CHEMISTRY AND PROPERTIES OF PERHYDROBENZO[4.5.6]CHOLESTANES

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by

CAMIELAH MOHAMED

Supervisors: Professor J. R. Bull
Dr. D. W. Gammon

Department of Chemistry
University of Cape town
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To my parents,
Amina and Abdurakieb Mohamed
and my husband,
Abdullatief Kerbelker

"On no soul doth God
Place a burden greater
Than it can bear.
It gets every good that it earns,
And it suffers every ill that it earns."
Surah ii, verse 286, Al Quran

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SUMMARY

The investigations undertaken include an intramolecular Michael-aldol approach and a cycloaddition approach to the synthesis of perhydrobenzo[4.5.6]cholestanes.

A reaction sequence has been developed to obtain the 3β-acetoxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5′(6′H)-one **85** in an optimised yield from the 3β-acetoacetoxy-Δ^4-6-ketone **71**. The key steps in the transformation involved base-treatment of the 3β-acetoacetoxy enone to give (2*R*)-2-(3β-hydroxy-6-oxo-5β-cholestan-4β-yl)-3-oxobutanoic acid 1,3′-lactone **79**, which was followed by lactone cleavage, decarboxylation and intramolecular aldol closure of the derived lactone to give 3β,6-dihydroxy-4α,4′,5β,6β-tetrahydrobenzo[4.5.6]cholestan-5′(6′H)-one **81**. Treatment of 3β-acetoxy-6-hydroxy-4α,4′,5β,6β-tetrahydrobenzo[4.5.6]cholestan-5′(6′H)-one **82** with HMPA and phosphoryl chloride gave the 3β-acetoxy-4α,4′,5β,6-tetrahydrobenzo[4.5.6]cholest-6-en-5′(6′H)-one **83** and the 4α,5α-$\Delta^{4'}$-isomer **85**. The formation of 3β-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5′(6′H)-one **80** when treating the lactone **79** with potassium hydroxide indicated an alternative reaction pathway to the 4α,5α-isomer. However conditions were not established for the isolation of the 4α,5α-$\Delta^{4'}$-isomer **80** in an appreciable yield.

Access to the 4α,5β-isomer was achieved by treating the 3β-acetoxy-6β-hydroxy derivative **82** with $BF_3.OEt_2$ which gave the 4α,5β-Δ^6-isomer **83** and 3β-acetoxy-4α,4′,5β,6-dihydrobenzo[4.5.6]cholestan-5′(6′H)-one **86**. Thionyl choride-pyridine treatment of the 3β-acetoxy-6β-hydroxy derivative **82** gave an inconclusive result including the formation of the expected 4α,5β-Δ^6-isomer **83**. The expected thermodynamic relationship between the 4α,5α-isomer **85** and the 4β,5α-isomer was confirmed by base equilibration of the 4α,5α-isomer into the 4β,5α-isomer.

Detailed 400 MHz ^{1}H and ^{13}C NMR data of key pentacyclic cholestanes enabled interpretations about their structural and conformational properties and related thermodynamic stabilities.

The Diels-Alder cycloaddition of 6-methylenecholest-4-en-3β-ol **96** with acrolein and methyl vinyl ketone gave 6′α-acetyl-4β,4′,5′β,6′-tetrahydrobenzo[4.5.6]cholestan-3β-yl acetate **97** and 3β,6′-epoxymethano-4β,4′,5′,6′-tetrahydrobenzo[4.5.6]cholestan-6′^{1}β-yl acetate **98** respectively. The structures of these cycloaddition products were determined with 400 MHz ^{1}H and ^{13}C NMR data which included NOE spectra. The 6′-H orientation in the cycloadduct **98** could however not be established unambiguously.

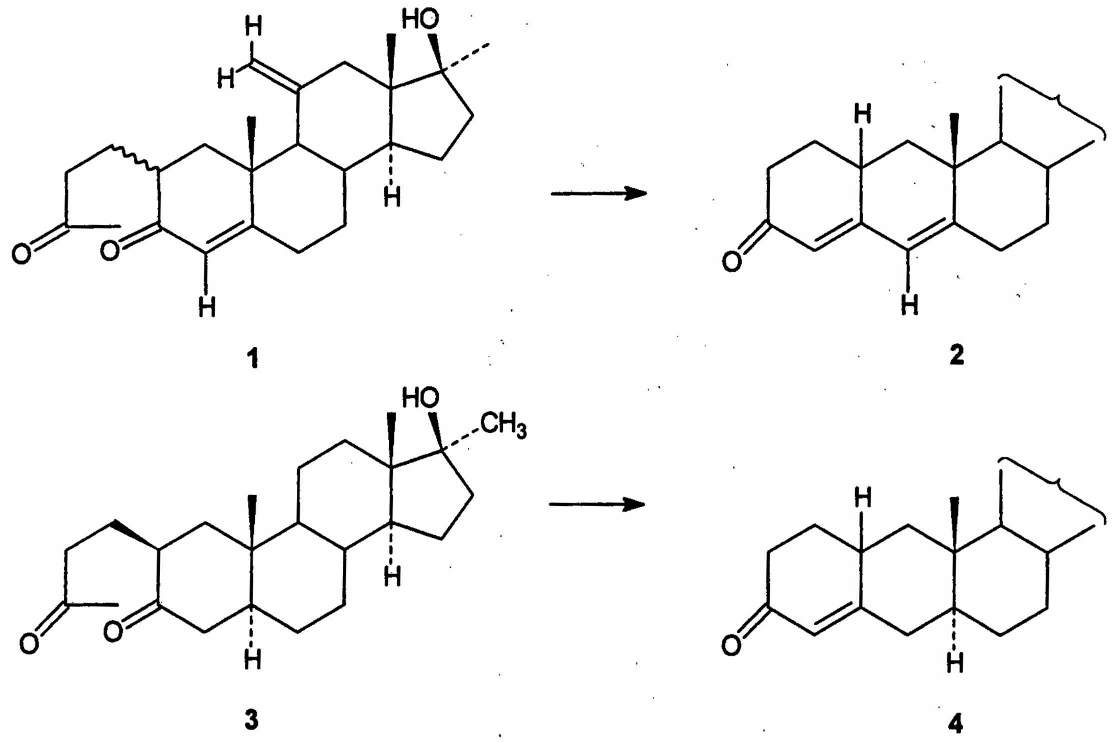
CHAPTER 1

INTRODUCTION

The molecular structure and associated conformational properties of steroid hormones are intimately linked to their activity and function. The characteristic actions of steroid hormones are known to be associated with the high-affinity binding of the specific steroid with specific receptor proteins in target tissues. For instance, it is known that compounds having a high affinity for estrogen, progestin and corticoid receptors display tight association between the receptor and the steroidal A ring whereas the structural feature common to compounds showing a high affinity for the progesterone receptor is the steroid ring system and the 4-en-3-one functionality.[1] An understanding of steroid-protein interactions and the conformational preferences of steroid molecules would therefore assist in the predictive design of molecules with increased biological activity.

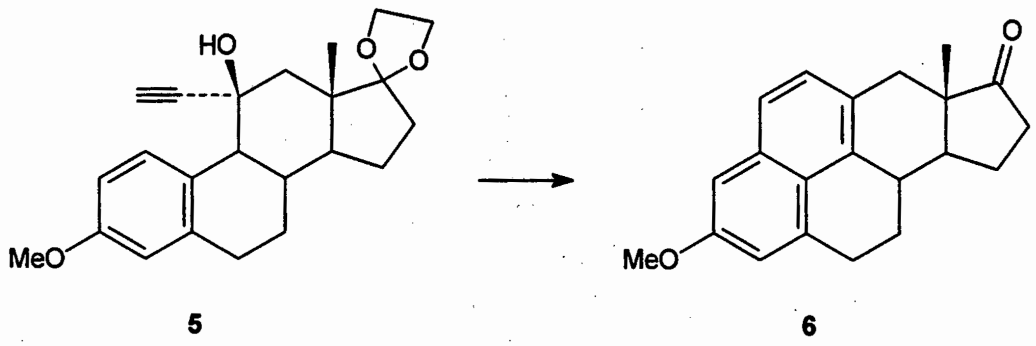
Thus, the construction of additional rings on the tetracyclic steroid nucleus could either increase or decrease the activity depending on whether the conformational changes induced in the molecule strengthen or weaken the steroid-protein interaction. Attention has been devoted to the addition of five-membered rings to the steroid nucleus.[2-6] This discussion will, however, be focussing on examples in which an additional six-membered ring has been added to the steroid skeleton.

Cooly and co-workers have synthesised modified steroidal hormones in which cyclohexenone rings were attached to C-2 and C-3 of the A ring.[7] The oxobutyl precursors **1** and **3** were treated with aqueous potassium hydroxide in dioxane to give the cyclised products **2** and **4**. These products however did not display significant biological activity.



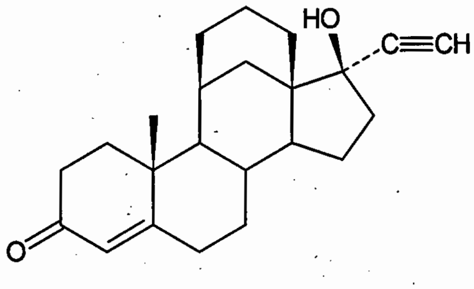
Scheme 1 Pentacyclic steroids with a cyclohexenone ring attached to C-2 and C-3

Pitt and co-workers introduced a 1,11-etheno-bridge into the steroid nucleus by treating the 11α-ethynyl-11β-hydroxy derivative **5** with tris(triphenylsilyl)vanadate which gave **6** (76%).[8]



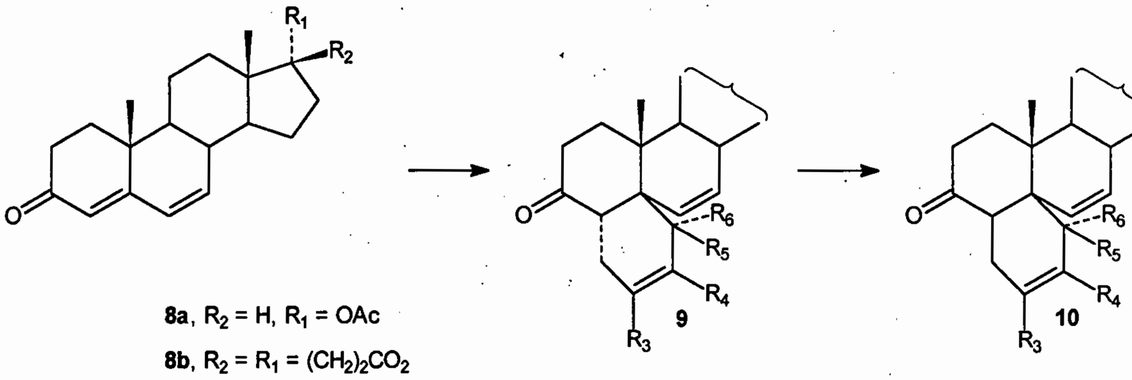
Scheme 2 Attachment of an etheno-bridge to C-1 and C-11

The 1,11-etheno-bridge in **6** did not increase its biological activity whereas the introduction of an 11β,13β-propano bridge in **7** did increase the biological activity significantly.[9]



7

Lenz studied the photocycloaddition of linear steroidal 3-keto-4,6-dienes with isoprene, butadiene and a range of substituted butadiene derivatives which gave products with a six-membered ring attached to C-4 and C-5 of the steroid nucleus.[10] The initial *trans*-adducts **9** could be epimerised to the *cis*-adducts **10**.

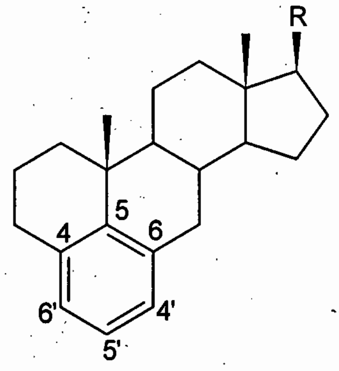


8a, $R_2 = H$, $R_1 = OAc$

8b, $R_2 = R_1 = (CH_2)_2CO_2$

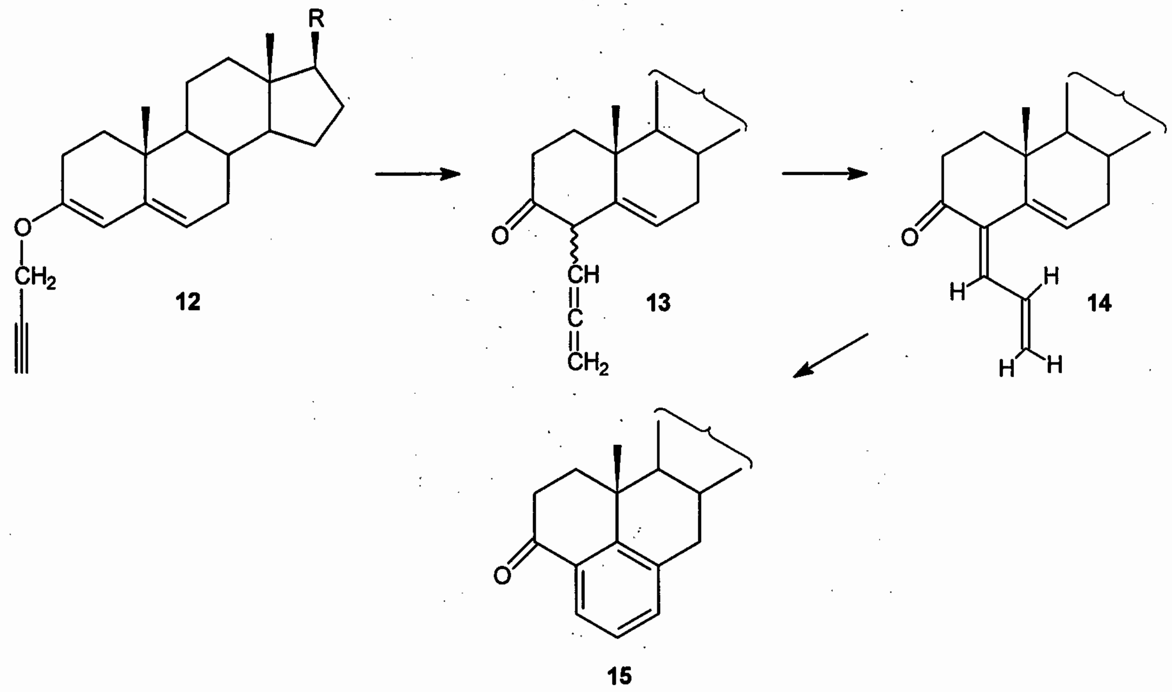
Scheme 3 Photocycloaddition of steroidal 3-keto-4-6-dienes to give a six-membered ring attached to C-4 and C-5

The addition of a fully unsaturated ring to C-4, C-5 and C-6 of the steroid nucleus to produce benzo[4.5.6]steroids of the type **11** has received considerable attention in the literature.[11-15] Some selected syntheses of benzo[4.5.6]steroids will be discussed here.



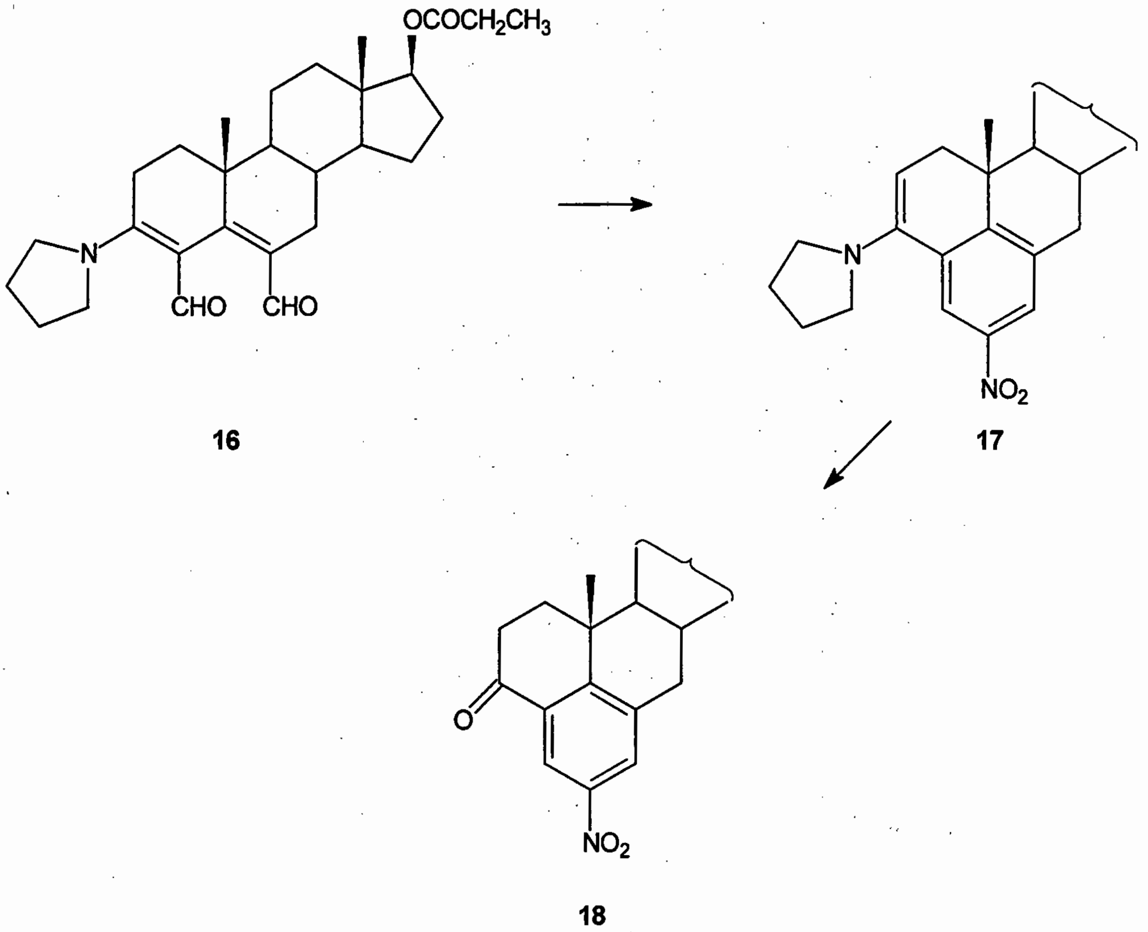
11

Gardi and co-workers have synthesised benzo[4.5.6]steroids by the rearrangement of propargyl enol ethers.[13] Steroidal propargyl enol ethers of the type **12** were refluxed in toluene to give epimeric mixtures of 3-oxo-4-allene derivatives **13**. Passage of the epimeric mixtures through alumina gave the 3-oxo-4-allylidene compounds **14**. Refluxing the enol ethers **12** or the derivatives **13** and **14** in pyridine in the presence of palladium on charcoal then gave the benzo[4.5.6]steroids **15**.



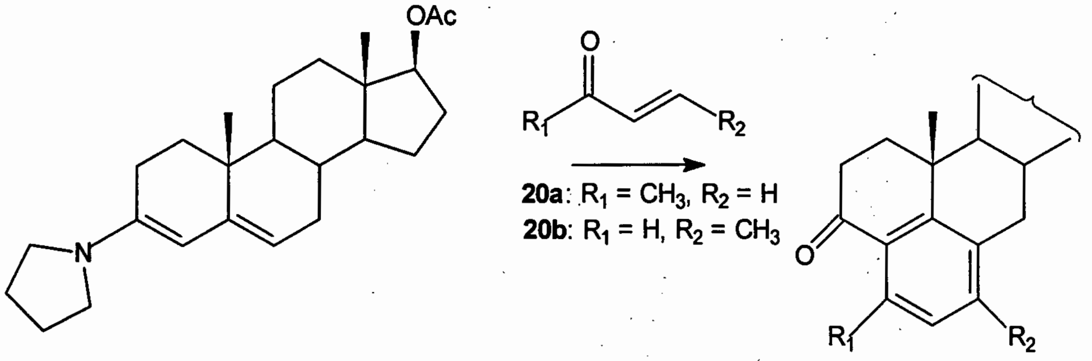
Scheme 4 Benzosteroids obtained from the rearrangement of propargyl enol ethers

Sciaky and Pallini have synthesised benzosteroids by treatment of the diformyl derivative **16** with nitromethane and sodium acetate in ethanol to give the nitrobenzene derivative **17** which was hydrolysed to the corresponding 3-keto steroid **18**.[14]



Scheme 5 Benzosteroids obtained from diformyl precursors

The most recently published work in the area of benzosteroid synthesis was reported by Manhas and co-workers.[15] This synthesis involves a single step annulation reaction of the steroidal dienamine **19** with methyl vinyl ketone **20a** or crotonaldehyde **20b** to give the benzo[4.5.6]steroids **21a** and **21b**.



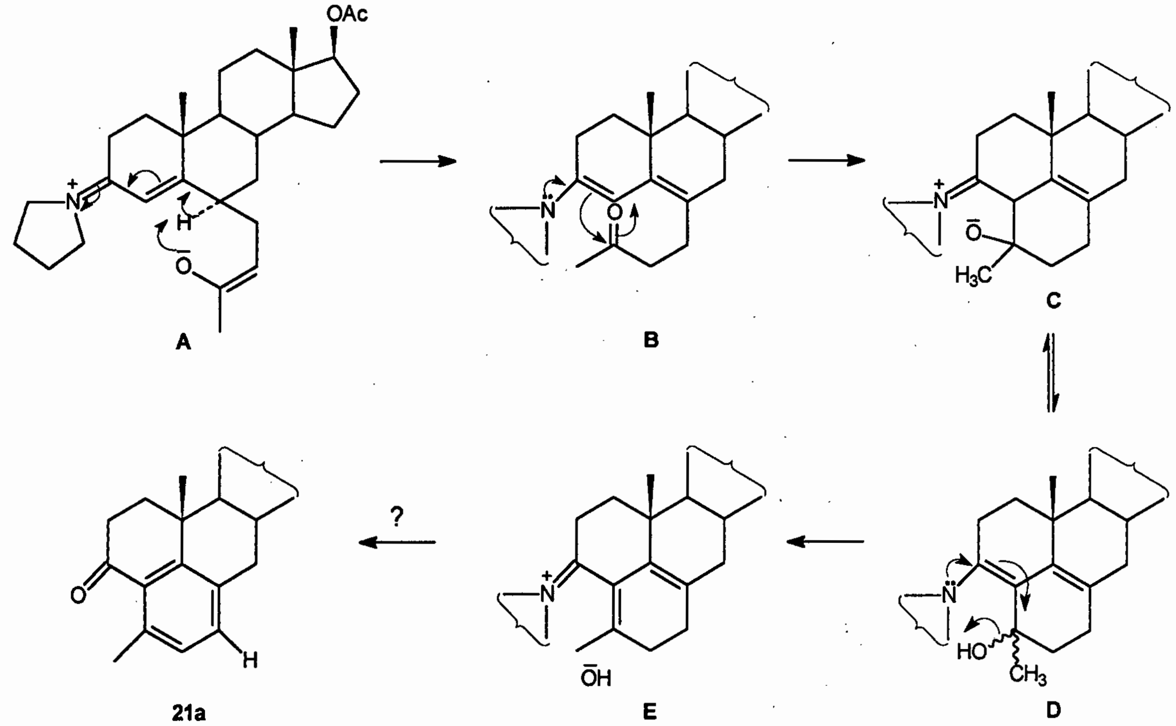
19

20a: $R_1 = CH_3$, $R_2 = H$
20b: $R_1 = H$, $R_2 = CH_3$

21a: $R_1 = CH_3$, $R_2 = H$
21b: $R_1 = H$, $R_2 = CH_3$

Scheme 6 Formation of benzosteroids from the reaction of dienamines with functionalised olefins

The mechanism[16] of formation of **21a** and **21b** involves an initial Michael type addition of the dienamine **19** via the γ-carbon to the electrophilic olefin **20a** or **20b** to give a dipolar intermediate **A** which is neutralised by proton transfer via a six-membered transition state to form **B**. The enamine then intramolecularly attacks the ketone group to give the intermediates **C** and **D** which are in equilibrium with each other. The formation of the products **21a** and **21b** is presumed to involve dehydrogenation and hydrolysis of the intermediate E. Aromatisation of ring E could either involve air oxidation during workup or a disproportionation process.



Scheme 7 Mechanism of formation of benzosteroids **21a** and **21b**

Formal saturation of benzo[4.5.6]steroids generates a family of perhydrobenzo[4.5.6]steroids in which rings A, B and E constitute a perhydrophenalene substructure. Dillen[17] has undertaken a conformational analysis of perhydrophenalene in order to assist in the understanding of the conformational behaviour of the related perhydrobenzo[4.5.6]steroids. Four isomeric configurations of perhydrophenalene are possible (See Fig. 1).

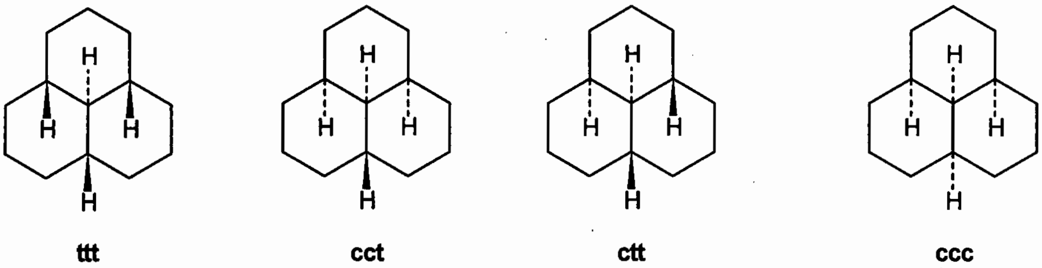


Figure 1 Schematic representation of the four isomers of perhydrophenalene: trans, trans, trans,; cis, cis, trans; cis, trans, trans; cis, cis, cis

Idealised models of the tricyclic ring system indicate that the lowest energy form of each isomer is dictated by the maximum number of chair-like conformations in each case. Flexibility within this system increases with the number of cis ring junctions if more then one ring occupies a boat conformation. The most stable conformation of each isomer is depicted in Fig. 2.

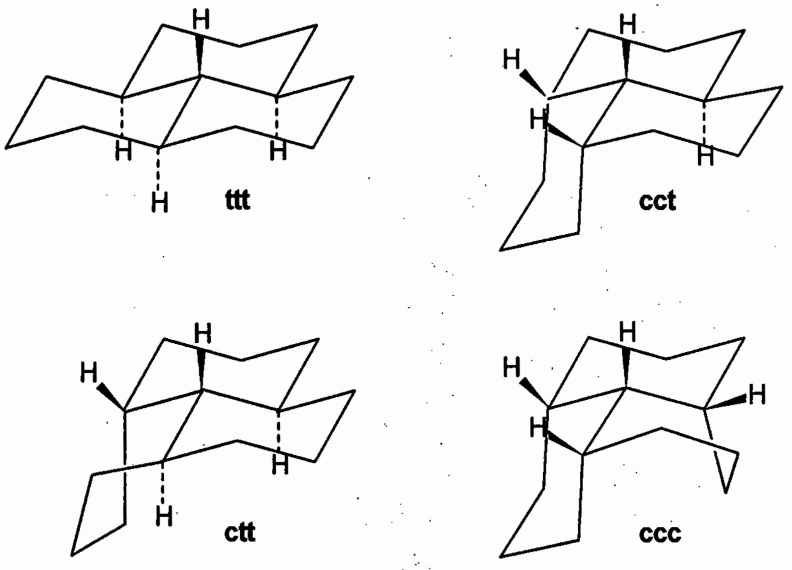
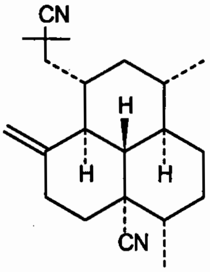
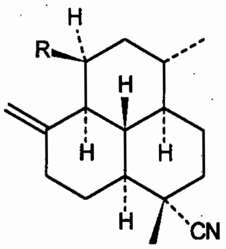


Figure 2 Lowest energy conformations of isomeric perhydrophenalenes

Unlike the perhydrobenzo[4.5.6]steroids which are not naturally occurring structures, the perhydrophenalene subunit is found in nature. Wells and co-workers have isolated the compounds **22** and **23** from the sponge *Hymeniacydon amphilecta.*[18] Both of these compounds occur as all-*trans* structures.

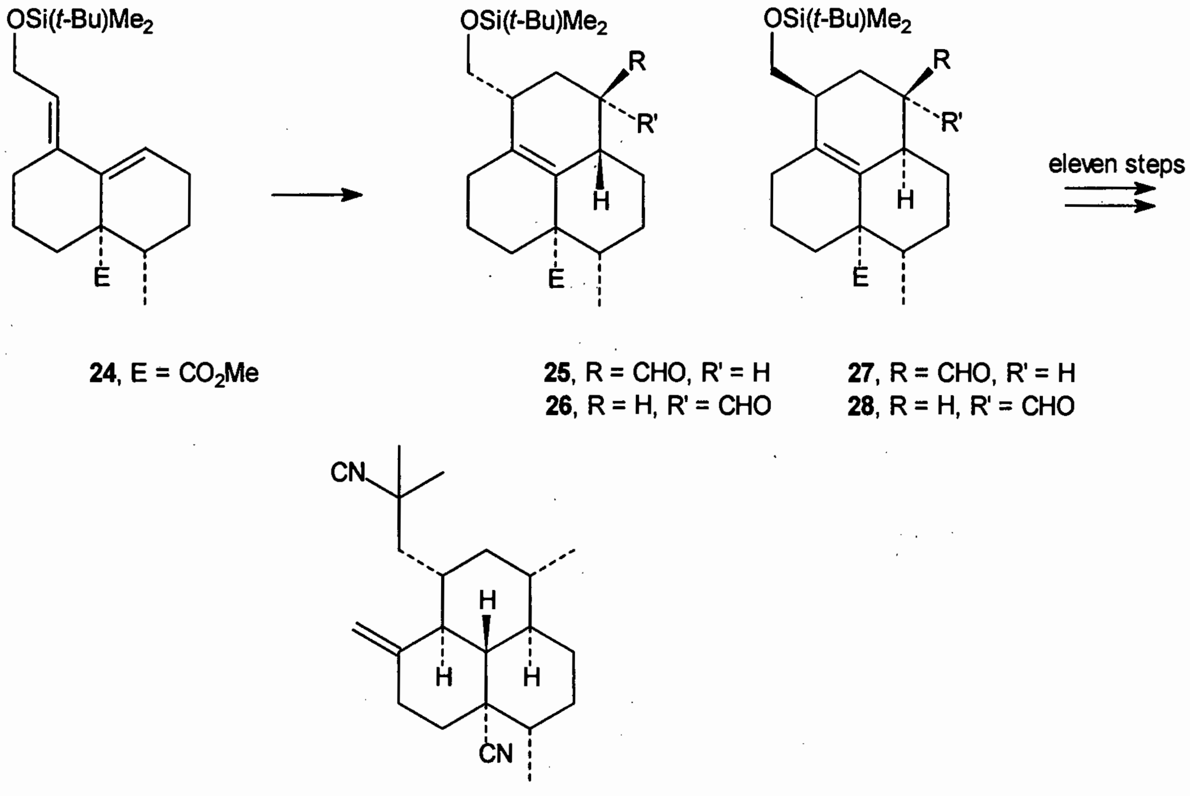


22



23

Piers and co-workers[19] have attempted a total synthesis of the diterpenoid **22**. A key step in the synthesis of the target molecule employed a Diels-Alder reaction of the diene **24** with propenal which gave a mixture of four cycloadducts (**25** - **28**). This mixture was subsequently treated with sodium methoxide in methanol to give a mixture of aldehydes **25** and **28** in a ratio of 3:7 respectively. Separation of this mixture gave the aldehydes **25** and **28** in yields of 29% and 58% respectively. The target compound **22** was obtained in an isolated yield of 8.3% via an eleven step sequence from the aldehyde **28**.



24, E = CO_2Me

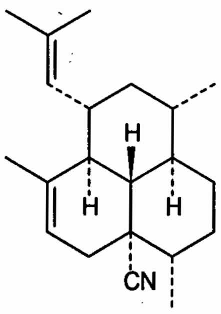
25, R = CHO, R' = H
26, R = H, R' = CHO

27, R = CHO, R' = H
28, R = H, R' = CHO

(+/-) - **22**

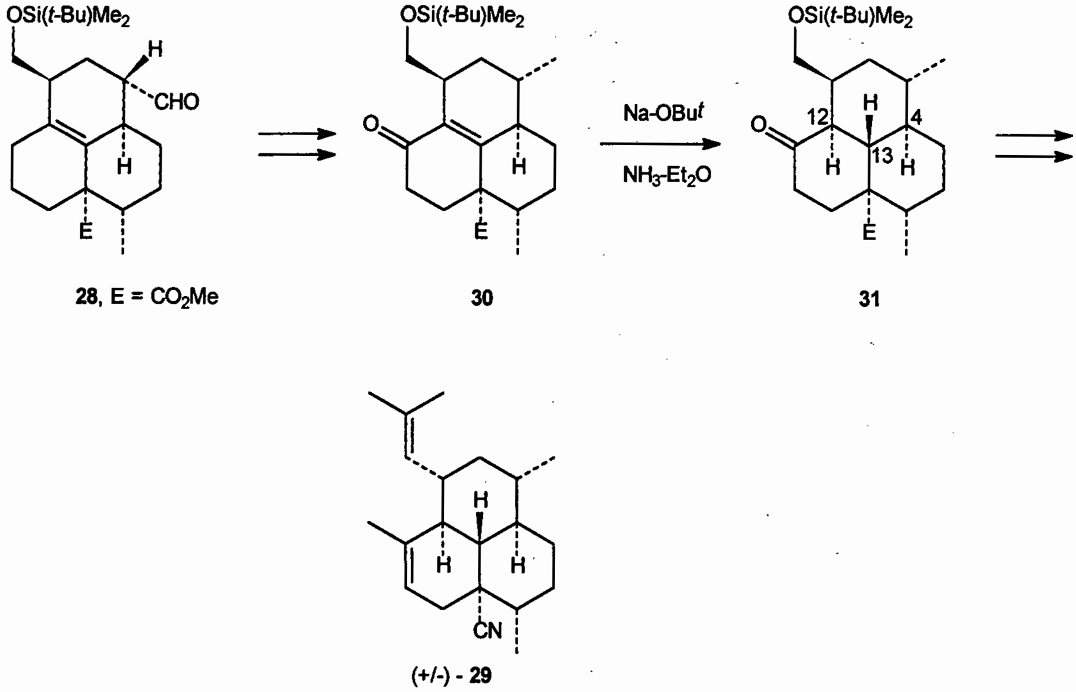
Scheme 8 Key synthetic steps in the synthesis of the diterpenoid natural product **22**

Piers and co-workers have also isolated the diterpenoid **29** and have reported[20] its total synthesis starting from a previously reported tricyclic ketone **31** which itself was obtained via the thermodynamically stable Diels-Alder adduct **28**.



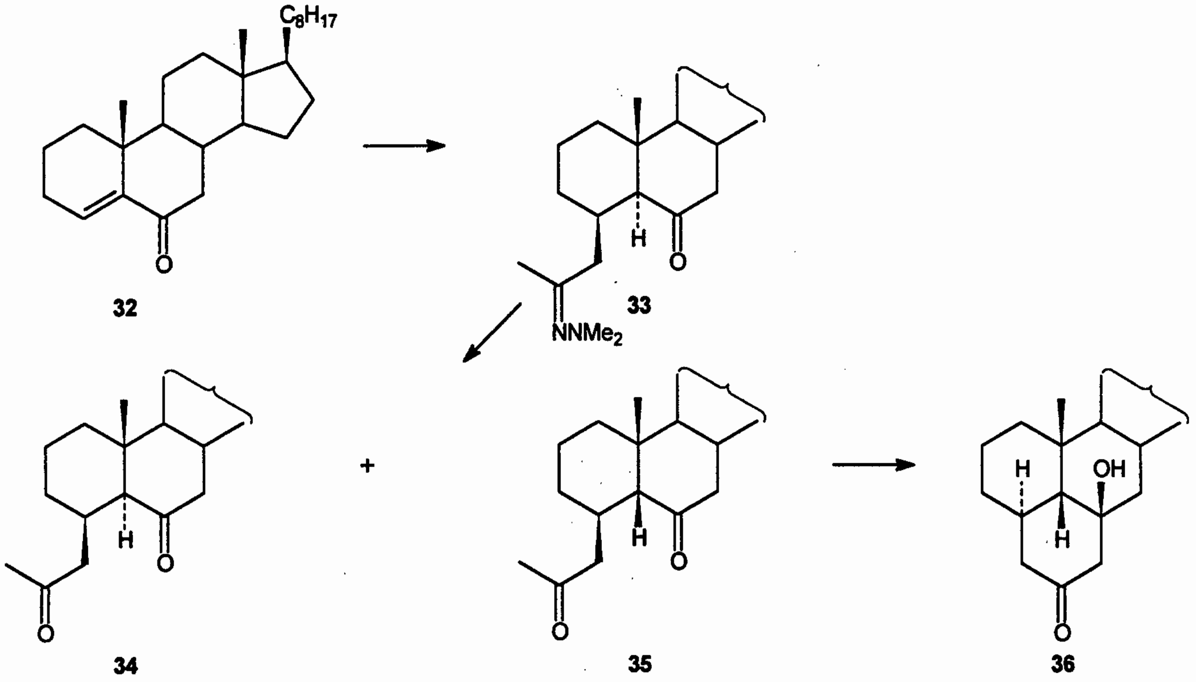
29

A key step in the formation of the ketone **31** involved stereoselective reduction of the enone **30** which gave the 12α,13β dihydro derivative **31**. The ketone **31** was ultimately converted to the diterpenoid natural product **29** (Scheme 9).



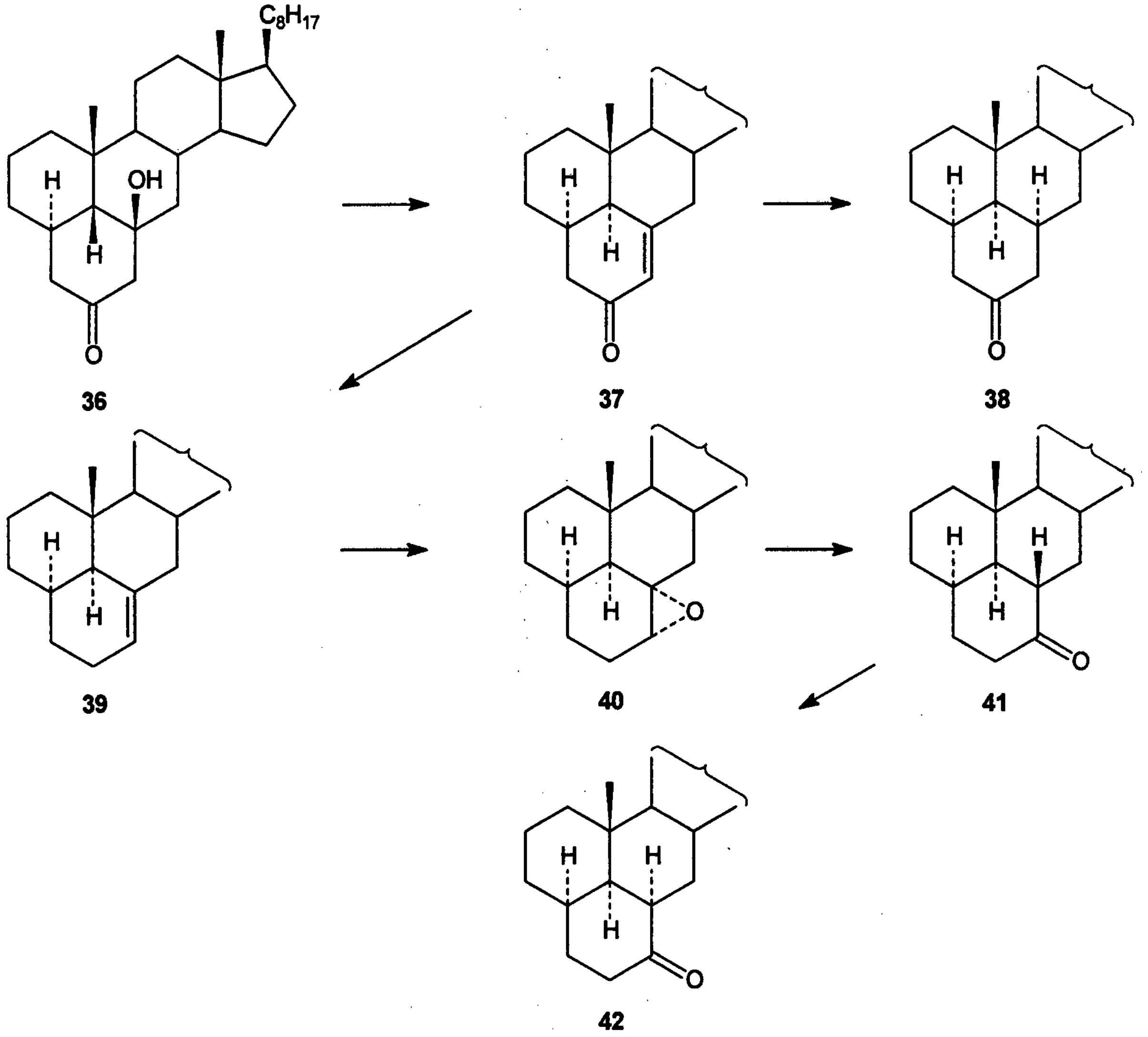
Scheme 9 Synthesis of the diterpenoid natural product **29**

The attachment of a partially or fully saturated six-membered ring fused to C-4, C-5 and C-6 of the steroid nucleus has received very limited attention. An early synthetic approach to perhydrobenzo[4.5.6]cholestanes utilised by Bull and co-workers[21] was based on the conjugate addition of a masked acetonyl equivalent to cholest-4-en-6-one **32** which gave the conjugate alkylation product **33** which was hydrolysed to give a mixture of 5α- and 5β-acetonyl derivatives **34** and **35**. Subsequent intramolecular aldol closure gave the pentacyclic steroid **36** through the intermediacy of **35**.



Scheme 10 Synthesis of perhydrobenzo[4.5.6]cholestanes using conjugate addition methodology

Subsequent work by Bull and co-workers involved transformations which gave rise to representative 4α,5α,6α- and 4α,5α,6β-isomers.[22] (See Scheme 11) Reduction of the enone **37** with palladium on calcium carbonate gave the 4α,5α,6α -5'-ketone **38**. An alternative means of reducing the $\Delta^{6(4')}$ bond in **37** involved treatment of the enone **37** with ethanedithiol and boron trifluoride diethyl etherate, followed by sodium-liquid ammonia reduction which gave the 4α,5α-olefin **39**. Entry into the 4α,5α,6β-series was obtained by epoxidation of **39** which gave the 6α,4'α-epoxide **40** as a major product. The epoxide **40** was isomerised with a catalytic amount of boron trifluoride diethyl etherate to give the 4α,5α,6β-isomer **41** which was quantitatively isomerised to the 4α,5α,6α-isomer **42**.



Scheme 11 Synthesis of isomeric 4α,5α,6α- and 4α,5α,6β-perhydrobenzo[4.5.6]cholestanes

This was followed by a molecular mechanics investigation of the hexahydrobenzo[4.5.6]androstanes isomeric at C-4, C-5 and C-6 which was undertaken to provide information about the the conformational preferences of the corresponding perhydrobenzo[4.5.6]cholestanes. The differences between this system and the perhydrophenalene ring system were the C-8, C-9 ring fusion which connects the remainder of the steroid skeleton and the 10β-methyl group. Within this ring system eight stereoisomers are possible. The minimum energy conformation for each possible stereoisomer is depicted in Fig. 3.

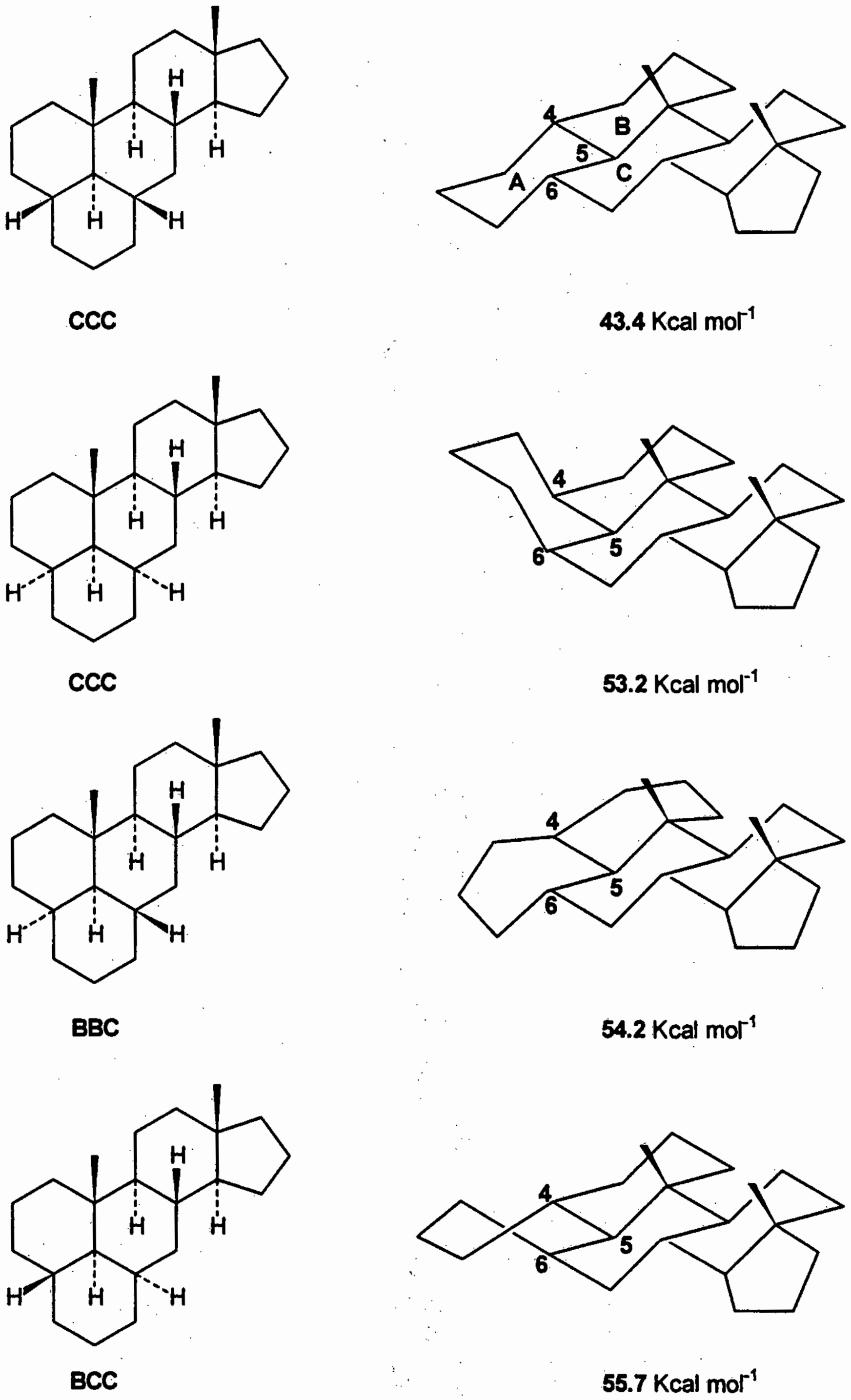
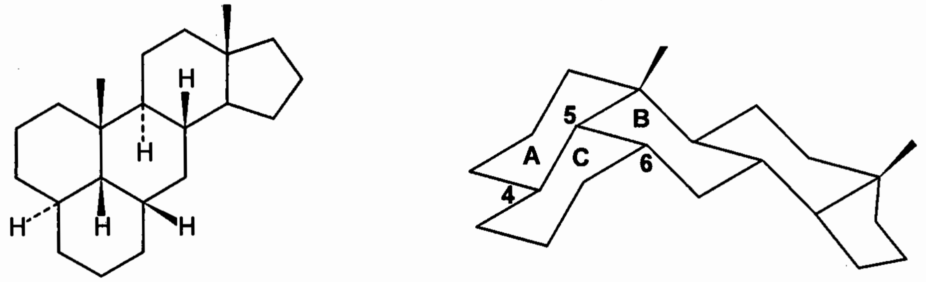
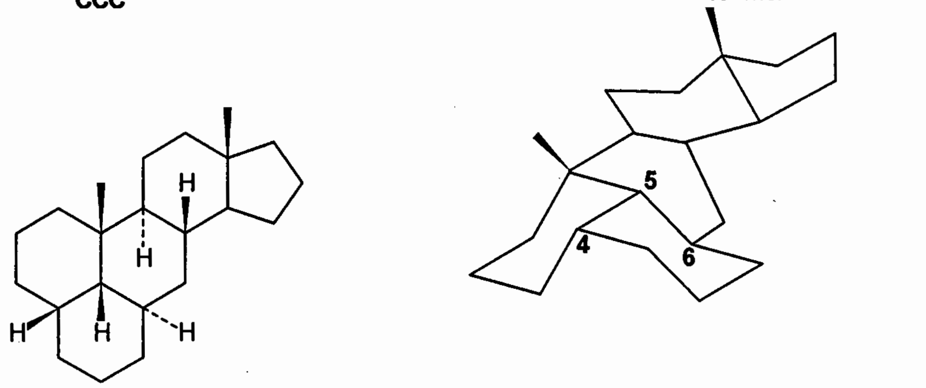


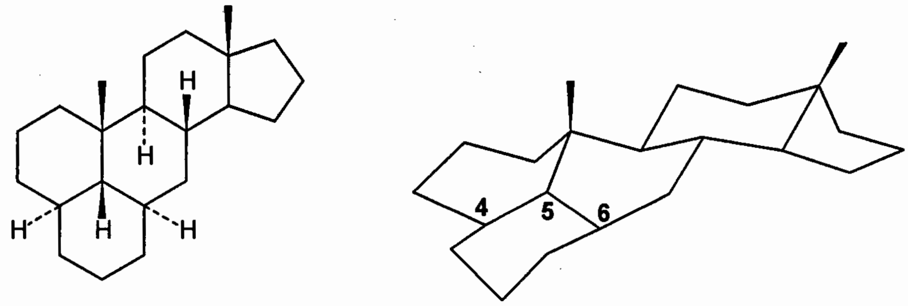
Figure 3 Minimum energy conformations for each possible stereoisomer of hexahydrobenzo[4.5.6]androstanes (5α-series)



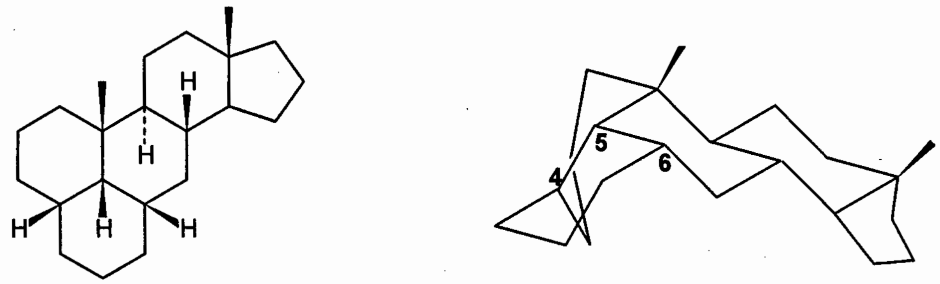
CCC **44.9** Kcal mol^{-1}



CCB **50.4** Kcal mol^{-1}



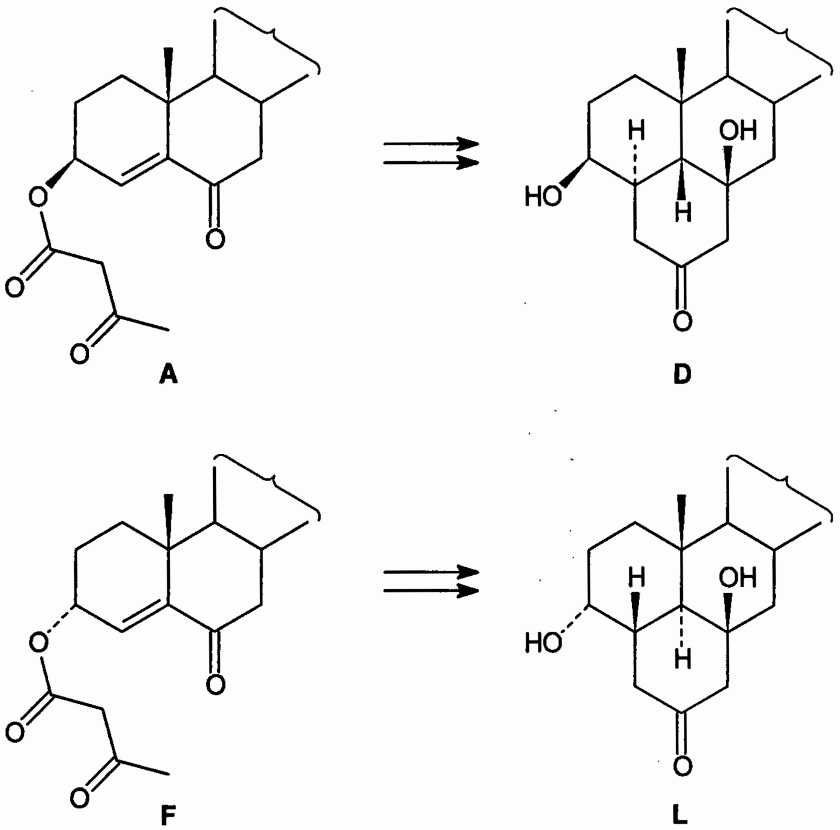
CBB **52.4** Kcal mol-1



CBC **55.2** Kcal mol^{-1}

Figure 3 (continued) Minimum energy conformations within the 5β-series

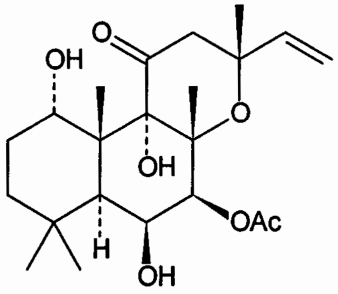
More recent work of Bull and co-workers focussed on the use of a sequential methodology for the synthesis of perhydrobenzo[4.5.6]cholestanes. The synthesis employed a sequential intramolecular Michael-aldol strategy in which the isomeric 3-acetoacetoxy enones **A** and **F** were converted into the corresponding dihydroxy hexahydrobenzo[4.5.6]cholestanes **D** and **L**.[23] The pertinent aspects of this investigation will be discussed at a later stage (Section **2.1**) in this dissertation.



A **D**

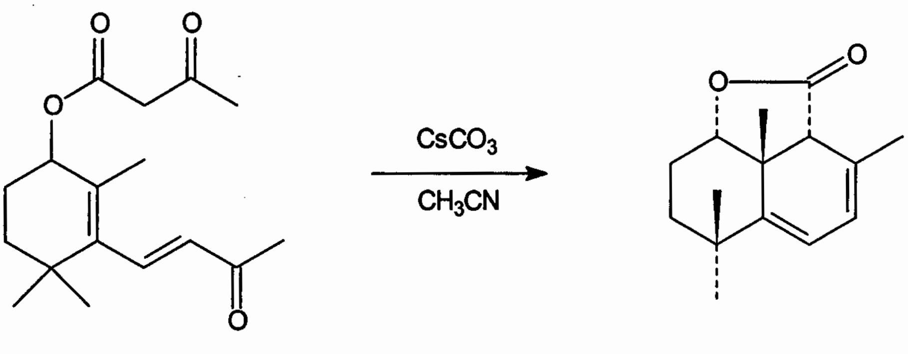
F **L**

The foregoing sequential methodology, which is receiving increasing attention,[24,25] aims to achieve greater conversion efficiency for multistep reaction processes. The utility of sequential intramolecular Michael-aldol methodology has been widely demonstrated in the synthesis of polycyclic molecules. Several of these processes are characterised by a high degree of stereocontrol in the formation of products. The versatility of the intramolecular Michael-aldol reaction is exemplified by the use of this reaction sequence for the synthesis of intermediates in the construction of the terpenoid natural product forskolin.

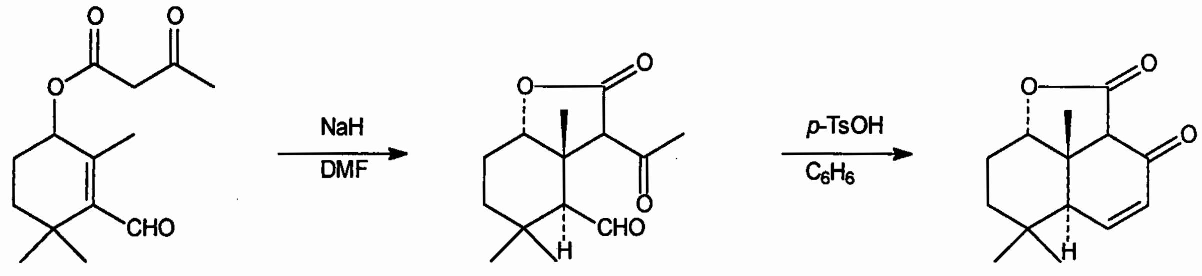


forskolin

Koft and co-workers have utilised a Michael-aldol strategy in the synthesis of the forskolin intermediate **44** starting from the acetoacetate precursor **43**.[26] The acetoacetate **43** was treated with cesium carbonate in acetonitrile to produce the tricyclic lactone **44** in a sequence of reactions which involved intramolecular Michael addition, aldol condensation, deacylation and olefin isomerisation.

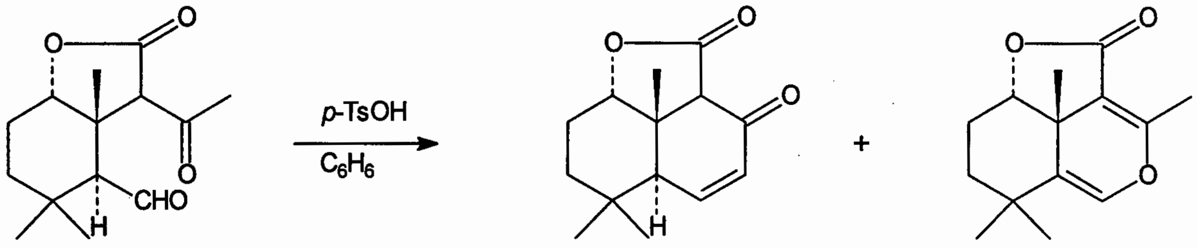


Wu and co-workers incorporated intramolecular Michael addition as a key step in the synthesis of the forskolin intermediate **47**.[27] The acetoacetate ester **45** was treated with sodium hydride in dimethylformamide to give stereocontrolled formation of the Michael adduct **46**. The lactone **46** was transformed into the tricyclic lactone **47** via an intramolecular aldol condensation with *p*-toluenesulfonic acid in benzene.



45 **46** **47**

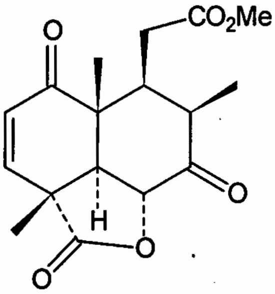
The same tricyclic lactone intermediate **47** was synthesised by Ruveda and co-workers using an approach in which the acetoacetate **45** was treated with potassium carbonate in ethanol to give the Michael adduct **46**, acidic treatment of which gave the lactone **47** (75%) and the dienol ether **48** (8%).[28]



46 **47** **48**

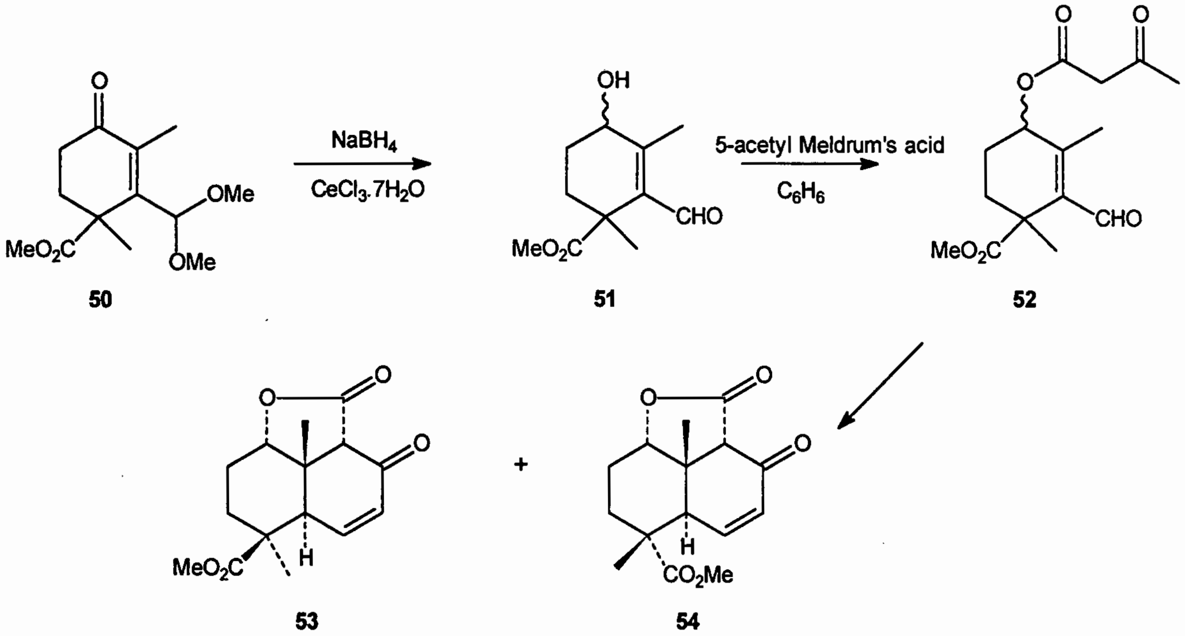
The conditions for the aldol condensation had to be carefully controlled so as to inhibit formation of the β, γ-unsaturated isomer of **47**. An improved synthesis of the lactone **47** was developed in which potassium *tert*-butoxide in refluxing benzene was used for the Michael addition. A dilute, refluxing solution of **46** and *p*-toluenesulfonic acid in 1, 2-dichloroethane gave the lactone **47** in an optimised yield with negligible formation of the β, γ-unsaturated ketone.

The intramolecular Michael-aldol reaction sequence has also been used in the synthesis of key intermediates of the terpenoid natural products nimbolide and deoxonimbolide.[29] For the synthesis of these natural products, studies were directed toward obtaining the lactone **49** which resembles the decalin portion of the natural product nimbolide.

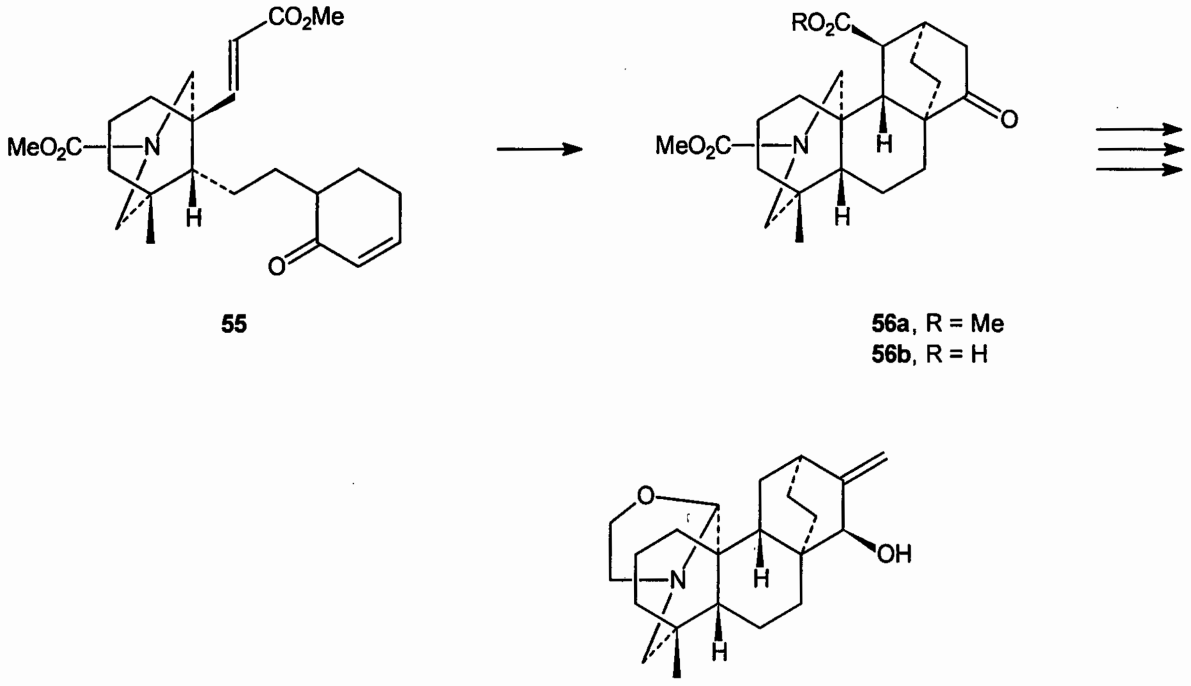


49

A key step in the synthesis of intermediates of the lactone **49** involved a Michael-aldol reaction of the mixture of keto esters **52** to give the lactones **53** and **54**. The β-keto esters **52** were synthesised by treatment of the epimeric alcohols **51** obtained from sodium borohydride reduction of the enone **50**, with 5-acetyl Meldrum's acid. The isomeric lactones **53** and **54** were obtained in a 2:1 ratio and were separated by column chromatography. It was assumed that both β-ketoesters **52** would undergo the intramolecular Michael addition at similar rates and that the ratio of isomeric lactones obtained was a result of the ratio of allylic alcohols produced in the reduction of the enone **50**.



Lastly, an elegant example in which the double Michael reaction has been employed for the synthesis of the diterpene alkaloid atisine **57**, illustrates the high degree of stereocontrol which is often possible when using the Michael reaction as part of a reaction sequence.[30] The precursor **55** was treated with lithium hexamethyldisilazide to give **56a** as the major product via a double Michael reaction. The *C*-methoxycarbonyl group of **56a** was hydrolysed to give **56b** which was ultimately converted to atisine **57**.



55

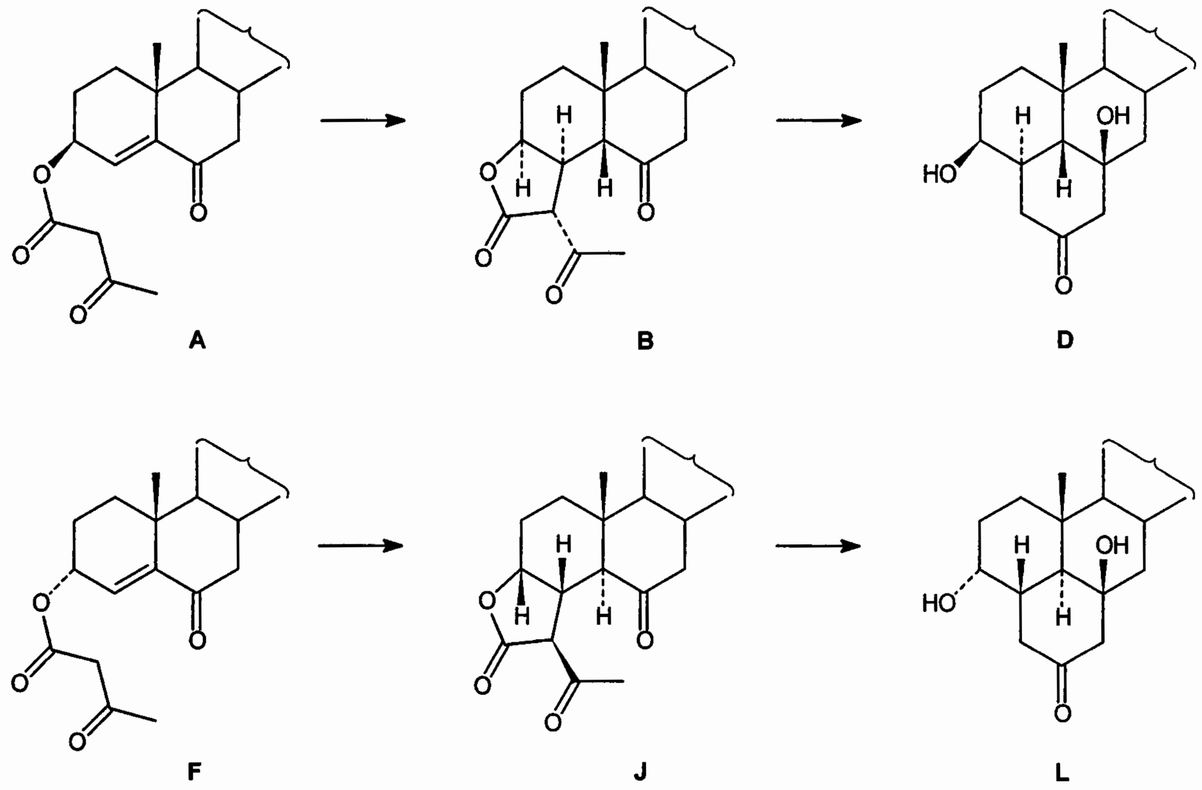
56a, R = Me
56b, R = H

57

The foregoing discussion and illustrative examples provide a context for the study described in the following sections, which describe an investigation into improved pathways to perhydrobenzo[4.5.6]cholestanes using Michael-aldol or Diels-Alder methodologies.

OBJECTIVES

The overall objective of this investigation is to study the intramolecular Michael-aldol reaction sequence for the stereocontrolled conversion of 3β- and 3α-acetoacetoxy cholest-4-en-6-ones (**A** and **F**) into perhydrobenzo[4.5.6]cholestanes (**D** and **L**) with a view to optimising key bond-forming steps through the modification of reaction conditions or the development of one-pot methodology.

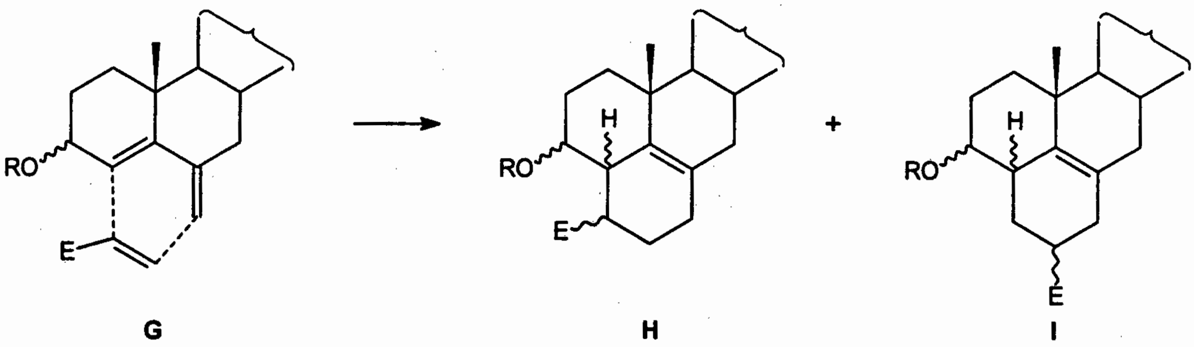


A **B** **D**

F **J** **L**

The initial investigation would be directed at obtaining optimised formation of the γ-lactone intermediates (**B** and **J**). Once the γ-lactone intermediates were obtained, improved one-pot conversions of **A** and **F** to **D** and **L** would be investigated. If efficient syntheses of the pentacyclic compounds (**D** and **L**) were achieved, their chemistry and conformational properties would be investigated. An examination of the conformational properties of **D** and **L** with the use of NMR spectroscopy would assist in further interpretation of the stereochemical relationships derived from earlier molecular mechanics correlations of the isomeric hydrocarbons.

An additional objective was to conduct preliminary investigations into an alternative strategy for elaborating perhydrobenzo[4.5.6]cholestanes via the Diels-Alder reaction of 6-methylene-cholest-4-enes with suitably functionalised dienophiles (**G**) to give the corresponding cycloadducts **H** and **I.**



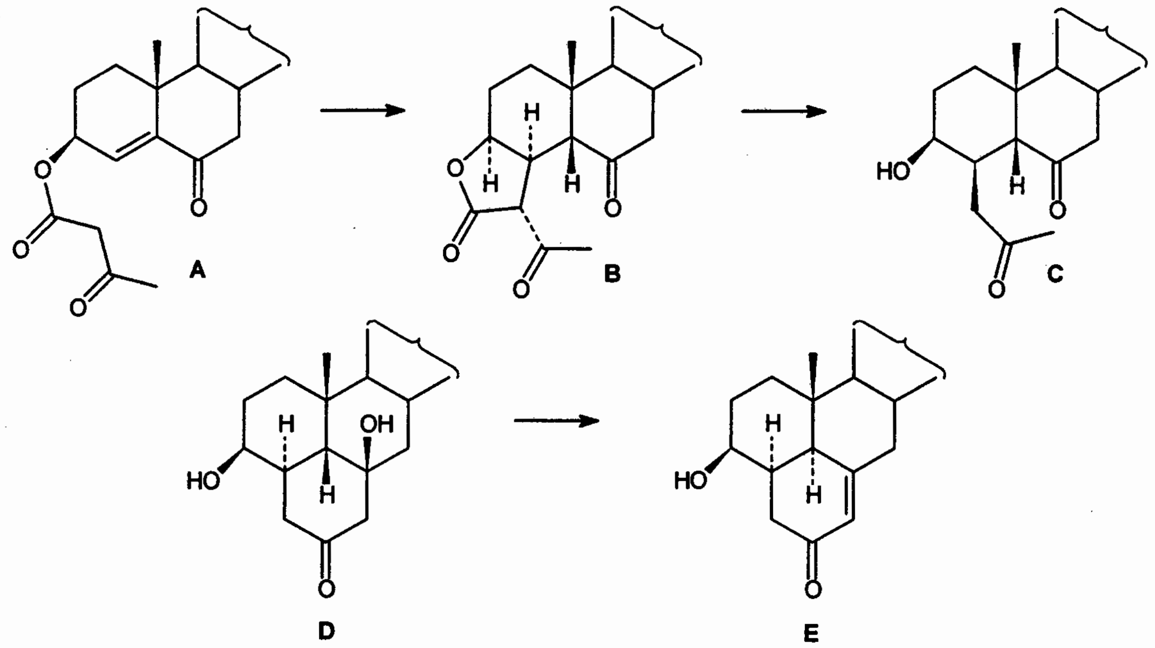
CHAPTER 2

AN INTRAMOLECULAR MICHAEL-ALDOL APPROACH TO PERHYDROBENZO[4. 5. 6]CHOLESTANES

2.1 Introduction

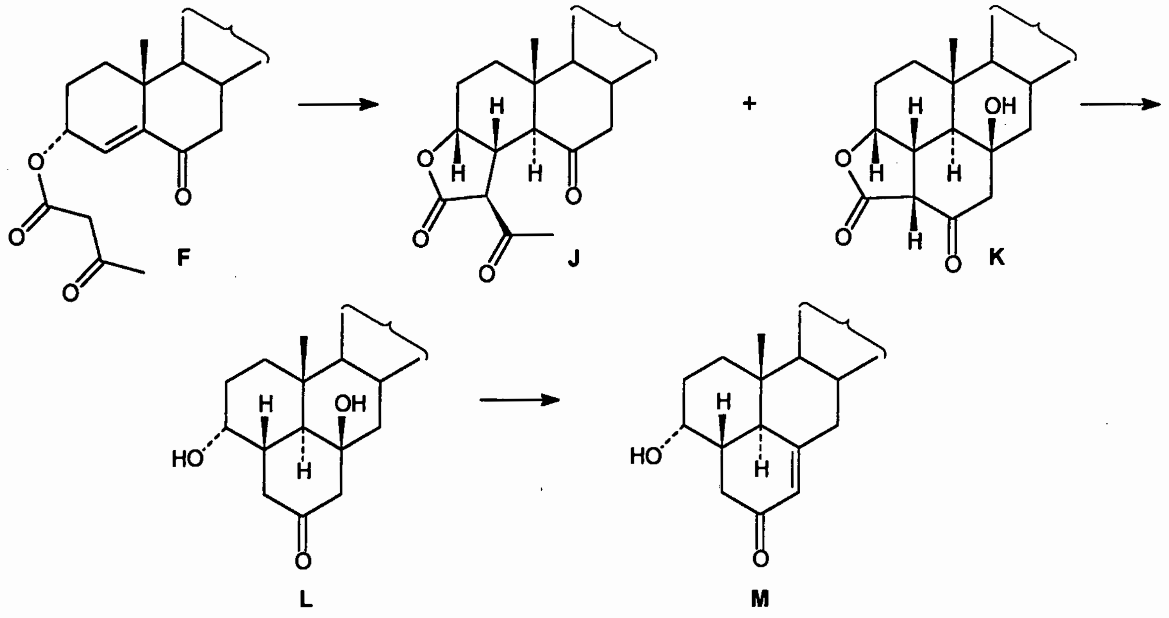
The ultimate objective of the initial investigation undertaken by Borry[23, 31] into the assembly of hexahydrobenzo[4.5.6]cholestanes was to develop efficient one-pot syntheses which would convert the 3β- and 3α-acetoacetoxy enones **A** and **F** stereoselectively into their respective pentacyclic enones **E** and **M**. The results of this earlier study are summarised here to provide the context for the continued investigation.

Thus, treatment of the 3β-acetoacetate **A** with mild base in ethanol produced the γ-lactone **B** with absolute control of the stereochemistry at C-4 and C-5.



Subjecting the γ-lactone **B** to refluxing potassium hydroxide in a mixture of dioxane and water afforded the dihydroxy 4α,5β pentacyclic steroid **D** and its precursor **C**. Subsequent lowering of the reaction temperature forced complete conversion of the 5β,4β-acetonyl precursor **C**, into **D**. Treating **D** with *p*-toluenesulfonic acid in refluxing benzene afforded the β-elimination product **E** in a low yield with the stereochemistry inverted at C-5.

In contrast, the 3α-acetoacetoxy enone **F** was subjected to potassium *tert*-butoxide in *tert*-butyl alcohol to yield the corresponding γ-lactone **J** with concomitant stereoselective formation of the bonds at C-4 and C-5, together with a secondary product **K** which resulted from intramolecular aldol closure of the γ-lactone **J**.



Treatment of **J** with aqueous potassium hydroxide in refluxing dimethylformamide or ethanol afforded both the dihydroxy 4β,5α pentacyclic steroid **L** and the derived β-elimination product **M**. Prolonged treatment of the mixture (**L** + **M**) with *p*-toluenesulfonic acid and mild heating forced conversion of **L** into the β-elimination product **M**.

The two reaction pathways differ in that in the 3β-series a product of intramolecular aldol closure analogous to compound **K** in the 3α-series, was not isolated. The β-elimination product **E** was synthesised less efficiently than the enone **M**, the formation of which also involved a sequential intramolecular Michael-aldol reaction, followed by β-elimination. The initial purpose in continuing the investigation was therefore to optimise the respective reaction pathways leading to the key pentacyclic compounds **D** and **L**, and to explore the scope for developing a one-pot methodology for their syntheses. With these products in hand, access to derived hexahydrobenzo[4.5.6]cholestanes would enable more detailed studies of their chemistry and conformational properties.

2.2 Synthesis of 3β- and 3α-hydroxycholest-4-en-6-ones

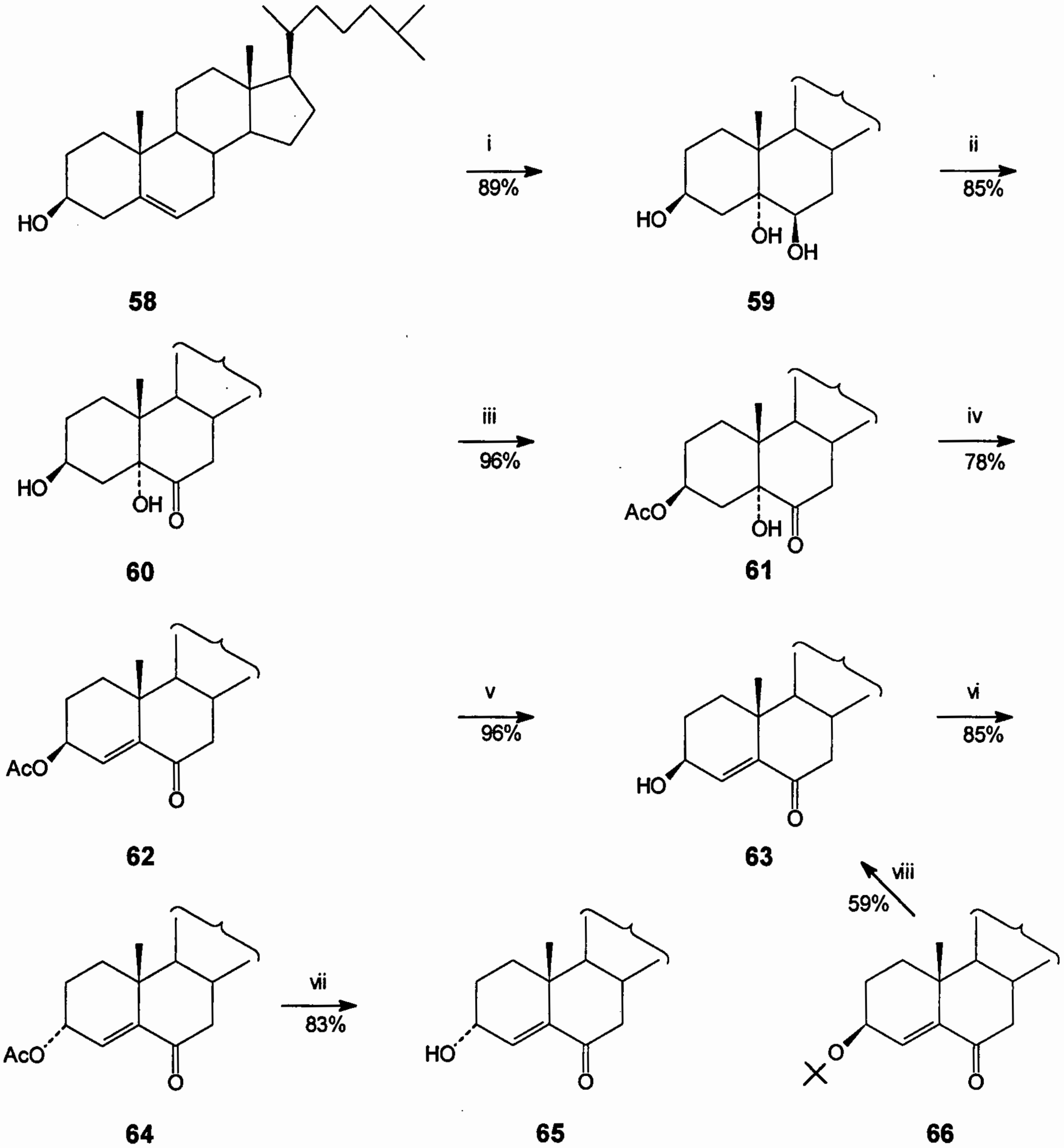
The synthetic route employed to produce the 3β- and 3α-hydroxy enones **63** and **65** was based on well established reactions cited in the literature.[32] This was also the route undertaken by Borry.[31] The synthesis proceeded as expected, although the yields obtained were slightly lower than those reported.[31] The 3β,5α,6β-triol **59** was prepared by treatment of cholesterol **58** with performic acid (generated *in situ* by the addition of hydrogen peroxide to a formic acid solution) which produced the 3β,5α,6β-triol 3,6-diformate as the major product which was hydrolysed with aqueous sodium hydroxide to give the triol **59**.[32]

Selective oxidation of **59** with N-bromosuccinimide in aqueous methanol-diethyl ether produced the 3β,5α-dihydroxy 6-ketone **60**,[33] which was chemoselectively acetylated to give the 3β-acetoxy-5α-hydroxy-6-ketone **61**. Treatment of **61** with thionyl chloride in pyridine at 0 °C followed by hydrolysis of the resultant 3β-acetoxy Δ^4-6-ketone **62** with aqueous base gave 3β-hydroxycholest-4-en-6-one **63**.

Access to the 3α-series was obtained via a Mitsunobu[31, 34] reaction of the 3β-hydroxy-Δ^4-6-ketone **63** which gave the 3α-acetoxy-Δ^4-6-ketone **64**. Hydrolysis of **64** gave the 3α-hydroxy-Δ^4-6-ketone **65**.

Although the foregoing reaction sequence provided a reliable route to the desired starting materials **63** and **65**, a recent report by Sica and co-workers[35] suggested a possible way of achieving more direct conversion of cholesteryl acetate into the precursor **61**.

With this in mind, a shortening of the synthetic route to starting material synthesis was attempted.



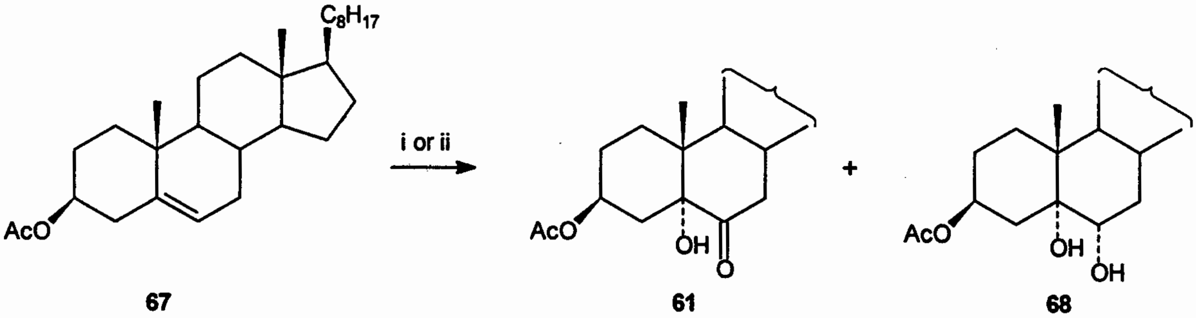
Scheme 12 *Reaction conditions*: i, HCOOH, H_2O_2; then NaOH; ii, NBS, MeOH-Et_2O-H_2O; iii, Ac_2O, C_5H_5N, -5 °C; v, 2M KOH, EtOH; vi, CH_3CO_2H, PPh_3, DEAD, C_6H_6; vii, K_2CO_3, MeOH; viii, 1M $TiCl_4$, CH_2Cl_2, -70 °C[†]

[†] 3β-Hydroxycholest-4-en-6-one was also obtained by the hydrolysis of available 3β-*t*-butoxycholest-4-en-6-one (see experimental section)

2.3 Attempted ruthenium tetroxide oxidation of cholesteryl acetate

Sica and co-workers[35] have reported the oxidation of selected tri- and tetra- substituted steroidal olefins using stoichiometric amounts of ruthenium tetroxide. They observed that treating cholest-5-en-3β-yl acetate with ruthenium tetroxide in aqueous acetone in the absence of a co-oxidant such as sodium metaperiodate, did not effect the expected oxidative scission of the Δ^5 bond but afforded a mixture of the known 3β-acetoxy-5-hydroxy-5α-cholestan-6-one **61**[32] and 5α-cholestane-3β,5,6α-triol 3-acetate **68**[36] in yields of 60% and 32% respectively. On the basis of this result the oxidation of cholesteryl acetate was attempted. It was anticipated that a successful large-scale ruthenium tetroxide oxidation of **67** and subsequent oxidation of the mixture (**61** + **68**) to increase the yield of the hydroxy-ketone **61** would eliminate the need for chromatography.

An initial attempt to conduct the oxidation in accordance with the described method,[35] using preformed ruthenium tetroxide in solution, failed. However, slow addition of a solution of **67** to a stoichiometric amount of $RuO_2.2H_2O$ in an acetone-water mixture did afford the expected 5α-hydroxy 6-ketone **61** in an increased yield (70%) to that reported, whereas the 5α,6α-diol **68** was obtained in a much lower yield (12%).



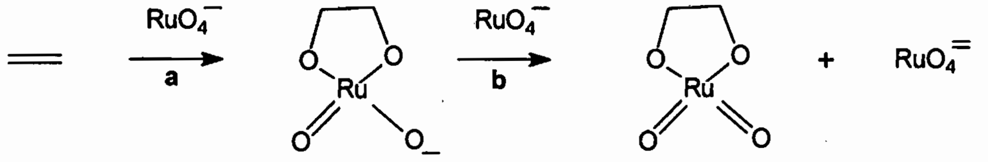
Scheme 13 *Reaction condtions*: i, $RuO_2.2H_2O$, acetone-water, $NaIO_4$, 25 °C: **61**, 70%; **68**, 12%
ii, As above, 0 °C: **67**, 39%; **61**, 14%; **68**, 18%

The 200 MHz ^{1}H NMR spectra of 5α-cholestan-3β-5,6α-triol 3-acetate **68** and 3β-acetoxy-5-hydroxy-5α-cholestan-6-one **61** were consistent with those reported in the

The 200 MHz ^{1}H NMR spectra of 5α-cholestan-3β-5,6α-triol 3-acetate **68** and 3β-acetoxy-5-hydroxy-5α-cholestan-6-one **61** were consistent with those reported in the literature.[35] For **68** the dd at δ3.63 (*J* 10.7 and 5.1 Hz) was distinctive for the axial 6β proton. The expected molecular ion (M$^+$ 462) for **68** was observed, as well as peaks at *m/z* 445 and 385 which correspond to the loss of an OH group in the first instance and subsequent loss of acetic acid. The molecular ion (M$^+$ 460) for **61** was also observed and a peak at *m/z* 460 corresponded to the loss of acetic acid.

In previous investigations of this methodology, the active oxidising reagent (ruthenium tetroxide) was either produced *in situ* in an aqueous acetone solution with the addition of a co-oxidant such as sodium metaperiodate,[37] or the precipitated ruthenium dioxide was separated from the active ruthenium tetroxide solution.[35] A two-phase system of carbon tetrachloride and water has also been utilised,[38] and the addition of acetonitrile to the aforementioned system is reported to improve the efficiency of the ruthenium tetroxide oxidising complex.[39]

In the context of the results reported by Sica and co-workers[35] the mechanism of the oxidation is assumed to entail stereoselective approach of the reagent from the α-face of the substrate.[35] A mechanism which has been proposed by Sharpless and co-workers[39] shows similarities to the potasssium permanganate oxidation mechanism of olefinic bonds. This involves reaction (**a**) of a perruthenate ion with the double bond to produce a cyclic ruthenium (v) species, followed by a fast reaction (**b**) with another perruthenate ion to give a cyclic ruthenium (vi) diester (equation 1).



(1)

The formation of the 5α-hydroxy ketone **61** is not unexpected since ruthenium tetroxide oxidation of a secondary steroidal alcohol in the α-orientation to give the corresponding ketone in an excellent yield has been reported.[37] What is puzzling is that the oxidation of the secondary 6α-hydroxyl group does not go to completion.

Our attempts to perform the oxidation at reduced temperature (i.e 0 °C) reduced the rate of oxidation and increased the yield of the cis-diol. Attempts at oxidising the total reaction product (**61**+ **68**) were investigated using Dess Martin[40] and Swern[41] oxidations. These methods however did not increase the yield of the desired hydroxy-ketone **61** but gave mixtures of products which were not isolated. This avenue was therefore not pursued.

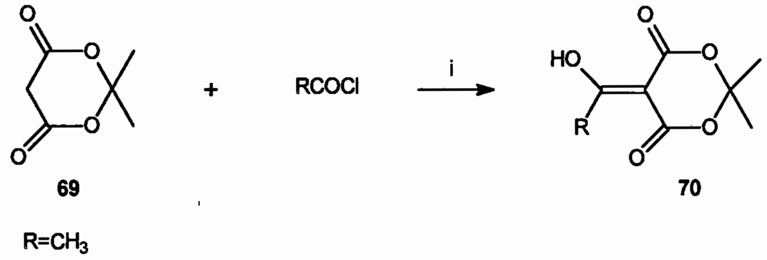
One of the major drawbacks of this oxidation procedure for large-scale synthesis is the volume of solvent which would be required in order to reproduce the reported results. An aqueous acetone solution of 120 cm^3 is required to oxidise only 150 mg of starting material. (i.e a 2.9 *mM* reaction solution)

At this stage it was decided to abandon the ruthenium tetroxide oxidative approach in favour of the conventional route described in section **2.2**, since time did not permit an investigation into the effect that varying the concentration of the reaction solution would have on the distribution of products.

2.4 Synthesis of 3-functionalised enone systems

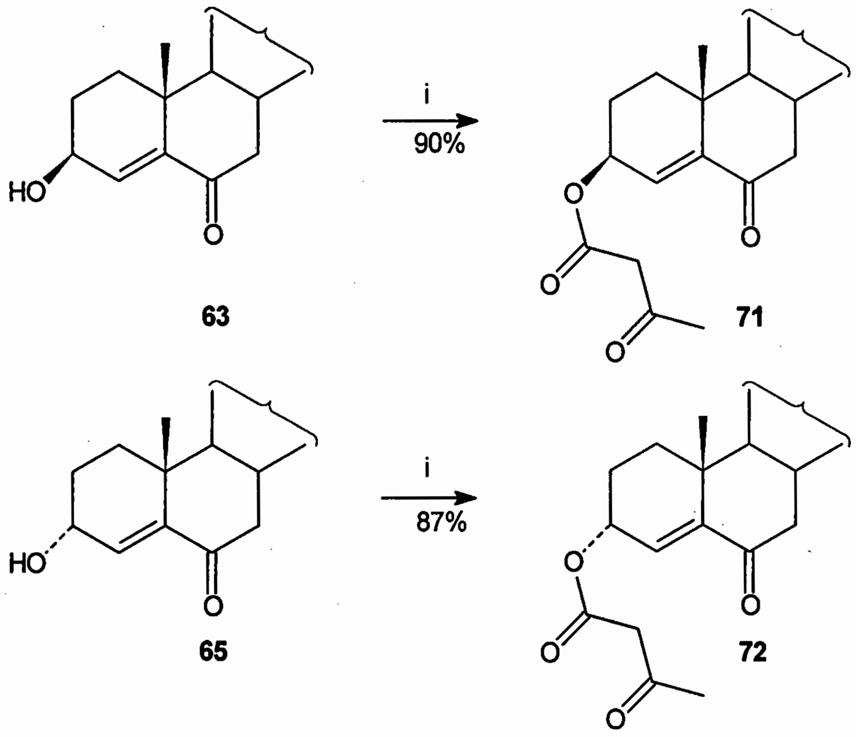
With the 3β- and 3α-hydroxycholest-4-en-6-ones **63** and **65** in hand, the synthesis of the 3β- and 3α-acetoacetoxycholest-4-en-6-ones was undertaken using a described method of acetoacetylation with 5-acetyl Meldrum's acid.[23, 31]

The reagent **70** was readily prepared from acetyl chloride and meldrum's acid **69** in dichloromethane and pyridine at 0 °C according to the described method, in which Meldrum's acid can be converted into a variety of 5-acyl derivatives[42] (Scheme 14).



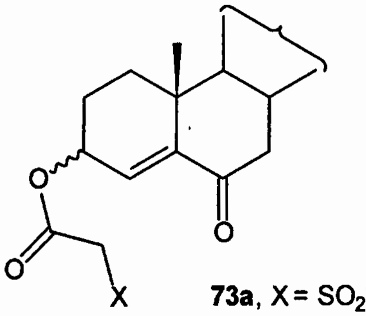
Scheme 14 *Reaction conditions*: i, Acetyl chloride, CH_2Cl_2, pyridine, 0 °C then 25 °C

Thus both 3β- and 3α-hydroxycholest-4-en-6-one **63** and **65** were treated with 5-acetyl Meldrum's acid in benzene to produce 3β-acetoacetoxycholest-4-en-6-one **71** (90%) and 3α-acetoacetoxycholest-4-en-6-one **72** (87 %) respectively.

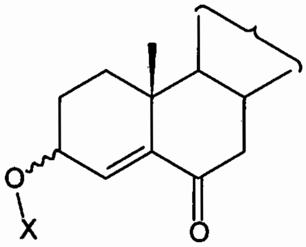


Scheme 15 *Reaction conditions*: i, 5-Acetyl meldrum's acid, benzene, 72 °C

At this stage it was anticipated that investigating the intramolecular Michael reactivity of a variety of 3-substituted enones of the type **73a** and **73b** would offer an alternative route to perhydrobenzo[4.5.6]cholestanes.

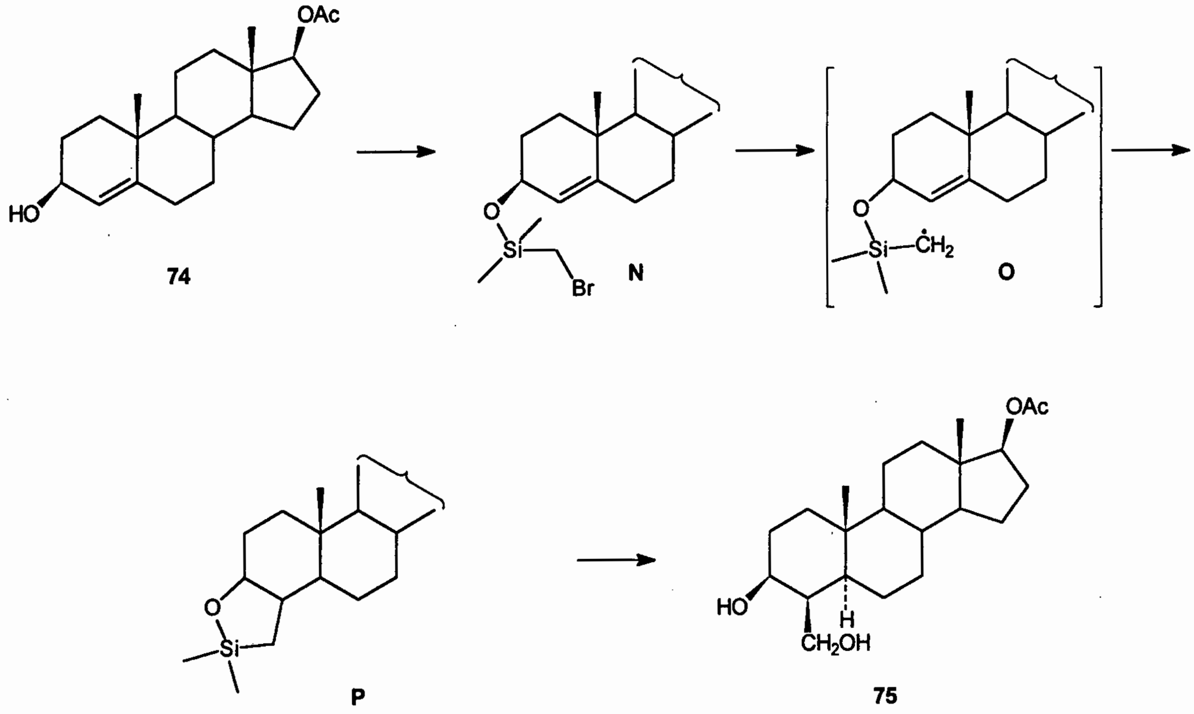


73a, X = SO_2Ph



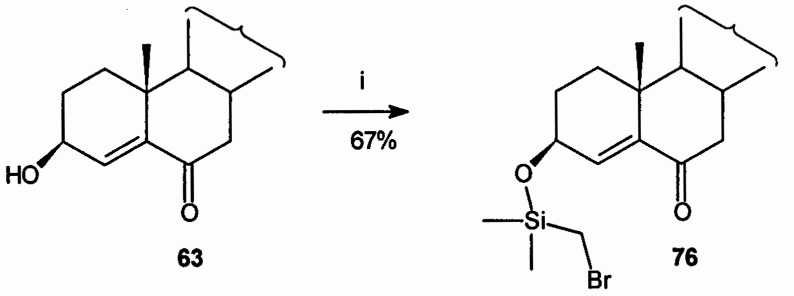
73b, X = SiR_2CH_2X

With this in mind, the methodology utilised by Hanson and co-workers[43] was attempted in which a 3β-(bromomethyl)dimethylsilyloxy derivative was synthesised as a precursor for the stereoselective insertion of an hydroxymethyl group into ring A of the steroid nucleus to form compound **75**. (See Scheme 16) This methodology was based on work done by Stork and co-workers[44] in which the initially formed 3β-(bromomethyl)dimethylsilyloxy derivative **N** was treated with AIBN and tributyltin hydride to form a siloxane **P** via radical closure of the intermediate **O**. The siloxane was not isolated but oxidatively cleaved with potassium fluoride and hydrogen peroxide in dimethylformamide to achieve stereocontrolled introduction of a hydroxymethyl group onto a predetermined vicinal position (Scheme 16).



Scheme 16 Synthesis of a 4β-hydroxymethyl androstane derivative via an intermediate siloxane

An attempted conversion of the 3β-hydroxy enone **63** into its derived 3β-(bromomethyl)dimethylsilyl ether with (bromomethyl)chlorodimethylsilane and imidazole at room temperature resulted in an incomplete conversion, the derived product **76** (67%) being contaminated with starting material even after passing the crude reaction product through a flash column. The product was however crystallised to purity from diisopropyl ether-methanol.



Scheme 17 *Reaction conditions*: i, $BrCH_2Si(Me)_2Cl$, benzene, imidazole, 25 °C, 24 h

Table 1 Diagnostic 400 MHz ^{1}H NMR data for **76**

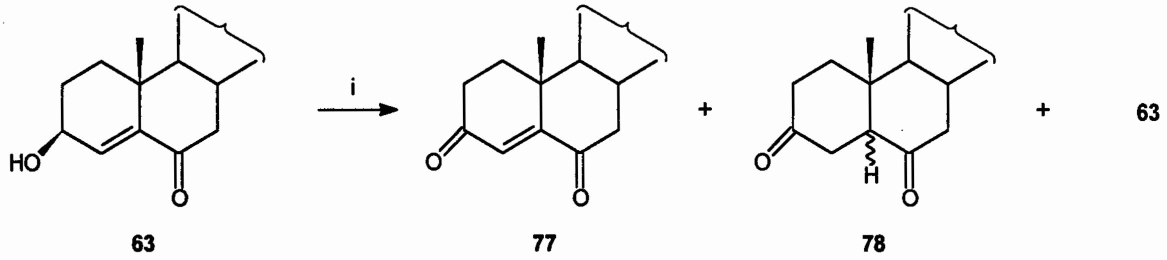
δ	Integration	Multi.	J (Hz)	Assignment
0.27	6H	s		1'-Si(Me)$_2$
2.47	2H	s		2'-CH$_2$
4.33	1H	ddd	10.0, 6.3, 2.2	3α-H
6.06	1H	t[‡]	2 × 1.8	4-H

The formation of **76** was confirmed by diagnostic ^{1}H NMR data (See Table 1) In particular, the ddd assigned to 3α-H displayed the expected multiplicity and coupling pattern for a proton incorporating an antiperiplanar coupling to 2β-H , a synclinal coupling to 2α-H and the smallest coupling constant (2.2 Hz) which could correspond to coupling to 4-H. The coupling to 4-H could not be verified by a correlation to 3α-H in the COSY spectrum. Infrared absorption at ν_{max} 1684 cm^{-1}, is characteristic for a carbonyl group in conjugation with a double bond, and the band at 1087 cm^{-1} was assigned to the Si-O stretch in the O-Si(Me)$_2$CH$_2$Br group. The expected molecular ion (M$^+$ 551) was not observed, but instead a peak at *m/z* 536 which corresponds to the loss of a methyl group was recorded. The (bromomethyl)dimethylsilyl ether derivative **76** was not stable to chromatography as prolonged exposure of the crude product to silica gel afforded only starting material with no trace of **76**. Attempts to encourage complete conversion to **76** by substituting triethylamine or Hünig's base for imidazole did not produce a clean conversion to the desired product. Prolonged exposure (2-3 days) of the starting material to imidazole and (bromomethyl)chlorodimethylsilane in benzene at room temperature was complicated by the formation of the Δ^4-3,6-diketone **77** (19%) and the 3,6-diketone **78** (65%).

[‡] It is not clear why this signal suggests possible coupling between 3α-H and 4-H (a correlation between 3α-H and 4-H was not found in the COSY spectrum of compound **76**)

The formation of **77** was confirmed by the presence of the expected molecular ion (M^+ 398) and infrared absorption at ν_{max} 1678 cm^{-1} which indicated the presence of a conjugated carbonyl group.

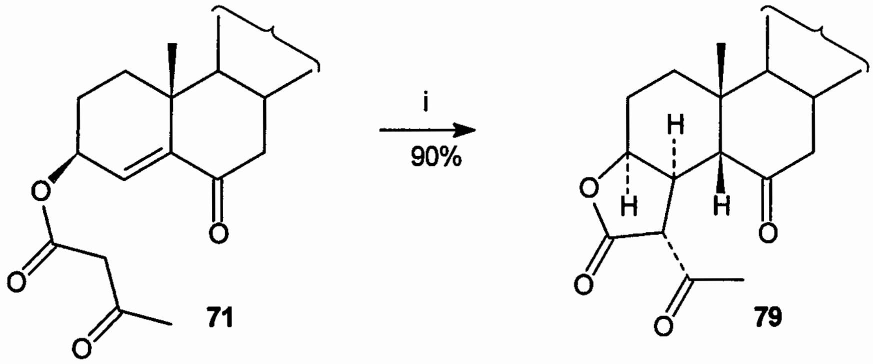
The formation of **78** was indicated by the appearance of two singlets in the ^{13}C spectrum at δ209.1 and 211.2 ppm assigned to C-3 and C-6 respectively, the signals falling within the range of carbonyl carbons for steroids (i.e 177-220 ppm),[45] and by an infrared absorption at ν_{max} 1718 cm^{-1}. The ^{13}C spectrum of **78** was assigned by comparison with the ^{13}C spectra of cholestan-3-one, 5α-cholestane and 5β-cholestane.[52]



Scheme 18 *Reaction conditions*: i, $BrCH_2Si(Me)_2Cl$, imidazole, benzene, 25 °C, 3 days: **77**, 19%; **78**, 65%; **63**, 8%

2.5 Base-mediated intramolecular Michael-aldol reaction of the 3β-acetoacetate 71

Treatment of the 3β-acetoacetoxy enone **71** with aqueous potassium carbonate in ethanol afforded the expected γ-lactone **79** (See Scheme 19). The properties were in agreement with those reported for this compound.[23] A 400 MHz ^1H NMR spectrum of the γ-lactone **79** displayed additional signals which were not resolved in the 200 MHz ^1H NMR spectrum. A ddd at δ2.24 (*J* 15.1, 4.9, 1.2 Hz) was assigned to 7β-H, which displayed *w*-coupling (1.2 Hz) to 5β-H through the 6-CO centre. A signal at δ1.82 (dd, *J* 12.1, 1.2 Hz) was assigned to 5β-H. This *w*-coupling was verified by a correlation between these two signals in the COSY spectrum. Four - bond diequatorial coupling constants of magnitudes 1.4 and 1.7 Hz through a carbonyl centre have been reported for 2, 4-disubstituted cyclohexanones.[46] A dd at δ 2.12 (*J* 15.2, 12.1 Hz) was assigned to 7α-H.

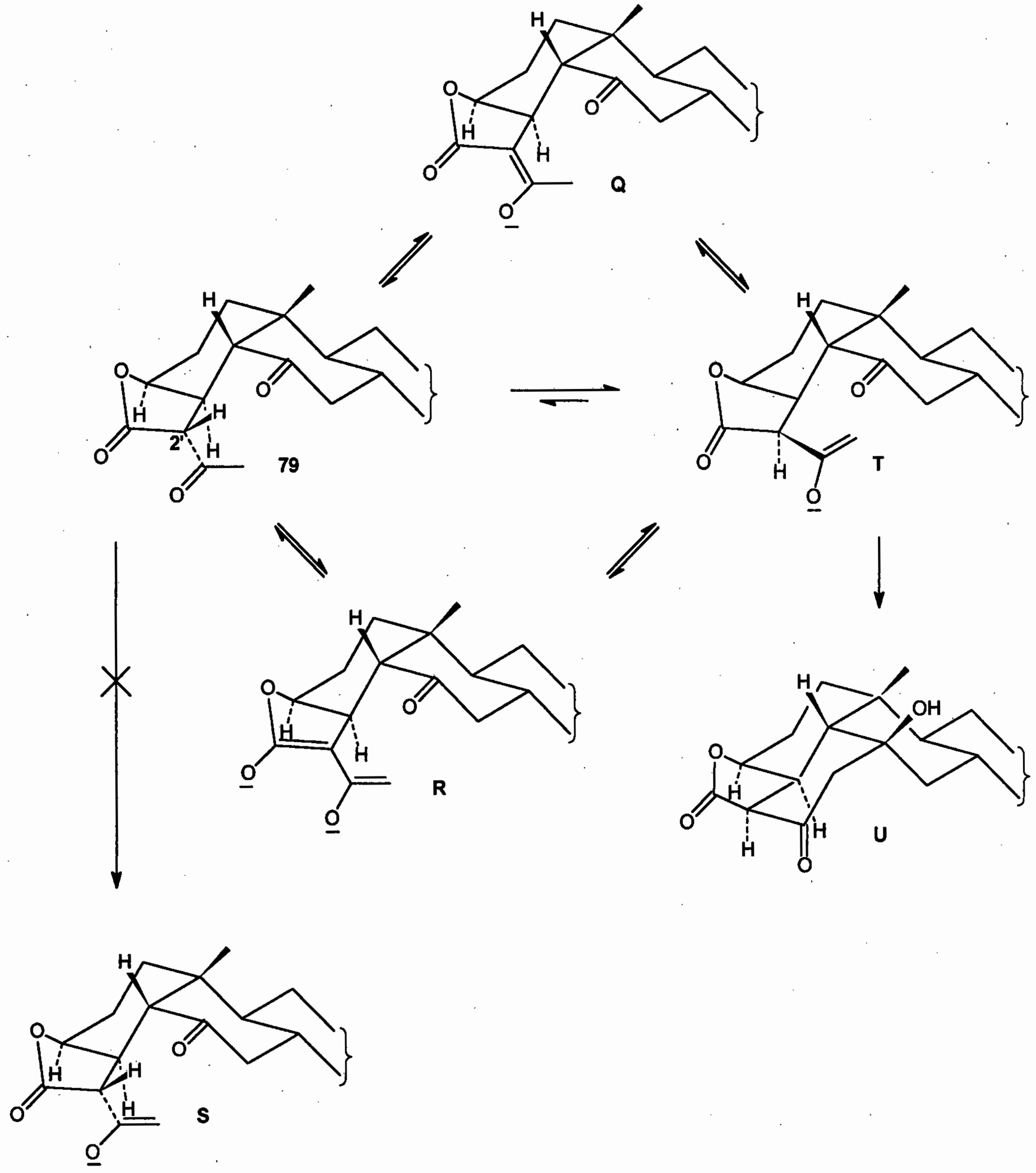


Scheme 19 *Reaction conditions*: i, Aqueous 1M K_2CO_3, ethanol, 67 °C

The 3β-acetoacetoxy appendage forces nucleophilic attack of the enolate from the β-face of the C4-C5 bond, and subsequent 5β-protonation produces the corresponding γ-lactone **79**. The aforementioned experimental conditions were identical to those employed by Borry,[23, 31] establishing the reproducibility of this reaction.

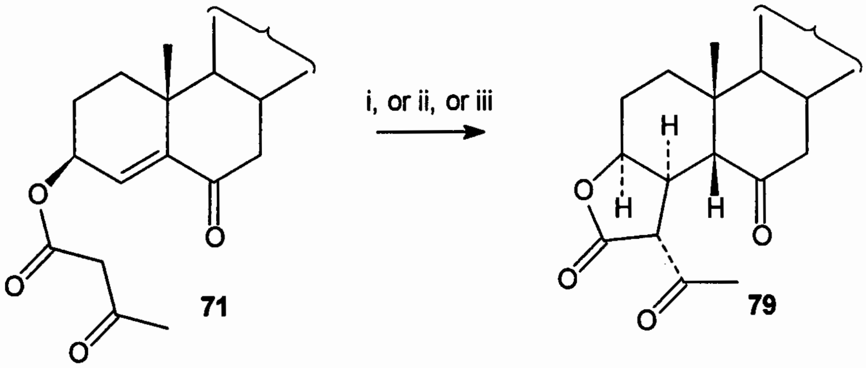
It was recognised in earlier work[23] that the 2'-acetyl group in **79** was unfavourably oriented for intramolecular aldol closure at C-6, and it was reasoned that conditions favouring the formation of enolate ions **Q** and **R** would allow epimerisation at C-2' to form the enolate ion **T** at the expense of the enolate ion **S**. Subsequent intramolecular aldol closure of **T** would form the aldol-closed product **U** whereas in **S** the acetyl group is not suitably aligned for intramolecular aldol closure at C-6.

The aldol-closed product with the lactone ring intact (**U**), was therefore not expected to form unless epimerisation at C-2' could occur. Since refluxing **79** in mild aqueous base over a prolonged period failed to produce a secondary aldol-closed product, it was reasoned that utilising a non-nucleophilic, hindered base might induce epimerisation at C-2' and consequently afford the aldol-closed product.



Scheme 20 Range of possible enolate ions from lactone **79**

With this in mind, the 3β-acetoacetate **71** was treated with potassium *tert*-butoxide (generated by sublimation) in tetrahydrofuran at 5 °C, and then the reaction mixture was allowed to warm to room temperature. However the γ-lactone **79** (76%) was the only product isolated. Alternatively, treating **71** with potassium *tert*-butoxide (generated *in situ* n *tert*-butyl alcohol) at 25 °C also produced the lactone **79** (78%) and treatment of **71** with aqueous 2*M*-potassium hydroxide in ethanol at 25 °C again afforded only the γ-lactone **79** (83%) (See Scheme 21).[§]

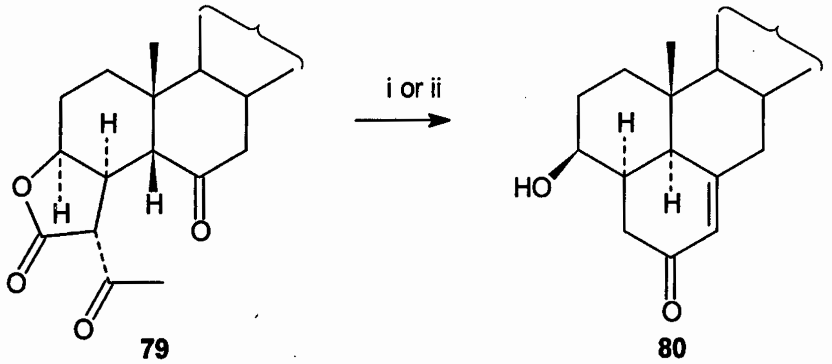


71 **79**

Scheme 21 *Reaction conditions*: i, KOBu^t (sublimed), THF, 5 °C, 25 °C: **79**, 76%
or ii, KOBu^t (generated in situ), *t*-BuOH, THF, 25 °C: **79**, 78%
or iii, aqueous 2M-potassium hydroxide, 25 °C, ethanol: **79**, 83%

However, prolonged treatment of the lactone **79** with aqueous potassium hydroxide in refluxing ethanol (21 h) did afford the pentacyclic conjugated enone **80** in a yield of only 19% accompanied by a mixture of less polar fractions (70 mg, 51% of the crude product isolated) (See Scheme 22). The 400 MHz ^{1}H NMR spectrum of **80** displayed diagnostic features which unequivocally confirmed its structure. The ddt at δ2.57 (*J* 15.4, 5.6, 2 × 5.4 Hz) was assigned to 4α-H which incorporated an antiperiplanar coupling to 6'β-H and three synclinal couplings to 3α-H, 5α-H and 6'α-H. The synclinal relationship of 4α-H to 5α-H was confirmed by the doublet assigned to the latter proton which displayed a coupling constant of 5.3 Hz. The region containing 6'α-H and 6'β-H was located by a crosspeak to 4α-H in the COSY spectrum. The dd at δ2.44 (*J* 17.5, 5.4 Hz) was assigned to 6'α-H and the dd at δ2.29 (*J* 17.3, 15.4 Hz) was assigned to 6'β-H. A dd at δ2.36 (*J* 12.4, 4.6 Hz) was assigned to 7β-H since this is the only other proton which is expected to give this multiplicity.

[§] An examiner has suggested the use of non-nucleophilic bases such as DBN or DBU for this step

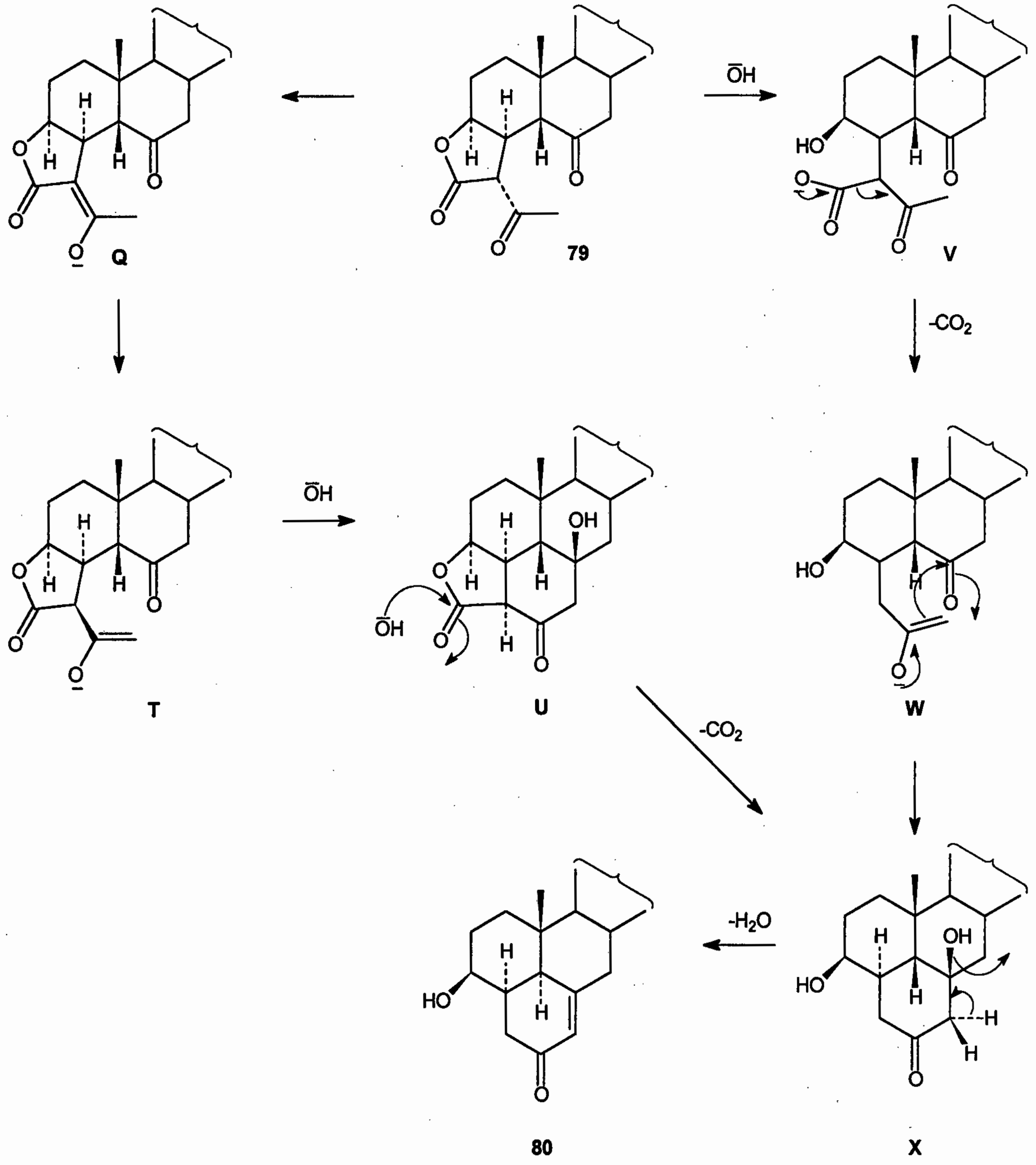


79 **80**

Scheme 22 *Reaction conditions*: i, aqueous 2M KOH, EtOH, reflux (21h): **80**, 19% + 70 mg of less polar mixed fractions (51% of crude product isolated) or ii, as above, reflux for 5 h, 6 °C (21 h): **92**, 27%

The conjugated enone **80** could only result from either a sequential process of lactone cleavage, decarboxylation, intramolecular aldol closure and finally β-elimination or another sequence of events involving epimerisation at C-2' to form the enolate ion **T**, intramolecular aldol closure at C-6 to form **U**, lactone cleavage and β-elimination. (See scheme 23)

The formation of the enone **80** indicated that the above reaction pathways were possible when using potassium hydroxide. An attempt was therefore made to optimise these experimental conditions in order to increase the yield of the β-elimination product **80**. Treating the lactone **79** with aqueous potassium hydroxide in refluxing ethanol followed by prolonged exposure of the reaction mixture to low temperature (6 °C, 21 h), served only to slightly increase the yield of the enone **80** (27%).



Scheme 23 Alternative pathways for the formation of the enone **80**[**]

These reaction conditions were not investigated further, but rather the method described by Borry[23, 31] in the synthesis of the 3β,6β-dihydroxy derivative **81** was attempted. The γ-lactone **79** was therefore refluxed with aqueous potassium hydroxide in a

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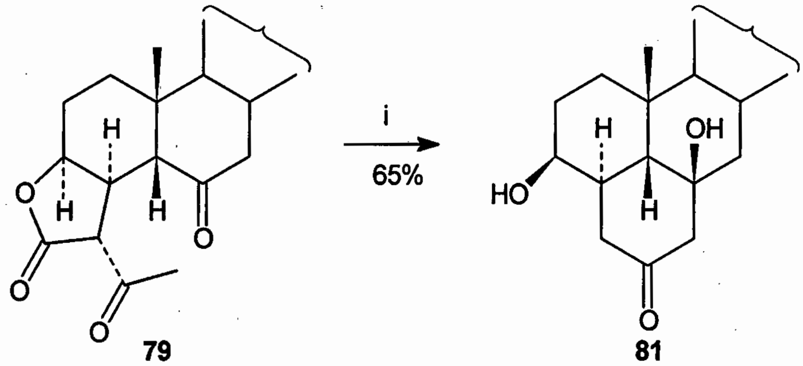
[**] Refer to **Scheme 29** for the possible pathways of isomerisation at C-5

mixture of dioxane and water until the starting material had disappeared, after which the reaction temperature was reduced (6 °C, 2 days) to afford **81** in an optimised yield of 65%. Reducing the temperature encourages the formation of the kinetic enolate of the lactone-cleaved and decarboxylated intermediate **W** (See Scheme 23) which subsequently undergoes intramolecular aldol closure at C-6 to form the 3β,6β-dihydroxy derivative **81**.[31]



79 **81**

Scheme 24 *Reaction conditions*: i,aqueous 2M-KOH, dioxane-water, reflux, 0 °C

The formation of **81** was confirmed by characteristic absorption bands at 1710 cm^{-1} which indicated the presence of a saturated carbonyl group, and at 3598 cm^{-1} which was assigned to the 3β- and 6β-hydroxyl groups. The structure of **81** was further confirmed by diagnostic 400 MHz NMR data (See Table 2 and Fig. 4).

Table 2 Diagnostic 400 MHz ^{1}H NMR data for **81**

δ	Integration	Multi.	*J* (Hz)	Assignment
3.75	1H	m	$W_{½}$ 10.0	3α-H
1.90-2.01	2H*	br. m.	$W_{½}$ 39.0	4α-H
1.86	1H	dd	11.9, 1.2	5β-H
2.10	1H	dt	13.3, 2 × 3.0	6'α-H
2.64	1H	td	2 × 13.2, 0.9	6'β-H
2.34	1H	dd	13.3, 2.6	4'α-H
2.56	1H	d	13.3, 1.2	4'β-H

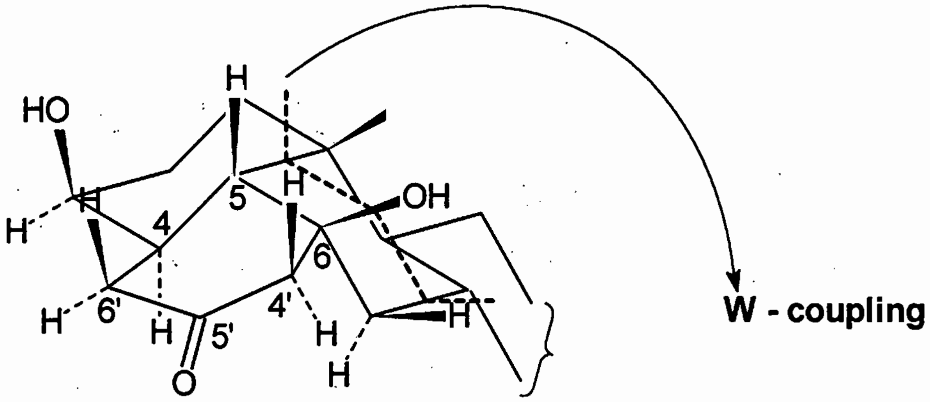
*- multiplet at δ1.90-2.01 overlaps with the signal of another proton which could not be unambiguously identified

The $W_{½}$ value (10.0 Hz) of the multiplet assigned to 3α-H can be reconciled with three synclinal couplings to 2β-H, 2α-H and 4α-H. The multiplet assigned to 4α-H was identified by crosspeaks to 3α-H, 5β-H, 6'α-H and 6'β-H in the COSY spectrum. The 4α-H assignment was supported by the presence of a crosspeak to C-4 in the HETCOR spectrum which was identified as a methine carbon at δ38.3 ppm in the DEPT spectrum. The 4α-H signal could however not be deciphered as it coincided with the signal of another proton which could not be unambiguously identified. The dd assigned to 5β-H (*J* 11.9, 1.2 Hz) showed evidence of possible *w*-coupling to 7β-H (1.2 Hz) and antiperiplanar coupling to 4α-H (11.9 Hz).

A dd at δ2.34 (*J* 13.3, 2.6 Hz) assigned to 4'α-H, could be ascribed to geminal coupling to 4'β-H (13.3 Hz) and *w*-coupling to 6'α-H (3.0 Hz). A dt at δ2.10 (*J* 13.3, 2 × 3.0 Hz), which was assigned to 6'α-H, displayed geminal coupling to 6'β-H (13.3 Hz), synclinal coupling to 4α-H (3.0 Hz) and complementary *w*-coupling to 4'α-H (3.0 Hz). The *w*-coupling between 4'α-H and 6'α-H was verified by a correlation between the assigned signals in the COSY spectrum.

A dd at δ2.56 (J 13.3, 1.2 Hz) was assigned to 4'β-H. This signal is consistent with geminal coupling to 4'α-H (13.3 Hz) and coupling to the axial 6'β-H proton (1.2 Hz).

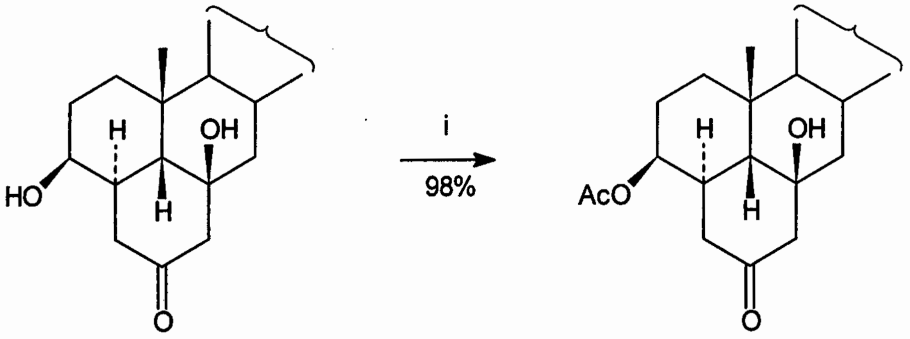
The td at δ2.64 (J 2 × 13.2, 0.9 Hz) which was assigned to 6'β-H, incorporated geminal coupling to 6'α-H (13.2 Hz), coupling to the antiperiplanar 4α-H proton (13.2 Hz) and a coupling constant of 0.9 Hz which was associated with coupling to the axial 4'β-H proton. Coupling between axial protons separated by four bonds has been reported for 2-substituted cyclohexanone systems.[47]



81

Figure 4 Three-dimensional perspective of **81** indicating possible *w*-coupling between 5β-H and 7β-H

Compound **81** was subsequently treated with acetic anhydride and pyridine to give the 3β-acetoxy-6β-hydroxy-5'-ketone **82** (98%) in an almost quantitative yield. An infrared absorption band at 1732 cm^{-1} correspondes to the acetoxy carbonyl group, while a band at 1719 cm^{-1} was assigned to the 5'-carbonyl group.



81 **82**

Scheme 25 *Reaction conditions*: i, Ac_2O, C_5H_5N, 25 °C

The 400 MHz ^{1}H NMR spectrum of **82** displayed similiar characteristics to the ^{1}H NMR spectrum of **81**. The dd at δ1.88 (*J* 11.8, 1.5 Hz) which was assigned to 5β-H, displayed possible *w*-coupling to 7β-H (1.5 Hz). The COSY spectrum however did not verify the *w*-coupling between 5β-H and 7β-H. The region for the 4α-H signal was located by a crosspeak for C-4 to a multiplet at δ1.97-2.07 in the HETCOR spectrum. This multiplet could not be resolved as it coincided with an additional signal which could be correlated, using HETCOR data, to either of the 7-H protons. The 4'α-H and 4'β-H protons displayed the expected multiplicities. The 6'β-H signal could however not be resolved and the acetate methyl singlet at δ2.09 obscured the 6'α-H signal.

2.6 Dehydration and isomerisation pathways of 3β,6-dihydroxy-4α,4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one

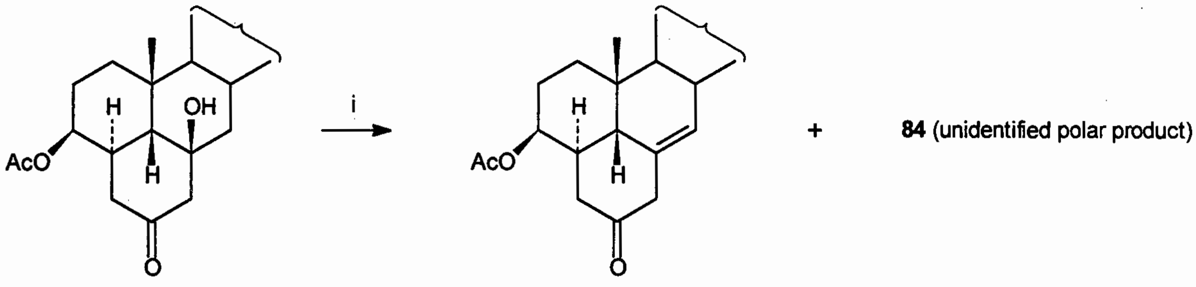
With the 3β,6β-dihydroxy pentacyclic compound **81** in hand, it was decided to focus attention on the chemistry of this pentacyclic product, more specifically the products that would result from different dehydration pathways and the isomerisation possibilities that would accompany the formation of these products. Borry had investigated the acid-mediated dehydration sequence of compound **81** in which *p*-toluenesulfonic acid treatment of **81** gave the enone **80** in an isolated yield of only 30% via the intermediate deconjugated

Δ^6-5'-ketone.[31] It was therefore necessary to investigate alternative reaction pathways which would produce the conjugated enone **80** more efficiently.

The Δ^6-isomer is the major product which is expected to form under conditions of E2 elimination since 7α-H is the only proton which is in an antiperiplanar arrangement with respect to the 6β-hydroxy group. In an E_2 elimination reaction this antiperiplanar arrangement allows the departing groups to be parallel to each other which facilitates maximum overlap of the p orbitals in the formation of the newly formed pi bond.[48] Formation of the $\Delta^{6(4')}$-isomer is expected to occur to a lesser extent, if at all, due to the anti-diequatorial disposition of 4'α-H and 6β-OH. Although it must be noted that in most cases introducing conjugation into a system contributes to its thermodynamic stability, an additional factor to consider would be to what extent accompanying ring strain due to conjugation would influence the formation of the expected isomers. The increased stability which often accompanies an increase in conjugation is due to a decrease in energy which results from lateral overlap between an increased number of p atomic orbitals.[48, 49]

2.6.1 Thionyl chloride -pyridine dehydration

On the basis of previous studies conducted on the 3-deoxy analogue of **81**,[21] it was expected that conventional dehydration of **82** in the presence of thionyl chloride-pyridine would result in preferential formation of the Δ^6-5'-ketone **83** mediated by the only antiperiplanar elimination pathway available to the 6β-hydroxy group. However the reaction of **82** with thionyl chloride-pyridine at 0 °C , resulted in the formation of a complex mixture, from which the Δ^6-5'-ketone **83** was isolated in poor yield (29%) by column chromatography. A more polar fraction (37mg, 23% of the crude product isolated) which appeared to be a single component on TLC, was isolated and crystallised from diisopropyl ether-methanol.



82 **83**

Scheme 26 *Reaction conditions*: i, $SOCl_2$, C_5H_5N, 0 °C: **83**, 29%; unidentified polar product; **84**, 23% of the crude product isolated

No other products could be accounted for except for mixtures of less polar products which eluted first during chromatography. The structure of the polar product could not be deduced from the available analytical and spectroscopic data. The 400 MHz ^{1}H NMR spectrum displayed no distinct signals which could be reconciled with plausible structures expected for this reaction. The key features of the ^{1}H NMR spectrum were a singlet at δ2.07 which could be associated with an acetate methyl group, two singlets and a set of doublets between δ0.68-0.89 which resembled the features expected for the aliphatic methyl groups of the cholestane skeleton and a set of triplets and a doublet between δ7.90-9.02 which resembled the peaks expected for pyridine (See Appendix for 400 MHz ^{1}H NMR spectrum of unidentified product). Infrared absorption at ν_{max} 1730 cm^{-1} could be assigned to an AcO functional group. Several mass spectra which were run at source temperatures between 180-230 °C displayed peaks at 486, 471 and 404. These masses could not be reconciled with any expected dehydration products.

The formation of the deconjugated enone **83** was indicated by diagnostic infrared absorption peaks and 400 MHz NMR data (See Table 3 and Fig. 5).

Table 3 Diagnostic 400 MHz ^{1}H NMR data for **83**

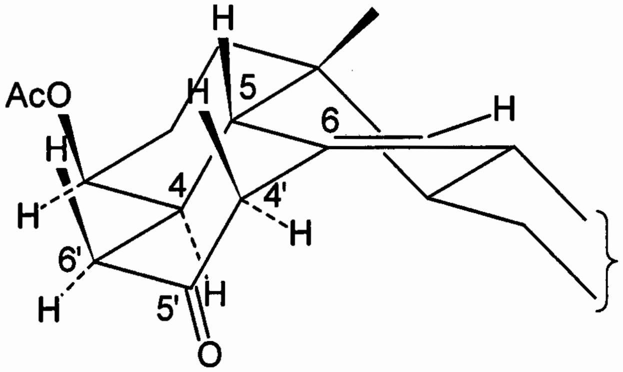
δ	Integration	Multi.	*J* (Hz)	Assignment
1.94	1H	d	11.0	5β-H
2.30	1H	ddd	14.9, 4.3, 1.9	6'α-H
2.37	1H	dd	14.9, 11.5	6'β-H
2.96	1H	dd	14.7, 1.9	4'β-H
3.05	1H	ddd	14.8, 3.6, 2.3	4'α-H
4.82	1H	m	$W_{½}$ 8.9	3α-H
5.29	1H	s		7-H

The deconjugated nature of **83** was confirmed by infrared absorption at ν_{max} 1715 cm^{-1} which is associated with the stretching frequency of an isolated carbonyl group. The 400 MHz ^{1}H NMR spectrum provided detailed information about the 6'-H and 4'-H protons. A ddd at δ2.30 (*J* 14.9, 4.3, 1.9 Hz) assigned to 6'α-H, was consistent with geminal coupling to 6'β-H (14.9 Hz), a smaller synclinal coupling to 4α-H (4.3 Hz) and possible *w*-coupling to 4'α-H (1.9 Hz). The ddd at δ3.04 (*J* 14.8, 3.6, 2.3 Hz) which was assigned to 4'α-H, displayed geminal coupling to 4'β-H (14.8 Hz), complementary *w*-coupling to 6'α-H (2.3 Hz), and a coupling constant of 3.6 Hz which could not be associated with any obvious coupling partner.

The dd at δ2.37 (*J* 14.9, 11.5 Hz) which was assigned to 6'β-H, incorporated geminal coupling to 6'α-H and a *trans*-diaxial vicinal coupling to 4α-H. The dd at δ2.96 (*J* 14.7, 1.9 Hz) assigned to 4'β-H, displayed geminal coupling to 4'α-H and allylic coupling (1.9 Hz) to 7-H. Complementary allylic coupling was not evident in the 7-H signal, but could be verified by a correlation between 7-H and 4'β-H in the COSY spectrum. The d at δ1.94 (*J* 11.0 Hz) was tentatively assigned to 5β-H on the basis of a crosspeak to C-5 in the

HETCOR spectrum. The C-5 assignment was supported by the presence of a singlet at δ47.4 ppm in the methine region of the DEPT spectrum. All the above assignments were verified by appropriate crosspeaks in the HETCOR spectrum.

Attempts directed at trying to increase the yields of the products isolated for this reaction were unsuccessful and the reaction conditions were therefore not investigated further.



83

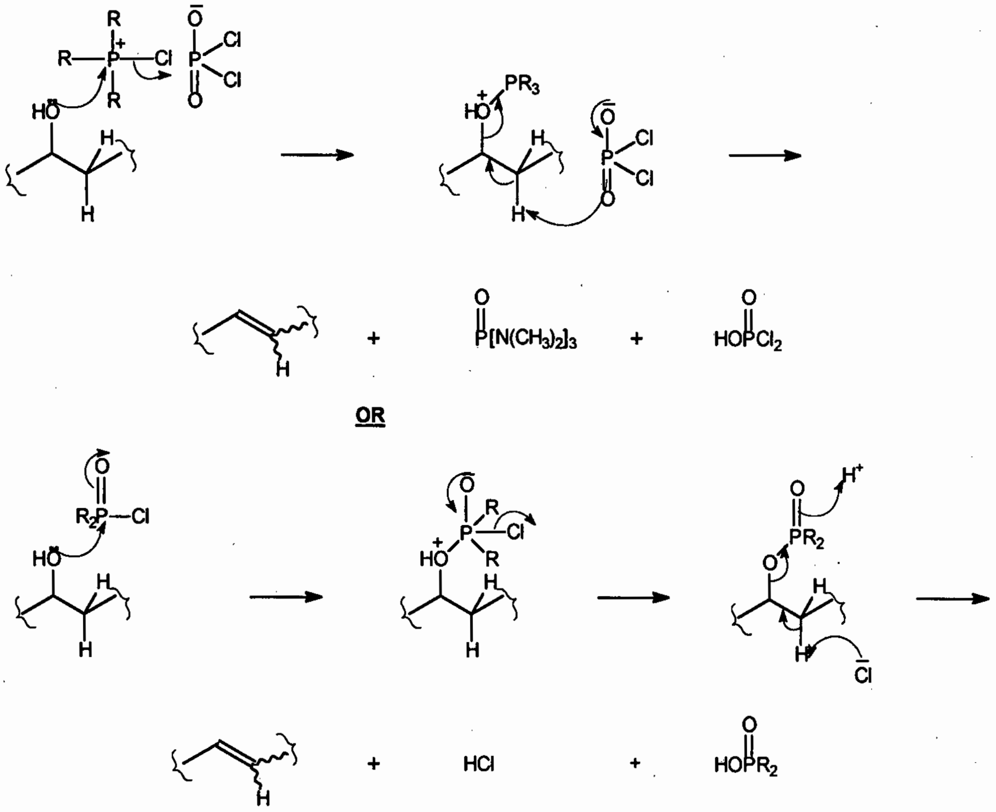
Figure 5 Three-dimensional perspective of the deconjugated enone **83**

2.6.2 $POCl_3$-HMPA Dehydration

Attention was consequently directed to alternative methods of dehydration. In the first instance the use of $POCl_3$-HMPA was considered. Trost and co-workers[50] have reported the successful use of this reagent for the dehydration of adducts of 1-lithiocyclopropyl phenyl sulfide. It was noted that addition of $POCl_3$ to a solution of the starting material in HMPA resulted in the formation of a white precipitate which eventually dissolved on warming of the reaction mixture. There has been some speculation as to the actual reagent involved in the dehydration and it has been suggested that a redistribution of groups occurs which either results in the intermediacy of a salt **Y** or the formation of N, N, N', N'-tetramethylphosphordiamidic chloride **Z**[50] (equation 2).

$$O{=}PCl_3 \quad + \quad O{=}P[N(CH_3)_2]_3 \longrightarrow \underset{\textbf{Y}}{[(CH_3)N]_3PCl^+PO_2Cl_2^-} \longrightarrow \underset{\textbf{Z}}{?O{=}P(Cl)[N(CH_3)_2]_2} \qquad \textbf{(2)}$$

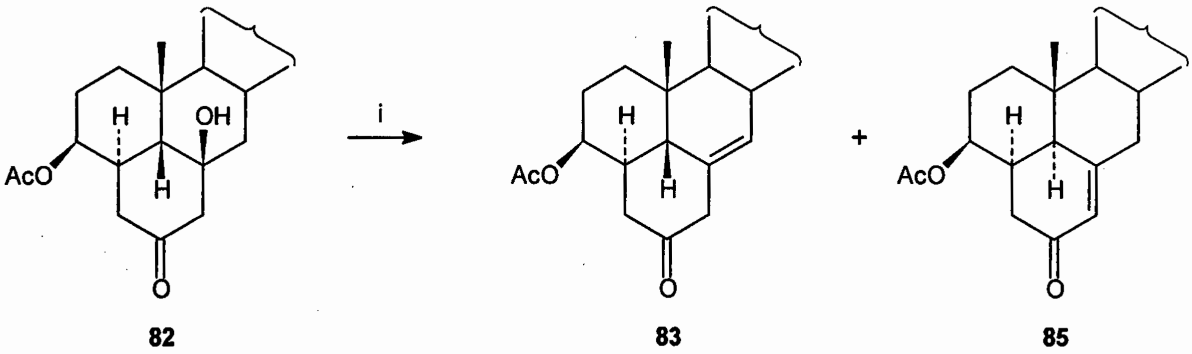
Either of the above-mentioned groups could participate in activating the hydroxyl group toward elimination. The authors have not offered any mechanistic interpretations; however a proposed mechanism for the interaction of an hydroxyl group with either the salt **Y** or the species **Z** is represented in **Scheme 27.** This process involves nucleophilic attack of the lone pair electrons of the hydroxyl oxygen on the phosphorus atom of either of the above-mentioned species to form an oxygen-phosphorous intermediate which releases anionic species that can act as bases for proton abstraction to form the double bond.



R=N(CH3)2

Scheme 27 Possible mechanisms of interaction of POCl3 and HMPA with a secondary hydroxyl group to form a double bond

A reaction in which $POCl_3$ was slowly added to a solution of **82** furnished, in good overall yield, a two-component mixture, which was readily separated by chromatography (Scheme 28). The minor less polar compound (12%) was shown to be the expected Δ^6-5'-ketone **83** and the major product (70%) was assigned the structure **85** on the basis of spectroscopic and analytical data.



Scheme 28 *Reaction conditions*: i, $POCl_3$, HMPA, 25 °C (1 h), 50-70 °C: **83**, 12%; **85**, 70%

The formation of the 4α,5α-enone **85** was indicated by characteristic infrared absorption at ν_{max} 1665 cm^{-1} (C=O) and 1634 cm^{-1} (C=C) which clearly demonstrated the presence of a conjugated enone moiety and the 4α,5α-configuration followed from diagnostic 400 MHz NMR data (See Table 4 and Fig. 6).

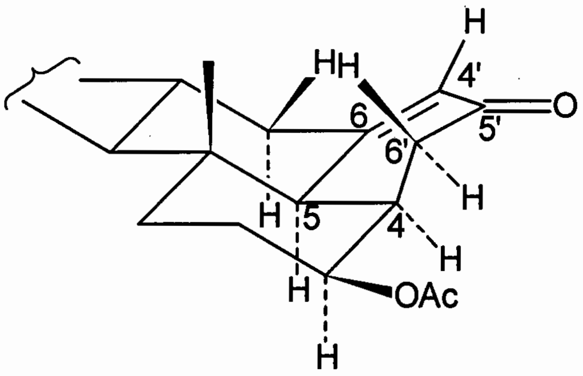
Table 4 Diagnostic 400 MHz ^{1}H NMR data for **85**

δ	Integration	Multi.	*J* (Hz)	Assignment
2.11	1H	d	5.5	5α-H
2.69	1H	dq	14.8, 3 × 5.9	4α-H
4.91	1H	dt*	11.7, 2 × 5.85	3α-H
2.26	1H	dd	17.6, 5.9	6'α-H
2.40	2H	dd	17.5, 14.5	6'β-H
5.96	1H	s		4'-H

*- signal is actually a ddd which appears as a dt due to equivalent synclinal coupling constants

The signals assigned to 3-H and 5-H fix both the axial orientation of 3α-H and the 5α-H. The dt assigned to 3α-H displayed an antiperiplanar coupling (11.7 Hz) to 2β-H and two synclinal couplings (2 × 5.85 Hz) which corresponded to coupling to 2α-H and another synclinal neighbour which consequently fixed the 4α-configuration. The synclinal relationship of 5α-H to 4α-H was verified by the coupling constant (*J* 5.5 Hz) of the d assigned to 5α-H. The dq assigned to 4α-H (14.8, 3 × 5.9 Hz) corresponded to one antiperiplanar coupling to 6'β-H (14.8 Hz) and three equivalent synclinal couplings to 5α-H, 3α-H and 6'α-H (5.9 Hz each). The signals assigned to 4α-H and the 6'-methylene protons were confirmed by distinct crosspeaks to 5α-H and the 6'-CH_2 protons in the former case and an isolated crosspeak to 4α-H in the latter case, in the COSY spectrum. The region containing the 6'α-H and 6'β-H protons integrates for three protons rather than the expected two protons. A crosspeak to C-7 in the HETCOR spectrum indicated that 7β-H or 7α-H could be located in this region. The assignments for 3α-H, 4α-H, 5α-H and the 6'-H protons were confirmed by appropriate crosspeaks to the relevant carbon signals in the

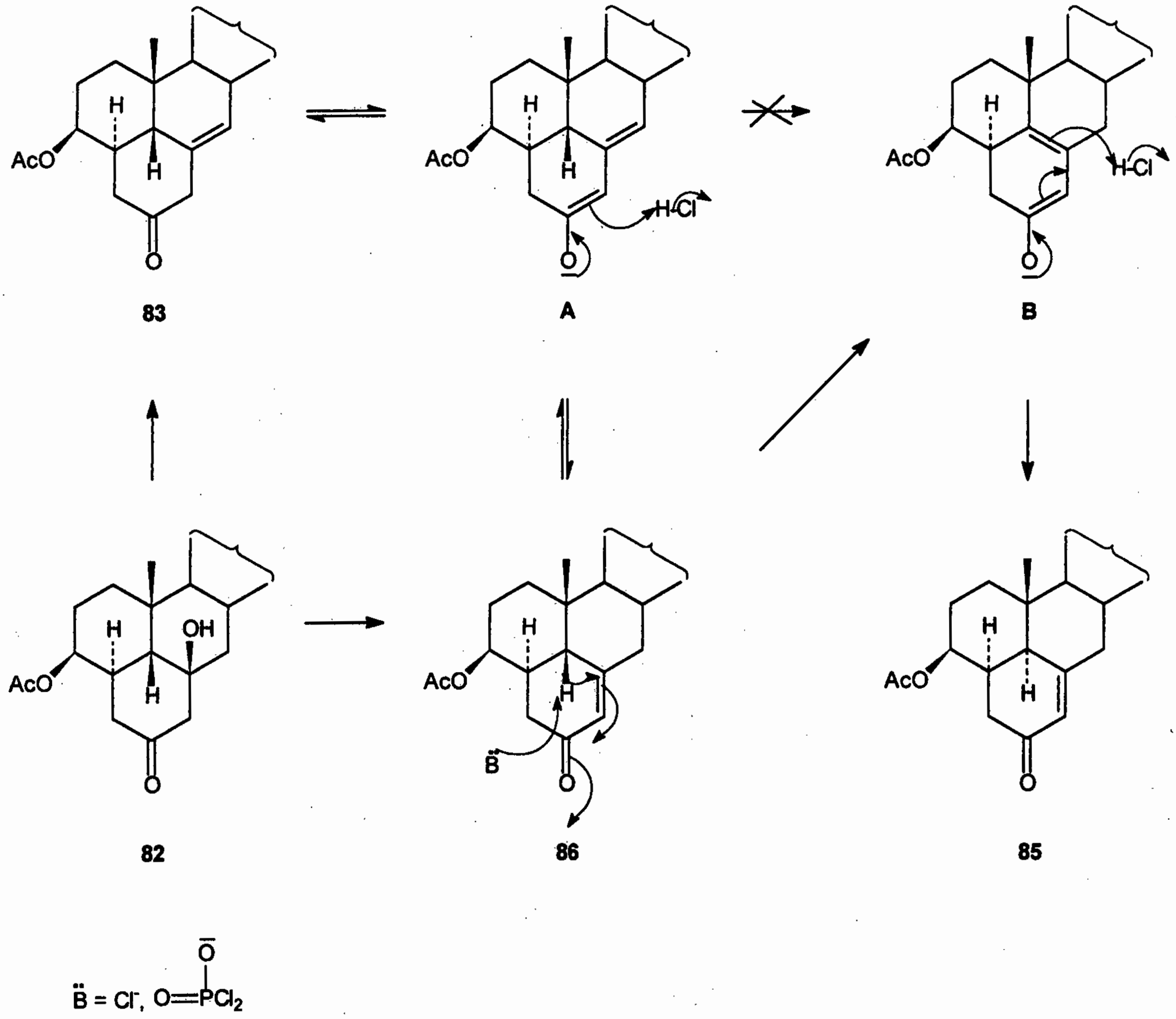
HETCOR spectrum. The multiplicities and coupling constants of the remaining ring E protons (i.e 6'α-H, 6'β-H and 4-H) follow from the 3α,4α,5α-configuration of **85**.



85

Figure 6 Three-dimensional perspective of the 4α,5α-enone **85**

Isomerisation of **82** at C-5 could either occur through the intermediacy of **83** if it is assumed that **83** is formed due to kinetically controlled dehydration at the expense of a syn elimination product, or subsequent to the formation of the 4α,5β-5'-ketone **86** (See Scheme 29). The $\Delta^{6,4'}$-dienolate ion **A** could form under alkaline conditions, however 5β-H abstraction from **A** followed by protonation at C-7 to form **B** would occur with difficulty since a model shows that the β-face of **A** is sterically hindered. Protonation at C-4' would lead to the reformation of **83** or alternatively, protonation at C-7 would produce the 4α,5β-enone **86**. Abstraction of 5β-H would give **B**, which on protonation from the less hindered α-face would produce the 4α,5α-enone **85**.



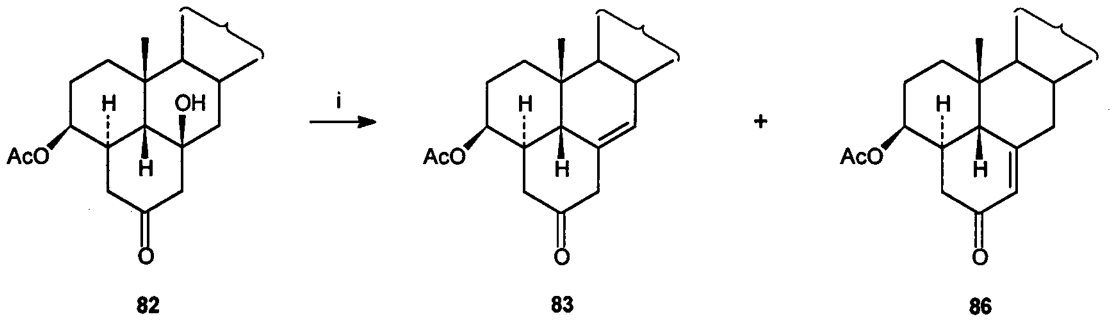
Scheme 29 Alternative pathways for the isomerisation of C-5 to form the 4α,5α-enone **85**

If preferential formation of the 4α,5β-enone **86** is accepted as the more plausible reaction pathway, it can be assumed that the reaction is not under kinetic control but rather proceeds to form the 4α,5β-enone **86** through the intermediacy of **83** or directly from **82** which then rapidly isomerises to the thermodynamically stable product, **85**.

2.6.3 BF_3.OEt_2 Dehydration

A report on the successful dehydration of a tertiary alcohol with BF_3.OEt_2 used in the asymmetric total synthesis of (+)-pentalene prompted an attempt to apply these conditions to the dehydration of the 6β-hydroxy-5'-ketone **82** in the hope that any products resulting from secondary reactions would be suppressed.[51]

Thus, the slow addition of $BF_3.OEt_2$ to **82** at 25 °C gave a separable mixture of the Δ^6-5'-ketone **83** (36%) and the $\Delta^{4'}$-5'-ketone **86** (52%) (Scheme 30). In contrast to the $POCl_3$-HMPA dehydration, concomitant epimerisation at C-5 did not occur. The infrared spectrum of compound **86** displayed characteristic absorption at ν_{max} 1659 cm^{-1} (5'-C=O) and 1615 cm^{-1} (C=C) which confirmed the conjugated enone functionality in ring E. The mass spectrum recorded peaks at *m/z* 482, which corresponds to the molecular ion, and *m/z* 422 which corresponds to the loss of acetic acid. The 4α,5β-configuration was established by diagnostic 400 MHz NMR data (See Table 5 and Fig. 7).



Scheme 30 *Reaction conditions*: i, $BF_3.OEt_2$, CH_2Cl_2, 25 °C: **83**, 36%; **86**, 52%

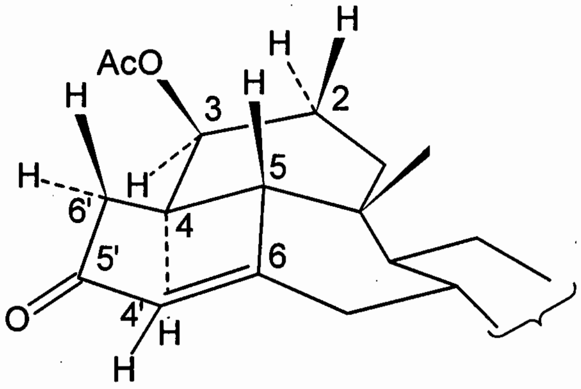
Table 5 Diagnostic 400 MHz ^{1}H NMR data for **86**

δ	Integration	Multi.	*J* (Hz)	Assignment
1.80	1H	dd	11.3, 2.2	5β-H
2.64	1H	ddt	15.8, 11.5, 2 × 2.1	4α-H
4.95	1H	ddd	8.0, 5.5, 4.1	3α-H
2.20	*	dd	17.3, 12.5	6'β-H
5.78	1H	t	2 × 2.1	4'-H

*- an accurate integral could not be ascertained as the 6'β-H signal coincided with other signals which could not be identified

If ring A of **86** occupies a chair conformation the signal assigned to 3α-H is expected to display three synclinal coupling constants. However one of the coupling constants exhibited by the assigned signal (*J* 8.0, 5.5, 4.1 Hz) does not coincide with the expected magnitude. This could be due to the fact that ring A is slightly distorted which allows for a dihedral angle greater than 60° between 3α-H and 2β-H and consequently a larger *J* value (8.0 Hz). The ddt at δ2.64 assigned to 4α-H, incorporated two antiperiplanar couplings to 5β-H (11.5 Hz) and 6'β-H (15.8 Hz) and two synclinal couplings to 3α-H and 6'α-H (2.1 Hz each). The assignment for 4α-H was supported by a crosspeak correlating C-4 and the assigned signal in the HETCOR spectrum. The C-4 signal was identified as a singlet at δ34.2 ppm in the methine region of the DEPT spectrum. Evidence of the 5β-configuration was indicated by the dd at δ1.80 (*J* 11.3, 2.2 Hz) assigned to 5β-H, which was located by a distinct crosspeak to 4α-H in the COSY spectrum. The large coupling constant (11.3 Hz) can be attributed to the near antiperiplanar relationship between 4α-H and 5β-H and the smaller coupling constant (2.2 Hz) can be attributed to allylic coupling to 4'-H. The signal assigned to 4'-H (*J* 2 × 2.1 Hz) displayed complementary allylic coupling to 5β-H and allylic coupling to either 7β-H or 7α-H. The region for the 6'β-H and 6'α-H protons was established by the presence of a distinct crosspeak to 4α-H in the COSY spectrum. A dd at δ2.20 (*J* 17.3, 12.5 Hz) in this region was assigned to 6'β-H. A signal which could be matched with the expected multiplicity for 6'α-H could however not be deciphered. The assignments for 3α-H and 4α-H were verified by appropriate crosspeaks to C-3 and C-4 in the HETCOR spectrum.

The mechanism of this reaction could possibly involve complexation of $BF_3.OEt_2$ to the lone pair electrons of the tertiary hydroxyl oxygen, thus activating the hydroxyl group toward elimination. Although treatment of **82** with $BF_3.OEt_2$ produced the expected Δ^6- and $\Delta^{4'}$- isomers **83** and **86**, these conditions were not conducive to subsequent isomerisation to form the thermodynamically stable 4α,5α-enone **85**.



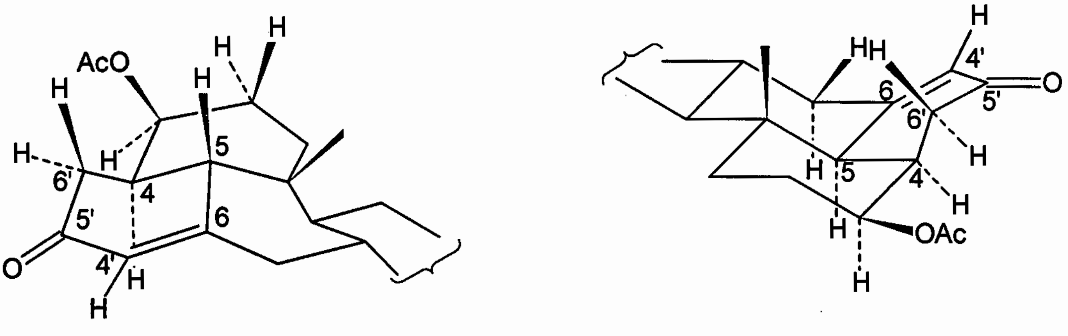
86

Figure 7 Three-dimensional perspective of the 4α,5β-enone **86**

2.7 Comparison of the thermodynamic stabilities of the 4α,5α- and 4α,5β-isomers

Although a model of the 4α,5α-enone **85** (See Fig. 8) shows the C4-C6' bond exhibiting a strong 1,3-diaxial interaction with the 10β-methyl group, rings A and B can be accommodated in chair-like structures which contributes to the thermodynamic stability of this product. Ring B occupies a slightly distorted chair conformation due to flattening as a result of the C6-C4'-C5' enone system in ring E.

Bull and co-workers[21] have reported on the ring conformations for the 3-deoxy derivative of **85**. Ring A is said to occupy a conformation which displays "a minor component of a half chair character" whereas ring B occupies a conformation which is "strongly distorted toward a half chair". These conclusions were based on X-ray crystallographic data.

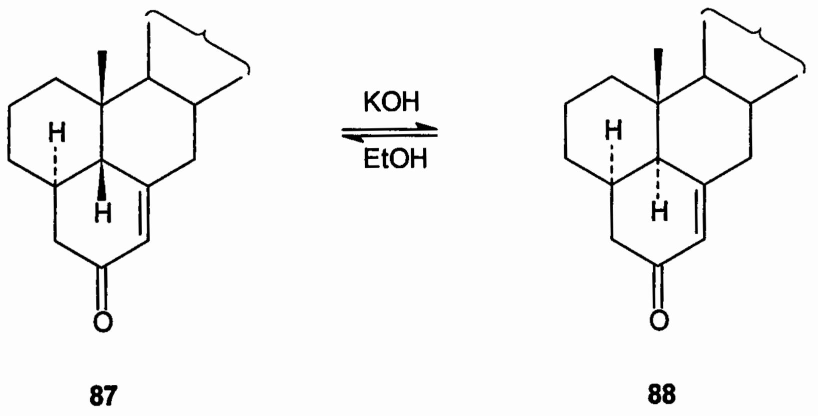


86 **85**

Figure 8 Three-dimensional perspectives of the $4\alpha,5\beta$-isomer **86** and the $4\alpha,5\alpha$-isomer **85**

A molecular model of the $4\alpha,5\beta$-enone **86** shows that if ring A occupied a chair conformation, ring B would be restricted to a boat conformation with ring E occupying a distorted half chair conformation due to resultant flattening of the C6-C4'-C5' enone system. However the 400 MHz ^{1}H NMR results for **86** suggest that ring A must exhibit some deviation from a perfect chair conformation. (See section **2.6.3** for explanation of signal assigned to 3α-H) Bull and co-workers[21] have in fact reported on the conformations of rings A, B and E for the 3-deoxy analogue of **86** which established that ring A does not occupy a chair conformation in the 3-deoxy derivative of **86**. (See Fig. 8 for three-dimensional perspectives of **86** and **85**) The X-ray crystallographic data for the analogous 3-deoxy compound revealed it to be a highly strained system in which ring A occupies a hybrid twist boat conformation, ring B a twist conformation and ring E a conformation which "closely approximates that of an envelope". Ring A is said to be deformed from a possible chair conformation ($^{1}C_{4}$) to a hybrid twist-boat conformation ($^{5}T_{1} + B_{4,1}$).

Based on the observations obtained from models of the $4\alpha,5\alpha$-enone **85** and the $4\alpha,5\beta$-enone **86** it is expected that the $4\alpha,5\alpha$-isomer **85** would be the thermodynamically more stable product, since in **86**, whichever conformation ring A assumes, at least one ring has to occupy a boat conformation. This thermodynamic relationship would be confirmed if it was established that the the $4\alpha,5\beta$-enone **86** would isomerise to the $4\alpha,5\alpha$-enone **85** under conditions of equilibration. This equilibration experiment was not performed, but it was noted that in analogous work Bull and co-workers[21] have established a thermodynamic relationship between the 3-deoxy-4α,5β-5'-ketone **87** and the 3-deoxy-4α,5α-5'-ketone **88** by treating **87** with ethanolic potassium hydroxide which converted to 4α,5α-isomer **88** (See scheme 31). In the related saturated isomers of these compounds the thermodynamic relationship between the 4α,5α,6β-4'-ketone **41** and the 4α,5α,6α-4'-ketone **42** was verified by quantitative isomerisation of **41** into **42** by treatment with methanolic potassium hydroxide[22] (See **Introduction**, pg 14). In the 4α,5α,6β-isomer **41**, ring E is constrained to adopt a twist-boat conformation.

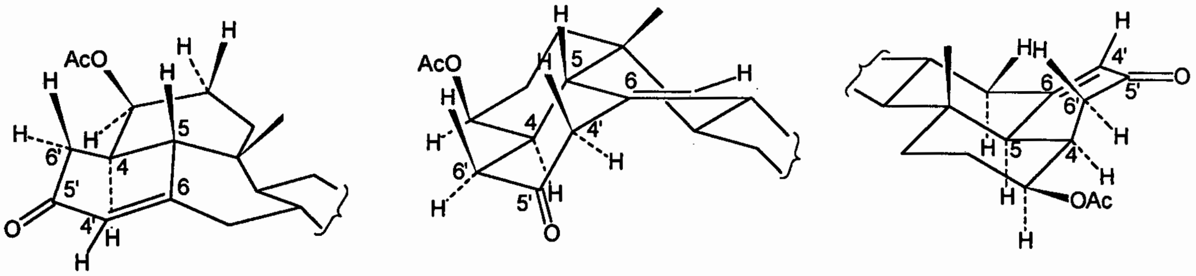


Scheme 31 Thermodynamic relationship between the 4α,5β-isomer **87** and the 4α,5α-isomer **88**

2.8 Analysis of selected ^{13}C assignments for the 4α,5α- and 4α,5β-isomers

The C-9 and C-19 ^{13}C assignments for the 5β- and 5α-isomers obtained from the dehydration experiments (**86**, **83** and **85**) were compared to 5β- and 5α-cholestane, and provided further support for their C-5 configurations[52] (See Table 6, pg 62) The C-9 chemical shift of the Δ^6-5'-ketone **83** was similar to that of 5β-cholestane whereas in the $\Delta^{4'}$-5'-ketone **86** the C-9 resonance displayed a considerable downfield shift (6.7 ppm). This could be due to deformation experienced by ring B in the 4α,5β-$\Delta^{4'}$-5'-ketone **86**. The C-19 chemical shifts for both **86** and **83** displayed upfield shifts (3.2 ppm) which is ascribed to the γ-orientation of the C4-C6' bond with respect to C-19 in both these compounds.[53, 54]

The upfield shift displayed by the C-5 signals for both **86** and **83** with respect to C-5 in 5β-cholestane is ascribed to the γ-effect of the axial 3β-acetoxy substituent. This effect is more pronounced in the Δ^6-5'-ketone **83** (See Fig. 9).



86 **83** **85**

Figure 9 Perspective representations of the 4α,5β- and 4α,5α-isomers

The C-19 chemical shift for the 4α,5α-enone **85** is deshielded compared to the C-19 chemical shift for 5α-cholestane (See Table 6 and Fig. 9) whereas the C-9 chemical shift does not differ significantly. This deshielding influence is ascribed to the *syn*-axial interaction of the C4-C6' bond with the 10β-methyl group whereas the small deviation in the C-9 chemical shift indicates that ring B does not exhibit excessive distortion.[53, 54] The ring A carbons display chemical shift variations which can be rationalised in terms of the effects of the equatorial 3β-acetoxy substituent and 4β-substitution of the C4-C6' bond. The C-10 and C-8 chemical shifts however experience appreciable downfield shifts which is contrary to what is expected for C-10 due to its γ-gauche orientation relative to the C4-C6' bond.[52] The downfield shift in C-8 could be due to the deformation caused in ring B by the C6-C4' olefinic bond.

	Carbon no.																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
5β-cholestane*	37.7	21.4	27.1	27.3	43.8	27.6	26.6	36.0	40.6	35.4	20.9	40.4	42.7	56.7	24.3	28.3	56.4
86	31.0	25.6	71.0	34.2	45.5	168.3	34.6	37.6	47.3	36.7	22.0	39.8	43.6	56.2	23.9	28.0	55.3
83	29.1	24.95	71.3	37.2	47.4	133.9	124.6	41.1	40.2	34.0	20.8	39.95	43.2	56.1	23.8	28.3	54.5
5α-cholestane*	38.7	22.2	26.9	29.1	47.1	29.1	32.3	35.6	54.9	36.3	20.9	40.2	42.6	56.7	24.2	28.3	56.4
85	35.4	23.0	73.2	34.6	49.5	165.5	34.6	39.5	56.0	40.7	20.95	39.4	43.0	56.7	24.1	28.1	56.1

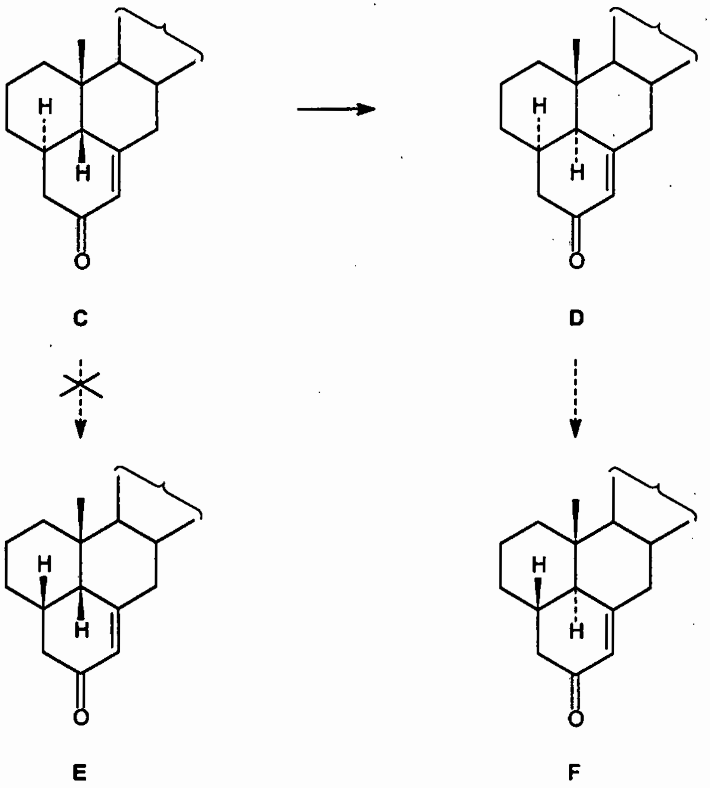
	Carbon no.														
	18	19	20	21	22	23	24	25	26	27	4'	5'	6'	AcO (CH_3)	AcO (C=O)
5β-cholestane*	12.1	24.3	35.8	18.7	36.2	23.9	39.5	28.0	22.5	22.8					
86	12.2	21.1	35.7	18.6	36.1	23.8	39.5	28.0	22.6	22.8	126.9	198.8	39.8	22.6	170.4
83	12.0	21.1	35.7	18.6	36.1	23.8	39.5	28.0	22.5	22.8	52.8	207.4	44.8	23.4	170.4
5α-cholestane*	12.1	12.3	35.8	18.7	36.3	23.9	39.5	28.0	22.6	22.8					
85	12.1	15.5	35.7	18.7	36.1	23.0	39.5	28.0	22.6	22.8	124.2	199.6	41.4	21.1	170.3

* - ^{13}C data taken from J. W. Blunt and J. B. Stothers, *Org. Magn. Res.*, 1977, **9**, 439

Table 6: Tabulated data for selected pentacyclic cholestanes and 5β- and 5α-cholestane

2.9 Equilibration to the 4β,5α-isomer

The results of the foregoing dehydration experiments suggest that the 4α,5β-isomer **86** may undergoe ready epimerisation at C-5 to give the 4α,5α-isomer **85**. The calculated steric energies between the corresponding isomers for perhydrobenzo[4.5.6]androstanes support this conclusion for the 4,5,5',6'-tetrahydrobenzo[4.5.6] series.[22] Furthermore it is inferred that a suitably functionalised derivative of the 4α,5α-isomer should be susceptible to epimerisation at C-4 to generate the thermodynamically most favoured 4β,5α-derivative **F** (See Scheme 32). Similarly it is expected that the 4α,5β-derivative **C** would resist epimerisation at C-4 to give the 4β,5β-derivative **E**, since this isomer should represent the thermodynamically least-favoured isomer of this series of compounds.



Scheme 32 Expected thermodynamic relationships between dihydrobenzo[4.5.6]cholestanes isomeric at C-4 and C-5

to 4α-H confirmed the retention of the 4α-H configuration by displaying an antiperiplanar coupling to 6'β-H (14.7 Hz) and two synclinal couplings to 6'α-H and 5α-H (7.1 Hz each). The d at δ2.98 (d, *J* 6.6 Hz) was assigned to 5α-H based on a crosspeak for C-5 in the HETCOR spectrum. The C-5 signal was identified as a singlet at δ49.0 ppm in the methine region of the DEPT spectrum. The mass spectrum displayed the expected molecular ion at M^+ 438.

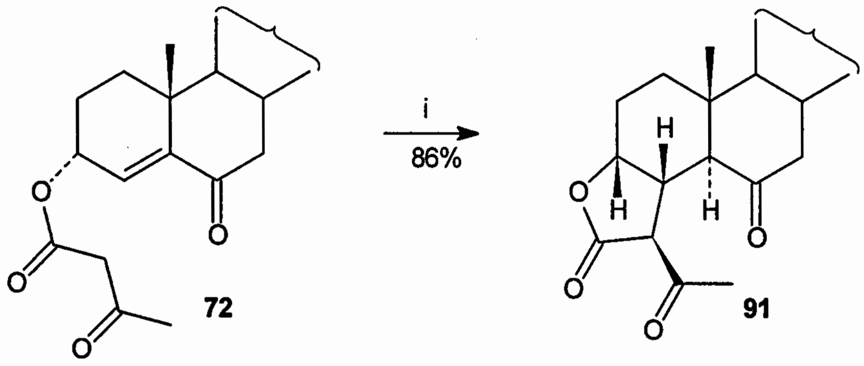
Epimerisation of C-4 was induced by subjecting the 4α,5α-diketone **89** to aqueous 2*M*-potassium hydroxide in ethanol under nitrogen which gave the expected 4β,5α-isomer **90** (95%). Infrared absorption at ν_{max} 1712 and 1669 cm^{-1} indicated the presence of the 3-oxo and 5'-oxo groups. Diagnostic 400 MHz NMR data confirmed inversion to the 4β,5α-isomer **90**. The td at δ2.98 (*J* 2 × 12.8, 3.6 Hz) assigned to 4β-H, incorporated two antiperiplanar couplings to 5α-H and 6'β-H and a synclinal coupling to 6'α-H. The antiperiplanar relationship between 5α-H and 4β-H was verified by the dt assigned to 5α-H (*J* 12.1, 2 × 1.9 Hz). The large coupling constant (12.1 Hz) is consistent with an antiperiplanar relationship to 4β-H and one smaller coupling constant (1.9 Hz) is due to allylic coupling to 4'-H. A t at δ5.88 (*J* 2 × 2.4 Hz) which was assigned to 4'-H, displayed complementary allylic coupling to 5α-H and allylic coupling to either 7β-H or 7α-H. The allylic coupling was verified by a correlation between the signals assigned to 5α-H and 4'-H in the COSY spectrum. The smaller coupling constant (3.6 Hz) of the dd at δ2.58 (*J* 16.3, 3.6 Hz) assigned to 6'β-H, could be reconciled with the synclinal coupling in the 4β-H signal and a dd at δ2.30 (*J* 16.4, 13.7 Hz) was assigned to 6'α-H.

The 4α,5α-isomer isomerised to the thermodynamically more stable 4β,5α-isomer as predicted. However no experiments were performed to verify the prediction that the 4α,5β-isomer **C** would not isomerise to the 4β,5β-isomer **E** but rather to the 4α,5α-isomer **D** or the 4β,5α-isomer **F**. (See Scheme 32)

2.10 Intramolecular reactivity of the 3α-acetoacetate

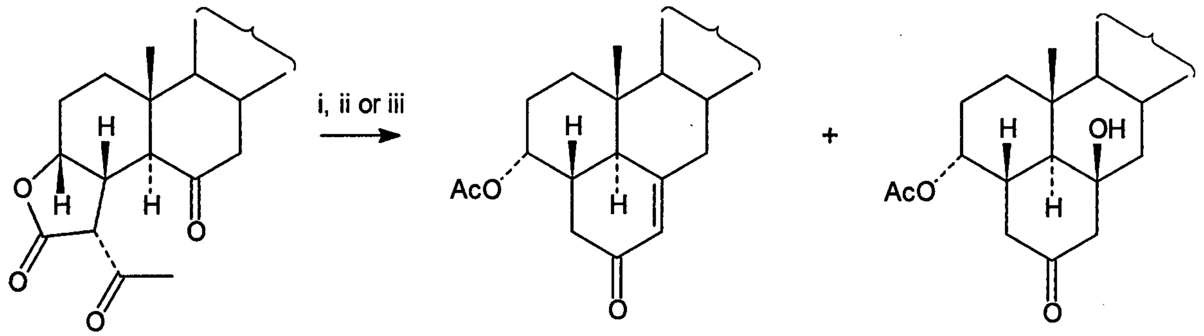
Base treatment of the 3α-acetoacetate **72** was expected to induce intramolecular Michael attack of the enolate at C-4 from the α-face to afford stereoselective formation of the 3β,4β-lactone **91**.[23]

Thus, treatment of **72** with potassium *tert*-butoxide (generated *in situ* in *tert*-butyl alcohol) at 40 °C produced the δ-lactone **91** (86%). The properties of this compound were consistent with the reported data.[23] Additional information displayed by the 400 MHz ^{1}H NMR spectrum of the lactone **91** were a t at δ1.95 (*J* 2 × 12.8 Hz) assigned to 7α-H, and a dd at δ2.35 (*J* 13.0, 4.3 Hz) which was assigned to 7β-H. The secondary product **K**, (See Introduction, Chapter 2) which was reported in earlier experiments[23] was not detected in this work.



Scheme 34 *Reaction conditions*: i, K-OBu^t, THF, *t*-Butyl alcohol, 40 °C

With the 3β,4β-lactone **91** in hand, the intramolecular aldol reactivity of this compound was investigated. As a starting point into this work it was decided to repeat the reported experimental conditions[31] for the formation of 3α,6β-dihydroxy-tetrahydrobenzo[4.5.6]cholestane **L** and the corresponding β-elimination product **M**. (See Introduction, Chapter 2) Treatment of the γ-lactone **72** with aqueous potassium hydroxide in either ethanol or a mixture of dimethylformamide and water followed by acetic anhydride and pyridine was reported to yield the acetylated derivatives of **92** and **93**[31] (See Scheme 35). Thus refluxing the lactone **91** with aqueous potassium hydroxide in dimethylformamide and water and acetylating the mixture afforded **92** (45%) and **93** (46%) in yields similar to those reported.[31]. The properties of **92** and **93** were consistent with the data reported for these compounds. The report also claims that performing the above reaction in ethanol produces a cleaner reaction and a more straightforward work-up procedure.[31] However when the δ-lactone **91** was refluxed with aqueous potassium hydroxide in ethanol only the 4β,5α-5'-ketone **93** (54%) was obtained (See Scheme 35). This result is contrary to what Borry[31] observed. No conclusions or observations on compounds obtained in the 3α-series could be made, as investigations attempted in this series were very preliminary.



91 **92** **93**

Scheme 35 *Reaction conditions*: i, DMF-H$_2$O, aqueous 2M-KOH, reflux; ii, Ac$_2$O, C$_5$H$_5$N, 25 °C: **92**, 45%; **93**, 46%
or iii, as above but EtOH as solvent: only **93**, 54%

2.11 Conclusions

The 4α,5α-enone **85** was obtained by introducing a variation into the β-elimination pathway with the use of HMPA and POCl$_3$ which induced epimerisation at C-5 to produce the 4α,5α-isomer. Improved methodology to obtain the key 3β,6β-dihydroxy pentacyclic product **81** was however not established. Thionyl chloride-pyridine mediated dehydration of the 4α,5β-isomer **82** was inconclusive, although the expected 4α,5β-Δ^6-5'-ketone **83** was obtained as a minor product (29%). The 4α,5β-isomer **86** was obtained by treating the 6β-hydroxy-5'-ketone **82** with $BF_3.OEt_2$ which gave **86** and the expected E2 elimination product **83**.

The 400 MHz ^{1}H and ^{13}C NMR data for the 3β,6β-dihydroxy derivative **81**, the 4α,5α-isomer **85** and the 4α,5β-isomers **83** and **86** provided detailed information about their structural and conformational properties. The observations obtained from the ^{1}H and ^{13}C data were used to speculate on the thermodynamic relationship between the 4α,5α-isomer **85** and the 4α,5β-isomer **86**. The expected thermodynamic relationship between the 4α,5α-isomer and the 4β,5α-isomer was verified by equilibration of the former into the latter using a reported reaction sequence.[31]

Investigations into the 3α-series were very preliminary and provided no new information on the chemistry and structural properties of the compounds in this series except for additional detail in the 400 MHz ^{1}H NMR spectra of the lactone **91** and the compounds **92** and **93**.

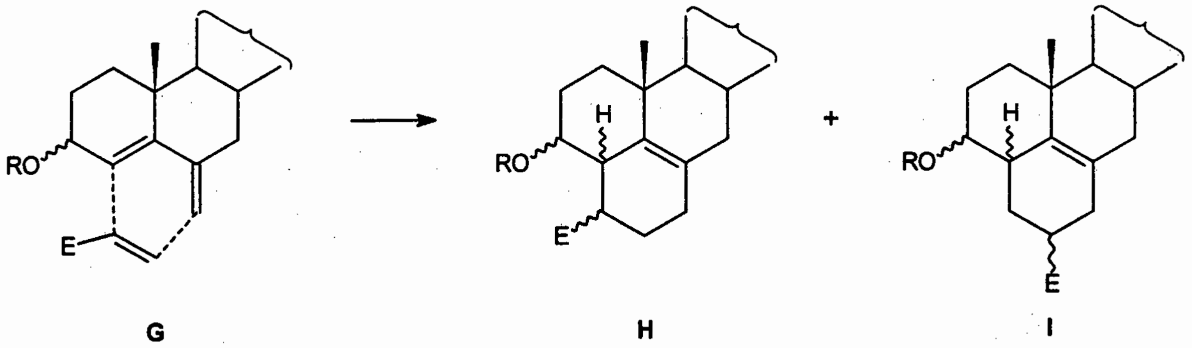
In conclusion it can be stated that an improved β-elimination pathway to obtain the 4α,5α-isomer **85** has been developed and that reaction conditions have been found to synthesise the 4α,5β-isomer **86** within the 3β-acetoacetate series.

CHAPTER 3

A CYCLOADDITION APPROACH TO PERHYDROBENZO[4.5.6]CHOLESTANES

3.1 Introduction

In an earlier part of this study an approach to the synthesis of perhydrobenzo[4.5.6]cholestanes involved the use of a sequential intramolecular Michael-aldol reaction sequence for the construction of ring E. An alternative approach was envisaged which involved the use of Diels-Alder methodology for the construction of ring E. This would require the preparation of a suitable diene in the ring A-ring B portion of the cholestane skeleton and the reaction of this diene with a variety of dienophiles to give the corresponding cycloadducts (**H** and **I**) which could be further manipulated depending on the functionality incorporated in the dienophile (See Scheme 36). Introduction of a substituent at the 3-position of the diene would provide an opportunity for possible interaction of the dienophile with the 3-substituent of the diene. The chosen dienophiles would have to exhibit functionality which could easily be removed or transformed to provide a handle for later manipulation of the cycloadducts formed. For instance, the formyl group from acrolein could be reduced or the phenylsulfonyl group from phenyl vinyl sulfone could be easily removed using standard procedures.



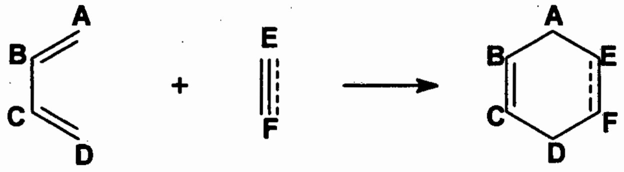
E = CHO, SO_2Ph etc.

Scheme 36 Schematic representation of the reaction of the diene and dienophile to form perhydrobenzo[4.5.6]cholestanes

This introductory section is followed by a brief discussion of the Diels-Alder reaction in order to address the various aspects of this reaction which are expected to influence the formation of the products expected. This is followed by a discussion of the cyloaddition reactions attempted and an interpretation of the results obtained.

3.2 The Diels-Alder cycloaddition reaction

The Diels-Alder reaction is a cycloaddition reaction which involves the interaction of a conjugated diene with a dienophile which may either be a double or a triple bond to form a six-membered adduct, the result of which is the formation of two sigma bonds in the product at the expense of two pi bonds in the starting materials.[55] (Scheme 37)

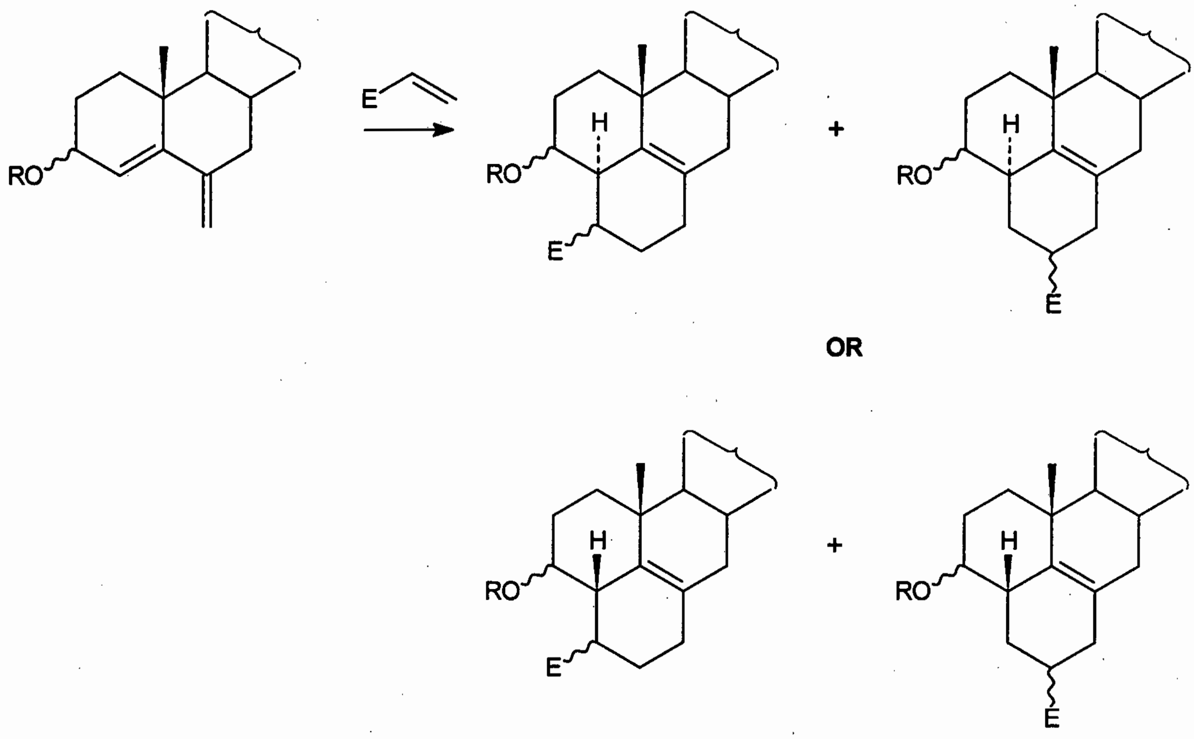


Scheme 37 Schematic representation of the Diels-Alder reaction

A large variety of dienes and dienophiles can be utilised in this reaction which adds to its synthetic scope in the production of different classes of compounds.

3.2.1 Regioisomeric and stereochemical possibilities

The possibility of different regioisomeric cycloadducts is introduced when unsymmetrical dienes react with unsymmetrical dienophiles. In the particular case with which we are concerned, interaction of the diene with an unsymmetrical dienophile could in principle produce eight different cycloadducts with an equal distribution of the two possible regioisomers expected for the alternative orientations of the dienophile (See Scheme 38).



Scheme 38 Different regioisomeric cycloadducts possible in the synthesis of perhydrobenzo[4.5.6]cholestanes

In terms of Frontier Molecular Orbital (FMO) theory,[49] the predominance of a particular regioisomer over another depends on a combination of steric factors, secondary orbital interactions and a matching of the magnitudes of the atomic orbital coefficients of the reacting centres of the diene and the dienophile. Substituents on either the diene or the dienophile alter the magnitudes of the atomic orbital coefficients which influences the regioselectivity. A contrasting theory which has also been used to predict regioselectivity is based on assuming that the transition state of the interacting species corresponds to a biradical. The preferred regioisomer is recognised by selecting the most stable biradical species.[56] The formation of several products are possible with the Diels-Alder reaction (See Scheme 38), however it is often found that certain products dominate over others. The formation of the dominant product is usually governed by two rules which are known as the *cis*-rule and the Alder *endo*-rule.[55]The cis-rule dictates that the relative configuration of both the diene and the dienophile are retained in the product. The *endo*-rule specifies that the diene and the dienophile will orientate themselves in parallel planes which allows for the maximum overlap of orbitals between the reacting partners to give the *endo*-product. The *exo*- or *endo*-approach of the dienophile to the diene would determine the orientation of the sustituent E. In terms of FMO theory the preference for *endo*-addition is explained by the interaction of secondary orbitals which do not lead to bond formation in the product, but have the effect of lowering the transition state energy[49] (See Scheme 38).

Another aspect of stereoselectivity is introduced when the top and bottom faces of the diene are not equivalent, which is the case with our particular diene. For this diene, one would expect the 10β-methyl group to direct the cycloadditions to the α-face of the diene so that the predominant product would have a 4β-H configuration. The role of the remainder of the steroid skeleton and the conformation of the diene moiety in directing the dienophile to either face of the diene cannot be predicted with certainty.

3.2.2 Reactivity and Mechanism

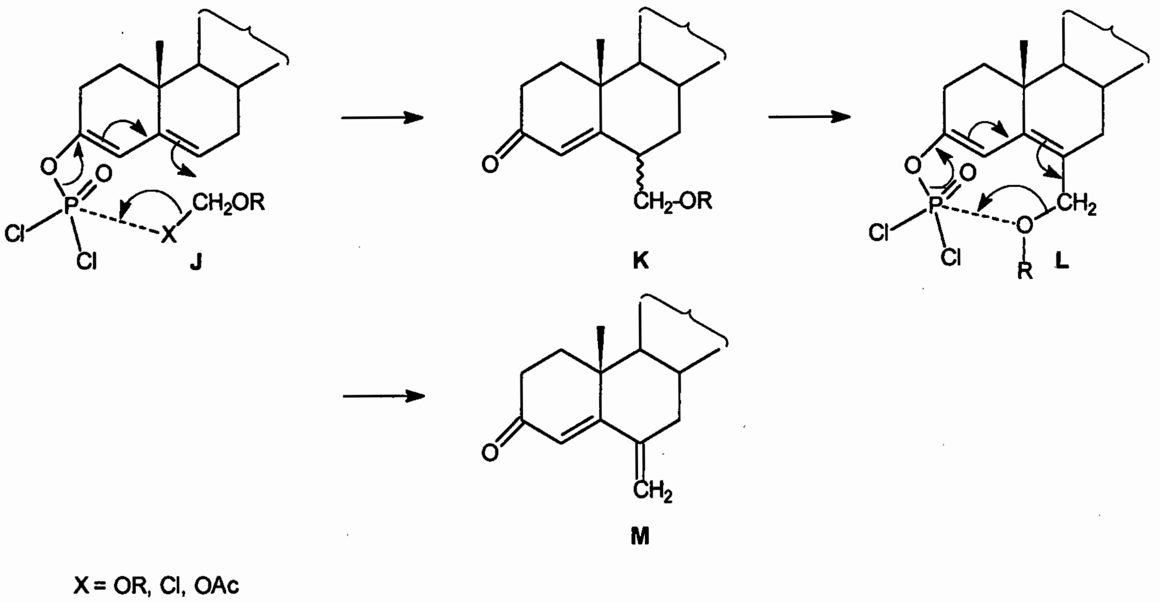
FMO theory explains the reactivity of the reacting partners in the Diels-Alder reaction in terms of the magnitude of the energy separation between the frontier orbitals.[49] Based on this interpretation Diels Alder reactions have been divided into three different classes, each of which have different orbital interactions which dominate in the interaction of the diene and the dienophile.[57] For a normal electron demand Diels-Alder reaction the dominant FMO interaction is the one involving the smaller energy difference which corresponds to the HOMO of the diene and the LUMO of the dienophile whereas for an inverse electron demand reaction, the dominant FMO interaction occurs between the LUMO of the diene and the HOMO of the dienophile. For a neutral electron demand reaction neither of the frontier orbitals dominate.

Although investigations suggest that the mechanism of the Diels-Alder reaction does not only fit one interpretation, most thermal cycloadditions can be described by a symmetry-allowed one-step mechanism. In some instances a two-step mechanism involving a biradical or zwitterion intermediate is also a strong possibility. In terms of FMO theory, the reaction is controlled by the suprafacial "in-phase" interaction of the molecular orbitals of the HOMO-LUMO pair closest in energy.[58]

3.3 Synthesis of an appropriate diene

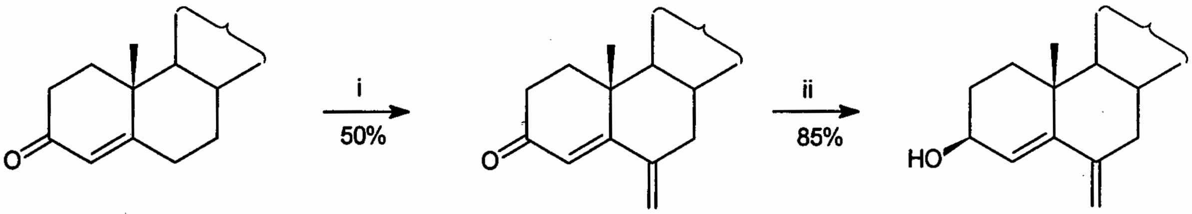
The envisaged diene was synthesised by utilising a γ-alkylenation procedure developed by Annen and co-workers[59] in which phosphoryl chloride, formaldehyde dimethyl or diethyl acetal, sodium acetate and chloroform were used to methylenate a variety of 3-oxo-Δ^4 steroids. The required acidity of the reaction medium could also be achieved by using *p*-toluenesulfonic acid or strong mineral acids, although the preferred conditions were the use of phosphoryl chloride with formaldehyde dimethyl or diethyl acetal. The yields of products ranged from 18 to 84%.

The authors have proposed a mechanism[59] for the γ-methylenation reaction which involves the formation of a phosphorus ester at the the 3-position followed by nucleophilic attack on the methylenating agent (**J**) to form the intermediate **K**, followed by reformation of the phosphorus (v) species and final 6-methylene bond formation to form **M** (Scheme 39).



Scheme 39 Proposed mechanism for 6-methylene formation

An experiment in which phosphoryl chloride was slowly added to cholest-4-en-3-one **94** and sodium acetate in chloroform and formaldehyde dimethyl acetal gave 6-methylene-cholest-4-en-3-one **95** (50%). (Scheme 40)



94 **95** **96**

Scheme 40 *Reagents and conditions*: i, $POCl_3$, CH_3COONa, $CHCl_3$, $CH_2(OMe)_2$, refux, 18 h
ii, $LiAlH_4$, THF, 5 °C

The formation of **95** was confirmed by infrared absorption at ν_{max} 1655 and 1602 cm^{-1} which indicated the presence of the conjugated enone moiety and the exo-methylene group.The 400 MHz ^1H NMR spectrum displayed a singlet at δ5.84, which was assigned to

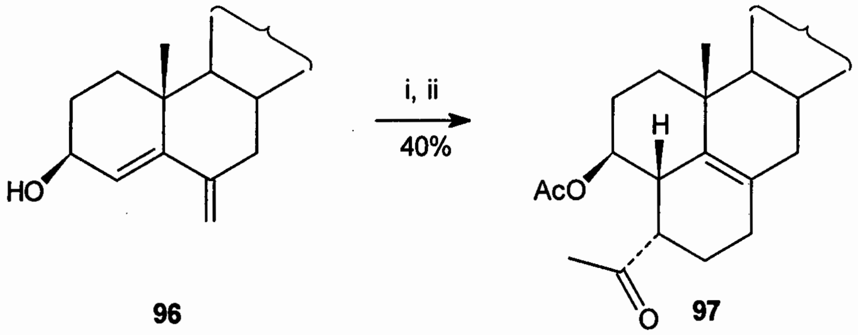
4-H, and a set of triplets at δ4.91 and δ5.03 (*J* 2 × 2.1 Hz) which were assigned to the exo-methylene protons. The set of triplets corresponded to geminal coupling between the 6'-H_2 protons and allylic coupling to 7β-H or 7α-H. The mass spectrum displayed the expected molecular ion at *m/z* 396.

Reduction of 6-methylene cholest-4-en-3-one **95** with lithium aluminium hydride gave 6-methylene-cholest-4-en-3β-ol **96** (83%). The formation of the reduced product was confirmed by diagnostic ^{1}H NMR data which included the appearance of a ddd at δ4.21 (*J* 10.0, 6.1 and 2.2 Hz) assigned to 3α-H, which incorporated antiperiplanar and synclinal couplings to 2β-H (10.0 Hz) and 2α-H (6.1 Hz) respectively, and the smallest coupling constant (*J* 2.2 Hz) could correspond to coupling to the olefinic 4-H. A triplet at δ5.48 (*J* 2 × 1.8 Hz) was assigned to 4-H based on a correlation between this signal and the signal assigned to 3α-H in the COSY spectrum. The mass spectrum displayed the expected molecular ion at *m/z* 398 and infrared absorption at ν_{max} 3598 cm^{-1} confirmed the presence of an hydroxyl group.

Although the first step in the synthesis afforded the desired compound in a low yield, its advantage was that the exo-methylene functionality was introduced in only one step whereas previous methods used to effect γ-methylenation within the steroid series involved multi-step procedures.[60, 61]

3.4 Cycloaddition reactions

A mixture of 6-methylenecholest-4-en-3β-ol **96** and methyl vinyl ketone was heated in benzene at 125 °C and the resultant product was treated with acetic anhydride and pyridine to give a mixture of cycloadducts as evidenced by an NMR of the crude product. However this did not reveal evidence of the presence of regio- or stereoisomers. The mixture was crystallised to purity to give a single cycloadduct **97** (40%) (Scheme 41).



Scheme 41 *Reagents and conditions*: i, C_6H_6, C_4H_6O, 125 °C; ii, Ac_2O, C_5H_5N, 25 °C

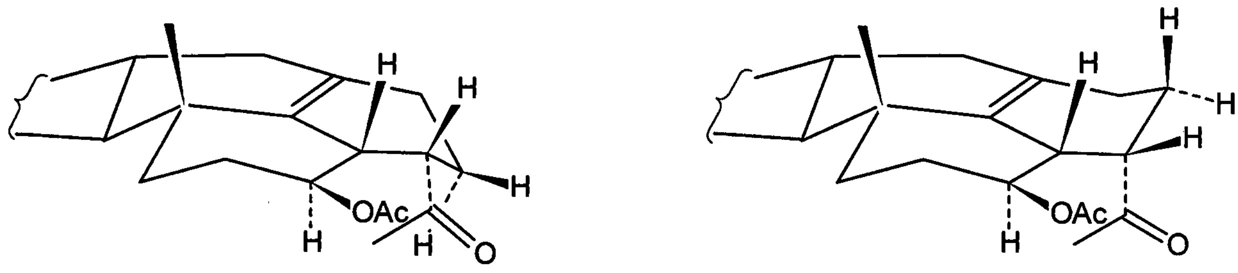
The structure of the cycloadduct **97** was established with the use of diagnostic 400 MHz NMR data (Table 7) which included NOE spectra of the product and characteristic infrared absorption peaks. Infrared absorption at ν_{max} 1729 and 1709 cm^{-1} indicated the presence of an acetoxy carbonyl group and an isolated carbonyl group respectively. The expected molecular ion (M^+ 510) was not observed, but a peak at *m/z* 450 which corresponds to the loss of acetic acid was noted.

Table 7 Diagnostic 400 MHz ^1H NMR data for **97**

δ	Integration	Multi.	*J* (Hz)	Assignment
4.65	1H	ddd	11.2, 10.0, 4.5	3α-H
3.08	1H	dd	11.2, 4.6	4β-H
2.46	1H	ddd	12.4, 5.1, 5.1	6'β-H

The regiochemistry of addition of methyl vinyl ketone to the diene **96** was established by a distinct correlation between the signal assigned to 4β-H and the signal assigned to 6'β-H in the COSY spectrum. The signal assigned to 4β-H was identified by its correlation to 3α-H in the COSY spectrum and its multiplicity (dd, *J* 11.2, 4.6 Hz) which indicated antiperiplanar coupling to 3α-H and synclinal coupling to another neighbour (4.6

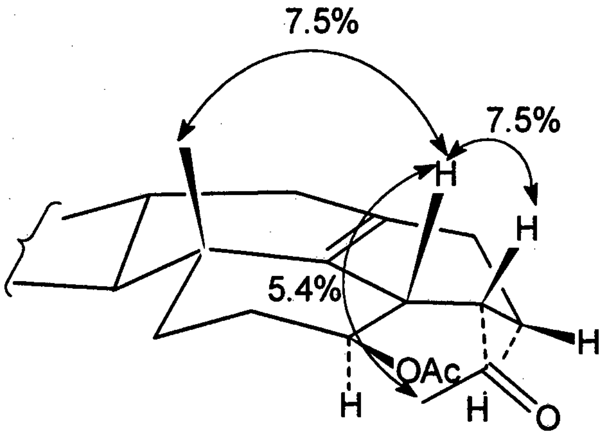
Hz). The assignment was supported by a correlation between the assigned signal and C-4 in the HETCOR spectrum, which was identified as a singlet at δ40.8 ppm in the methine region of the DEPT spectrum. The 4β-H configuration was confirmed by an enhancement of the 10β-CH_3 singlet (7.5%) upon irradiation of the dd at δ3.08 assigned to 4β-H. The signal assigned to 6'β-H was identified by a crosspeak to 4β-H in the COSY spectrum. The assignment was supported by a correlation between the assigned signal and C-6', which was identified as a singlet at δ51.1 ppm in the methine region of the DEPT spectrum. It's multiplicity (ddd, *J* 12.4, 5.1, 5.1 Hz) indicated one antiperiplanar coupling (12.4 Hz) and two synclinal couplings (5.1 Hz each) which can be reconciled with the structure **97** and not the structure **N** in which ring E occupies an alternative conformation (See Fig. 10).



97 **N**

Figure 10 Possible conformations of ring E for cycloadduct **97**

The 6'β-H and 6'-Ac orientations were verified by the enhancements of the 6'β-H signal (7.5%) and the singlet assigned to the methyl group of the 6'-Ac substituent (5.4%) upon irradiation of the signal assigned to 4β-H which indicates spatial interaction between the 6'-Ac methyl group and 4β-H (See Fig. 11). This interaction would not be possible for the structure **N** in which the 6'-Ac group is directed toward the α-face.



97

Figure 11 Cycloadduct **97** showing established NOE interactions

The 4β-H configuration indicates that the dienophile has approached the diene from the α-face. The orientation of the 6'-Ac substituent could only result from endo-approach of methyl vinyl ketone to the diene **96**. (See Fig. 12)

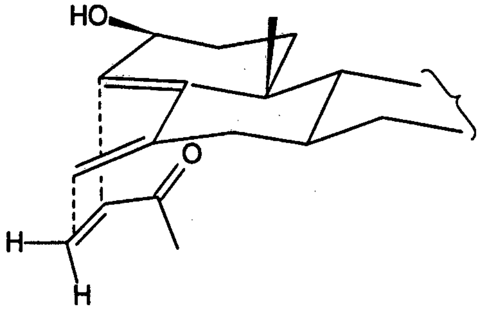
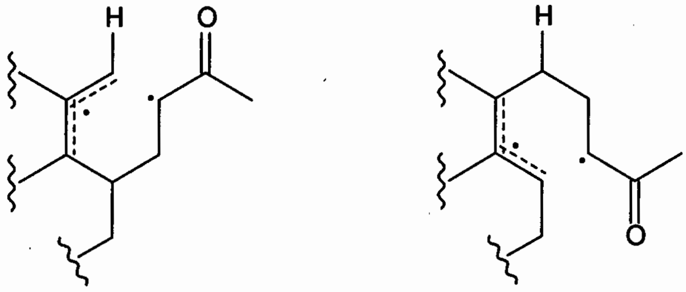


Figure 12 Endo-approach of methyl vinyl ketone to the diene **96**

The methyl ketone moiety of methyl vinyl ketone could be considered both as a group which increases the conjugation of the dienophile and as an electron-withdrawing group. If the hydroxyl group of the diene is assumed to have very little effect on the C-4 terminus of the diene, preference for the formation of an "ortho" cycloadduct is expected. This is in agreement with the general rule which states that there is a preference for "ortho" adduct formation in the interaction of a 1-C or 1-Z substituted diene with a C-, Z- or X-substituted dienophile where C is associated with an increase in conjugation, Z an electron-withdrawing group and X, an electron-releasing group.[49] This rule is based on the changes which the frontier orbitals and atomic orbital coefficients experience upon introduction of substituents to both the diene and the dienophile. Another theory which can also be used to predict regioselectivity in Diels-Alder reactions assumes that the transition state of the reacting partners corresponds to a biradical.[56] For the diene **96** and methyl vinyl ketone simplified representations of the biradical species expected for the alternative orientations of the dienophile are represented in Fig. 13.

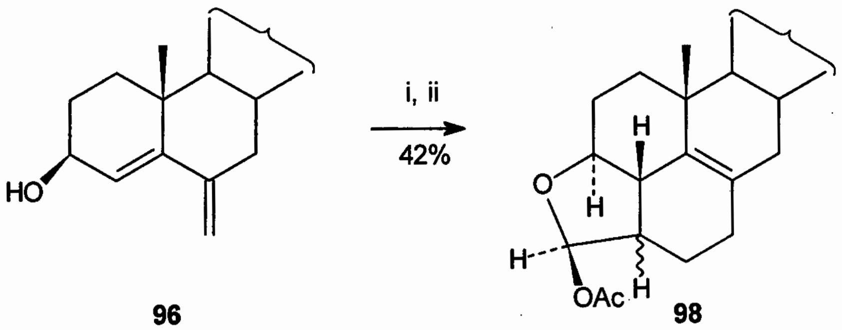


O **P**

Figure 13 Alternative biradical species for the diene **96** and methyl vinyl ketone

In the structure **P**, the radical centres are stabilised by more alkyl substituents than in **O**, and this theory also predicts a preference for "ortho" adduct formation.

A subsequent cycloaddition in which the diene **96** and acrolein were maintained at 95 °C (sealed tube) for one day and the resultant product treated with acetic anhydride and pyridine gave the cycloadduct **98** (42%), accompanied by other unidentified material (Scheme 42).



96 **98**

Scheme 42 *Reagents and conditions*: i, C_6H_6, C_3H_4O, 90-95 °C; ii, Ac_2O, C_5H_5N, 25 °C

The structure of the cycloadduct **98** was determined with the use of 400 MHz NMR data (^1H and ^{13}C) which included NOE spectra, although the 6'-H configuration could not be unambiguously ascertained. Infrared absorption at ν_{max} 1736 cm^{-1} indicated the presence of the acetoxy carbonyl group and the mass spectrum displayed the expected molecular ion at *m/z* 496.

Table 8 Characteristic ^1H NMR signals for cycloadduct **98**

δ	Integration	Multi.	*J* (Hz)	Assignment
3.41	1H	td	2 × 11.1, 3.5	3α-H
2.36	1H	dd	10.6, 8.8	4β-H
2.25	1H	ddd	13.5, 8.1, 5.2	6'α-H
5.90	1H	d	1.0	$6'^1$α-H

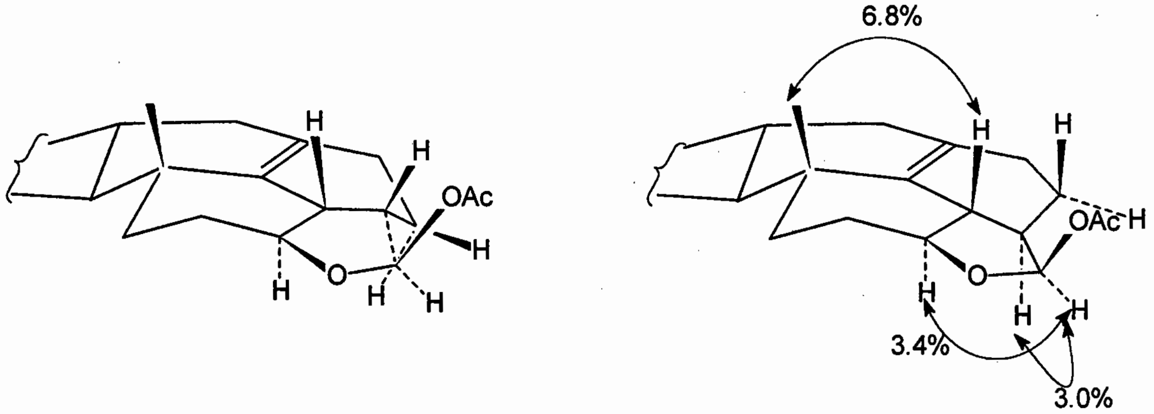
The regiochemistry of the cycloadduct was evident from secondary hemiacetal formation, and was verified by the connectivity pattern in the COSY spectrum, which correlated 3α-H to 4β-H and 4β-H to the signal assigned to 6'-H. The acetylated hemi-acetal moiety was indicated by a methine carbon at δ103.4 in the DEPT spectrum which could be correlated to $6'^1$-H by the presence of a crosspeak to this proton in the HETCOR spectrum.

The signal assigned to 4β-H was identified by a crosspeak to 3α-H in the COSY spectrum. The assignment was supported by a correlation between the assigned signal and the singlet assigned to C-4 at δ42.2 ppm in the methine region of the DEPT spectrum. The 4β-H orientation was confirmed by the enhanced 10β-CH_3 signal (6.8%) upon irradiation of the signal assigned to 4β-H. The multiplicity of the 4β-H signal (dd, *J* 10.6, 8.8 Hz) could be reconciled with antiperiplanar coupling to 3α-H (10.6 Hz); however the magnitude of the remaining coupling constant (8.8 Hz) was not conclusive enough to be associated with either an antiperiplanar or a synclinal coupling.

The signal assigned to 6'-H was identified by a correlation to 4β-H in the COSY spectrum. The assignment was supported by a correlation between the assigned signal and a methine carbon resonating at δ43.2 in the DEPT spectrum. The multiplicity of the 4β-H signal indicated coupling to an antiperiplanar neighbour (13.5 Hz), a synclinal neighbour (5.2 Hz) and a coupling constant of 8.1 Hz which indicated the relationship of 6'-H to 4β-H.

The alternative 6'-H orientations represented in structures **Q** and **R** could be reconciled with most of the 400 MHz ^{1}H NMR data (See Fig. 14). For structure **Q**, the dihedral angle between 6'β-H and $6'^1$α-H closely approximates 90° which would account for the signal assigned to $6'^1$α-H (d, *J* 1.0 Hz). Structure **Q** would result from *endo*-approach of acrolein to the diene **96**. However a definite enhancement of the 6'β-H signal upon irradiation of the 4β-H signal would be expected for this structure but this is not observed in the NOE spectra for this product. Structure **R** is consistent with the NOE interactions established (See Fig. 14), however the signal assigned to $6'^1$α-H (d, *J* 1.0 Hz) cannot be reconciled with the dihedral angle between 6'α-H and $6'^1$α-H. An optimised molecular model of structure **R** approximates the dihedral angle at 28° which has an associated coupling constant of *ca* 5 Hz according to the Karplus curve.

Structure **R** could only result from *exo*-approach of acrolein to the diene **96**. Based on the result obtained for methyl vinyl ketone, there is no obvious reason why acrolein should approach the diene in an *exo*-fashion. Thus, in the absence of the NOE results, structure **Q** would be the favoured structure for the cycloadduct **98**.



Q **R**

Figure 14 Depiction of alternative orientations for 6'-H showing the established NOE interactions which are consistent with structure **R**

If structure **Q** is accepted as the more plausible structure, *endo*-approach of acrolein to the diene **96** would produce an initially formed intermediate **S** in which the 6'α-formyl group is favourably oriented for intramolecular attack by the 3β-hydroxyl group to give the hemi-acetal derived cycloadduct **T**. Acetylation of **T** would produce the cycloadduct **98**.

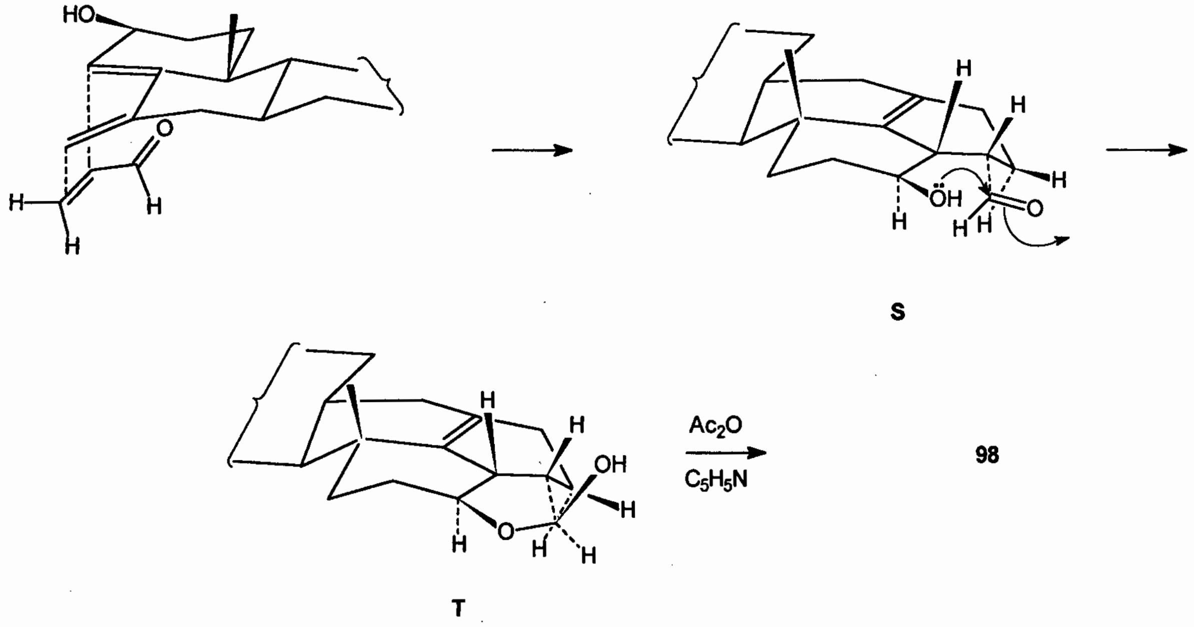


Figure 15 *Endo*-approach of acrolein to the diene **96**

Using the same reasoning as with methyl vinyl ketone (See Fig. 13), the "ortho" adduct is predicted for this cycloaddition reaction.[56]

3.5 Conclusions

In both the cycloadditions attempted, it can be concluded that the 10β-methyl group directs the approach of the respective dienophiles from the α-face of the diene **96**. The role of the diene in directing the approach of the dienophiles cannot be speculated upon, however for a similar diene system having one double bond exocyclic, an X-ray crystallographic study established that the diene moiety is twisted away from coplanarity to the extent of about 54°.[68] In the apparent absence of the NOE interaction expected between 4β-H and 6′β-H for the structure **Q**, the proposed structure for **98** was based on the assumption of preferred *endo*-approach of acrolein in the cycloaddition reaction. The existing spectroscopic evidence however does not permit unambiguous assignment of the structure.

If the reaction conditions for this methodology were optimised, this synthetic approach would considerably shorten the synthesis of perhydrobenzo[4.5.6]cholestanes compared to the intramolecular Michael-aldol approach, as only one step is required to attach a three-carbon appendage to form the initial dihydrobenzo[4.5.6]steroid.

CHAPTER 4

EXPERIMENTAL

General

Spectra were recorded as follows: Infrared spectra were recorded on a Perkin-Elmer 983 spectrometer in chloroform solutions. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-200 (200MHz spectrophotometer) and Varian VXR-400 (400 MHz spectrophotometer) using tetramethylsilane as an internal standard in deuteriochloroform solutions unless otherwise specified. Mass spectra were determined on a VG micromass 16F spectrometer operating at 70ev with an ion source temperature of 170-200 °C. Optical rotations were determined in chloroform solutions using a Perkin-Elmer 141 polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1}.

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

All reactions were monitored by TLC using aluminium-backed silica gel plates in various solvent systems. Once developed the plates were viewed using an ultraviolet lamp (wavelength 254nm) then sprayed with a solution of ceric ammonium sulphate in 5*M*-sulphuric acid and heated in an oven at 200°C. 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 desribed below:

Tetrahydrofuran, benzene, diethyl ether: dried over sodium wire and distilled from sodium wire prior to use, using benzophenone as an indicator.

Acetic anhydride: fractionally distilled

Pyridine: dried over calcium hydride and fractionally distilled over calcium hydride or potassium hydroxide.

Ethanol: refluxed over magnesium turnings and iodine and fractionally distilled.

Dichloromethane and chloroform: dried over phosphorous pentoxide and distilled.

Glacial acetic acid: fractionally distilled.

5α-Cholestane-3β,5,6β-triol 59

Cholesterol **58** (20 g, 52 mmol) and 88% formic acid (200 cm^3) were heated to 80 °C with stirring. After 2 h an oily layer separated and solidified. After 6 h of stirring the reaction mixture was cooled to 25 °C. Aqueous 30% hydrogen peroxide (20 cm^3) was added in one portion. The reaction mixture was stirred at 25 °C for 2 days. Boiling water (300 cm^3) was added and the mixture was cooled to 25 °C after which it was cooled in an ice-water bath for 30 min. The precipitated colourless solid was washed with water, collected and pre-dried on a Buchner filter after which it was dried under reduced pressure in a vacuum desiccator for 2 days. The colourless precipitate was dissolved in boiling methanol (800 cm^3). The solution was cooled, aqueous 25% sodium hydroxide (20 cm^3) was added, and the resulting mixture was then boiled for 40 min. The hot reaction mixture was filtered and the filtrate was acidified with 3*M*- hydrochloric acid, and diluted with water (215 cm^3). The resulting precipitated solid was collected, the first portion of the filtrate being refiltered, and washed with water. The solid was dried under vacuum at 60 °C for 24 h to give the triol **59** (19.42 g, 89%), mp 169-173 °C; $[\alpha]_D$ +14 (*c* 1.2) (lit.,[32] mp 168-173 °C; $[\alpha]_D$ +13).

3β, 5-Dihydroxy-5α-cholestan-6-one 60

5α-Cholestane-3β,5,6α-triol (19.94 g, 47.5 mmol) was dissolved in a mixture of methanol (65 cm^3), water (65 cm^3) and diethyl ether (390 cm^3). The mixture was warmed to allow solution of the triol and then cooled to room temperature. *N*-Bromosuccinimide (12.38 g, 71.2 mmol) was added in one portion. Within 10 min the reaction mixture changed from yellow to a dark-red colour. The oxidation was complete in 2 h. The reaction mixture was transferred to a separating funnel and the organic layer was washed with 5% sodium metabisulphite, 5% sodium hydroxide, water and 3*M*-hydrochloric acid. The precipitated solid was collected on a Buchner filter and washed with water. The solid was dried in a vacuum desiccator over phosphorous pentoxide to give 3β,5-dihydroxy-5α-cholestan-6-one **60** (16.77g, 85%), mp 228-232 °C (from methanol); $[\alpha]_D$ -32 (*c* 1.0) (lit.,[32] mp 229-231 °C; $[\alpha]_D$ -32).

3β-Acetoxy-5-hydroxy-5α-cholestan-6-one 61

3β-5-Dihydroxy-5α-cholestan-6-one (16.77 g, 40.1 mmol) was treated with pyridine (50 cm^3) and acetic anhydride (46 cm^3). After 11 h the acetylation was complete. A large excess of water was added causing precipitation of a colourless solid. The precipitated solid was collected and washed with large amounts of water until the smell of pyridine was no longer detectable. The solid was dried in a vacuum desiccator under reduced pressure over calcium chloride for 2 days, affording 3β-acetoxy-5-hydroxy-5α-cholestan-6-one **61** (17.64 g, 96%), mp 228-232 °C (from methanol); $[\alpha]_D$ -58; (lit,[32] mp 232-233 °C; $[\alpha]_D$ -57); ν_{max} 3578 (OH), 1709 (AcO) cm^{-1}; δ_H (200 MHz) 0.63 (3H, s, 13β-CH_3), 0.92 (3H, s, 10β-CH_3), 2.02 (3H, s, 3β-AcO), 2.75 (1H, t, *J* 2 × 12.3 Hz, 7α-H), 2.81 (1H, s, exch. by D_2O, OH), 5.03 (1H, sept, *J* 2 × 10.8, 2 × 5.4 Hz, 3α-H); (Found: C, 75.3; H, 10.5%; M^+, 460. $C_{29}H_{48}O_4$ requires C, 75.7; H, 10.4%; *M*, 460)

3β-Acetoxycholest-4-en-6-one 62

3β-Acetoxy-5-hydroxy-5α-cholestan-6-one (5.09 g, 11.1 mmol) was dissolved in pyridine (23 cm^3) under nitrogen. Thionyl chloride (3.4 cm^3 , 47 mmol) was added dropwise over 15 min at -5 °C. The reaction mixture was poured into an acidified (HCl) ice-water solution and extracted with ethyl acetate (3 × 150 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate and brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure affording a crude product (4.64 g). The crude product was flash-chromatographed on silica gel using ethyl acetate-toluene (5:95) as eluent to give 3β-acetoxycholest-4-en-6-one **62** (3.83g, 78%), mp 107-111 °C (from methanol); $[\alpha]_D$ -48 (*c* 1.0) (lit.,[62] mp 110 °C; $[\alpha]_D$ -48); ν_{max} 1727 (AcO), 1684 (C=O), 1633 (C=C) cm^{-1}; δ_H (200 MHz) 0.69 (3H, s, 13β-CH_3), 1.02 (3H, s, 10β-CH_3), 2.06 (3H, s, 3β-AcO), 5.32 (1H, ddd, *J* 9.8, 6.1, 2.2 Hz, 3α-H), 6.07 (1H, t, *J* 2 × 1.9 Hz, 4-H) (Found: C, 78.4; H, 11.0%, M^+, 442. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.4%, *M*, 442).

3β-Hydroxycholest-4-en-6-one 63

(a) 3β-Acetoxycholest-4-en-6-one (3.4 g, 7.7 mmol) was dissolved in dry ethanol (215 cm^3). Aqueous 2*M*-potassium hydroxide was added to the ethanolic solution . After 10 min the reaction was neutralised with 3*M*-hydrochloric acid. The mixture was partly evaporated, diluted with water, and extracted with ethyl acetate (3 × 100 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate and brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give 3β-hydroxycholest-4-en-6-one **63** (2.96 g, 96%), mp151-154 °C (from acetone); $[\alpha]_D$ -14 (*c* 1.1) (lit.,[62] mp 152 °C, $[\alpha]_D$ -12); ν_{max} 3414 (OH),1681 (C=O), 1626 (C=C) cm^{-1}; δ_H (200 MHz) 0.69 (3H, s, 13β-CH_3), 1.00 (3H, s, 10β-CH_3), 2.16 (1H, s, exch. by D_2O, OH), 4.22 (1H, m, $W_{½}$ 15.3 Hz, 3α-H), 6.14 (1H, s, 4-H) (Found: C, 80.6; H, 11.2; M$^+$, 400. $C_{27}H_{44}O_2$ requires C, 81.0; H, 11.0; *M*, 400).

(b) 3β-*t*-Butoxycholest-4-en-6-one **66** (150 mg, 0.33 mmol) (mp 238-240 °C, lit.,[22] mp 237-239 °C, $[\alpha]_D$ -44) was dissolved in dry dichloromethane at -70 °C. 1*M*-Titanium tetrachloride in dichloromethane (0.49 cm^3, 0.49 mmol) was added dropwise. The reaction mixture was allowed to stir at -70 °C for 23 min and then allowed to warm to -40 °C. After 40 min water was added resulting in a white precipitate in the reaction flask. The mixture was extracted with dichloromethane (1 × 25 cm^3, 1 × 15 cm^3, 1 × 10 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate and brine, and dried ($MgSO_4$), affording a crude product (117 mg). The crude product was flash chromatographed on silica gel using ethyl acetate-toluene as eluent (30:70) to give 3β-hydroxycholest-4-en-6-one **63** (97mg, 74%), mp 152-154 °C (from acetone).

(c) The foregoing reaction was repeated on 3β-*t*-butoxycholest-4-en-6-one (3.14 g, 6.89 mmol) at -70 °C. Work-up and chromatography of the crude product (2.76 g) gave compound **63** (1.63 g, 59%), mp 150-153 °C (from acetone).

3α-Acetoxycholest-4-en-6-one 64

Glacial acetic acid (2.1 cm^3, 37 mmol), triphenylphosphine (4.79 g, 18.3 mmol) and diethyl azodicarboxylate were added successively to 3β-hydroxycholest-4-en-6-one (2.92 g, 7.3 mmol) dissolved in dry benzene (118 cm^3). After 17 h additional triphenylphosphine (0.96 g, 3.65 mmol) and diethyl azodicarboxylate (0.57 cm^3, 3.65 mmol) were added. The reaction proceeded to completion after this addition. The mixture was filtered, and the filtrate was partially evaporated under reduced pressure, benzene was added and the precipitated triphenylphosphine was collected. The benzene layer was washed with saturated aqueous sodium hydrogen carbonate and brine, and dried ($MgSO_4$). The crude product was chromatographed on silica gel using ethyl acetate-toluene (4:96) as eluent affording 3α-acetoxycholest-4-en-6-one **64** (2.74 g, 85%), mp 88-91 °C (from methanol); [α]$_D$ +97 (*c* 1.0) (lit.,[63] mp 90 °C; [α]$_D$ +94); ν_{max} 1723 (AcO), 1682 (C=O) and 1627 (C=C) cm^{-1}; δ_H (200 MHz) 0.70 (3H, s, 13β-CH_3), 0.88 (3H, s, 10β-CH_3), 2.03 (3H, s, 3α-AcO), 2.57 (1H, dd, *J* 15.1, 3.3 Hz, 7β-H), 5.27 (1H, m, $W_{1/2}$ 10.9 Hz, 3β-H), 6.21 (1H, d, *J* 4.8 Hz, 4-H) (Found: C 78.5; H, 10.9%; M$^+$, 442. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.4%, *M*, 442).

3α-Hydroxycholest-4-en-6-one 65

Solid potassium carbonate (1.22 g, 8.3 mmol) was added to 3α-acetoxycholest-4-en-6-one (1.3 g, 2.94 mmol) in methanol (82 cm^3). After 47 min water was added and the mixture was filtered. The filtrate was partially evaporated under reduced pressure and extracted with ethyl acetate (3 × 75 cm^3). The combined organic extracts were washed with brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure affording a crude product (1.12 g). The crude product was chromatographed on silica gel (112 g) using ethyl acetate-toluene (20:80) as eluent to give 3α-hydroxycholest-4-en-6-one **65** (980 mg, 83%), mp 123-125 °C; [α]$_D$ +62 (c 1.0) (lit.,[63] mp 124-125 °C; [α]$_D$ +62); ν_{max} 3604 (OH), 1681 (C=O), 1626 (C=C) cm^{-1}, δ_H (200 MHz) 0.69 (3H, s, 13β-CH_3), 0.91 (3H, s, 10β-CH_3), 1.58 (1H, s, OH), 4.23 (1H, m, $W_{1/2}$ 10.6 Hz, 3α-H), 6.26 (1H, d, *J* 4.5 Hz, 4-H), 1.28 (1H, dd, *J* 14.9, 3.1 Hz, 7β-H) (Found: C, 81.0; H, 11.2%, M$^+$, 400. $C_{27}H_{44}O_2$ requires C, 81.0; H, 11.0%; *M*, 400).

Ruthenium tetroxide oxidation of cholesteryl acetate

(a) $RuO_2.2H_2O$ (92 mg, 0.55 mmol) was added to a stirring solution of sodium metaperiodate (652 mg, 3.1 mmol) in acetone-water (120 cm^3, 5:1). This was allowed to stir at 25 °C for 1h. Cholest-5-en-3β-yl acetate (150 mg, 0.35 mmol) dissolved in acetone (50 cm^3) was added dropwise to this mixture over a 55 min period. The reaction was quenched by the addition of propan-2-ol (10 cm^3).The resulting mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane and the organic layer was washed with brine and dried (MgSO4). The solvent was evaporated under reduced pressure to give a crude product (153 mg) which was flash chromatographed on silica gel (15 g) using ethyl acetate-toluene (7:93) as eluent to give 3β-acetoxy-5-hydroxy-5α- cholestan-6-one **61** (107 mg, 70%), mp 228-232 °C (from methanol). Further elution with ethyl acetate- toluene (25:75) gave 5α-cholestane-3β,5,6α-triol 3-acetate **68** (18 mg, 12%), mp 173-175 °C (from ethyl acetate-toluene); $[\alpha]_D$ +6 (*c* 1.0) (lit.,[36] mp 170.5-171.5 °C); ν_{max} 3454 (OH), 3558 (OH), 1718 (AcO) cm^{-1}; δ_H (200 MHz) 0.63 (3H, s, 13β-CH_3), 0.96 (3H, s, 10β-CH_3), 2.01 (3H, s, 3β-AcO), 3.63 (1H, dd, *J* 10.7, 5.1 Hz, 6β-H), 5.11 (1H, sept, *J* 2 × 10.6, 2 × 5.3 Hz, 3α-H) (Found: C, 75.3; H, 10.9%; M$^+$ 462. $C_{29}H_{50}O_4$ requires C, 75.3; H, 10.8%; *M*, 462).

(b) $RuO_2.H_2O$ (180 mg, 1.1 mmol) was added to sodium periodate (1.30 g, 6.1 mmol) in an acetone-water (240 cm^3, 5:1) solution at 0 °C. Cholesteryl acetate (300 mg, 0.70 mmol) dissolved in acetone (50 cm^3) was added dropwise at 0 °C. After 5 h the reaction mixture was worked-up as described above. The crude product (279 mg) was chromatographed on silica gel (28 g) using ethyl acetate-toluene (5:95) as eluent, to give cholesteryl acetate (starting material) **67**, (108 mg, 39%); further elution with ethyl acetate-toluene (7:93) gave the 5α-hydroxy-6-ketone **61** (40 mg, 14%), mp 229-233 °C (from methanol). Final elution with ethyl acetate-toluene (20:80) gave the 5α,6α-diol **68** (49mg, 18%), mp 173-177 °C (from ethyl acetate-hexane).

5-Acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione 70

Acetyl chloride (3.2 cm^3, 44.3 mmol) dissolved in dichloromethane (30 cm^3) was added dropwise to 2,2-dimethyl-1,3-dioxane-4,6-dione (5.76 g, 40 mmol) in dichloromethane (50 cm^3) and pyridine (64 cm^3). The reaction mixture was stirred at 0 °C under nitrogen for 1 h and then allowed to stir at 25 °C for 21 h after which the mixture was partly evaporated under reduced pressure. Benzene was added to increase pyridinium hydrochloride precipitation. The mixture was filtered and the solvent evaporated under reduced pressure to give a crystalline residue which was dissolved in toluene and filtered through Celite. The residue was co-evaporated with toluene several times in order to remove residual pyridine. A crude product (6.46 g) was afforded which was crystallised from benzene-hexane to give **70**, m. p. 80-82 °C, (lit.,[64] mp 80-81 °C).

3β-Acetoacetoxycholest-4-en-6-one 71

A mixture of 3β-hydroxycholest-4-en-6-one (4.0 g, 10 mmol) and 5-acetyl-2,2-dimethyl-1,3 dioxane-4,6-dione (2.23 g, 12.0 mmol) was stirred in dry benzene (21 cm^3) under nitrogen at 72 °C. After 1 h and 20 min the reaction mixture was cooled to 25 °C, water was added and the benzene layer was collected. The aqueous phase was extracted with ethyl acetate (2 × 60 cm^3, 1 × 100 cm^3). The combined organic extracts were washed with brine, dried ($MgSO_4$) and the solvent evaporated under reduced pressure to afford a crude product (4.99 g). The crude product was flash chromatographed on silica gel (249 g) using ethyl acetate-toluene (7:93) as eluent to give 3β-acetoacetoxycholest-4-en-6-one **71** (4.5 g, 93%), mp 123-126 °C; $[\alpha]_D$ -50 (*c* 1.0) (lit.,[23] mp 119-122 °C, $[\alpha]_D$ -49.5); δ_H (400 MHz) 0.69 (3H, s, 13β-CH_3), 0.85 (6H, 2 × d, *J* 6.6, 6.7 Hz, 26-CH_3 and 27-CH_3), 0.91 (3H, d, *J* 6.4 Hz, 21-CH_3), 1.01 (3H, s, 10β-CH_3), 2.26 (3H, s, 4'-CH_3), 2.55 (1H, dd, *J* 15.3, 3.6 Hz, 7β-H), 3.45 (2H, s, 2'-CH_3), 5.38 (1H, ddd, *J* 10.2, 6.3, 2.2 Hz, 3α-H), 6.04 (1H, t, *J* 2 × 2.1 Hz, 4-H); δ_C (100 MHz) 11.9 (q, C-18), 18.6 (q, C-21), 19.5 (q, C-19), 20.7 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 23.9 (t, C-2), 24.0 (t, C-15), 28.0 (t, C-16 and d, C-25), 30.2 (q, C-4'), 34.1 (d, C-8), 34.3 (t, C-1), 35.7 (d, C-20), 36.1 (t, C-22), 38.3 (s, C-10), 39.3 (t, C-24), 39.4(t, C-12), 42.6 (s, C-13), 46.3 (t, C-2′), 50.1 (t, C-7), 51.2 (d, C-9), 57.0 (d, C-17), 56.6 (d, C-14), 70.4 (d, C-3), 127.7 (d, C-4), 148.5 (s, C-5), 166.8 (s, C-1'), 200.2 (s, C-6), 202.4 (s, C-3') (Found: C, 77.1; H, 10.2, M$^+$, 484. $C_{31}H_{48}O_4$ requires C, 76.9, H; 9.9; *M*, 484).

3α-Acetoacetoxycholest-4-en-6-one 72

3α-Hydroxycholest-4-en-6-one (1.26 g, 3.15 mmol) was dissolved in anhydrous benzene (12 cm^3) under nitrogen, and 5-acetyl Meldrum's acid (0.7 g, 3.8 mmol) was added. The reaction mixture was stirred at 70-75 °C for 2 h. The mixture was then cooled to 25 °C and diluted with benzene (20 cm^3).The benzene layer was washed with water (10 cm^3), brine (10 cm^3), diluted with ethyl acetate, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (1.51 g) which was chromatographed on silica gel using ethyl acetate-toluene (6:94) as eluent, to give the 3α-acetoacetate **72** as an oil (1.32 g, 86%), $[\alpha]_D$ +86 (*c* 1.0) (lit.,[23] $[\alpha]_D$ +131); ν_{max} 1740 (1'-C=O), 1714 (3'-C=O), 1685 (6-C=O), 1631 (C=C) cm^{-1}; δ_H (200 MHz) 0.69 (3H, s, 13β-CH_3), 0.95 (3H, s, 10β-CH_3), 2.25 (3H, s, 4'-CH_3), 2.56 (1H, dd, *J* 14.9, 3.1 Hz, 7β-H), 3.43 (2H, s, 2'-CH_2), 5.27 (1H, br. m, $W_{½}$ 11.2 Hz, 3β-H), 6.16 (1H, d, *J* 4.9 Hz, 4-H); δ_C (50 MHz) 11.9 (q, C-18), 18.3 (q, C-19), 18.6 (q, C-21), 21.3 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-2), 23.9 (t, C-23), 24.2 (t, C-15), 28.0 (t, C-16 and d, C-25), 30.1 (q, C4′), 30.7 (t, C-1), 34.0 (d, C-8), 35.7 (d, C-20), 36.1 (t, C-22), 38.7 (s, C-10), 39.3 (t, C-12), 39.4 (t, C-24), 42.6 (s, C-13), 46.4 (t, C-7), 50.2 (t, C-2′), 50.9 (d, C-9), 56.0 (d, C-17), 56.6 (d, C-14), 66.8 (d, C-3), 125.1 (d, C-4), 151.4 (s, C-5), 166.3 (s, C-1'), 200.3 (s, C-6), 203.2 (s, C-3') (Found: M$^+$, 484. $C_{31}H_{48}O_4$ requires *M*, 484).

(Bromomethyl)chlorodimethylsilane treatment of 3β-hydroxycholest-4-en-6-one

(a) 3β-Hydroxycholest-4-en-6-one (200 mg, 0.5 mmol) and imidazole (80 mg, 1.2 mmol) was dissolved in dry benzene (6 cm^3). (Bromomethyl)chlorodimethylsilane (0.34 cm^3, 2.5

mmol) was added dropwise at 25 °C. The reaction mixture was stirred under nitrogen for 24 h and then diluted with benzene (60 cm^3). The benzene layer was washed with saturated aqueous sodium hydrogen carbonate (10 cm^3) and brine, and dried (MgSO$_4$). The solvent was evaporated under reduced pressure affording a crude product (343 mg). The crude product was flash chromatographed on silica gel using ethyl acetate-toluene (10:90) as eluent to give *3β-[(bromomethyl)dimethylsilyloxy]cholest-4-en-6-one* **76** (167 mg, 61%), mp 128-131 °C (from diisopropyl ether-methanol); $[\alpha]_D$ -31.2 (*c* 1.0); ν_{max} 1684 (C=O), 1087 (O-SiMe$_2$) cm^{-1}, δ_H (400 MHz) 0.27 (6H, s, 1'-SiMe$_2$), 0.68 (3H, s, 13β-CH$_3$), 0.85 (6H, 2 × d, *J* 6.5, 6.7 Hz, 26-CH$_3$ and 27-CH$_3$), 0.90 (3H, d, *J* 6.5 Hz, 21-CH$_3$), 0.99 (3H, s, 10β-CH$_3$), 2.47 (2H, s, 2'-CH$_2$), 4.33 (1H, ddd, *J* 10.0, 6.3, 2.2 Hz, 3α-H), 6.06 (1H, t, *J* 2 × 1.8 Hz, 4-H); δ_C (100 MHz) -2.5 (2 × q, 1'-SiMe$_2$), 11.9 (q, C-18), 16.2 (t, C-2'), 18.6 (q, C-21), 19.8 (q, C-19), 20.8 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 23.9 (t, C-15), 28.0 (d, C-25 and t, C-16), 28.4 (t, C-2), 34.0 (d, C-8), 34.8 (t, C-1), 35.7 (d, C-20), 36.1 (t, C-22), 38.1 (s, C-10), 39.3 (t, C-24), 39.4 (t, C-12), 42.6 (s, C-13), 46.3 (t, C-7), 51.2 (d, C-9), 56.0 (d, C-17), 56.7 (d, C-14), 68.4 (d, C-3), 133.0 (d, C-4), 146.2 (s, C-5), 202.5 (s, C-6) (Found: C, 65.6; H, 9.5%; M$^+$-15, 536. C$_{30}$H$_{51}$O$_2$SiBr requires C, 65.3; H, 9.3%; *M*, 551). Further elution with ethyl acetate-toluene (30:70) gave 3β-hydroxycholest-4-en-6-one (starting material) **63** (57 mg, 29%).

(b) 3β-Hydroxycholest-4-en-6-one (200 mg, 0.5 mmol) and imidazole (73 mg, 1.1 mmol) was dissolved in dry benzene (6.5 cm^3) with warming. (Bromomethyl)chlorodimethylsilane (0.32 cm^3, 2.4 mmol) was added dropwise at 25 °C. The reaction mixture was stirred under nitrogen for 3 days and diluted with benzene (40 cm^3). The benzene layer was washed with saturated aqueous sodium hydrogen carbonate and brine, and dried (MgSO$_4$). The solvent was evaporated under reduced pressure to give a crude product (304 mg). The crude product was flash chromatographed on silica gel using ethyl acetate-toluene (4:96) as eluent to give cholest-4-en-3,6-dione **77** (38 mg, 19%), mp 127-129 °C (lit.,[65] mp 124-125 °C, $[\alpha]_D$ -40) ν_{max}1678 (C=O) cm^{-1}, δ_H (400 MHz) 0.71 (3H, s, 13β-CH$_3$), 0.85 (6H, 2 × d, *J* 6.6 Hz, 26-CH$_3$ and 27-CH$_3$), 0.91 (3H, d, *J* 6.6 Hz, 21-CH$_3$), 1.15 (3H, s, 10β-CH$_3$), 6.18 (1H, s, 4-H) (Found: M$^+$, 398. $C_{27}H_{42}O_2$ requires *M*, 398), followed by cholestane-3,6-dione **78** (129 mg, 65%), mp 168-172 °C (from benzene-hexane); $[\alpha]_D$ +3.6 (*c* 1.0) (lit.,[66] mp 171-172 °C, $[\alpha]_D$ +1.8); ν_{max} 1718 (C=O) cm^{-1}, δ_H (400 MHz) 0.69 (3H, s, 13β-CH$_3$), 0.86 (6H, 2 × d, *J* 6.7 , 6.6 Hz, 26-CH$_3$ and 27-CH$_3$), 0.91 (3H, d, *J* 6.6 Hz, 21-CH$_3$), 0.95 (3H, s, 10β-CH$_3$); δ_C (100 MHz) 12.0 (q, C-18), 12.6 (q, C-19), 18.6 (q, C-21), 21.7 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 24.0 (t, C-15), 28.0 (d, C-25 and t, C-12), 35.7 (d, C-20), 36.1 (t, C-22), 37.0 (t, C-7), 37.4 (t, C-2), 38.0 (d, C-8), 38.1 (t, C-1), 39.4 (t, C-24), 39.5 (t, C-16), 41.2 (s, C-10), 43.0 (s, C-13), 46.6 (d, C-4), 53.5 (d, C-9), 56.1 (d, C-14), 56.6 (d, C-17), 57.5 (d, C-5), 209.1 (s, C-3), 211.2 (s, C-6) (Found: C, 81.0; H, 11.3%; M$^+$, 400. $C_{27}H_{44}O_2$ requires C, 81.0; H, 11.0%; *M*, 400). Further elution with ethyl acetate-toluene (30:70) gave 3β-hydroxycholest-4-en-6-one **63** (starting material), (16 mg, 8%).

(2*R*)-2-(3β-Hydroxy-6-oxo-5β-cholestan-4β-yl)-3-oxobutanoic acid 1, 3'-lactone 79

(a) Aqueous 1*M*-potassium carbonate (0.83 cm^3, 0.8 mmol,) was added to 3β-acetoacetoxycholest-4-en-6-one (200 mg, 0.4 mmol) dissolved in ethanol at 67 °C. After 1 h the mixture was cooled to 25 °C and filtered. The filtrate was partially evaporated under reduced pressure and water was added. The mixture was extracted with ethyl acetate (3 × 25 cm^3). The combined organic extracts were washed with 1.5*M*-hydrochloric acid (10 cm^3) and saturated aqueous sodium hydrogen carbonate (10 cm^3). The neutralised organic layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure affording a crude product (192 mg). The crude product was chromatographed on silica gel using ethyl acetate-toluene (4:96) as eluent to give (2*R*)-2-(3 β-hydroxy-6-oxo-5β-cholestan-4β-yl)-3-oxobutanoic acid 1,3'-lactone **79** (160 mg, 80%); mp 132-136 °C (from ethyl acetate-methanol); $[\alpha]_D$ 1.1 (*c* 1.0) (lit.,[23] mp 130-133 °C, $[\alpha]_D$ 1.0); ν_{max} 1775 (1'-C=O), 1717 (6-C=O), 1697 (3'-C=O) cm^{-1};δ_H (400 MHz) 0.65 (3H, s, 13 β-CH$_3$), 0.91 (3H, s, 21-CH$_3$), 0.86 (3H, s, 10β-CH$_3$), 1.82 (1H, dd, *J* 12.1, 1.2 Hz, 5β-H), 1.99 (1H, dq, *J* 15.6, 3 × 3.6 Hz, 2β-H), 2.12 (1H, dd, *J* 15.2, 12.1 Hz, 7α-H), 2.24 (1H, ddd, *J* 15.1, 4.9, 1.2 Hz, 7β-H), 3.06 (1H, dd, *J* 12.0, 5.1 Hz, 4α-H), 2.32 (3H, s, 4'-CH$_3$), 3.57 (1H, s, 2'-H), 4.62-4.65 (1H, br. m., $W_{½}$ 11.8 Hz, 3α-H); δ_C (50 MHz) 11.9 (q, C-18), 18.6 (q, C-21), 20.9 (t, C-11), 21.7 (t, C-2), 22.5 (q, C-26), 22.8 (q, C-27), 23.2 (q, C-19), 23.8 (t, C-23), 23.8 (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), 37.5 (s, C-10), 39.4 (t, C-12 and t, C-24), 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), 58.9 (d, C-5), 62.4 (d, C-2'), 77.4 (d, C-3), 170.9 (s, C-1'), 199.2 (s, C-3'), 212.2 (s, C-6) (Found: C, 76.9; H, 10.1%; M^+, 484. $C_{31}H_{48}O_4$ requires C, 76.9; H, 9.9%; *M*, 484).

(b) Freshly sublimed potassium *tert*-butoxide (46 mg, 0.41 mmol) was added to 3β-acetoacetoxycholest-4-en-6-one (200 mg, 0.41 mmol) dissolved in dry tetrahydrofuran under nitrogen. This was allowed to stir between -5 and 5 °C for 2 h after which the reaction mixture was warmed to 25 °C. 3*M*-Hydrochloric acid was added to the reaction mixture after prolonged reaction (21 h) at 25 °C. The mixture was partially evaporated under reduced pressure and diluted with water, and extracted with ethyl acetate (1 × 25 cm^3, 2 × 20 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (5 cm^3) and brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure affording a crude product (177 mg) which was flash chromatographed on silica gel (18 g) using ethyl acetate-toluene (5:95) as eluent to give the γ-lactone **79** (151mg, 76%); mp 131-135 °C (from ethyl acetate-methanol).

(c) Potassium (42 mg, 1.1 mmol) was dissolved in dry *tert*-butyl alcohol under reflux. 3β-Acetoacetoxycholest-4-en-6-one (200 mg, 0.41 mmol) was dissolved in dry tetrahydrofuran (10 cm^3) and added dropwise to the aforementioned solution at 25 °C. After 1 h and 25 min the reaction mixture was neutralised with 3*M*-hydrochloric acid. The mixture was partially evaporated under reduced pressure and diluted with water (10 cm^3). The mixture was extracted with ethyl acetate (1 × 25 cm^3, 2 × 15 cm^3). The combined extracts were washed with 1.5*M*-hydrochloric acid (10 cm^3), water and saturated aqueous sodium hydrogen carbonate. The neutralised organic layer was dried ($MgSO_4$) and the solvent evaporated under reduced pressure affording a crude product (196 mg). The crude product was chromatographed on silica gel using ethyl acetate-toluene (4:96) as eluent to give **79** (155mg, 78%), mp 131-134 °C (from acetone-methanol).

(d) 3β-Acetoacetoxycholest-4-en-6-one (100 mg, 0.21 mmol) dissolved in ethanol (8 cm^3) and tetrahydrofuran (3 cm^3) was added dropwise to a stirring mixture of aqueous ethanolic 2*M*-potassium hydroxide (0.21 cm^3, 0.42 mmol) over a 25-minute period. After 8 min 3*M*-hydrochloric acid (1.4 cm^3, 4.2 mmol) was added and the mixture was partially evaporated under reduced pressure and diluted with water. The mixture was extracted with ethyl acetate (1 × 25 cm^3, 2 × 15 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (5 cm^3) and brine, and dried ($MgSO_4$). The crude product was chromatographed on silica gel using ethyl acetate-toluene (4:96) as eluent to give the γ-lactone **79** (83mg, 83%), mp 129-133 °C (from acetone-methanol).

3β-Hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one 80

(a) (2*R*)-2-(3β-hydroxy-6-oxo-5β-cholestan-4β-yl)-3-oxobutanoic acid 1,3'-lactone (288 mg, 0.60 mmol) was dissolved in ethanol (18 cm^3). Aqueous 2*M*-potassium hydroxide was added dropwise. The reaction mixture was refluxed for 21 h after which aqueous 1*M*-hydrochloric acid was added to neutralise the mixture. The mixture was cooled to room temperature and partially evaporated under reduced pressure. Water was added, and the pH was adjusted from 5 to 1. The mixture was extracted with ethyl acetate (3 × 15 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (5 cm^3) and water, and brine.The solvent was evaporated affording a crude product (137 mg). This was chromatographed on silica gel using ethyl acetate-toluene (50:50), affording a mixture of the least polar fractions (70 mg, 51% of the crude product obtained) and 3β-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one **80** (50 mg, 19%), mp 168-172 °C (from acetone); $[\alpha]_D$ +115.5 (*c* 0.9) (lit.,[23] mp 169-172 °C, $[\alpha]_D$ +105); ν_{max} 3692 (OH), 1655 (C=O), 1630 (C=C) cm^{-1}; δ_H (400 MHz) 0.64 (3H, s, 13β-CH_3), 0.88 (3H, d, *J* 6.4 Hz, 21-CH_3), 0.94 (3H, s, 10β-CH_3), 2.00 (1H, dt, *J* 12.5 Hz, 2 × 3.3 Hz), 2.05 (1H, d, *J* 5.3 Hz, 5α-H), 2.29 (1H, dd, *J* 17.3, 15.4 Hz, 6'β-H), 2.36 (1H, dd, *J* 12.4, 4.6 Hz, 7β-H), 2.44 (1H, d, *J* 17.5, 5.4 Hz, 6'α-H), 2.55 (1H, dq, *J* 15.4, 3 × 5.5 Hz, 4α-H), 3.87 (1H, ddd, *J* 11.9, 6.4, 5.1 Hz, 3α-H), 5.91 (1H, s, 4'-H); δ_C (100 MHz) 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), 24.1 (t, C-15), 26.6 (t, C-2), 28.0 (d, C-25), 28.1 (t, C-16), 34.0 (t, C-7), 35.6 (t, C-1), 35.7 (d, C-20), 36.1 (t, C-22), 38.1 (d, C-4), 39.4 (t, C-12), 39.5 (t, C-24), 39.6 (d, C-8), 40.8 (s, C-10), 41.4 (t, C-6'), 43.0 (s, C-13), 49.8 (d, C-5), 56.1 (d, C-9 and d, C-17), 56.8 (d, C-14), 71.3 (d, C-3), 124.3 (d, C-4'), 165.8 (s, C-6), 200.2 (s, C-5) (Found: C, 81.9; H, 11.4%; M^+, 440. $C_{30}H_{48}O_2$ requires C, 81.8; H,10.9%; *M*, 440).

(b) (2*R*)-2-(3β-hydroxy-6-oxo-5β-cholestan-4β-yl)-3-oxobutanoic acid 1,3'-lactone (100 mg, 0.21 mmol) was dissolved in ethanol with heating. Aqueous 2*M*-potassium hydroxide was added dropwise and the reaction mixture was refluxed for 5 h. The reaction mixture was stirred at 6 °C for 21 h after which 3*M*-hydrochloric acid (0.5 cm^3) was added. The mixture was partially evaporated under reduced pressure, water was added and the pH was adjusted to 1. The mixture was extracted with ethyl acetate (3 × 10 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (5 cm^3) and brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (47 mg) which was chromatographed on silica gel using ethyl acetate-toluene (50:50) as eluent, affording 3β-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5′(6′H)-one **80** (25 mg, 27%).

(c) Aqueous 2*M*-potassium hydroxide (7 cm^3) was added dropwise to 3β-acetoxy-4α, 5α-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one (250 mg, 0.52 mmol) in ethanol (25 cm^3) at 25 °C. After 10 min 1*M*-hydrochloric acid (8 cm^3) was added, the mixture was partially evaporated under reduced pressure and water (10 cm^3) was added. The aqueous phase was extracted with ethyl acetate (3 × 40 cm^3). The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate (10 cm^3) and brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (225 mg). Flash chromatography on silica gel using ethyl acetate-toluene (50:50) as eluent gave 3β-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5′(6′H)-one **80** (210 mg, 92%).

3β, 6-Dihydroxy-4α, 4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one 81

(a) (2*R*)-2-(3β-hydroxy-6-oxo-5β-cholestan-4β-yl)-3-oxobutanoic acid 1, 3'-lactone (417 mg, 0.86 mmol) was dissolved in dioxane (22.4 cm^3) and water (1.6 cm^3). Aqueous 2*M*-potassium hydroxide (4.3 cm^3, 8.6 mmol) was added at once. The reaction mixture was cooled to 25 °C and stirred at 6 °C for 2 days. The mixture was neutralised with 1*M*-hydrochloric acid (4.5 cm^3), partially evaporated under reduced pressure, and water was added. The pH was adjusted to 5 by the addition of 1*M*-hydrochloric acid. The mixture was extracted with ethyl acetate (3 × 20 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate and brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (292 mg). This was chromatographed on silica gel using ethyl acetate-toluene (60:40) as eluent, to give 3β,6-dihydroxy-4α,4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one **81** (258 mg, 65%), mp 185-189 °C (from methanol); $[\alpha]_D$ +2.9 (*c* 1.0) (lit.,[23] mp 180-183 °C, $[\alpha]_D$ +3); ν_{max} 3598 (3 and 6-OH), 1710 (5′-C=O) cm^{-1}; δ_H (400 MHz) 0.66 (3H, s, 13β-CH_3), 0.88 (3H, d, *J* 6.8 Hz, 21-CH_3), 0.89 (6H, 2 × d, *J* 6.4 and 6.8 Hz, 26-CH_3 and 27-CH_3), 1.01 (3H, s, 13β-CH_3), 1.86 (1H, dd, *J* 11.9, 1.2 Hz, 5β-H), 1.90-2.01 (2H, br. m., $W_{½}$ *ca* 39Hz, 4α-H), 2.10 (1H, dt, *J* 13.3, 2 × 3.0 Hz, 6'α-H), 2.34 (1H, dd, *J* 13.2, 2.6 Hz, 4'α-H), 2.56 (1H, dd, *J* 13.3, 1.2 Hz, 4'β-H), 2.64 (1H, td, *J* 2 × 13.2, 0.9 Hz, 6'β-H), 3.75 (1H, m, $W_{½}$ 10.0 Hz, 3α-H); δ_C (100 MHz) 12.15 (q, C-18), 18.7 (q, C-21), 21.0 (t, C-11), 22.5 (q, C-26), 22.8 (q, C-27), 23.9 (t, C-23), 24.1 (t, C-15), 27.2 (q, C-19), 28.0 (d, C-25), 28.3 (t, C-2 and t, C-16), 31.0 (t, C-1), 32.4 (d, C-8), 35.8 (d, C-20), 36.1 (t, C-22), 36.7 (s, C-10), 38.3 (d, C-4), 39.2 (t, C-7), 39.5 (t, C-24), 40.0 (t, C-12), 40.6 (d, C-9), 42.9 (s, C-13), 45.2 (t, C-6'), 50.4 (d, C-5), 56.0 (d, C-17), 56.3 (d, C-14), 58.3 (t, C-4'), 69.6 (d, C-3), 76.6 (s, C-6), 208.8 (s, C-5') (Found: C, 78.5; H, 11.2 %; M$^+$, 458. $C_{30}H_{50}O_3$ requires C, 78.6; H, 10.9%, *M*, 458).

(b) 3β-Acetoacetoxycholest-4-en-6-one (1.02 g, 2.1 mmol) was dissolved in absolute ethanol (56 cm^3). Aqueous 1*M*-potassium carbonate (3.9 cm^3, 3.9 mmol) was added dropwise and the mixture was stirred between 65-70 °C. After completion (TLC), the mixture was filtered and the filtrate was partially evaporated under reduced pressure. The filtrate was diluted with water (10 cm^3) and extracted with ethyl acetate (3 × 25 cm^3). The combined organic extracts were washed with 3*M*-hydrochloric acid (10 cm^3). The neutralised organic layer was washed with brine and dried (MgSO$_4$). The solvent was evaporated under reduced pressure to give a crude product (967 mg). The crude product was refluxed in a mixture of dioxane (61.6 cm^3) and water (4.4 cm^3) after which aqueous 2*M*-potassium hydroxide (11.9 cm^3, 23.8 mmol) was added dropwise. The mixture was refluxed for 4.5 h after which it cooled to 25 °C and stirred for 2.5 days at 5 °C. The reaction mixture was neutralised with 1*M*-hydrochloric acid (12 cm^3), partially evaporated under reduced pressure and water was added.The pH was adjusted to 5 and the mixture was extracted with ethyl acetate (3 × 75 cm^3). The combined organic extracts were washed with brine and dried (MgSO$_4$).The solvent was evaporated under reduced pressure to give a crude product (742 mg).This was chromatographed on silica gel using ethyl acetate-toluene (60:40) as eluent, to give 3β,6-dihydroxy-4α,4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one **81** (520 mg, 51%).

3β-Acetoxy-6-hydroxy-4α, 4', 5β, 6β-tetrahydrobenzo[4. 5. 6]cholestan-5'(6'H)-one 82
3β,6-Dihydroxy-4α,4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one (320 mg, 0.64 mmol) was dissolved in pyridine (5 cm^3) and acetic anhydride (11.7 cm^3). The reaction mixture was allowed to stir for 24 h. On completion (TLC), the mixture was cooled to 0 °C and saturated aqueous sodium hydrogen carbonate (5 cm^3) was added. The mixture was extracted with ethyl acetate (3 × 20 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate. The neutralised organic layer was washed with brine (10 cm^3) and dried (MgSO$_4$). Pyridine was co-evaporated with toluene. The solvent was evaporated under reduced pressure to give a crude product (377 mg) which was flash chromatographed on silica gel using ethyl acetate-toluene (30:70) as eluent, to

give **82** (343 mg, 98%), mp 96-99 °C; $[\alpha]_D$ +16.2 (*c* 1.0) (lit.,[31] mp 95-97 °C, $[\alpha]_D$ +14); ν_{max} 3591 (OH), 1732 (AcO), 1719 (5′-C=O) cm^{-1}; δ_H (400 MHz) 0.67 (3H, s, 13β-CH$_3$), 0.85 (6H, 2 × d, *J* 6.5 Hz, 26-CH$_3$ and 27-CH$_3$), 0.89 (3H, d, *J* 6.5 Hz, 21-CH$_3$), 1.24 (3H, s, 10β-CH$_3$), 1.88 (1H, dd, *J* 11.8, 1.5 Hz, 5β-H), 2.09 (3H, s, AcO), 2.36 (1H, dd, *J* 13.3, 2.6 Hz, 4'α-H), 2.58 (1H, dd, *J* 13.4, 1.0 Hz, 4'β-H), 4.85 (1H, m, $W_{½}$ 7.9 Hz, 3α-H); δ_C (100 MHz) 12.1 (q, C-18), 18.7 (q, C-21), 21.0 (t, C-11), 21.2 (q, AcO), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 24.1 (t, C-15), 24.9 (t, C-2), 27.2 (q, C-19), 28.0 (d, C-25), 28.2 (t, C-16), 31.7 (t, C-1), 32.4 (d, C-8), 35.8 (d, C-20), 36.1 (t, C-22), 36.6 (s, C-10), 36.8 (d, C-4), 39.2 (t, C-7), 39.5 (t, C-24), 40.0 (t, C-12), 40.7 (d, C-9), 42.9 (s, C-13), 44.6 (t, C-6'), 51.1 (d, C-5), 55.9 (d, C-17), 56.3 (d, C-14), 58.2 (t, C-4'), 72.5 (d, C-3), 76.2 (s, C-6), 170.4 (s, AcO), 207.4 (s, C-5') (Found: C, 76.6; H, 10.6%; M$^+$, 500. $C_{32}H_{52}O_4$ requires C, 76.8; H, 10.4, *M*, 500).

Dehydrations of 3β-Acetoxy-6-hydroxy-4α,4′,5β,6β-tetrahydrobenzo[4.5.6]cholestan-5′(6′H)-one

(a) 3β-Acetoxy-6-hydroxy-4α, 4', 5β, 6β-tetrahydrobenzo[4. 5. 6]cholestan-5'(6'H)one (181 mg, 0.36 mmol) was dissolved in pyridine (1 cm^3) at 0 °C under nitrogen. Thionyl chloride (0.13 cm^3, 1.79 mmol) dissolved in pyridine (0.13 cm^3) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and at 25 °C for an additional 30 min. The reaction mixture was poured into an acidified (HCl) water-ice mixture, and extracted into ethyl acetate (3 × 15 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (10 cm^3) and brine. The neutralised organic layer was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give a crude product (160 mg). The crude product was chromatographed on silica gel using ethyl acetate-toluene (5:95) as eluent, to give 3β-acetoxy-4α,4',5β,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'H)-one **83** (51 mg, 29%), mp 134-137 °C (from methanol); $[\alpha]_D$ +47 (*c* 1.0) (lit.,[31] mp 112-118 °C, $[\alpha]_D$ +47); ν_{max} 1732 (AcO), 1715 (C=O) cm^{-1}; δ_H (400 MHz) 0.68 (3H, s, 13β-CH$_3$), 0.96 (3H, s, 10β-CH$_3$), 0.90 (3H, d, *J* 6.4 Hz, 21-CH$_3$), 0.86 (6H, 2 × d, *J* 6.6 Hz, 26-CH$_3$ and 27-CH$_3$), 1.94 (1H, d, *J* 11.0 Hz, 5β-H), 2.30 (1H, ddd, *J* 14.9, 4.3, 1.9 Hz, 6′α-H), 2.37 (1H, dd, *J* 14.9, 11.5 Hz, 6′β-H), 2.96 (1H, dd, *J* 14.7, 1.9 Hz, 4′β-H), 3.03-3.07 (1H, ddd, *J* 14.8, 3.6, 2.3 Hz, 4′α-H), 4.82 (1H, m, $W_{½}$ 8.9 Hz, 3α-H), 5.29 (1H, s, 7-H); δ_C (100 MHz) 12.0 (q, C-18), 18.6 (q, C-21), 20.8 (t, C-11), 21.1 (q, C-19), 22.5 (q, C-26), 22.8 (q, C-27), 23.4 (q, AcO), 23.8 (t, C-23), 23.8 (t, C-15), 24.95 (t, C-2), 28.0 (d, C-25), 28.3 (t, C-16), 29.1 (t, C-1), 34.0 (s, C-10), 35.7 (d, C-20), 36.1 (t, C-22), 37.2 (d, C-4), 39.5 (t, C-24), 39.95 (t, C-12), 40.2 (d, C-9), 41.1 (d, C-8), 43.2 (s, C-13), 44.8 (t, C-6′), 47.4 (d, C-5), 52.8 (t, C-4′), 54.5 (d, C-17), 56.1 (d, C-14), 71.3 (d, C-3), 124.6 (d, C-7), 133.9 (s, C-6), 170.4 (s, AcO), 207.4 (s, C-5′) (Found: C, 79.7; H, 10.7 %; M^+, 482. $C_{32}H_{50}O_3$ requires C, 79.7; H, 10.4%; *M*, 482). Further elution with methanol-dichloromethane (10:90) afforded an unknown product **84** (36 mg), mp 206-210 °C (from diisopropyl ether-methanol); ν_{max} 1730 cm^{-1}; δ_H (400 MHz) 0.68 (3H, s, 13β-CH_3), 0.85 (6H, 2 × d, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 0.89 (3H, s, 10β-CH_3), 2.56 (1H, d), 2.92 (1H, t, *J* 2 × 11.8 Hz), 3.09 (1H, d, *J* 12.1 Hz), 3.85 (1H, d, *J* 11.0 Hz), 5.01 (1H, s), 5.67 (1H, s), 7.92 (2H, t, *J* 2 × 7.3 Hz), 8.32 (1H, t, *J* 2 × 7.7 Hz), 9.01 (2H, d, *J* 6.1 Hz).

(b) 3β-Acetoxy-6-hydroxy-4α,4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one (448 mg, 0.89 mmol) was dissolved in hexamethylphosphoric triamide (6 cm^3) with heating. Phosphoryl chloride (0.31 cm^3, 3.40 mmol) was added dropwise at 25 °C. The mixture was allowed to stir at 25 °C for 1 h and at 50-70 °C until completion (TLC). Saturated aqueous sodium hydrogen carbonate was added to the reaction mixture contained in an ice-water bath. The aqueous phase was extracted with ethyl acetate (3 × 70 cm^3). The combined organic extracts were washed with 3*M*-hydrochoric acid, and brine and dried ($MgSO_4$). The solvent was evaporated, and the residue obtained was dried under reduced pressure at 35-50 °C for 8 h. The residue was chromatographed on silica gel using ethyl acetate-toluene (6:94) as eluent, to give a mixture of least polar fractions (26 mg), followed by 3β-acetoxy-4α,4',5β,6-tetrahydrobenzo[4.5.6]cholest-6-en-5'(6'H)-one **83** (52 mg, 12%). Further elution gave *3β-acetoxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one* **85** (302 mg, 70%), mp 158-162 °C (from diisopropyl ether-methanol); $[\alpha]_D$ +82 (*c* 0.9); ν_{max} 1634

(C=C), 1665 (C=O), 1707 (AcO) cm^{-1}; δ_H (400 MHz) 0.63 (3H, s, 13β-CH_3), 0.85 (6H, 2 × d, 6.6 and 6.4 Hz, 26-CH_3 and 27-CH_3), 0.89 (3H, s, 10β-CH_3), 2.03 (3H, s, AcO), 2.11 (1H, d, *J* 5.5 Hz, 5α-H), 2.26 (1H, dd, *J* 17.6, 5.9 Hz, 6'α-H), 2.40 (2H, dd, *J* 17.5, 14.5 Hz, 6'β-H), 2.69 (1H, dq, *J* 14.8, 3 × 5.9 Hz, 4α-H), 4.91 (1H, dt, *J* 11.7, 2 × 5.85 Hz, 3α-H), 5.96 (1H, s, 4'-H); δ_C (100 MHz) 12.1 (q, C-18), 15.5 (q, C-19), 18.7 (q, C-21), 20.95 (t, C-11), 21.1 (q, AcO), 22.6 (q, C-26), 22.8 (q, C-27), 23.0 (t, C-2), 23.0 (t, C-23), 24.1 (t, C-15), 28.0 (d, C-25), 28.1 (t, C-16), 34.6 (t, C-7), 35.1 (d, C-4), 35.4 (t, C-1), 35.7 (d, C-20), 36.1 (t, C-22), 39.4 (t, C-12), 39.5 (t, C-24), 39.5 (d, C-8), 40.7 (s, C-10), 41.4 (t, C-6'), 43.0 (s, C-13), 49.5 (d, C-5), 56.0 (d, C-9), 56.1 (d, C-17), 56.7 (d, C-14), 73.2 (d, C-3), 124.2 (d, C-4'), 165.5 (s, C-6), 170.3 (s, AcO), 199.6 (s, C-5') ((Found: C, 79.6; H, 10.7 %, M$^+$, 482. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.4%; *M*, 482).

(c) 3β-Acetoxy-6-hydroxy-4α,4',5β,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one (389 mg, 0.79 mmol) was dissolved in anhydrous dichloromethane (5 cm^3) under nitrogen. Boron trifluoride diethyl etherate (0.21 cm^3, 1.71 mmol) was added dropwise at 25 °C. The reaction mixture was stirred at 25 °C for 2.5 h after which saturated aqueous sodium hydrogen carbonate (10 cm^3) was added to the reaction mixture contained in an ice-water bath. The aqueous phase was extracted with ethyl acetate (3 × 70 cm^3). The combined organic extracts were washed with brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (384 mg) which was chromatographed on silica gel using ethyl acetate-toluene (7:93) as eluent to give the Δ^6-5'-ketone **83** (136 mg, 36%), followed by *3β-acetoxy-4α,5β,-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one* **86** (196 mg, 52%) as an oil, $[\alpha]_D$ +45.3 (*c* 0.9); ν_{max}1732 (AcO), 1659 (C=O), 1615 (C=C) cm^{-1}; δ_H (400 MHz) 0.68 (3H, s, 13β-CH_3), 0.84 (6H, 2 × d, *J* 6.5, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 0.87 (3H, d, *J* 6.4 Hz, 21-CH_3), 1.11 (3H, s, 10β-CH_3), 1.80 (1H, dd, *J* 11.3, 2.2 Hz, 5β-H), 1.97 (1H, dt, *J* 12.7, 3.4 Hz, 1α-H or 1β-H), 2.04 (3H, s, AcO), 2.20 (dd, *J* 17.3, 12.5 Hz, 6'β-H), 2.64 (1H, ddt, *J* 15.8 , 11.5, 2 × 2.1 Hz, 4α-H), 4.95 (1H, ddd, *J* 8.0, 5.5, 4.1 Hz, 3α -H), 5.78 (1H, t, *J* 2 × 2.1 Hz, 4'-H); δ_C (100 MHz) 12.2 (q, C-18), 18.6 (q, C-21), 21.1 (q, C-19), 22.0 (t, C-11), 22.6 (q, C-26), 22.6 (q, AcO), 22.8 (q, C-27), 23.8 (t, C-23), 23.9 (t, C-15), 25.6 (t, C-2), 28.0 (t, C-16), 28.0 (d, C-25), 31.0 (t, C-1), 34.2 (d, C-4), 34.6 (t, C-7), 35.7 (d, C-20), 36.1 (t, C-22), 36.7 (s, C-10), 37.6 (d, C-8), 39.5 (t, C-24), 39.8 (t, C-6' and t, C-12), 43.6 (s, C-13), 45.5 (d, C-5), 47.3 (d, C-9), 55.3 (d, C-17), 56.2 (d, C-14), 71.0 (d, C-3), 126.9 (d, C-4'), 168.3 (s, C-6), 170.4 (s, AcO), 198.8 (s, C-5') (Found: C, 79.4; H, 10.6%; M^+, 482. $C_{32}H_{50}O_3$ requires C, 79.7; H, 10.4%; *M*, 482).

4α,5α-Dihydrobenzo[4.5.6]cholestane-3,5'(6'H)-dione 89

Pyridine (0.2 cm^3, 2.5 mmol) and dichloromethane (2.5 cm^3) was added to 3β-hydroxy-4α,5α-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one (75 mg, 0.17 mmol) in a three-necked round bottom flask under nitrogen. Chromium trioxide (114 mg, 1.14 mmol) was added and the reaction mixture was stirred at 25 °C for 30 min. On completion (TLC), the flask was rinsed with dichloromethane. The organic layer was washed with aqueous 2*M*-potassium hydroxide (10 cm^3), 1*M*-hydrochloric acid (15 cm^3), saturated aqueous sodium hydrogen carbonate (10 cm^3), and brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (70 mg) which was flash chromatographed on silica gel using ethyl acetate-toluene (30:70) affording 4α,5α-dihydrobenzo[4.5.6]cholestane-3,5'(6'H)-dione **89** (66 mg, 89%), mp 133-137 °C (from acetone-methanol); $[\alpha]_D$ +57.2 (*c* 1.0) (lit.,[31] mp 126-128 °C, $[\alpha]_D$ +58); ν_{max} 1707 (3-C=O), 1662 (5′-C=O), 1631 (C=C) cm^{-1}; δ_H (400 MHz) 0.67 (3H, s, 13β-CH_3), 0.86 (6H, 2 × d, *J* 6.6 Hz , 26-CH_3 and 27-CH_3), 0.91 (3H, d, *J* 6.4 Hz, 21-CH_3), 1.03 (3H, s, 10β-CH_3), 2.39-2.47 (br. m, *W* 31.6 Hz, 6'α-H or 6'β-H), 2.49 (1H, d, *J* 6.6 Hz, 5α-H), 2.56 (1H, dd, *J* 12.1, 5.5 Hz, 7β-H), 2.98 (1H, dt, *J* 14.6, 2 × 7.1 Hz, 4α-H), 5.98 (1H, s, 4'-H), δ_C (100 MHz) 12.0 (q, C-18), 14.7 (q, C-19), 18.6 (q, C-21), 21.2 (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), 28.1 (t, C-16), 35.4 (t, C-2), 35.7 (d, C-20), 35.9 (t, C-22), 36.1 (t, C-1), 36.9 (t, C-12), 39.0 (d, C-8), 39.4 (t, C-7), 39.5 (t, C-24), 40.6 (s, C-10), 41.6 (t, C-6′), 42.9 (s, C-13), 45.7 (d, C-4), 49.0 (d, C-5), 55.2 (d, C-9), 56.1 (d, C-17), 56.5 (d, C-14), 124.4 (d, C-4′), 163.4 (s, C-6), 196.5 (s, C-5′), 211.2 (s, C-3) (Found: C, 81.7; H, 10.6%, M^+, 438. $C_{30}H_{46}O_2$ requires C, 82.2; H, 10.5%, *M*, 438).

4β,5α-Dihydrobenzo[4. 5.6]cholestan-3,5'(6'H)-dione 90

Aqueous 2*M*-potassium hydroxide (0.5 cm^3) was added to 4α,5α-dihydrobenzo[4.5.6]cholestan-3,5'(6'H)-one (200 mg, 0.46 mmol) in ethanol (12 cm^3) at 25 °C under nitrogen. After 30 min solid carbon dioxide was added, and the mixture was taken up into ethyl acetate (*ca* 100 cm^3), washed with brine (15 cm^3) and dried ($MgSO_4$).The solvent was evaporated under reduced pressure and the crude product (205 mg) was flash chromatographed on silica gel using ethyl acetate-toluene (40:60) as eluent affording 4β,5α-dihydrobenzo[4.5.6]cholestan-3,5'(6'H)-dione **90** (190 mg, 95%), mp 129-132 °C (from methanol); $[\alpha]_D$ -86.6 (c 1.0) (lit.,[31] mp 133-136 °C, $[\alpha]_D$ -84); ν_{max} 1712 (3-C=O), 1669 (5′-C=O), 1617 (C=C) cm^{-1}; δ_H (400 MHz) 0.69 (3H, s, 13β-CH_3), 0.86 (6H, 2 × d, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 0.91 (3H, d, *J* 6.4 Hz, 21-CH_3), 1.05 (3H, s, 10β-CH_3), 2.21 (1H, dt, *J* 12.1, 2 × 1.9 Hz, 5α-H), 2.30 (1H, dd, *J* 16.4, 13.7 Hz, 6'α-H), 2.42 (1H, ddd, *J* 15.0, 5.7, 2.2 Hz, 7β-H), 2.58 (1H, dd, *J* 16.3, 3.6 Hz, 6'β-H), 2.98 (1H, td, *J* 2 × 12.8, 3.4 Hz, 4β-H), 5.88 (1H, t, *J* 2 × 2.4 Hz, 4'-H); δ_C (100 MHz) 12.0 (C-18), 13.1 (C-19), 18.7 (C-21), 21.2 (C-11), 22.5 (C-26), 22.8 (C-27), 23.8 (C-23), 24.0 (C-15), 28.0 (C-25), 28.1 (C-16), 34.1 (C-8), 35.7 (C-20), 36.1 (C-1), 36.2 (C-22), 37.5 (C-12), 37.6 (C-10), 38.0 (C-7), 39.3 (C-2), 39.5 (C-24), 39.7 (C-6′), 42.6 (C-4), 44.7 (C-13), 52.6 (C-5), 52.7 (C-9), 56.1 (C-17), 56.5 (C-14), 126.4 (C-4'), 161.7 (C-6), 198.1 (C-5'), 208.8 (C-3) (Found: C, 82.3; H, 10.8%; M$^+$, 438. $C_{30}H_{46}O_2$ requires C, 82.2; H, 10.5%; *M*, 438).

(2*S*)-2-(3α-hydroxy-6-oxo-5α-cholestan-4α-yl)-3-oxobutanoic acid 1,3'-lactone 91

3α-Acetoacetoxycholest-4-en-6-one (192 mg, 0.40 mmol) dissolved in tetrahydrofuran (4 cm^3) was added dropwise to a solution of potassium *tert*-butoxide [generated *in situ* from potassium metal (14 mg, 0.36 mmol)] in *tert*-butyl alcohol (6 cm^3) at 40 °C. After 3.5 h 3*M*-hydrochloric acid (3 cm^3) was added, water was added and the aqueous phase was extracted with ethyl acetate (1 × 30 cm^3, 2 × 25 cm^3). The combined organic extracts were washed with 1*M*-hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate, and brine and dried ($MgSO_4$).The solvent was evaporated affording a crude product (169 mg) which was chromatographed on silica gel using ethyl acetate-toluene (5:95) as eluent to give the lactone **91** (165 mg, 86%), mp 164-167 °C (from acetone-methanol); $[\alpha]_D$ -11 (*c* 1.1) (lit.,[23] mp 155-157 °C, $[\alpha]_D$ -11); ν_{max} 1772 (1'-C=O), 1714 (6-C=O) cm^{-1}; δ_H (400 MHz) 0.64 (3H, s, 13β-CH_3), 0.85 (6H, 2 × d, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 0.90 (3H, d, *J* 6.6 Hz, 21-CH_3), 0.73 (3H, s, 10β-CH_3), 1.95 (1H, t, *J* 2 × 12.8 Hz, 7α-H), 2.17 (1H, d, *J* 12.6 Hz, 5α-H), 2.35 (1H, dd, *J* 13.0, 4.3 Hz, 7β-H), 2.36 (3H, s, 4'-CH_3), 3.01 (1H, dd, *J* 11.8, 4.5 Hz, 4β-H), 3.21 (1H, s, 2'-H), 4.72 (1H, td, *J* 2 × 4.5, 2.2 Hz , 3β-H); δ_C (100 MHz) 12.0 (q, C-18), 12.7 (q, C-19), 18.6 (q, C-21), 21.1 (t, C-11), 22.3 (t, C-2), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-15), 23.9 (t, C-23), 27.95 (t, C-16), 28.0 (d, C-25), 29.5 (q, AcO), 30.9 (t, C-1), 34.8 (d, C-4), 35.6 (d, C-20), 36.0 (t, C-22), 38.2 (d, C-8), 39.3 (t, C-12), 39.4 (t, C-24), 41.5 (s, C-10), 42.9 (s, C-13), 47.1 (t, C-7), 53.3 (d, C-9), 56.0 (d, C-14), 56.6 (d, C-17), 57.1 (d, C-5), 64.1 (d, C-2′), 78.0 (d, C-3), 172.4 (s, C-1′), 200.2 (s, C-3′), 209.8 (s, C-6) (Found: C, 76.6; H, 10.2; M^+, 484. $C_{31}H_{48}O_4$ requires C, 76.9; H, 9.9; *M*, 484).

Aqueous potassium hydroxide treatment of the 3β,4β-lactone 91

(a) Aqueous 2*M*-potassium hydroxide (1.7 cm^3) was added dropwise at 55 °C to (2*S*)-2-(3α-hydroxy-6-oxo-5α-cholestan-4α-yl)-3-oxobutanoic acid 1, 3’-lactone (145 mg, 0.30 mmol) in dry dimethylformamide (10 cm^3) and water (1.7 cm^3). The reaction mixture was refluxed for 2h after which it was cooled to 25 °C, diluted with water (10 cm^3) and acidified with 1*M*-hydrochloric acid (6cm^3). The mixture was extracted with diethyl ether (3 × 50 cm^3). The combined organic extracts were washed with 1*M*-hydrochloric acid (10 cm^3), water, saturated aqueous sodium hydrogen carbonate (10 cm^3), and brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure affording a crude product (153 mg). The crude product was dissolved in pyridine (2 cm^3) and acetic anhydride (4.7 cm^3) and the mixture was stirred for 3h. The reaction was quenched in an ice-water bath by the addition of saturated aqueous sodium hydrogen carbonate (10 cm^3). The aqueous phase was extracted with ethyl acetate (3 × 40 cm^3). The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate (10 cm^3), and brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure giving a crude product (160 mg) which was chromatographed on silica gel using ethyl acetate-toluene (8:92) as eluent , affording 3α-acetoxy-4β,5α-dihydrobenzo[4.5.6]cholestan-5'(6'H)-one **92** (65 mg, 45%); mp 182-185 °C; $[\alpha]_D$ -51.7 (*c* 0.9) (lit.,[23] mp 180-182 °C, $[\alpha]_D$ -50) ; ν_{max} 1732 (AcO), 1660 (C=O), 1615 (C=C) cm^{-1}; δ_H (400 MHz) 0.71 (3H, s, 13β-CH_3), 0.86 (6H, 2 × d, *J* 6.6, 6.4 Hz, 26-CH_3 and 27-CH_3), 0.91 (3H, d, *J* 6.6 Hz, 21-CH_3), 0.78 (3H, s, 10β-CH_3), 2.07 (3H, s, AcO), 2.47 (1H, dd, *J* 15.8, 4.5 Hz, 6'β-H), 4.95 (1H, m, $W_{1/2}$ 7.2 Hz, 3β-H), 5.82 (1H, t, *J* 2 × 2.3 Hz, 4'-H); δ_C (100 MHz) 12.0 (q, C-18), 12.6 (q, C-19), 18.6 (q, C-21), 20.8 (t, C-11), 21.2 (q, AcO), 22.5 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 23.95 (t, C-15), 26.0 (t, C-16), 28.0 (d, C-25), 28.1 (t, C-2), 31.9 (t, C-1), 34.1 (d, C-4), 35.4 (d, C-20), 35.7 (d, C-8), 36.1 (t, C-22), 37.3 (s, C-10), 39.5 (t, C-12 and t, C-24), 39.7 (t, C-7), 40.2 (t, C-6'), 42.6 (s, C-13), 46.7 (d, C-5), 52.7 (d, C-9), 56.1 (d, C-17), 56.7 (d, C-14), 71.3 (d, C-3), 125.5 (d, C-4'), 164.0 (s, C-6), 170.3 (s, AcO), 199.1 (s, C-5') (Found: C, 79.4; H, 10.8%; M^+, 482. $C_{32}H_{50}O_3$ requires C, 79.7; H, 10.4%; *M*, 482); followed by 3α-acetoxy-6-hydroxy-4β,4',5α,6β-tetrahydrobenzo[4.5.6]cholestan-5'(6'H)-one **93** (70 mg, 46%), mp 168-171 °C; $[\alpha]_D$ -23 (*c* 0.9) (lit.,[23] mp 167-170 °C, $[\alpha]_D$ -22); δ_H (400 MHz) 0.69 (3H, s, 13β-CH_3), 0.86 (6H, 2 × d, *J* 2 × 6.6 Hz, 26-CH_3 and 27-CH_3), 0.91 (3H, d, *J* 6.6 Hz, 21-CH_3), 1.04 (3H, s, 10β-CH_3), 2.09 (3H, s, AcO), 1.66 (1H, d, *J* 12.1 Hz, 5α-H), 2.21 (1H, dd, *J* 13.3, 1.9 Hz, 4'β-H), 2.43 (1H, d, *J* 13.6 Hz, 4'α-H), 4.92 (1H, q, *J* 3 × 2.9 Hz, 3β-H); δ_C (100 MHz) 12.1 (q, C-18), 15.9 (q, C-19), 18.7 (q, C-21), 20.8 (t, C-11), 21.3 (q, AcO), 22.5 (q, C-26), 22.8 (q, C-27), 23.9 (t, C-23), 24.2 (t, C-15), 26.0 (t, C-16), 28.0 (d, , C-25), 28.2 (t, C-1), 31.4 (d, C-8), 34.6 (t, C-2), 35.8 (d, C-20), 36.2 (t, C-22 and d, C-4), 36.9 (s, C-10), 39.5 (t, C-24), 39.8 (t, C-12), 42.7 (s, C-13), 43.3 (t, C-7), 43.4 (t, C-6'), 48.1 (d, C-5), 54.2 (d, C-9), 55.9 (d, C-17), 56.3 (d, C-14), 56.8 (t, C-4'), 72.3 (d, C-3), 75.6 (s, C-6), 170.4 (s, AcO), 209.2 (s, C-5') (Found: C, 76.6; H, 10.7%; M^+, 500. $C_{32}H_{52}O_4$ requires C, 76.8; H, 10.4%; *M*, 500).

(b) The lactone **91** (108 mg, 0.22 mmol) was dissolved in ethanol (7.4 cm^3) and water (1.2 cm^3) and aqueous 2*M*-potassium hydroxide was added dropwise. The mixture was refluxed under nitrogen for 1.5 h and cooled to 25 °C. 1*M*-Hydrochloric acid (5 cm^3) was added, the mixture was partially evaporated under reduced pressure and water (8 cm^3) was added. The aqueous phase was extracted with ethyl acetate (3 × 25 cm^3), the combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (10 cm^3), and brine and dried ($MgSO_4$). The crude product (75 mg) was dissolved in pyridine (3 cm^3) and acetic anhydride (7 cm^3) and stirred at 25 °C for 24 h. Saturated aqueous sodium hydrogen carbonate was added and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (80 mg) which was flash chromatographed on silica gel using ethyl acetate-hexane (8:92) as eluent to give **93** (60 mg, 54%).

6-Methylenecholest-4-en-3-one 95

Cholest-4-en-3-one (6 g, 15.6 mmol) and sodium acetate (6 g, 73.1 mmol) were flame-dried under nitrogen in a three-necked 1-litre round bottom flask. Chloroform (180 cm^3) and formaldehyde dimethyl acetal (180 cm^3) was added and the mixture was refluxed with vigorous stirring. Freshly distilled phoshoryl chloride (30 cm^3) was added dropwise. The reaction mixture was refluxed for 18h, cooled to 25 °C and saturated aqueous sodium hydrogen carbonate (*ca* 30g in 300 cm^3) was added dropwise to the reaction mixture contained in an ice-water bath. The organic layer was separated, washed with water (100 cm^3), and brine (100 cm^3) and dried ($MgSO_4$). The solvent was evaporated and excess solvent was removed under reduced pressure and in a vacuum oven at 58 °C to give a crude product (12.6 g) which was flash chromatographed on silica gel using ethyl acetate-hexane (5:95) as eluent, affording 6-methylenecholest-4-en-3-one **95** (3.1 g, 50%), mp 118-121 °C (from hexane); $[\alpha]_D$ +252 (*c* 0.9) (lit.,[67] mp 122 °C, $[\alpha]_D$ +251); ν_{max} 1655 (C=O), 1602 (C=C) cm^{-1}; δ_H (400 MHz) 0.70 (3H, s, 13β-CH_3), 0.86 (6H, 2 × d, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 0.91 (3H, d, *J* 6.6 Hz, 21-CH_3), 1.08 (3H, s, 10β-CH_3), 5.84 (1H, s, 4-H), 4.91and 5.03 (1H each, 2 × t, *J* 2 × 2.1 Hz, 6'-H_2); δ_C (100 MHz) 11.9 (q, C-18), 17.1 (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), 24.1 (t, C-15), 28.0 (d, C-25), 28.1 (t, C-12), 33.8 (t, C-7), 35.1 (t, C-2), 35.7 (d, C-8 and d, C-20), 36.1 (t, C-22), 39.1 (s, C-10), 39.5 (t, C-1 and t, C-24), 40.1 (t, C-16), 42.4 (s, C-13), 52.5 (d, C-9), 56.1 (d, C-14), 56.2 (d, C-17), 113.8 (t, C-6'), 121.5 (d, C-4), 146.4 (s, C-5), 169.4 (s, C-6), 199.9 (s, C-3) (Found: C, 84.7; H, 11.5 %; M^+, 396. $C_{28}H_{44}O$ requires C, 84.8; H, 11.1%; *M*, 396).

6-Methylenecholest-4-en-3β-ol 96

6-Methylene-cholest-4-en-3-one (3.74 g, 9.4 mmol) dissolved in dry tetrahydrofuran (130 cm^3) was added dropwise to a vigorously stirred suspension of lithium aluminium hydride (0.72 g, 18.87 mmol) in tetrahydrofuran (278 cm^3) at 5 °C. The reaction mixture was stirred at 25 °C for 2 h. Saturated aqueous ammonium chloride (65 cm^3) was added at 0 °C, and brine (100 cm^3) was added. The aqueous layer was extracted with dichloromethane (3 × 200 cm^3). The dichloromethane layer was washed with brine (200 cm^3), and dried ($MgSO_4$). The crude product was chromatographed on silica gel using ethyl acetate-hexane (10:90) as eluent to give *6-methylenecholest-4-en-3β-ol* **96** (3.1 g, 83%), mp 124-127 °C (from ethyl acetate); $[\alpha]_D$ +130 (*c* 0.9); ν_{max} 3598 (OH) cm^{-1}; δ_H (400 MHz) 0.67 (3H, s, 13β-CH_3), 0.86 (3H, 2 × d, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 0.90 (3H, d, *J* 6.6 Hz, 21-CH_3), 0.94 (3H, s, 10β-CH_3), 2.33 (1H, dd, *J* 13.1, 3.9 Hz, 7β-H), 4.21 (1H, ddd, *J* 10.0, 6.1, 2.2 Hz, 3α-H), 4.60 and 4.81 (1H each, 2 × t, *J* 2 × 2.4 Hz, 6'-H_2), 5.48 (1H, t, *J* 2 × 1.8 Hz, 4-H); δ_C (100 MHz) 12.0 (q, C-18), 18.7 (q, C-19), 19.0 (q, C-21), 21.0 (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-12), 29.1 (t, C-2), 34.7 (t, C-7), 35.7 (d, C-20), 36.1 (d, C-8), 36.1 (t, C-22), 37.8 (s, C-10), 39.5 (t, C-24), 39.7 (t, C-1), 40.7 (t, C-16), 42.5 (s, C-13), 53.2 (d, C-9), 56.1 (d, C-17), 56.4 (d, C-14), 67.7 (d, C-3), 109.3 (t, C-6'), 123.1 (d, C-4), 148.2 (s, C-5), 149.4 (s, C-6) (Found: C, 84.0; H, 12.0%; M^+, 398. $C_{28}H_{46}O$ requires C, 84.4; H, 11.6%, *M*, 398)

6'α-Acetyl-4β,4',5'β,6'-tetrahydrobenzo[4.5.6]cholestan-3β-yl acetate 97

6-Methylenecholest-4-en-3β-ol (250 mg, 0.63 mmol), benzene (5 cm^3) and methyl vinyl ketone (0.42 cm^3) was placed in a sealed tube and heated at 120-125 °C for 24 h. The crude product (388 mg) was dissolved in pyridine (6 cm^3) and acetic anhydride (14 cm^3) was added. This solution was stirred at 25 °C for 3 days after which saturated aqueous sodium hydrogen carbonate was added. The aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate, and brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (376 mg) which was flash chromatographed on silica gel using ethyl acetate-hexane (25:75) as eluent affording material which comprised a mixture of cycloadducts (162 mg). Recrystallisation from ethyl acetate-methanol afforded **97** (130 mg, 40 %), mp 200-204 °C (from ethyl acetate-methanol); $[\alpha]_D$ -91 (*c* 1.0); ν_{max} 1729 (AcO), 1709 (C=O) cm^{-1}; δ_H (400 MHz) 0.68 (3H, s, 13β-CH_3), 0.91 (3H, d, *J* 6.5 Hz, 21-CH_3), 0.86 (6H, 2 × d, *J* 6.6 Hz, 26-CH_3 and 27-CH_3), 1.09 (3H, s, 10β-CH_3), 1.91 (3H, s, AcO), 2.23 (3H, s, 6′α-CH_3CO), 2.46 (1H, ddd, *J* 12.4, 5.1, 5.1 Hz, 6'β-H), 3.08 (1H, dd, *J* 11.2, 4.6 Hz, 4β-H), 4.65 (1H, ddd, *J* 11.2, 10.0, 4.5 Hz, 3α-H); δ_C (100 MHz) 11.8 (q, C-18), 18.7 (q, C-21), 19.1 (t, C-4'), 19.5 (q, C-19), 20.7 (q, AcO), 21.4 (t, C-11), 22.6 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 24.3 (t, C-15), 28.0 (d, C-25), 28.8 (t, C-2), 29.1 (q, C6'-$\underline{\mathit{CH_3}}$CO), 30.9 (t, C-5'), 31.7 (d, C-8), 35.8 (d, C-20), 36.2 (t, C-22), 37.6 (t, C-7), 38.0 (t, C-1), 38.1 (s, C-10), 39.5 (t, C-24), 39.7 (t, C-12), 40.8 (d, C-4), 42.2 (s, C-13), 50.6 (d, C-9), 51.1 (d, C-6'), 56.1 (d, C-17), 56.6 (d, C-14), 73.8 (d, C-3), 130.9 (s, C-6), 133.2 (s, C-5), 169.8 (s, AcO), 210.3 (s, C6'-$CH_3\underline{\mathit{CO}}$) (Found: C, 80.0; H, 10.8%; M^+-60, 450. $C_{34}H_{54}O_3$ requires C, 80.0; H, 10.6%; *M*, 510).

3β,6'-Epoxymethano-4β,4',5',6'-tetrahydrobenzo[4.5.6]cholestan-6'^{1}β-yl acetate 98

6-Methylenecholest-4-en-3β-ol (200 mg, 0.50 mmol), benzene (4 cm^3) and acrolein (0.2 cm^3) were heated in a sealed tube at 90-95 °C for 24 h. The crude product (239 mg) was flash chromatographed on silica gel using ethyl acetate-hexane (20:80) as eluent affording a single product (TLC), (115 mg). This was dissolved in pyridine (3 cm^3) and acetic anhydride (7.2 cm^3) and stirred at 25 °C for 24 h. Saturated aqueous sodium hydrogen carbonate (15 cm^3) was added. The aqueous phase was extracted with ethyl acetate, and the combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate, and brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a crude product (239 mg) which was flash chromatographed on silica gel using ethyl acetate-hexane (10:90) as eluent affording **98**, (105 mg, 42%), mp 155-159 °C (from acetone); $[\alpha]_D$ -35 (*c* 0.9); ν_{max} 1736 (C=O), 3685 (OH) cm^{-1}; δ_H (400 MHz) 0.67 (3H, s, 13β-CH$_3$), 0.86 (6H, 2 × d, *J* 6.7, 6.5 Hz, 26-CH$_3$ and 27-CH$_3$), 0.91 (3H, d, *J* 6.7 Hz, 21-CH$_3$), 2.09 (3H, s, AcO), 2.25 (1H, ddd, *J* 13.5, 8.1, 5.2 Hz, 6'α-H or 6'β-H), 2.36 (1H, dd, *J* 10.6, 8.8 Hz, 4β-H), 3.41 (1H, td, *J* 2 × 11.1, 3.5 Hz, 3α-H), 5.90 (1H, d, *J* 1.0 Hz, 6$^{'1}$α-H); δ_C (100 MHz) 11.8 (q, C-18), 18.7 (q, C-21), 19.9 (q, C-19), 21.3 (q, AcO), 21.4 (t, C-11), 21.9 (t, C-5'), 22.6 (q, C-26), 22.8 (q, C-27), 23.8 (t, C-23), 24.4 (t, C-15), 28.0 (d, C-25), 28.2 (t, C-16), 28.5 (t, C-2), 29.0 (t, C-4′), 32.5 (d, C-8), 35.8 (d, C-20), 36.2 (t, C-7), 36.7 (t, C-22), 37.9 (s, C-10), 39.5 (t, C-24), 39.5 (t, C-1), 39.7 (t, C-12), 42.2 (d, C-4), 42.2 (s, C-13), 43.2 (d, C-6′), 50.6 (d, C-9), 56.2 (d, C-17), 56.6 (d, C-14), 84.8 (d, C-3), 103.4 (d, C-6$^{'1}$), 127.2 (s, C-6), 132.6 (s, C-5), 170.3 (s, AcO) (Found: C, 80.0; H, 10.8 %, M$^+$, 496. C$_{33}$H$_{52}$O$_3$ requires C, 79.8; H, 10.5%, *M*, 496).

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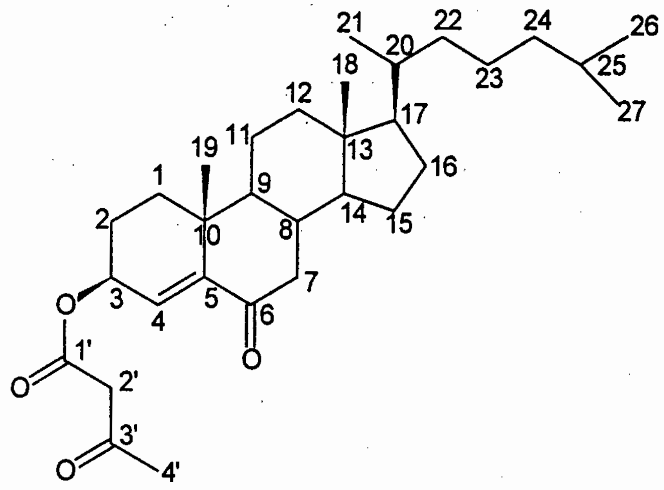
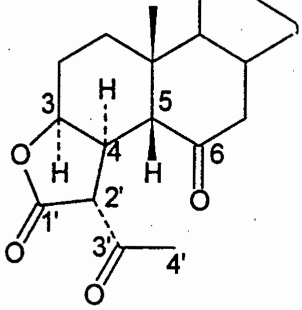
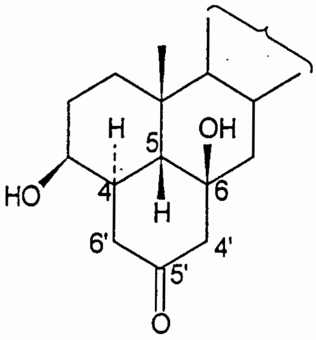
CHAPTER 5

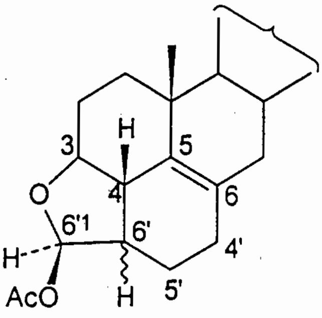
APPENDIX

The steroids represented in this dissertation are named according to the rules of IUPAC nomenclature for organic compounds. The relevant numbering for structures in which extended numbering was necessary are indicated below.

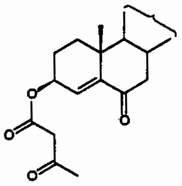
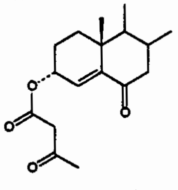
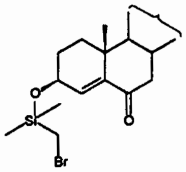
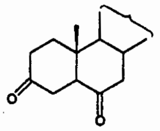




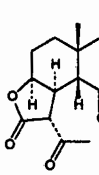
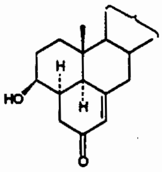
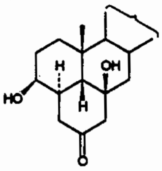
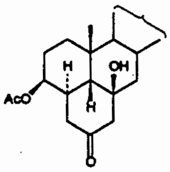


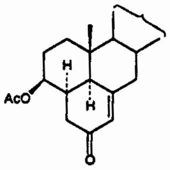
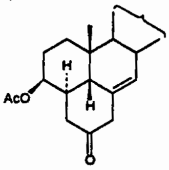
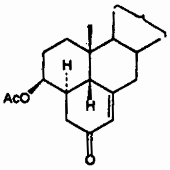
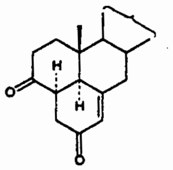


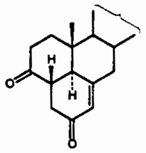
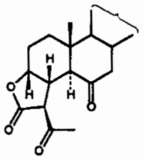
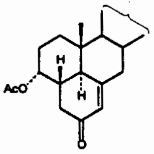
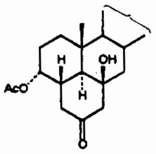
TABULATED ^{13}C NMR DATA

	δC (ppm)			
LOCANT	**71**	**72**	**76**	**78**
				
C1	34.3	30.7	34.8	38.1
C2	23.9	23.8	28.4	37.4
C3	70.4	66.8	68.4	209.1
C4	127.7	125.1	133.0	46.6
C5	148.5	151.4	146.2	57.5
C6	200.2	200.3	202.5	211.2
C7	50.1	46.4	46.3	37.0
C8	34.1	34.0	34.0	38.0
C9	51.2	50.9	51.2	53.5
C10	38.3	38.7	38.1	41.2
C11	20.7	21.3	20.8	21.7
C12	39.4	39.3	39.4	28.0
C13	42.6	42.6	42.6	43.0
C14	56.6	56.6	56.7	56.1
C15	24.0	24.2	23.9	24.0
C16	28.0	28.0	28.0	39.5
C17	57.0	56.0	56.0	56.6
C18	11.9	11.9	11.9	12.0
C19	19.5	18.3	19.8	12.6
C20	35.7	35.7	35.7	35.7
C21	18.6	18.6	18.6	18.6
C22	36.1	36.1	36.1	36.1
C23	23.8	23.9	23.8	23.8
C24	39.3	39.4	39.3	39.4
C25	28.0	28.0	28.0	28.0
C26	22.5	22.5	22.5	22.5
C27	22.8	22.8	22.8	22.8
C1'	166.8	166.3	-2.5[a]	-
C2'	46.3	50.2	16.2	-
C3'	202.4	203.2	-	-
C4'	30.2	30.1	-	-

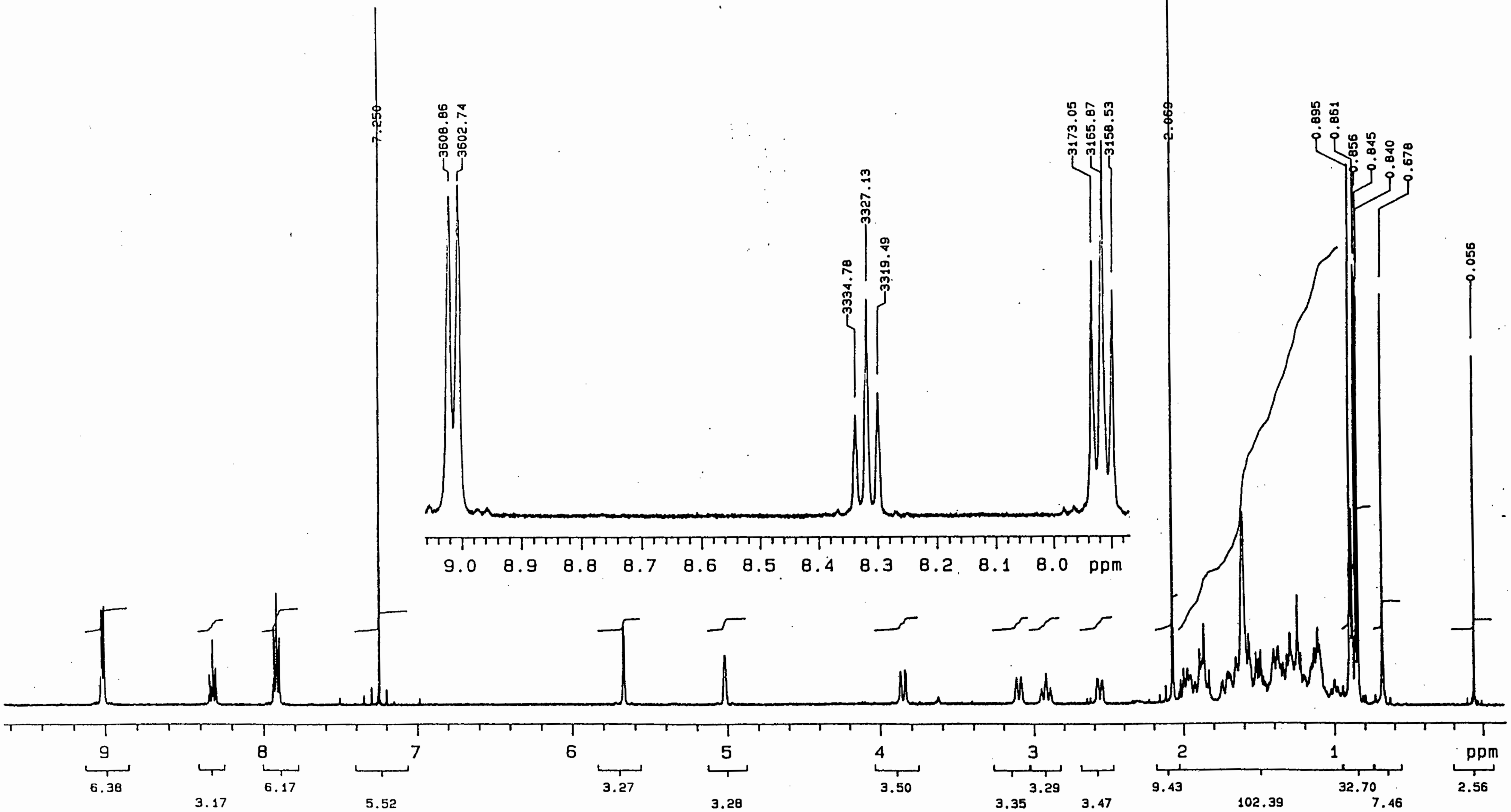
a - actually 2 distinct signals at -2.46 and -2.55 ppm

	δC (ppm)			
LOCANT	**79**	**80**	**81**	**82**
				
C1	28.3	35.6	31.0	31.7
C2	21.7	26.6	28.3	24.9
C3	77.4	71.3	69.6	72.5
C4	36.7	38.1	38.3	36.8
C5	58.9	49.8	50.4	51.1
C6	212.2	165.8	76.6	76.2
C7	42.7	34.0	39.2	39.2
C8	36.5	39.6	32.4	32.4
C9	40.8	56.1	40.6	40.7
C10	37.5	40.8	36.7	36.6
C11	20.9	21.0	21.0	21.0
C12	39.4	39.4	40.0	40.0
C13	43.0	43.0	42.9	42.9
C14	56.1	56.8	56.3	56.3
C15	23.8	24.1	24.1	24.1
C16	28.0	28.1	28.3	28.2
C17	56.6	56.1	56.0	55.9
C18	11.9	12.1	12.15	12.1
C19	23.2	15.6	27.2	27.2
C20	35.6	35.7	35.8	35.8
C21	18.6	18.6	18.7	18.7
C22	36.0	36.1	36.1	36.1
C23	23.8	23.8	23.9	23.8
C24	39.4	39.5	39.5	39.5
C25	27.9	28.0	28.0	28.0
C26	22.5	22.5	22.5	22.5
C27	22.8	22.8	22.8	-
C1′	170.9	-	-	-
C2′	62.4	-	-	-
C3′	199.2	-	-	-
C4′	28.7	124.3	58.3	58.2
C5′	-	200.2	208.8	207.4
C6′	-	41.4	45.2	44.6
AcO (CH_3)	-	-	-	21.2
AcO (C=O)	-	-	-	170.4

δC (ppm)				
LOCANT	**85**	**83**	**86**	**89**
				
C1	35.4	29.1	31.0	36.1
C2	23.0	24.95	25.6	35.4
C3	73.2	71.3	71.0	211.2
C4	34.6	37.2	34.2	45.7
C5	49.5	47.4	45.5	49.0
C6	165.5	133.9	168.3	163.4
C7	34.6	124.6	34.6	39.4
C8	39.5	41.1	37.6	39.0
C9	56.0	40.2	47.3	55.2
C10	40.7	34.0	36.7	40.6
C11	20.95	20.8	22.0	21.2
C12	39.4	39.95	39.8	36.9
C13	43.0	43.2	43.6	42.9
C14	56.7	56.1	56.2	56.5
C15	24.1	23.8	23.9	24.1
C16	28.1	28.3	28.0	28.1
C17	56.1	54.5	55.3	56.1
C18	12.1	12.0	12.2	12.0
C19	15.5	21.1	21.1	14.7
C20	35.7	35.7	35.7	35.7
C21	18.7	18.6	18.6	18.6
C22	36.1	36.1	36.1	35.9
C23	23.0	23.8	23.8	23.8
C24	39.5	39.5	39.5	39.5
C25	28.0	28.0	28.0	28.0
C26	22.6	22.5	22.6	22.5
C27	22.8	22.8	22.8	22.8
C1'	-	-	-	-
C2'	-	-	-	-
C3'	-	-	-	-
C4'	124.2	52.8	126.9	124.4
C5'	199.6	207.4	198.8	196.5
C6'	41.4	44.8	39.8	41.6
AcO (CH_3)	21.1	23.4	22.6	-
AcO (C=O)	170.3	170.4	170.4	-

δC (ppm)				
LOCANT	**90**	**91**	**92**	**93**
				
C1	36.1	30.9	31.9	28.2
C2	39.3	22.3	28.1	34.6
C3	208.8	78.0	71.3	72.3
C4	42.6	34.8	34.1	36.2
C5	52.6	57.1	46.7	48.1
C6	161.7	209.8	164.0	75.6
C7	38.0	47.1	39.7	43.3
C8	34.1	38.2	35.7	31.4
C9	52.7	53.3	52.7	54.2
C10	37.6	41.5	37.3	36.9
C11	21.2	21.1	20.8	20.8
C12	37.5	39.3	39.5	39.8
C13	44.7	42.9	42.6	42.7
C14	56.5	56.0	56.7	56.3
C15	24.0	23.8	23.95	24.2
C16	28.1	27.95	26.0	26.0
C17	56.1	56.6	56.1	55.9
C18	12.0	12.0	12.0	12.1
C19	13.1	12.7	12.6	15.8
C20	35.7	35.6	35.4	35.8
C21	18.7	18.6	18.6	18.7
C22	36.2	36.0	36.1	36.2
C23	23.8	23.9	23.8	23.9
C24	39.5	39.4	39.5	39.5
C25	28.0	28.0	28.0	28.0
C26	22.5	22.5	22.5	22.5
C27	22.8	22.8	22.8	22.8
C1′	-	172.4	-	-
C2′	-	64.1	-	-
C3′	-	200.2	-	-
C4′	126.4	29.5	125.5	56.8
C5′	198.1	-	199.1	209.2
C6′	39.7	-	40.2	43.4
AcO (CH_3)	-	-	21.2	21.3
AcO (C=O)	-	-	170.3	170.4

400 MHz ^{1}H NMR spectrum of unidentified product obtained from the thionyl chloride-pyridine dehydration



Expanded methyl region of unidentified product