, 10ß-CH_3), 2.05 (3H, s, AcO), 2.22 (1H, dd, *J* 14 and 4 Hz, 7α-H), 5.00 (1H, m, $W_{1/2}$ 6 Hz, 3ß-H), and 5.38 (1H, br.s, $W_{1/2}$ 10 Hz, 4'-H); δ_C 12.0 (q, C-18), 12.2 (q, C-19), 18.7 (q, C-21), 20.9 (t, C-11), 21.3 (q, AcO), 22.55 (q, C-26), 22.8 (q, C-27), 23.85 (t, C-23), 24.1 (t, C-15), 25.3 (t, C-6'), 25.0 (t, C-5'), 26.7 (t, C-2), 28.0 (d, C-25), 28.2 (t, C-16), 32.5 (t, C-1), 34.6 (d, C-4), 35.8 (d, C-20), 36.2 (t, C-22), 36.9 (s, C-10), 37.05 (d, C-8), 39.5 (t, C-7), 39.8 (t, C-24 and t, C-12), 42.5 (s, C-13), 46.1 (d, C-5), 53.3 (d, C-9), 56.2 (d, C-17), 56.7 (d, C-14), 73.2 (d, C-3), 120.7 (d, C-4'), 137.2 (s, C-6), and 171.0 (s, AcO); (Found: C, 81.9; H, 11.3%; M^+, 468. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%; *M*, 468), followed by 3α-*acetoxy*-4ß,4',5α,6α-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**100**) (143 mg, 72%), m.p. 192–195°C (from ethyl acetate-toluene); $[\alpha]_D$ –12° (*c* 1.0); υ_{max} 1728 (AcO), and 1705 (C=O) cm^{-1}; δ_H 0.64 (3H, s, 13ß-CH_3), 0.86 (3H, s, 10ß-CH_3), 2.07 (3H, s, AcO), and 4.82 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H); δ_C 12.1 (q, C-18), 12.9 (q, C-19), 18.7 (q, C-21), 20.9 (t, C-11), 21.2 (q, AcO), 22.55 (q, C-26), 22.8 (q, C-27), 23.9 (t, C-23), 24.1 (t, C-15), 26.1 (t, C-2), 28.0 (d, C-25), 28.2 (t, C-16), 32.6 (t, C-1), 34.9 (d, C-4), 35.8 (d, C-20), 36.15 (t, C-22), 36.5 (d, C-8 and s, C-10), 39.5 (t, C-24 and d, C-17),

72.0 (d, C-3), 170.4 (s, AcO), and 210.7 (C-5'); (Found: C, 79.3; H, 11.1%; M^+, 484. $C_{32}H_{52}O_3$ requires C, 79.3; H, 10.8%; *M*, 484), and finally 4ß,5α,5'ß,6'-tetrahydrobenzo[4.5.6]cholestane-3α,5'α-diol 3-acetate (**101**) (16 mg, 8%). A mixed melting point with the product (**101**), obtained from the attempted conjugate reduction with MeCu/DIBAL-HMPA was undepressed, and all the other data were identical.

(b) A solution of 3α-acetoxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**96**) (200 mg, 0.415 mmol) in tetrahydrofuran (10 ml), was hydrogenated with 5% palladium on calcium carbonate (30 mg). The suspension was stirred at 20°C for 24 h under hydrogen, after which the catalyst was removed by filtration through Celite. The filtrate was evaporated under reduced pressure to give a residue (200 mg), which was crystallised from ethyl acetate-methanol, to afford 3α-acetoxy-4ß,4',5α,6α-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**100**) (186 mg, 92%).

Attempted conjugate reduction with DIBAL-H/MeCu-HMPA

As a milder method for the conjugate reduction of enone (**96**), the following procedure[43] was attempted. A 1.6*M*–solution of methyllithium (0.23 ml, 0.5 mmol) in diethyl ether was added dropwise to a stirred suspension of copper(I) iodide (95 mg, 0.5 mmol) in dry tetrahydrofuran (8 ml) at −78°C (dry ice-acetone) under nitrogen. The resultant yellow precipitate was stirred for 5 min. Hexamethylphosphoric triamide (1.0 ml, 6 mmol), and a 1.4*M*–solution of diisobutylaluminium hydride (4.3 ml, 6 mmol) in toluene were added successively. The mixture was stirred for 30 min at −78°C. A solution of 3α-acetoxy-4ß,5a-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**96**) (150 mg, 0.31 mmol) in dry tetrahydrofuran (1 ml) was added. After 1 h at −78°C, no reaction had taken place (t.l.c.), and the reaction mixture was allowed to warm to

20°C. After 2 h at 20°C, the starting material had been consumed (t.l.c.). Aqueous 0.5*M*-hydrochloric acid (5 ml) was added, followed by diethyl ether (5 ml). The aqueous phase was back-extracted with diethyl ether (5 ml). The combined organic phase was washed with water (3 x 5 ml), and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a residue (195 mg), which was adsorbed on silica gel (18 g), and eluted with ethyl acetate-toluene (3:7), to afford 4ß,5α,5'ß,6'-*tetrahydrobenzo*[4.5.6]*cholestane*-3α,5'α-*diol* 3-*acetate* (**101**) (100 mg, 66%), m.p. 80–82°C (from methanol); $[\alpha]_D$ −32° (*c* 1.0); υ_{max} 3597 (OH) and 1729 (AcO) cm^{-1}; δ_H 0.64 (3H, s, 13ß-CH_3), 0.88 (3H, s, 10ß-CH_3), 2.05 (3H, s, AcO), 4.28 (1H, br.m, $W_{1/2}$ 20 Hz, 5'ß-H), 5.00 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H), and 5.30 (1H, s, 4'-H); δ_C 11.9 (q, C-18), 12.1 (q, C-19), 18.6 (q, C-21), 20.7 (t, C-11), 21.2 (q, AcO), 22.5 (q, C-26), 22.7 (q, C-27), 23.8 (t, C-23), 24.0 (t, C-15), 26.5 (t, C-2), 27.9 (d, C-25), 28.1 (t, C-16), 32.3 (t, C-1), 34.3 (d, C-4), 34.7 (d, C-20), 35.7 (d, C-8), 36.1 (t, C-7, and t, C-22), 36.6 (s, C-10), 39.1 (t, C-12), 39.4 (t, C-24), 39.6 (t, C-6'), 42.4(s, C-13), 45.8 (d, C-5), 53.1 (d, C-9), 56.1 (d, C-17), 56.6 (d, C-14), 67.9 (d, C-5'), 72,5 (d, C-3), 125.3 (d, C-4'), 139.3 (s, C-6), and 170.7 (s, AcO) (Found: C, 79.5; H, 11.0%; M^+, 484. $C_{32}H_{52}O_3$ requires C, 79.3; H, 10.8%; *M*, 484). Further elution with ethyl acetate-toluene (3:2) afforded 4ß,5α,5',6'-*tetrahydrobenzo*[4.5.6]*cholestane*-3α,5'α-*diol* (**109**) (40 mg, 29%), m.p. 195–198°C (from methanol); $[\alpha]_D$ −17° (*c* 0.8); υ_{max} 3610 (2 x OH) cm^{-1}; δ_H 0.64 (3H, s, 13ß-CH_3), 0.88 (3H, s, 10ß-CH_3), 3.85 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H), 4.3 (1H, m, $W_{1/2}$ 20 Hz, 5'ß-H), and 5.33 (1H, s, 4'-H); δ_C 11.9 (q, C-18), 12.0 (q, C-19), 18.6 (q, C-21), 20.7 (t, C-11), 22.5 (q, C-26), 22.7 (q, C-27), 23.7 (t, C-23), 24.0 (t, C-15), 27.9 (d, C-25), 28.1 (t, C-16), 29.4 (t, C-2), 31.7 (t, C-1), 34.4 (d, C-4), 35.7 (d, C-20), 36.0 (t, C-22), 36.1 (d, C-8), 36.6 (t, C-7), 36.8 (s, C-10), 39.2 (t, C-12), 39.4 (t, C-24), 39.6 (t, C-6'), 42.4 (s, C-13), 44.7 (d, C-5), 53.2 (d, C-9), 56.1 (d, C-

17), 56.6 (d, C-14), 68.35 (d, C-3), 70.0 (d, C-5'), 125.2 (d, C-4'), and 139.7 (s, C-6) (Found: C, 81.45; H, 11.6%; M^+, 442. $C_{30}H_{50}O_2$ requires C, 81.4; H,11.4%; *M*, 442).

3α-*Hydroxy-4ß,4',5α,6α-tetrahydrobenzo*[4.5.6]*cholestan-5'(6'H)-one* (102)

Aqueous 2*M*-potassium hydroxide (1 ml) was added dropwise to a stirred solution of 3α-acetoxy-4ß,4',5α,6α-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**100**) (100 mg, 0.21 mmol) in ethanol (20 ml) at 20° under nitrogen. After 3 h at 20°C, the starting material (**100**) had been consumed (t.l.c.). The reaction mixture was neutralised with solid carbon dioxide, filtered, and the solvent was removed under reduced pressure to give a residue (96 mg), which was adsorbed on silica gel (2 g), and eluted with ethyl acetate-toluene (1:3), to afford 3α-*hydroxy*-4ß,4',5α,6α-*tetrahydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**102**) (85 mg, 93%), m.p. 147–150°C (from methanol), $[\alpha]_D$ +11° (*c* 1.2); υ_{max} 3594 (OH) and 1702 (C=O) cm^{-1}; δ_H 0.64 (3H, s, 13ß-CH_3), 0.88 (3H, s, 10ß-CH_3), 2.19 (2H, br.m, $W_{1/2}$ 45 Hz, 6'ß-H and 4'ß-H), 2.61 (t, *J* 2 x 13 Hz, 6'α-H), and 3.70 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H); δ_C 12.0 (q, C-18), 12.7 (q, C-19), 18.6 (q, C-21), 20.85 (t, C-11), 22.45 (q, C-26), 22.7 (q, C-27), 23.7 (t, C-23), 24.0 (t, C-15), 27.0 (d, C-25), 28.1 (t, C-16), 29.6 (t, C-2), 31.9 (t, C-1), 34.8 (d, C-4), 35.7 (s, C-10), 36.1 (d, C-20), 36.6 (d, C-22), 36.9 (d, C-8), 39.4 (t, C-24), 39.8 (t, C-12), 40.5 (t, C-7), 41.1 (d, C-6), 42.5 (s, C-13), 24.3 (t, C-4'), 46.65 (d, C-5), 48.9 (t, C-6'), 54.2 (d, C-9), 56.1 (d, C-17), 56.2 (d, C-14), 69.0 (d, C-3), and 212.1 (s, C-5'); (Found: C, 81.25;H, 11.2%; M^+, 442. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%; *M*, 442).

***Catalytic hydrogenation of the* 3α-*hydroxy-enone* (95)**

A solution of 3α-hydroxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**95**) (300 mg, 0.68 mmol) in tetrahydrofuran (20 ml) was hydrogenated with 5% palladium on calcium carbonate (120 mg). The suspension was stirred at 20°C for 24 h under hydrogen. The catalyst was removed by filtration through Celite. The filtrate was evaporated under reduced pressure to give a residue (300 mg), which was adsorbed on silica gel (18 g), and eluted with ethyl acetate-toluene (1:3 / 1:1) to afford 3α-hydroxy-4ß,4',5α,6α-tetrahydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**102**) (252 mg, 84%), followed by 4ß,4',5α,5'α,6α,6'-*hexahydrobenzo*[4.5.6]*cholestan*-3,5'-*diol* (**103**) (42 mg, 14%), m.p. 197–200°C (from acetone); $[\alpha]_D$ +11° (*c* 0.8), δ_H 0.63 (3H, s, 13ß-CH_3), 0.86 (3H, s, 10ß-CH_3), 3.55 (1H, br.m, $W_{1/2}$ 25 Hz, 5'α-H), and 3.72 (1H, m, $W_{1/2}$ 8 Hz, 3ß-H); δ_C 12.1 (q, C-18), 13.0 (q, C-19), 18.7 (q, C-21), 21.0 (t, C-11), 22.6 (q, C-26), 22.8 (q, C-27), 23.9 (t, C-23), 24.2 (t, C-15), 28.0 (d, C-25), 28.3 (t, C-16), 29.3 (t, C-2), 32.3 (t, C-1), 33.4 (d, C-8), 34.9 (d, C-6), 35.8 (d, C-20), 36.05 (s, C-10), 36.2 (t, C-22), 38.0 (d, C-4), 39.0 (t, C-4'), 39.5 (t, C-24), 39.9 (t, C-12), 40.1 (t, C-7), 42.6 (s, C-13), 43.8 (t, C-6'), 47.0 (d, C-5), 54.5 (d, C-9), 56.3 (d, C-17), 56.4 (d, C-14), 69.8 (d, C-5), and 69.9 (d, C-3) (Found: C, 81.2; H, 11.9%; M^+, 444. $C_{30}H_{52}O_2$ requires C, 81.0; H, 11.8%; *M*, 444).

***Oxidation of* 3α-*hydroxy-4ß,4',5α,6α-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one* (102)**

Chromium trioxide (150 mg, 1.5 mmol) was added in one portion to a stirred solution of pyridine (235 mg, 3 mmol) in dichloromethane (4 ml). The flask was stoppered with a drying tube and the deep burgandy solution was stirred for 15 min at 20°C. At the end of this period, a solution of the starting material (**102**) (110 mg, 0.25 mmol) in dichloromethane (2 ml) was added at once. After 10 min, the reaction

mixture was decanted. The tarred flask was rinsed with two more portions of dichloromethane, and the combined organic phase was washed with aqueous 5% sodium hydroxide, 1*M*-hydrochloric acid, aqueous saturated sodium hydrogen carbonate, and brine. The organic phase was dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure to give a residue (110 mg), which was adsorbed on silica gel (3 g), and eluted with toluene, to afford 4ß,4',5α,6α-*tetrahydrobenzo*[4.5.6]*cholestan*-3,5'(6'*H*)-*dione* (**104**) (99 mg, 90%), m.p. 128–131°C (from methanol); $[\alpha]_D$ +17° (*c* 1.0); υ_{max} 1710 (2 x C=O) cm^{-1}; δ_H 0.69 (3H, s, 13ß-CH_3), 1.1 (3H, s, 10ß-CH_3), and 2.7 (1H, dt, *J* 2 x 12.4 and 4.7 Hz, 4ß-H); δ_C 12.05 (q, C-18), 13.0 (q, C-19), 18.65 (q, C-21), 21.3 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 24.1 (t, C-15), 28.0 (d, C-25), 28.2 (t, C-16), 34.8 (d, C-6), 35.75 (d, C-20), 36.1 (t, C-22), 36.65 (s, C-10), 37.75 (d, C-8), 37.9 (t, C-1), 39.3 (t, C-2), 39.5 (t, C-24),39.7 (t, C-12), 40.3 (t, C-7), 40.6 (t, C-4'), 42.6 (s, C-13), 48.4 (t, C-6'), 48.5 (d, C-5), 54.0 (d, C-4), 55.3 (d, C-9), 56.0 (d, C-17), 56.2 (d, C-14), 209.7 (s, C-5'), and 210.3 (s, C-3); (Found: C, 81.5; H, 11.2%; M^+, 440. $C_{30}H_{48}O_2$ requires C, 81.8; H, 11.0%; *M*, 440).

***lithium-liquid ammonia reduction of the 3α-hydroxy-enone* (95)**

An oven-dried flask was immersed in a dry ice-acetone cooling bath and filled with liquid ammonia. A few lumps of sodium were added, the cooling bath was removed, and the dry ammonia was condensed (200 ml) in an oven-dried reaction flask, equipped with a dry ice-acetone condensor. After the destillation, freshly cut lithium metal (350 mg) was added in small portions to the dry liquid ammonia. Tetrahydrofuran (80 ml) was added in portions (10 ml) to the solution, followed by t-butyl alcohol (3.5 ml). The mixture was left stirring at reflux (-33°C) for 15 min, after which a solution of 3α-hydroxy-4ß,5α-

dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**95**) (450 mg) in tetrahydrofuran (8 ml) was added to the lithium solution through a rubber septum. After 30 min, the reaction was quenched with methanol, and the reaction flask was left open overnight to evaporate the ammonia. Water was added, and the mixture was partly evaporated under reduced pressure. The mixture was then taken into ethyl acetate (300 ml), and was washed with aqueous 1*M*-hydrochloric acid. The organic phase was further washed with water, aqueous saturated sodium hydrogen carbonate, and brine. The organic phase was dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure to give the crude 4ß,4',5α,5'α,6α,6'-hexahydrobenzo[4.5.6]cholestan-3α,5'-diol (**103**) (450 mg, 98%). A mixed melting point with authentic material was undepressed.

Oxidation of this product with CrO_3-py-dichloromethane, afforded (**104**) (90%). A mixed melting point with authentic material was undepressed.

4ß,5α-*Dihydrobenzo*[4.5.6]*cholestane*-3,5'(6'H)-*dione* (107)

Chromium trioxide (280 mg, 2.71 mmol) was added to a stirred solution of pyridine (430 mg, 5.4 mmol) in dry dichloromethane (7 ml). The flask was stoppered with a drying tube and the solution was stirred for 15 min at 20°C. A solution of 3α-hydroxy-4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**95**) (200 mg, 0.45 mmol) in dry dichloromethane (3 ml) was added in one portion. After 20 min, the starting material had been consumed (t.l.c.), and the reaction mixture was decanted into dichloromethane (10 ml). The flask was rinsed with dichloromethane (2 x 10 ml). The combined organic phase was washed with aqueous 2*M*–sodium hydroxide, aqueous 1*M*–hydrochloric acid, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, and dried ($MgSO_4$). The solvent was removed

under reduced pressure to give 4ß,5α-*dihydrobenzo*[4.5.6]*cholestane*-3,5'(6'H)-*dione* (**106**) (208 mg, 90%), m. p. 133–136°C (from methanol); $[\alpha]_D$ −84° (*c* 1.0); υ_{max} 1713 (3-C=O), 1669 (5'-C=O), and 1613 (C=C) cm^{-1}; δ_H 0.69 (3H, s, 13ß-CH_3), 1.05 (3H, s, 10ß-CH_3), 2.98 (1H, ddd, *J* 13.0, 12.9, and 3.8 Hz, 4ß-H), and 5.92 (1H, s, 4'-H); δ_C 11.95 (q, C-18), 13.0 (q, C-19), 18.6 (q, C-21), 21.1 (t, C-11), 22.5 (q, C-26), 22.7 (q, C-27), 23.7 (t, C-23), 24.0 (t, C-15), 27.9 (d, C-25), 28.1 (t, C-16), 34.1 (d, C-8), 35.6 (d, C-20), 36.0 (t, C-1), 36.2 (t, C-22), 37.4 (t, C-12), 37.5 (s, C-10), 38.0 (t, C-7), 39.3 (t, C-2), 39.4 (t, C-24), 39.6 (t, C-6'), 42.5 (d, C-4), 44.6 (s, C-13), 52.5 (d, C-5), 52.7 (d, C-9), 56.0 (d, C-17), 56.4 (d, C-14), 126.4 (d, C-4'), 161.6 (s, C-6), 198.0 (s, C-5'), and 208.8 (s, C-3) (Found: C, 82.4; H, 10.6%; M^+, 438. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%; *M*, 438).

4α,5α-*Dihydrobenzo*[4.5.6]*cholestane*-3,5'(6'H)-*dione* (106)

Chromium trioxide (116 mg, 1.16 mmol) was added to a stirred solution of pyridine (184 mg, 2.32 mmol) in dry dichloromethane (3 ml). The flask was stoppered with a drying tube, and the reaction mixture was stirred for 15 min at 20°C. A solution of 3ß-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**91**) (85 mg, 0.19 mmol) in dry dichloromethane (2 ml) was added in one portion. After 10 min at 20°C, the starting material had been consumed (t.l.c.), and the reaction mixture was decanted into dichloromethane (10 ml). The flask was rinsed with dichloromethane (2 x 10 ml). The combined organic phase was washed with aqueous 2*M*–sodium hydroxide, aqueous 1*M*–hydrochloric acid, and aqueous saturated sodium hydrogen carbonate. The neutralised organic phase was washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 4α,5α-*dihydrobenzo*[4.5.6]*cholestane*-3,5'(6'H)-*dione* (**106**) (75 mg, 88%), m.p. 126–128°C (from acetone-methanol); $[\alpha]_D$ +58° (*c* 0.9); υ_{max} 1705 (3-C=O),

1663 (5'-C=O), and 1631 (C=C) cm^{-1}; δ_H 0.68 (3H, s, 13ß-CH_3), 1.00 (3H, s, 10ß-CH_3), 3.00 (1H, dt, *J* 13.9 and 2 x 7 Hz, 4α-H), and 5.98 (1H, s, 4'-H); δ_C 12.0 (q, C-18), 14.65 (q, C-19), 18.6 (q, C-21), 21.1 (t, C-11), 22.5 (q, C-26), 22.7 (q, C-27), 23.7 (t, C-23), 24.1 (t, C-15), 27.9 (d, C-25), 28.0 (t, C-16), 35.3 (t, C-2), 35.6 (d, C-20), 35.8 (t, C-22), 36.0 (t, C-1), 36.9 (t, C-12), 39.0 (d, C-8), 39.3 (t, C-7), 39.4 (t, C-24), 40.55 (s, C-10), 41.55 (t, C-6'), 42.9 (s, C-13), 45.7 (d, C-4), 49.0 (d, C-5), 55.2 (d, C-9), 56.0 (d, C-17), 56.5 (d, C-14), 124.4 (d, C-4'), 163.4 (s, C-6), 196.5 (s, C-5'), and 211.1 (s, C-3) (Found: C, 82.0; H, 10.8%; M^+, 438. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%; *M*, 438).

Alkaline equilibration

4α,5α-Dihydrobenzo[4.5.6]cholestan-3,5'(6'*H*)-dione (**106**) (100 mg, 0.23 mmol) was treated with aqueous 2*M*–potassium hydroxide (0.2 ml) in ethanol (5 ml) at 20°C under nitrogen. After 15 min at 20°C, solid carbon dioxide was added and the mixture was taken into ethyl acetate (50 ml), washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 4ß,5α-dihydrobenzo[4.5.6]cholestan-3,5'(6'*H*)-dione (**107**) (94 mg, 94%).

***5'-Hydroxybenzo*[4.5.6]*cholestan*-3-*one* (108)**

(a) 4α,5α-*dihydrobenzo*[4.5.6]*cholestan*-5'(6'H)-*one* (**106**) (250 mg, 0.57 mmol) was treated with aqueous 2*M*–potassium hydroxide (0.5 ml) in ethanol (10 ml) at 20°C. After 3 h at 20°C, solid carbon dioxide was added, and the mixture was extracted into ethyl acetate (50 ml). The organic phase was washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 5'-*hydroxybenzo*[4.5.6]*cholestan*-3-*one* (**108**) (219 mg, 88%), m.p. 237–239°C (from ethyl acetate-methanol); $[\alpha]_D$ +2.5° (*c* 0.8); υ_{max} 3590 (OH),

1669 (3-C=O), and 1606 (C=C) cm^{-1}; δ_H 0.73 (3H, s, 13ß-CH_3), 1.17 (3H, s, 10ß-CH_3), 5.45 (1H, s, exch. by D_2O, OH), 6.77 (1H, d, *J* 2.95 Hz, 4'-H), and 7.33 (1H, d, *J* 2.95 Hz, 6'-H) (Found: C, 82.4; H, 10.0%; M^+, 436. $C_{30}H_{44}O_2$ requires C, 82.5; H, 10.2%; *M*, 436).

(b) 4ß,5α-dihydrobenzo[4.5.6]cholestan-5'(6'*H*)-one (**107**) (250 mg, 0.57 mmol) was treated with aqueous 2*M*–potassium hydroxide (0.5 ml) in ethanol (10 ml) at 20°C. After 2 h at 20°C, solid carbon dioxide was added, and the mixture was extracted into ethyl acetate (50 ml). The organic phase was washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 5'-hydroxybenzo[4.5.6]-cholestan-3-one (**108**) (229 mg, 92%).

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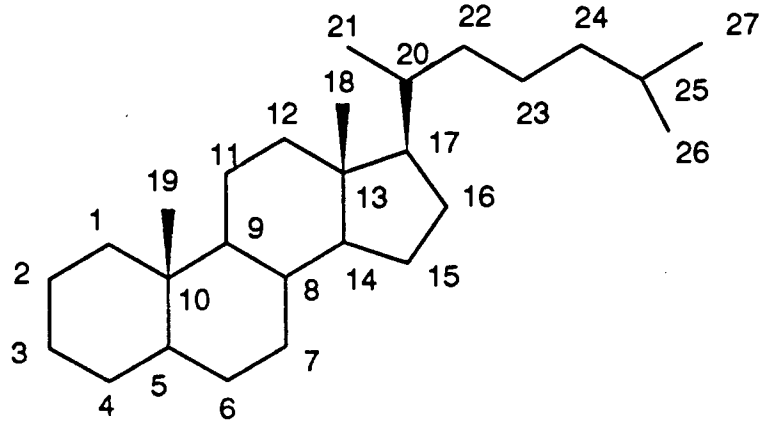
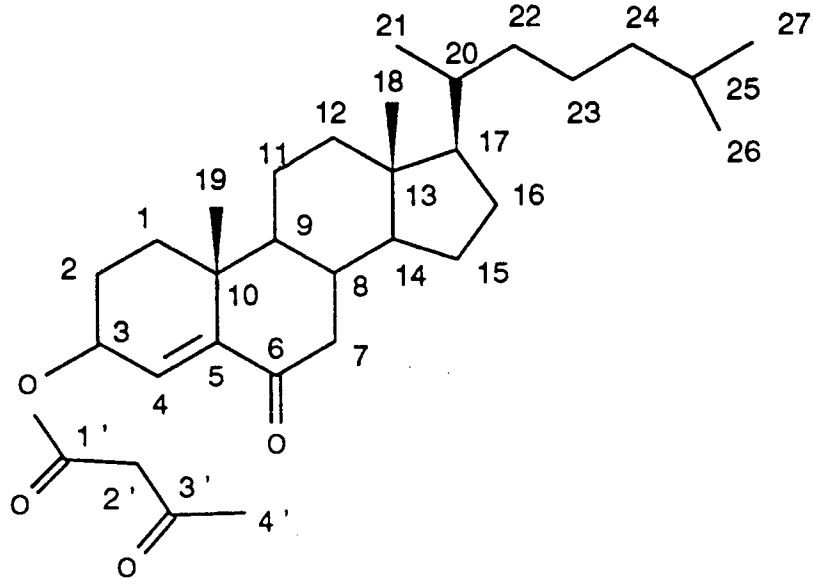
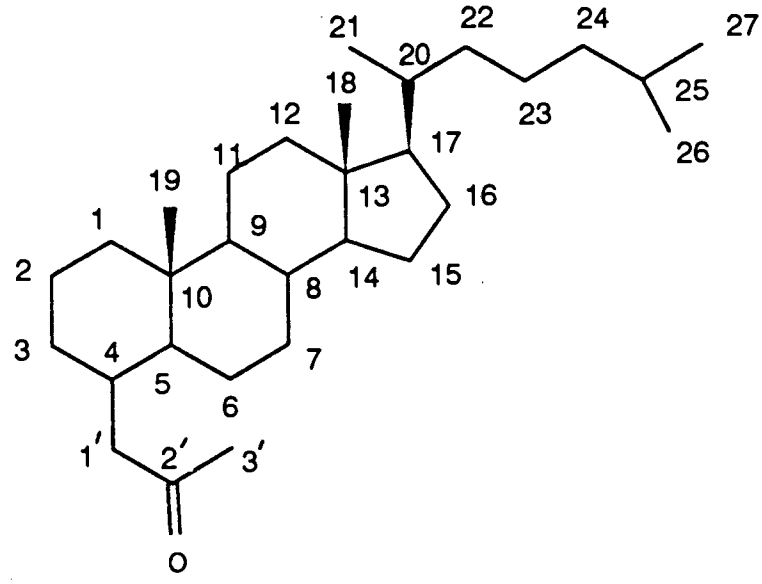
Appendix

Numbering of the steroids in this dissertation was done according to the IUPAC rules. The general steroidal skeletons which require additional numbering are represented.



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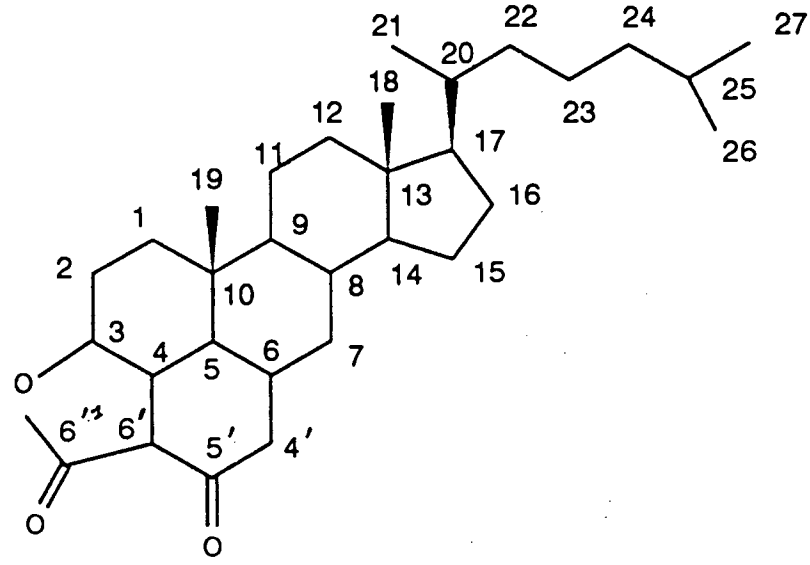
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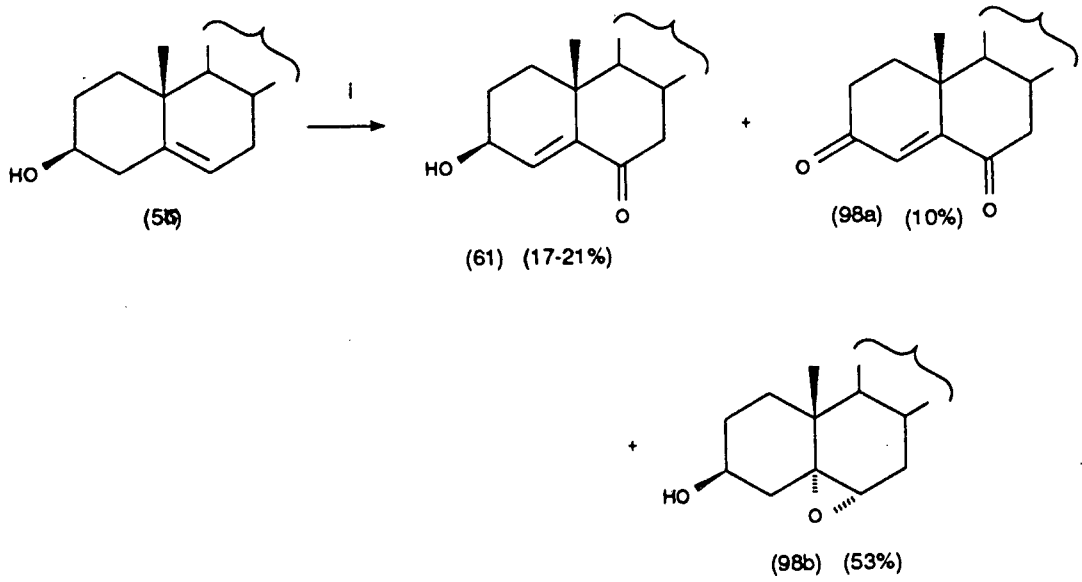
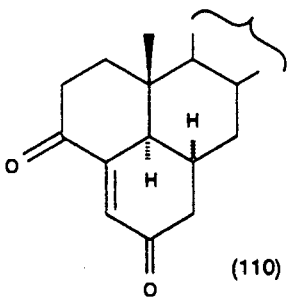
Representation of the reaction course of the attempted one-step oxidation of cholesterol (**56**)[22]. The numbering is in accordance with the phase of the project, during which the attempts were made.



Reagents and conditions: i, Ag_2CrO_4, I_2, py, CH_2Cl_2, 0°C, 1h, then 20°C, 1h.

The final alkaline equilibration of compound (**104**) could unfortunately only be carried out on a small scale, insufficient for full characterisation of the product involved. However, ^1H and ^{13}C NMR spectroscopy gave rather good quality spectra, despite the fact that the recordings were carried out on crude, noncrystallised material (6 mg). From these spectra, the product was formulated as 4',5α,5',6α-tetrahydrobenzo[4.5.6]cholestane-3,5'(6'*H*)-dione (**110**). ^1H displayed an olefinic proton at δ 6.6, while in the ^{13}C NMR spectrum, two conjugated carbonyl signals at δ 200.1 and 200.7 were notable, as well as the signals for the C(4) and C(6') at δ 153.8 and 128.3. This product must

have arisen from the oxidation of (**104**), since it is very difficult to totally exclude oxygen from the reaction mixture at micro-scale.



(110)