A Thesis

entitled

"Functionalisation of Non-Activated Positions in Steroids"

Submitted in part fulfilment of the requirements

for admittance to the degree of

Doctor of Philosophy

in

The University of Glasgow

by

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The work was carried out during the tenure of an S.E.R.C. Research Studentship, which is gratefully acknowledged.

Conventions, Nomenclature and Samples

In drawing of structures, the stereochemistry is not implied unless specifically indicated; a thickened or dotted bond donates a substituent located respectively above or below the plane of the paper.

Trivial name	Systematic name
lanosterol	5α-lanosta-8,24-dien-3β-ol
24,25-dihydrolanosterol	5α-lanost-8-en-3β-ol
5α-cholestan-3α-yl nicotinate	5α-cholestan-3α-yl pyridine-3-carboxylate
cortisone	17α,21-dihydroxypregn-4-ene-3,11,20-trione

Samples - sources of reference compounds

5α-cholest-2-ene)
) from Steraloids
19-hydroxycholest-5-en-3β-yl acetate)

cholest-5-ene - prepared by Dr. G. Steel

5α-cholest-7-en-3β-ol - from Ikapharm

5α-cholest-8(14)-en-3β-ol - from Makor

3β-acetoxy-5α-cholestan-7-one and
3β-acetoxy-5α-cholestane-6,7-dione were prepared by Dr. I.V. Ekhato

24(R,S)-hydroxycholest-5-en-3β-yl acetate and

25-hydroxycholesterol - donated by Dr. J. Redpath, Organon

5β-cholan-24-oic acid and

5β-cholan-24-ol - prepared by Dr. R.A. Anderson

polyporenic acid A was a gift to C.J.W. Brooks from G.W. Elson, Akers Research Laboratory

agnosteryl acetate was donated by Dr. G.F. Woods, Organon

dihydroagnosteryl acetate was donated by Prof. Sir Derek Barton.

The following samples were obtained from Prof. D.N. Kirk, MRC Steroid Reference Collection:

3β-acetoxy-5α-androstan-16-one

3β-acetoxy-5α-androstan-11-one

3β-acetoxy-5α-androstan-7-one

3β-acetoxy-5α-androstan-12-one

5α-androstan-17-one

5α,14α-androstan-15-one.

List of Abbreviations

Reagents etc.

AIBN - azobisisobutyronitrile

DBU - 1,8-diazabicyclo[5.4.0]undec-7-ene

mcpba - metachloroperbenzoic acid

THF - tetrahydrofuran

LAH - lithium aluminium hydride

CAN - ceric ammonium nitrate

BSTFA - N,O-bis(trimethylsilyl)-trifluoroacetamide

TMSIm - N-trimethylsilylimidazole

DMAP - 4-dimethylaminopyridine

DMSO - dimethylsulphoxide

PCC - pyridinium chlorochromate

DEAD - diethylazodicarboxylate

pyr - (C_5H_4N), pyridyl group

Techniques etc

TLC - thin layer chromatography

GLC - gas liquid chromatography

GC-MS - gas chromatography mass spectrometry

amu - atomic mass units

$\underline{I}$ - Kováts retention value

I.R. - infrared spectrum

NMR - nuclear magnetic resonance spectrum

D.E.P.T.- distortionless enhancement by polarisation transfer

C.O.S.Y.- correlated spectroscopy

2D - 2-dimensional

CI - chemical ionisation

EI - electron impact

General Experimental Procedures

Thin-layer chromatography (TLC) was carried out on glass plates (5 x 20 cm or 20 x 20 cm), coated with silica gel 60 F_{254} (supplier Merck) using 0.25 mm layer for analytical purposes. Spot detection was obtained by spraying with 1% (w/v) $Ce(SO_4)_2$ in 10% H_2SO_4 (v/v), then heating in an oven (~ 100°C) for a few minutes.

Gas-liquid chromatography (GLC) was performed on a Perkin-Elmer F33 using a 1% OV-1 (methyl siloxane) packed column (6 ft). Nitrogen was used as the carrier gas at 40 ml/min. Samples were dissolved in EtOAc (1-5 mg/ml) and aliquots were injected using a 10 µl syringe (supplier Hamilton). Capillary GLC was performed on a Hewlett-Packard 5880 gas chromatograph. Column: Cp-Sil 5CB 25 m x 0.32 mm ID, film thickness 0.11 µm. Helium carrier gas was used at 2 ml min^{-1}. A split injector was used (50:1).

n-Alkanes were used to standardise the retention data. The values (I) are Kováts retention indices.

General extraction

The material was extracted with the appropriate solvent, then the extracts were dried over anhydrous $MgSO_4$ or Na_2SO_4. After filtration, the solvent was removed under reduced pressure in a rotary evaporator.

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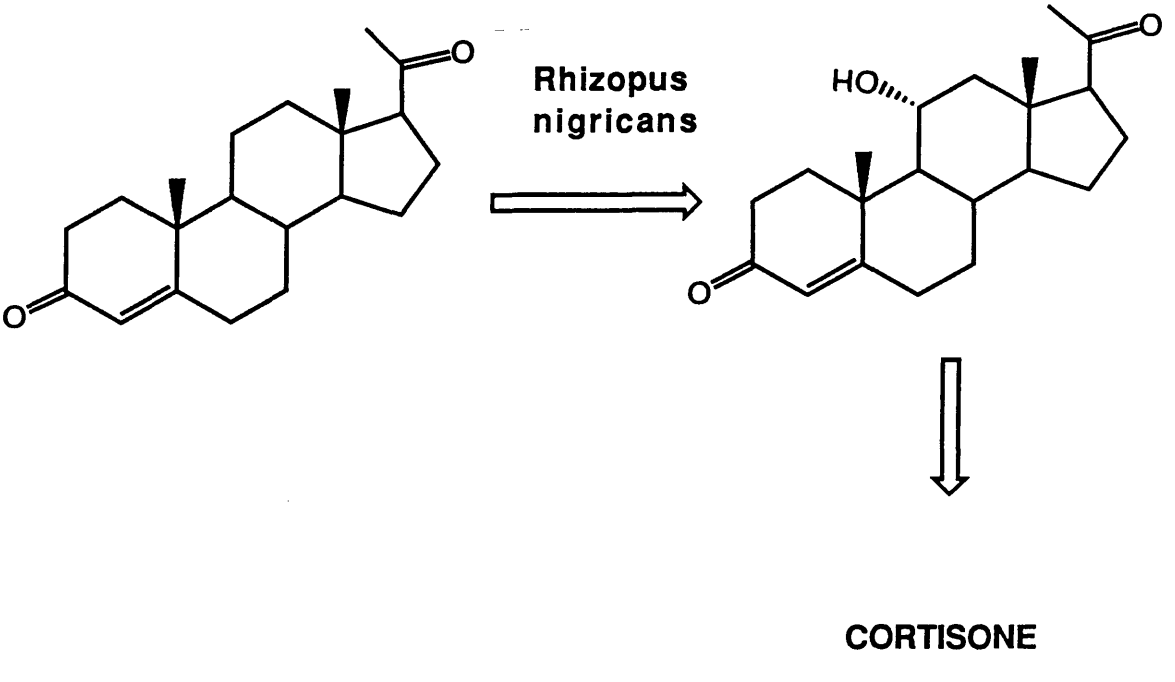
Summary

The work described in this thesis attempted to introduce functionality into non-activated positions in readily available steroids. Section 3.1 describes the ceric ammonium nitrate (CAN) oxidation of 25-hydroxy-5α-lanost-8-en-3β-yl acetate. Oxidation mainly occurs at the allylic positions and this was confirmed by the CAN oxidation of 5α-lanost-8-en-3β-yl acetate. An interesting nitrogenous compound was produced in which the starting material incorporated the solvent acetonitrile. CAN oxidation of 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate gave only dehydration products. Section 3.4 describes the functionalisation of the C-30 methyl group in 7α-hydroxy-5α-lanostan-3β-yl acetate using various reagents. Long range functionalisation, using radical relay chlorinations developed by Breslow, was attempted on the bile acid derivative, 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate. Chlorination mainly occurred at the C-14 tertiary position. CrO_3 oxidations of 5α-androstan-3β-yl acetate and 5α-cholestan-3β-yl acetate were performed in order to compare the selectivity of the oxidations. 5α-Cholestan-3β-yl acetate produced degraded steroids whereas 5α-androstan-3β-yl acetate gave the reported Δ^{14}-16-ketone as the major product.

Section 1: Introduction

Remote Functionalisation

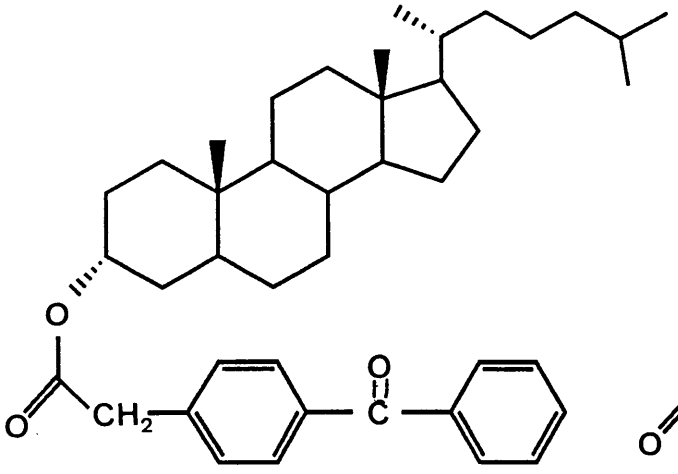
Remote functionalisation was introduced by Breslow in 1969[1] and involves generation of a functional group at a site too remote from existing functional groups for the convenient use of "normal" available reaction methods. In this work, Breslow initiated a program to introduce certain enzymatic principles into the design of specific organic functionalisation reactions. The essential idea was that the selectivity of enzymatic reactions is determined in a large part by geometric demands of the reagent, rather than by the intrinsic reactivity pattern of the substrate. Enzymes are able to carry out some remarkably selective reactions, for example, in the manufacture of corticosteroids industrially, the oxygen atom in ring C is commonly introduced by microbiological fermentation.



The high selectivity of enzymatic processes makes this a very efficient and selective oxidation of an otherwise unactivated position in the steroid. This is in marked contrast to the usual synthetic chemical style, in which functional group manipulation is used to adjust the substrate reactivity so as to produce the desired result, perhaps even with resort to more brutal chemical conditions. In the past, the effects of proximity have led to selective attack on unactivated and otherwise unreactive chemical positions. The Barton reaction,[2] for example, involves the production of a reactive heteroatom radical in a molecule which then, by intramolecular attack on a hydrogen atom located six atoms away, initiates functionalisation of a position which is not chemically activated in the usual sense. However, in Breslow's methods, a functional group within the substrate is used to attack a particular atom. Furthermore, the entropy factor favours intramolecular over intermolecular attack. Therefore, it seems possible to carry out a directed attack on a particular atom, provided the reagent and substrate (one molecule) are held fairly rigidly so that the process is not hopelessly improbable. Thus, a process in which a rigid reagent is attached to a substrate, allowing directed functionalisation of that substrate at a relatively large distance from the point of attachment, has been termed "remote oxidation" by Breslow.

1.1 Remote Oxidation of Steroids by Photolysis of Attached Benzophenone Groups[3,4,5,6,7]

The reagents which were explored in detail are derivatives of benzophenone which carry carboxyl groups, so that they can be temporarily attached to steroidal alcohols as esters. On irradiation, the benzophenone is excited to its triplet state, in which the oxygen atom is capable of attacking unactivated C-H bonds. This process was first explored using flexible substrates,[1] but such a procedure introduces a number of special difficulties because of the randomness of the initially attacked position. The benzophenone-4-acetic acid ester of 5α-cholestan-3α-ol (1) gives strikingly specific steroid functionalisation. Photochemical dehydrogenation is directed exclusively into ring D, affording the Δ^{14} olefin (2) (55% yield),[6] in which the double bond is quite remote from the original functionality. Isotope labelling studies[7] show the reaction sequence (see p. 6). Molecular models are consistent with this process, which is not only regiospecific in its introduction of the 14,15 double bond, but also stereospecific in respect of the hydrogen atom it removes at C-15. The selectivity is induced by the geometry of the system, specifically the matching of C-3 oxygen to C-14 hydrogen distance in the parent substrate with the carbonyl-to-ketone distance in the attached reagent. Other products identified were two lactones (3 and 4) formed by removal of hydrogen atoms at C-7 and C-12 and coupling of the resulting diradicals.



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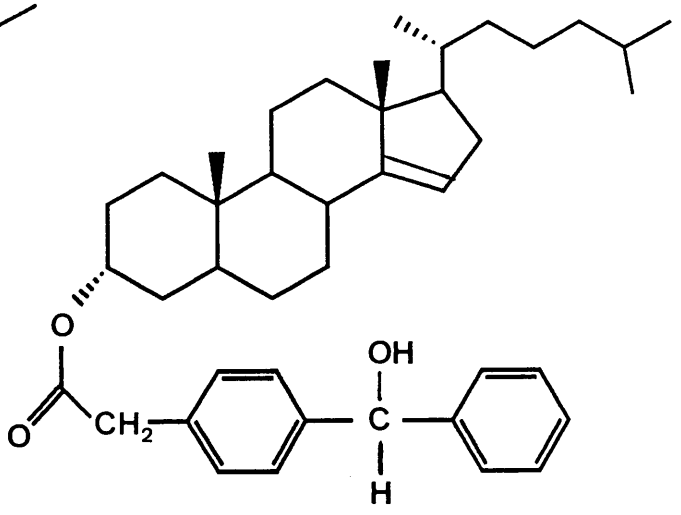

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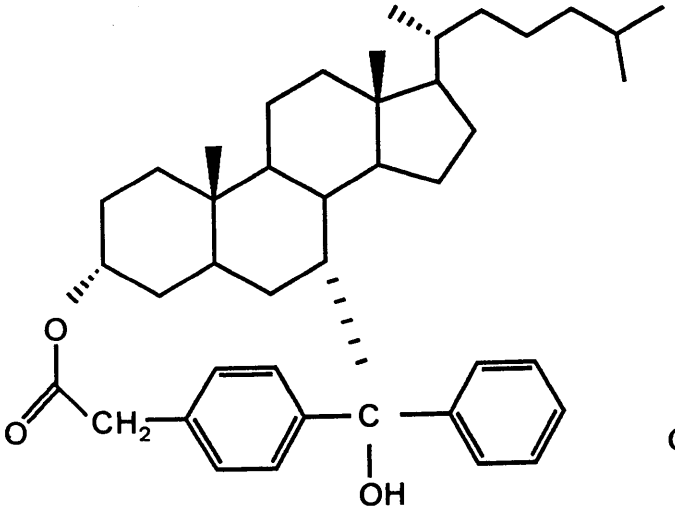

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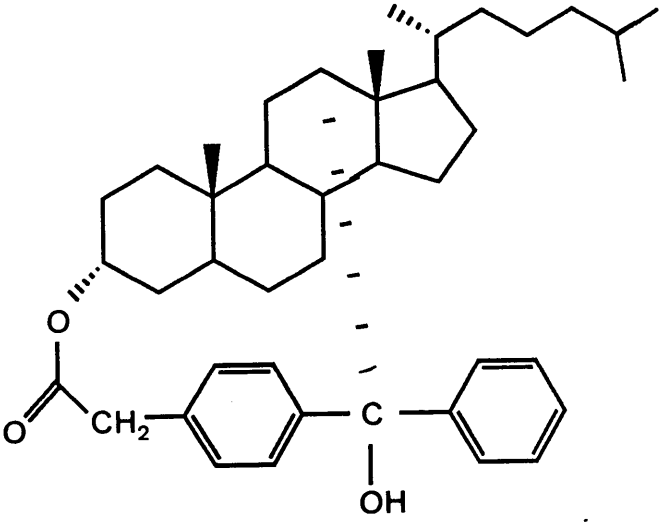

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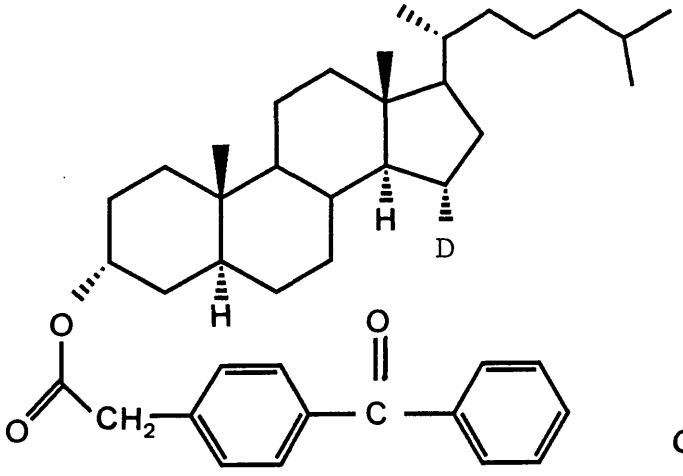
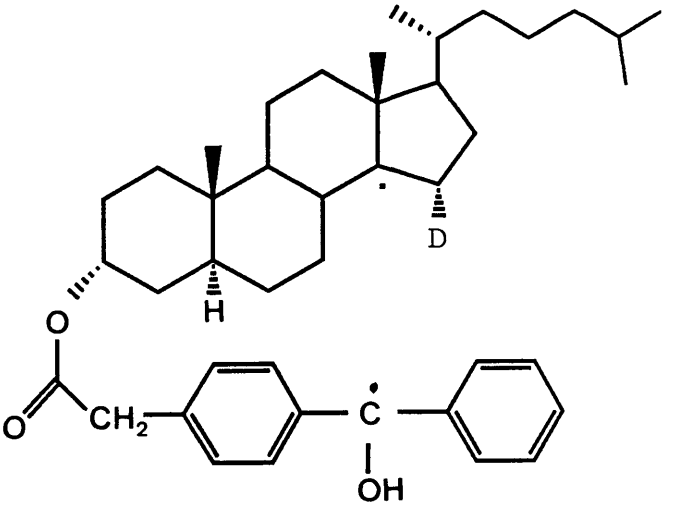
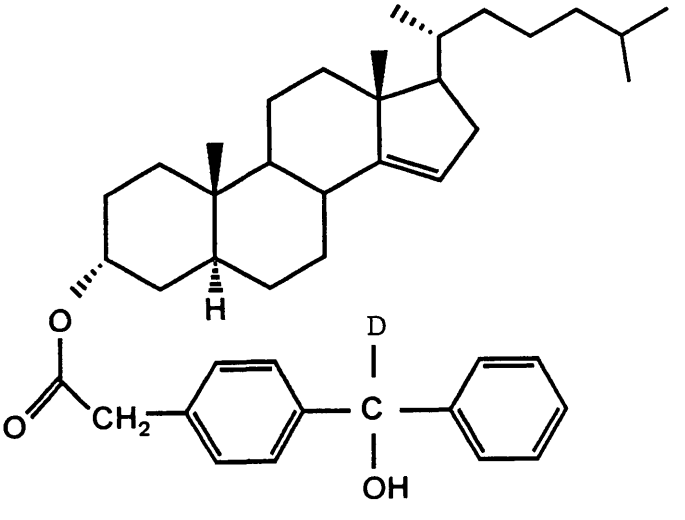
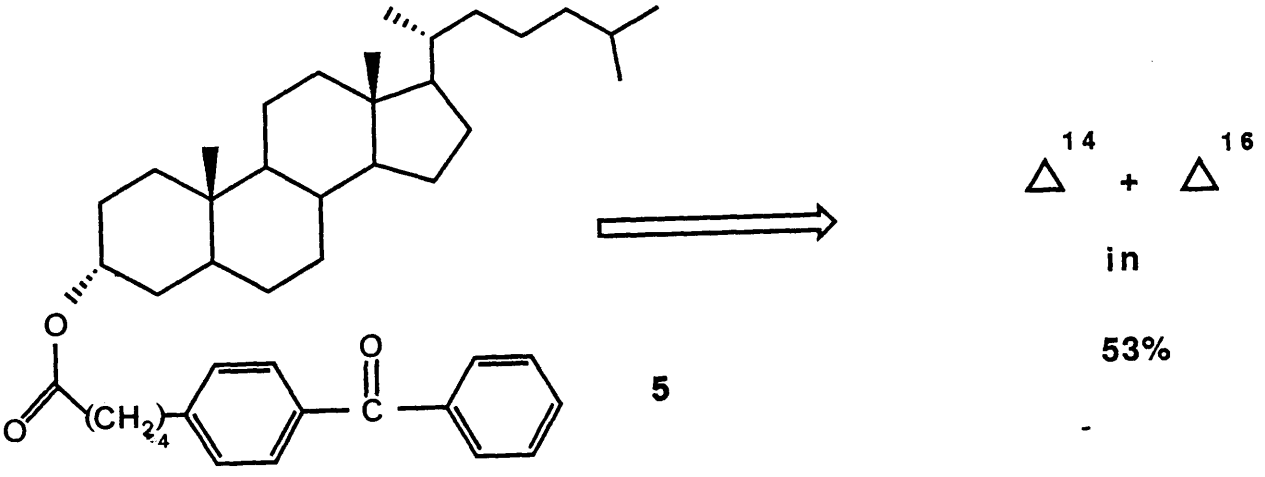
Reaction Sequence



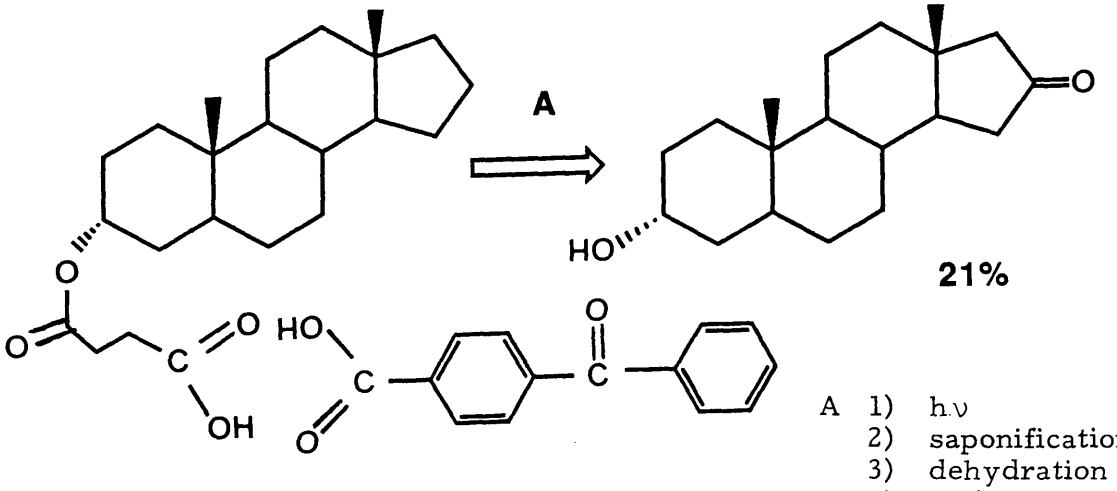


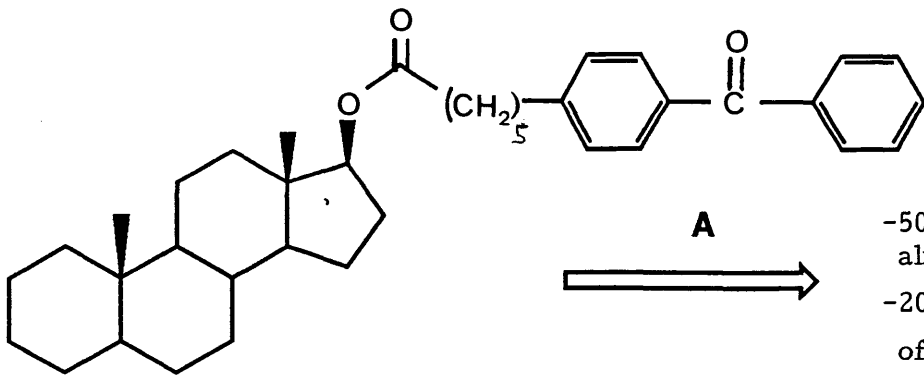


Longer chain benzophenone reagents were examined mainly by Breslow et al.[6] In work analogous to that of Breslow, Baldwin et al.[8] found that with the longer chain reagent of compound 5, attack was seen on an even more remote hydrogen at C-17, although the flexibility of the link still permitted some attack at C-14.



In another study,[4] the hydrogen-bonded complex 6 (with two hydrogen bonds, in non-polar media) underwent rather selective attack on photolysis, but due to residual freedom of motion in the reagent-substrate complex, this led to some randomness of attack.





A

-50% unfunctionalised steroid

-20% 3.3:1.0 mixture of $\Delta^{9(11)}$- and Δ^{14}-5α-androstan-17β-ols

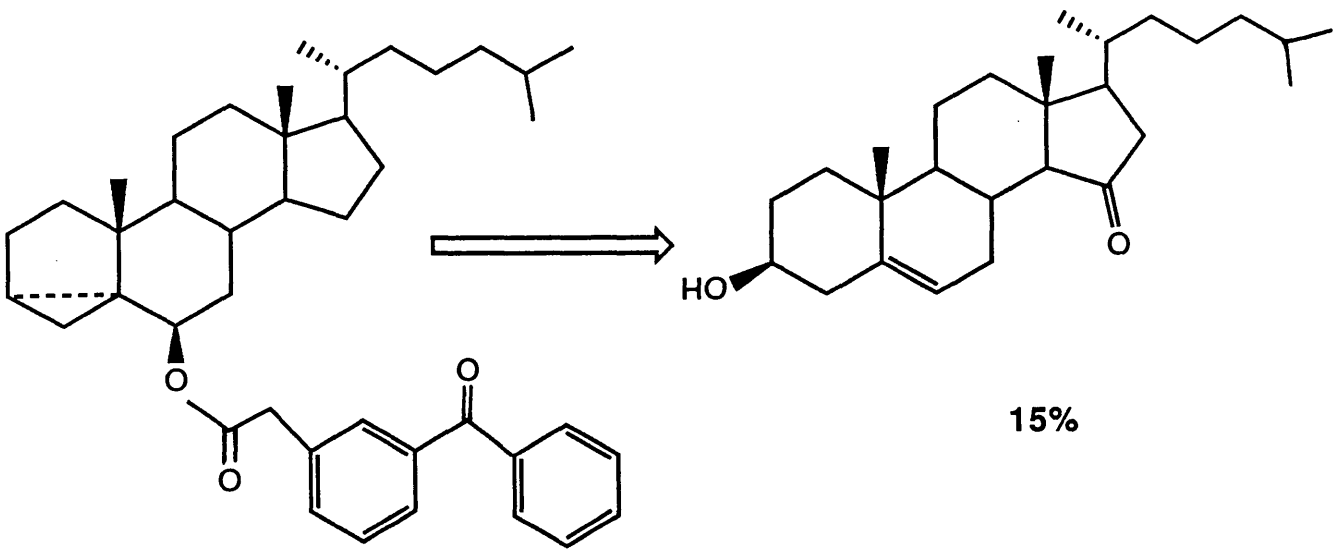
7

A 1) hν
2) $Pb(OAc)_4$/hydrolysis

Benzophenone Attached to Groups on Ring D

When the benzophenone-4-hexanoate (7) of 5α-androstan-17β-ol was photolysed in 1,1,2-trifluorotrichloroethane, an intramolecular functionalisation reaction occurred. In (7), the ester is originally on the β-side of the steroid, but it is able to curl under and permit the benzophenone to attack the α-face.

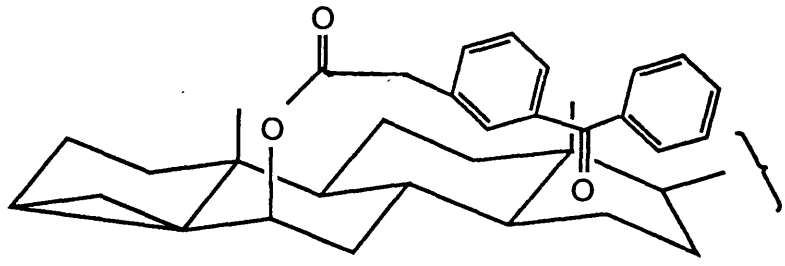
However, recent work[9] has shown that remote functionalisation can even be achieved on the steroid β-face. For example 6β-(3'-benzoylphenyl)acetoxy-3α,5α-cyclocholestane (8) was prepared from the parent 6β-ol ("i-cholesterol") and m-benzoylphenyl acetic acid, then irradiated.



8

15%

After ca. 6h, two unstable photo-products were formed, which were directly reduced by LAH, acetylated, and dehydrated by thionyl chloride; then successive ruthenium(VIII) oxide cleavage and acid treatment afforded 15-ketocholesterol. The achievement of this selectivity probably reflects the ability of the starting material to adopt the following conformation:

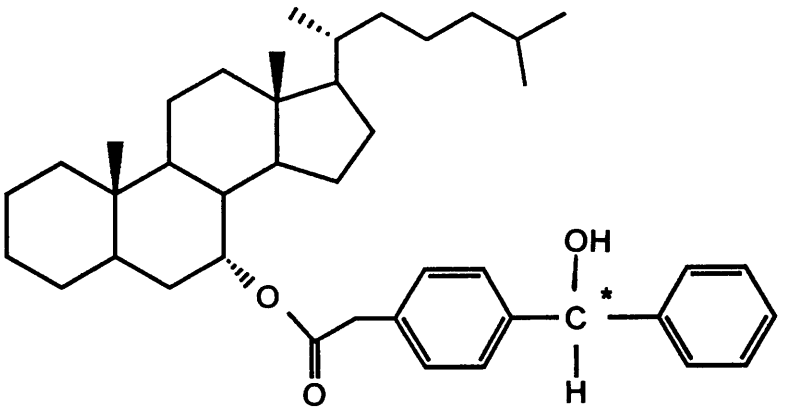


i.e. the σ-bond of 15β-H can easily be made coplanar with the benzophenone carbonyl.

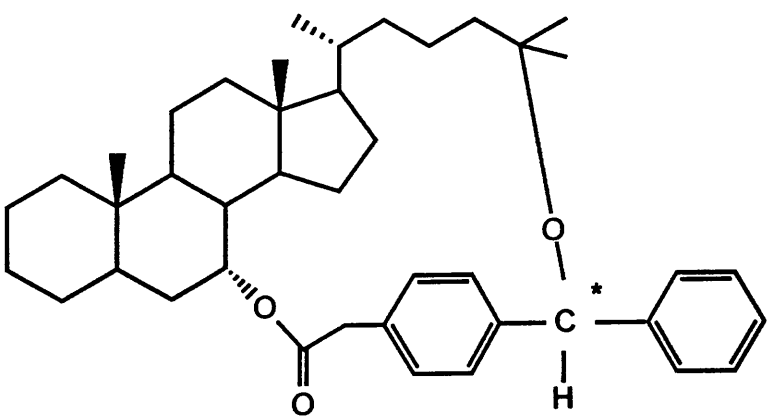
In all these cases mentioned, the residual freedom of motion in the reagent-substrate "complex" may lead to some randomness of attack. Moreover, the other difficulty is that benzophenone photochemistry, with a quantum yield of ca. 0.2 is not attractive for large-scale synthetic work.

Recently, Suginome et al.[10] have reported a two-step long-range intramolecular hydroxylation of the C-25 position in the cholestane side chain. The reaction was based on long-range intramolecular (1,20 H-atom transfer) hydrogen abstraction by alkoxy radicals generated by irradiation of hypoiodites. Thus, 5α-cholestan-7α-yl-4-(hydroxyphenylmethyl)phenyl acetate was prepared by reduction of

SCHEME 1



$HgO / I_2 / hv$



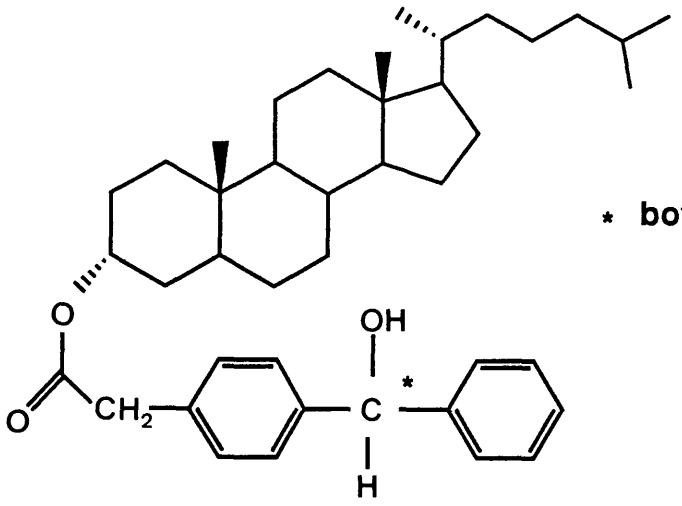
4.3% (R) or (S) at C^*

3.8% (R) or (S) at C^*

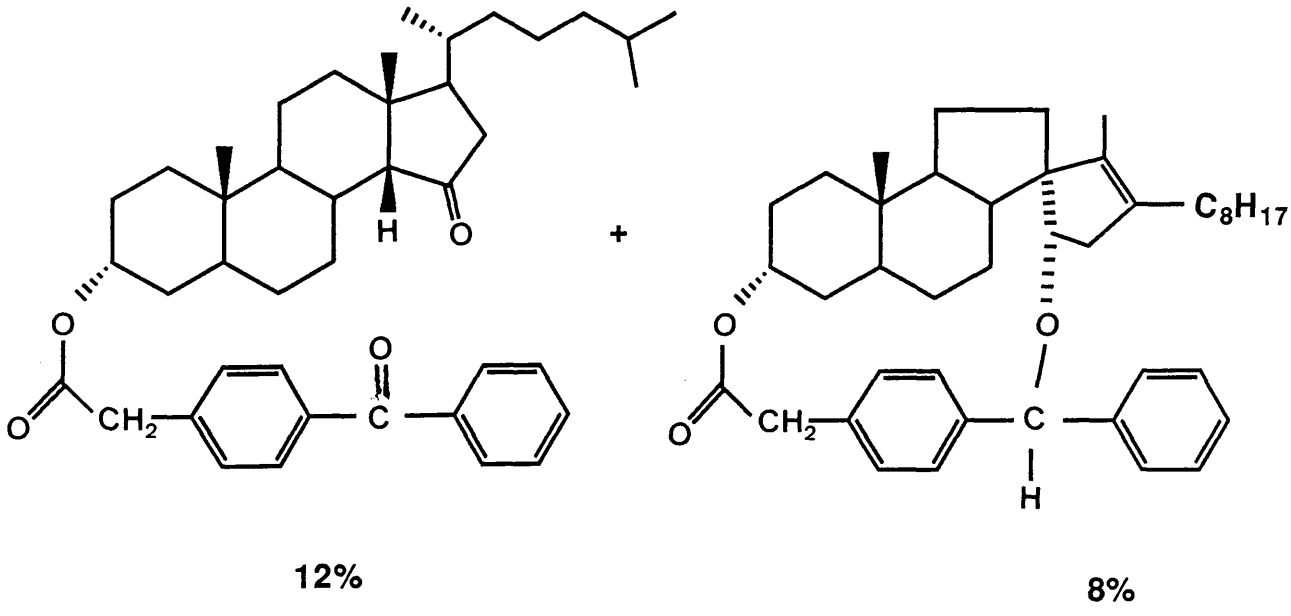
the corresponding benzophenone derivative. The mixture of epimeric esters was converted to the hypoiodites using 3 equiv. of HgO/I_2. The solution was then irradiated for 7h to give the macrocyclic ether lactones, as shown (Scheme 1), which can be clearly reduced to 5α-cholestane-7α,25-diol using $Na/NH_{3(\ell)}$. The same group have also developed a one-step procedure for introducing a carbonyl group into the C-15 position of a 5α-androstane skeleton in 20% yield[11] and into a 5α-cholestane skeleton in 12% yield.[12] Both reactions involve long-range intramolecular hydrogen abstraction via benzhydryl alkoxy radicals. When 5α-cholestan-3α-yl-4-(hydroxyphenylmethyl)phenyl acetate (Scheme 2) was subjected to the long-range hydroxylation conditions, the expected 15-ketone was produced in 12% yield, together with a macrocyclic ether lactone with a rearranged steroid skeleton obtained in 8% yield. The mechanism through which the macrocyclic ether lactone and the ketone are produced is outlined in Scheme 3. The alkoxy radical generated from the hypoiodite by irradiation abstracts the C-14 hydrogen to give the C-14 radical. One-electron oxidation and loss of H^+ gives the Δ^{14}-intermediate. The existence of this intermediate Δ^{14}-alkene was proved by the fact that both the macrocyclic ether lactone and 15-ketone (Scheme 3) were obtained when the intermediate alkene was exposed to the hydroxylation conditions.

Reaction of the Δ^{14}-alkene with iodoxy radical (IO·) followed by one-electron oxidation of the resulting C-14 radical, gives the 14β-H 15-ketone. However, long-range intramolecular addition of the alkoxy radical to the carbon-carbon double bond can also occur, followed by one-electron oxidation to give the C-14 carbonium ion which

SCHEME 2



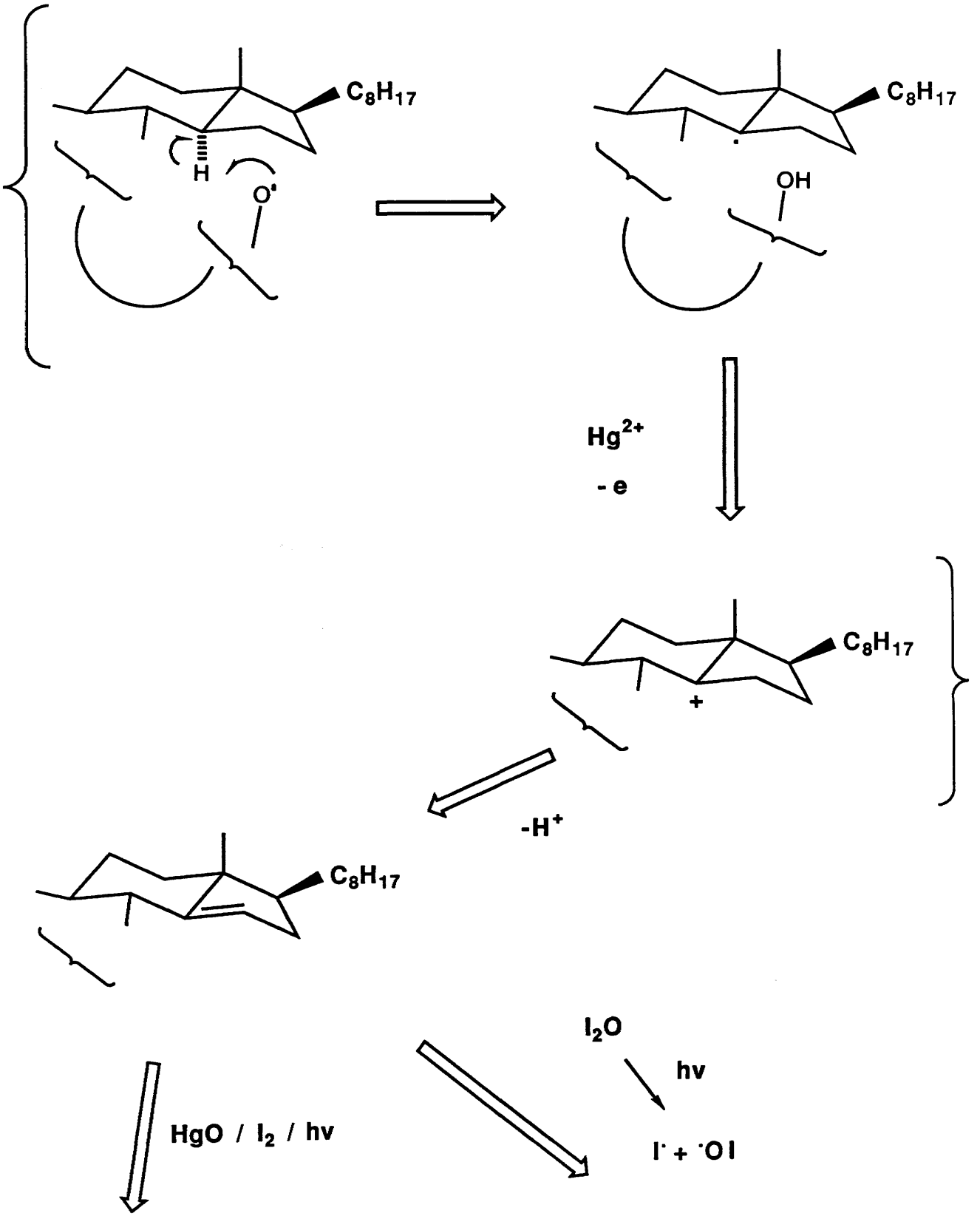
HgO / I_2 / hv

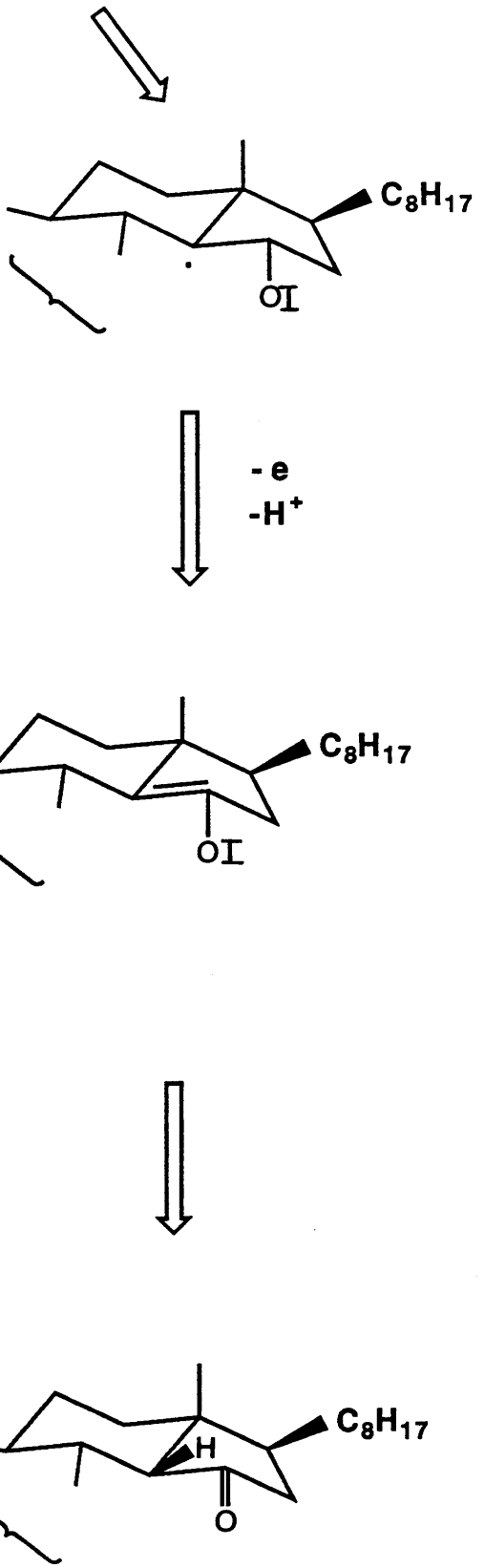


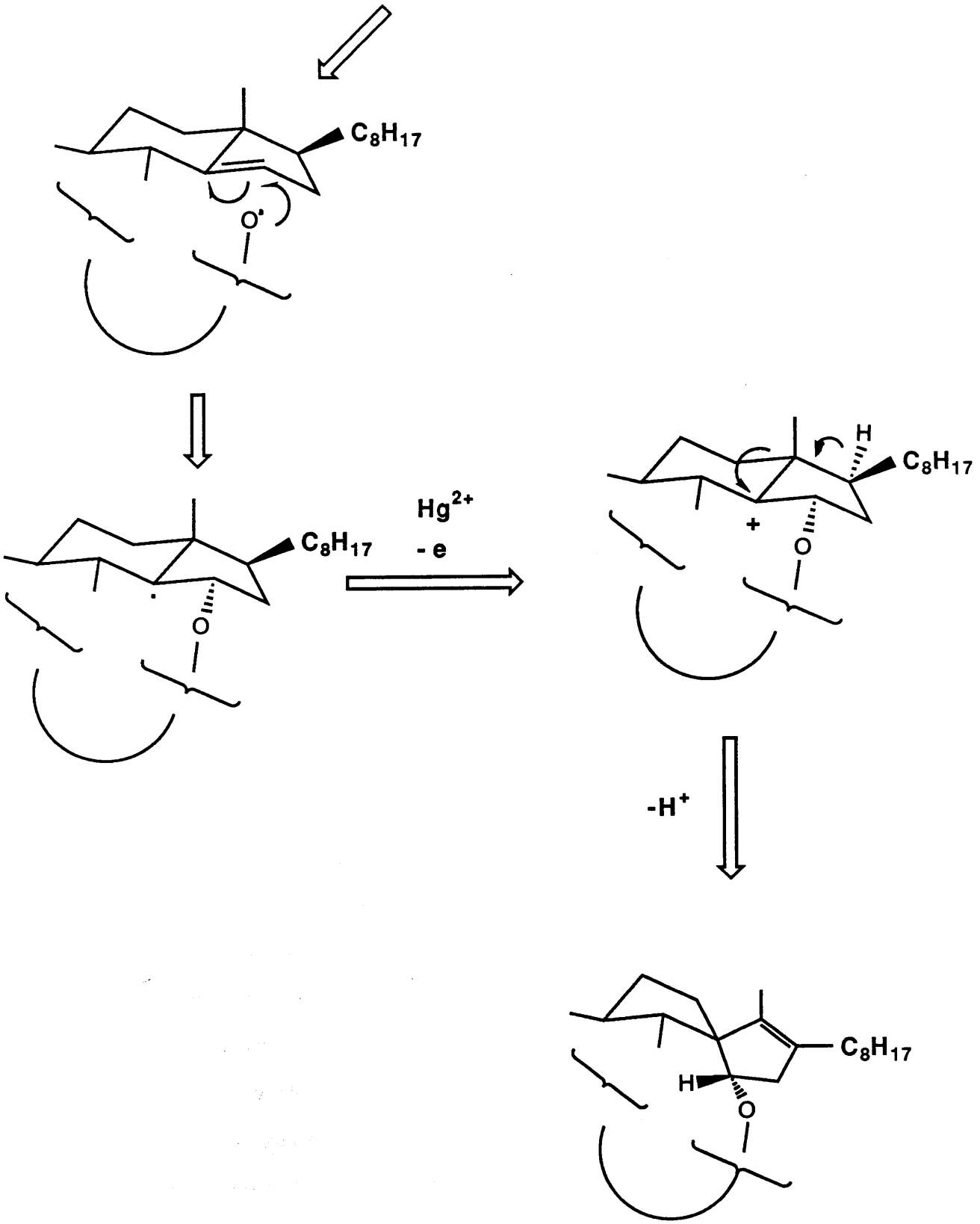
12%

8%

SCHEME 3




C8H17
OI
- e
-H+
C8H17
OI
C8H17
H
O


C8H17
O'
C8H17
O
Hg2+
- e
H
C8H17
+
O
-H+
C8H17
H
O

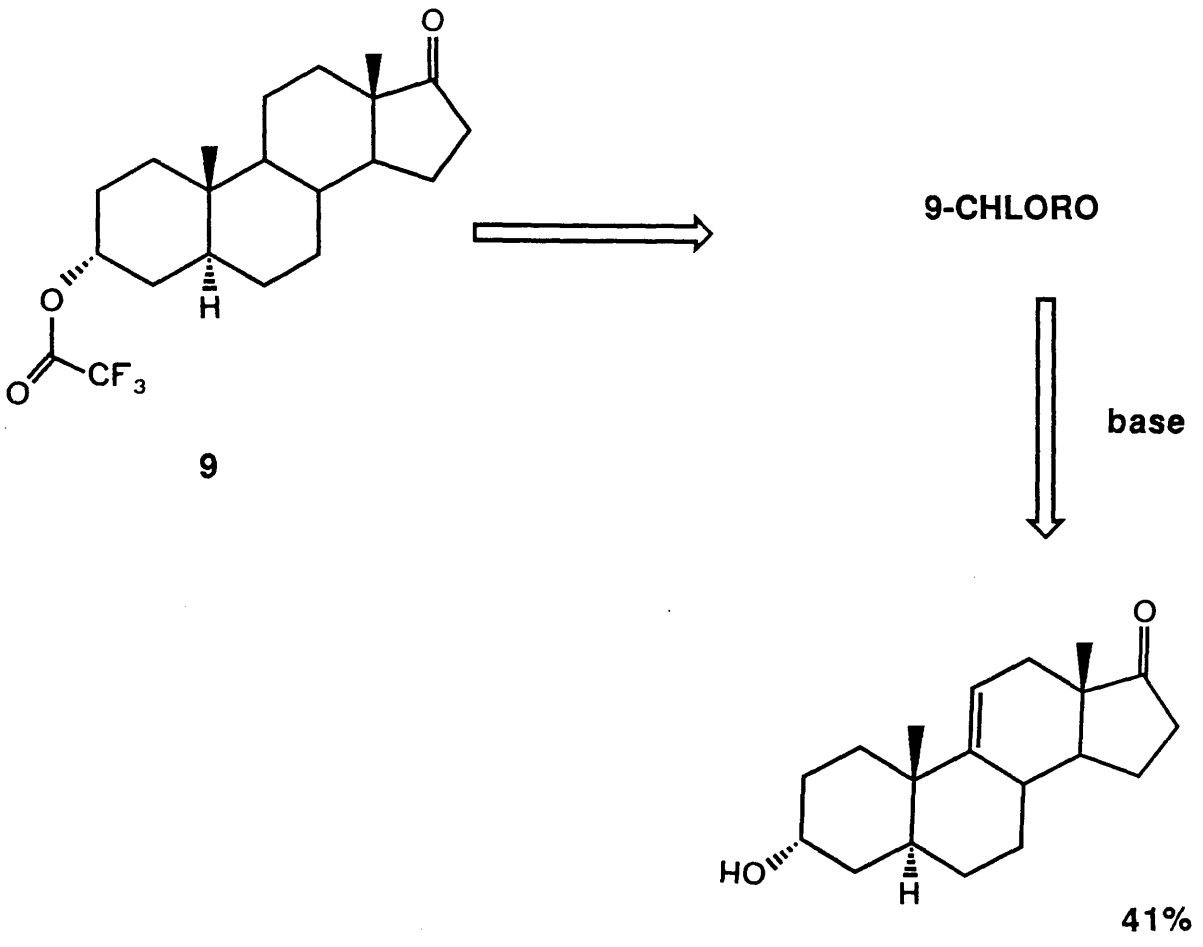
can undergo Wagner-Meerwein rearrangement to give the macrocyclic ether lactone.

1.2 Selective Halogenation of Steroids Using Attached Aryl Iodide Templates[13]

In a search for a suitable rigid free radical halogenating agent, Breslow was drawn to phenyliodine dichloride, which has great selectivity for tertiary hydrogens compared with secondary or primary C-H bonds.[14]

Unattached $PhIC\ell_2$

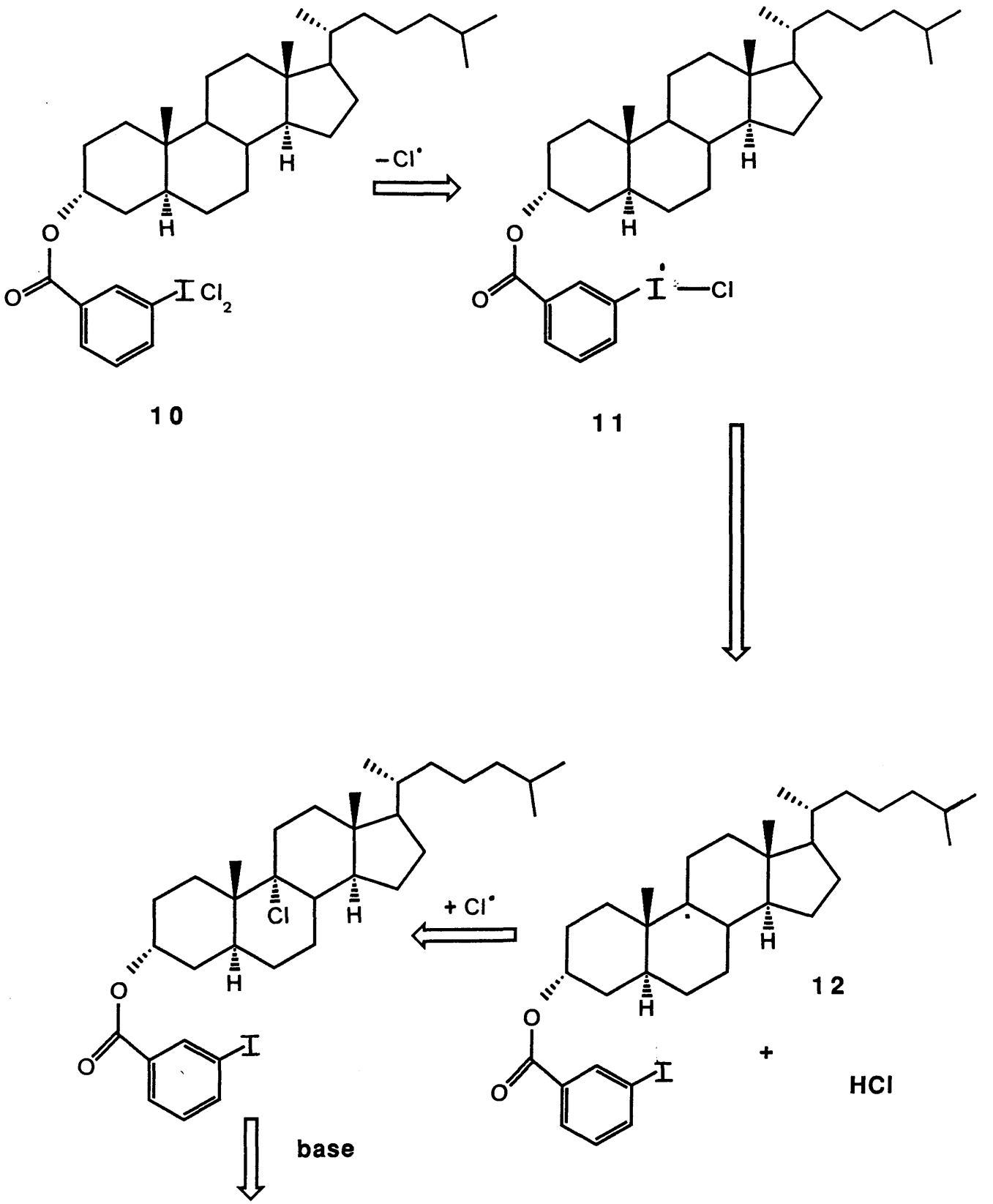
A short study of the selectivity of unattached phenyliodine dichloride in steroid functionalisation was undertaken. However, $PhIC\ell_2$ with various steroids in non-aromatic solvents, such as $CH_2C\ell_2$, gave no appreciable amount of halogenation of the steroid when the free radical process was initiated by photolysis. It was thought that $PhIC\ell_2$ was undergoing a light-induced self-decomposition. Steroids can be halogenated, however, using aromatic solvents such as benzene or chlorobenzene. For instance, 5α-cholestan-3β-yl acetate was halogenated largely at carbons 9 and 14, demonstrating attack on tertiary α-hydrogens, specifically those axial on 6-membered rings away from any polar substituents. It was this polar effect that Breslow used to direct halogenation, with such a random reagent, specifically into the C-9 position of androsterone trifluoroacetate (9), resulting in the introduction of the synthetically important 9(11) double bond.[15]

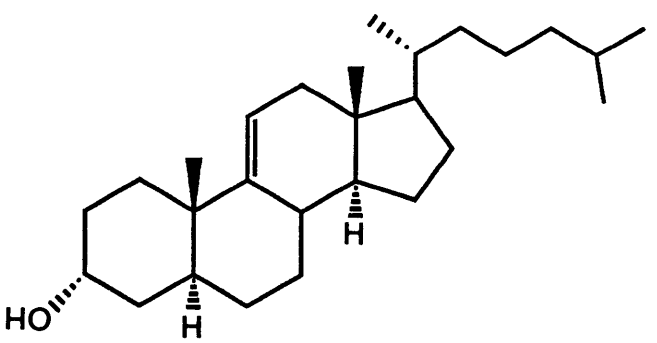


Attached Aryl Iodine Dichlorides

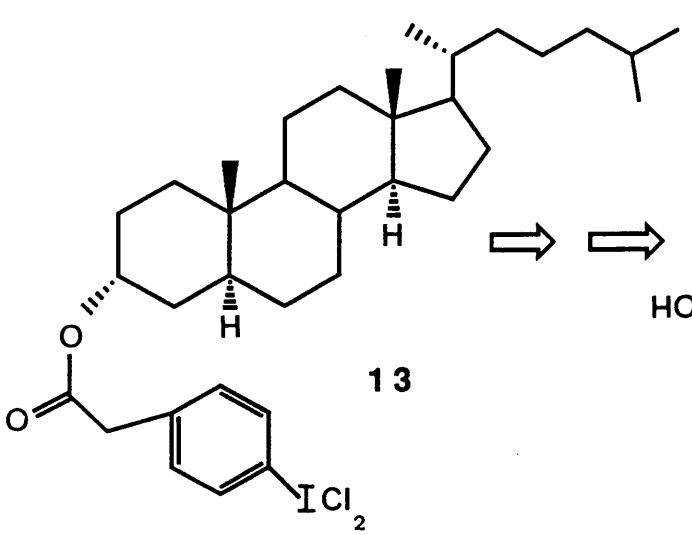
The m-iodobenzoate ester of 5α-cholestan-3α-ol was prepared, and on reaction with $C\ell_2$ in the dark gave the attached iododichloride (10). Brief irradiation with a sunlamp initiated a free-radical chain reaction (Scheme 4). The product of the reaction was found to be exclusively chlorinated at C-9 of the steroid; base hydrolysis removed the m-iodobenzoic acid, and produced the 9(11)-unsaturated steroid in 43% yield after acetylation. However, 35% of 5α-cholestan-3α-ol was recovered (as acetate) in addition to 9% of Δ^{14}-5α-cholestanol along with 2% of Δ^{5}-olefinic products. Androstane was included in this reaction as a control. It was halogenated to the extent of 20%, therefore intermolecular halogenations are accompanying the selective intramolecular process. This result was expected from an examination of molecular models or from a calculation of distances, since the attached chlorine atom of 11 can be located

SCHEME 4




HO
H
H

directly under the C-9 hydrogen. Also, after hydrogen abstraction, the intermediate radical 12 must collide with a second mole of substrate (10) to complete the chlorination and regenerate intermediate 11. The p-iodophenylacetate of 5α-cholestan-3α-ol when converted to its dichloride (13) and submitted to free radical initiating conditions performed selective chlorination at C-14 as predicted.[15]



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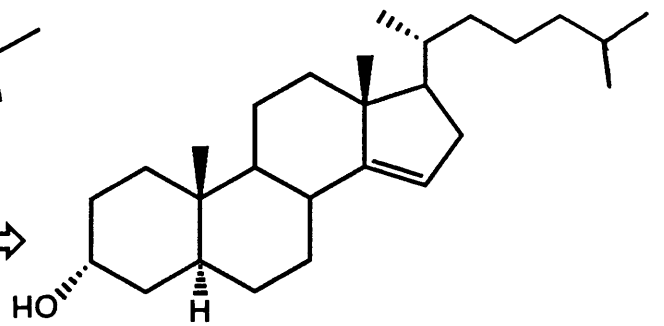

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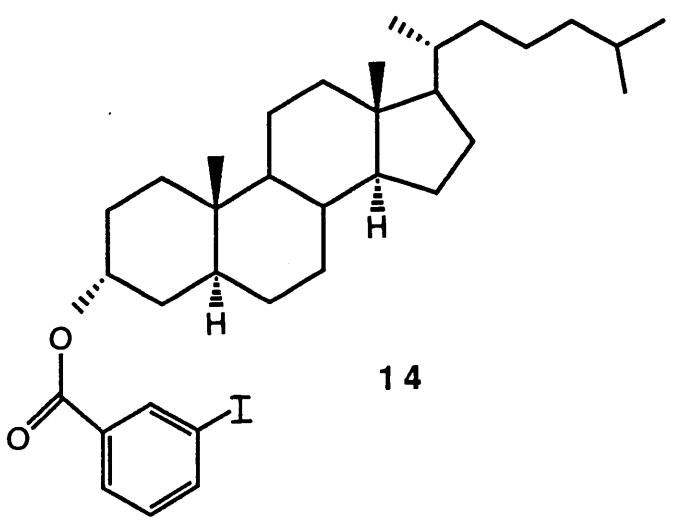
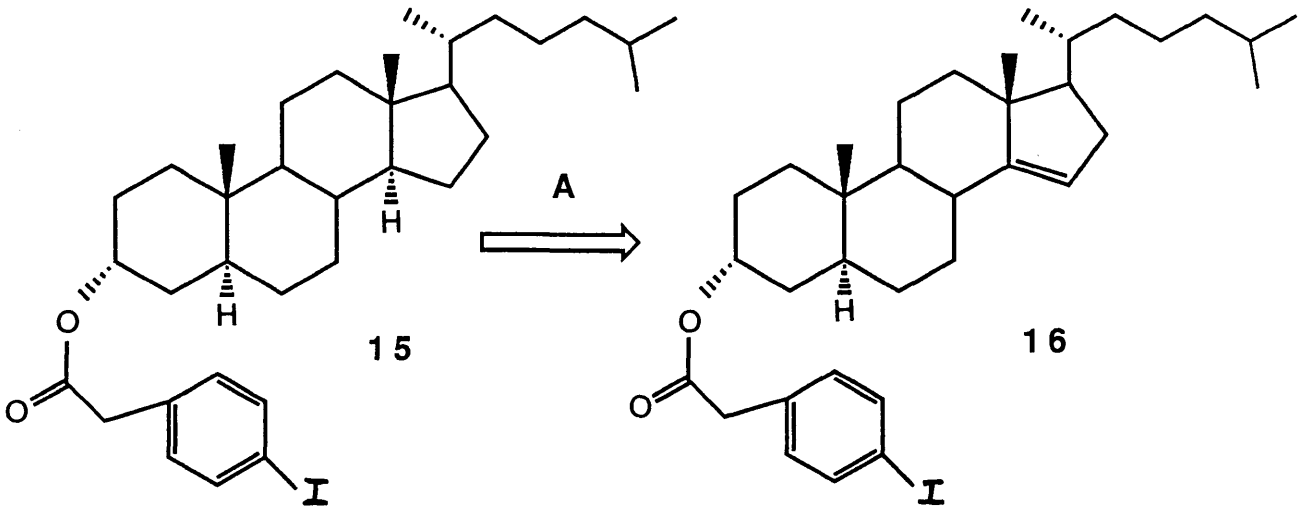


14

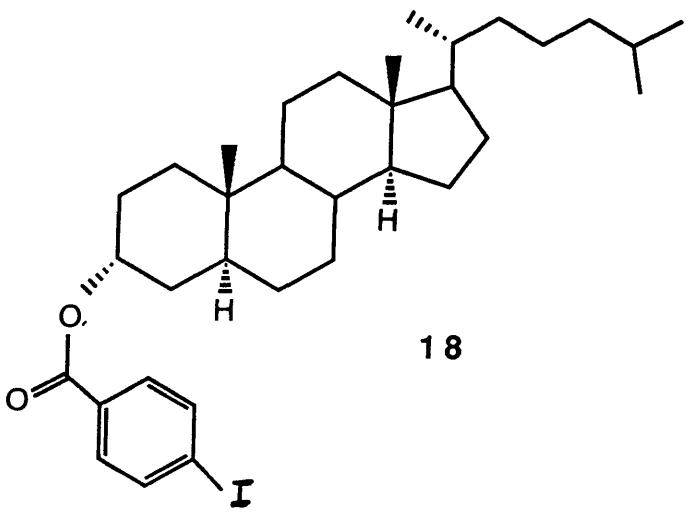
Radical Relay Chlorination under Template Control[13,16,17]

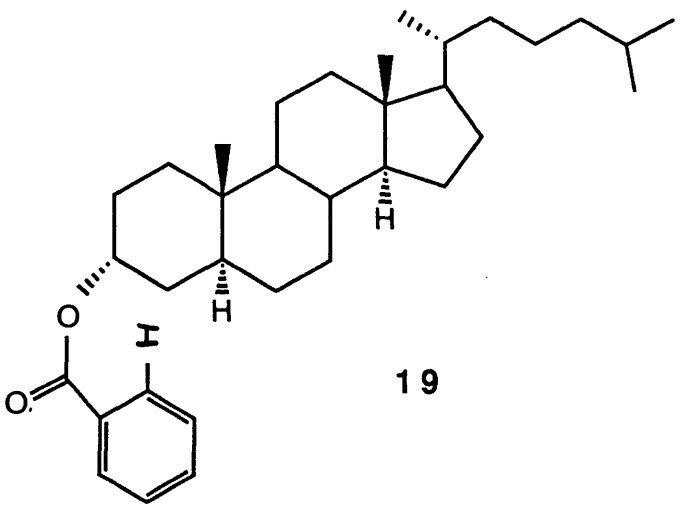
This mechanism generates species 11 by external transfer of a chlorine atom to the aryl iodide itself, thus by-passing the necessity for the preparation of an aryliodine dichloride. This chlorine atom could then be relayed to the correct hydrogen atom of the substrate. The m-iodobenzoate group of 14 would have then acted, not as a reagent, but as a template in directing the attack. In this radical relay mechanism, halogenating agents can be used, such as $C\ell_2$, $SO_2C\ell_2$, and $PhIC\ell_2$. The chlorine atom donors derived from these reagents would be, respectively, $C\ell^{\cdot}$, $SO_2C\ell^{\cdot}$, and $PhIC\ell^{\cdot}$. One of these would then approach the substrate (14) and transfer the chlorine to 14 so as to generate the species 11. The external halogenating agent thus involves an additional step when compared to the attached aryliodine dichloride mechanism. This two-step process is however preferred since there is an entropy advantage similar to that possessed by many hydrolytic enzymes, in that they do not use a water molecule to attack the substrate directly but, instead, use an enzymatic group to make an intermediate, and then hydrolyse that intermediate in a second step. Welzel et al.[18] have shown that when 5α-cholestan-3α-yl p-iodophenylacetate (15) is used under radical relay conditions, this results in an 80% yield of 16 without the need for subsequent base hydrolysis. This yield is much higher than previously reported by Breslow and co-workers.[13] However, the latter research group employed as substrate the attached dichloride and then used base hydrolysis to perform saponification and dehydrochlorination, thus characterising the product as the Δ^{14}-olefin (17).



A 1) $PhIC\ell_2$/hr
2) DBU

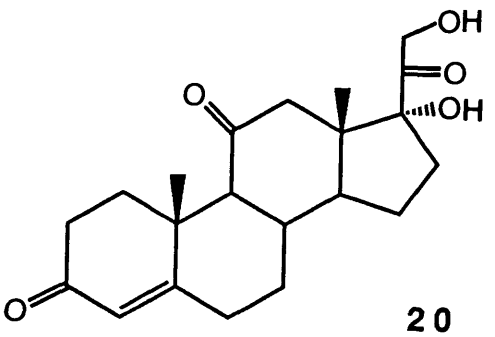
Under radical relay conditions 5α-cholestan-3α-yl p-iodobenzoate (18) gave no steroid functionalisation. Models have shown that the p-iodobenzoate ester is held in a V-shape and that the chlorine atom attached to the iodine cannot reach the steroid. Also, the o-iodobenzoate of 5α-cholestan-3α-ol (19) did not undergo any functionalisation due to steric crowding which prevented the adoption of a reactive conformation.





19

The 9(11)-olefins produced by selective halogenation and subsequent dehydrochlorination are of great interest in the synthesis of corticosteroids. Breslow has used the template-directed radical-relay reaction to advantage in the synthesis of cortisone[13,15] (20). The 17α-m-iodobenzoate (21) was prepared and, under radical relay conditions, halogenation at C-9 occurred. This same template also promoted halogenation at C-9 when attached to C-3; in both cases the template has to bridge one six-membered ring plus one extra bond, therefore the geometry of attack is similar. Furthermore, the radical relay process gives selective attack at the unactivated C-9 position, irrespective of several types of functionality in ring A.



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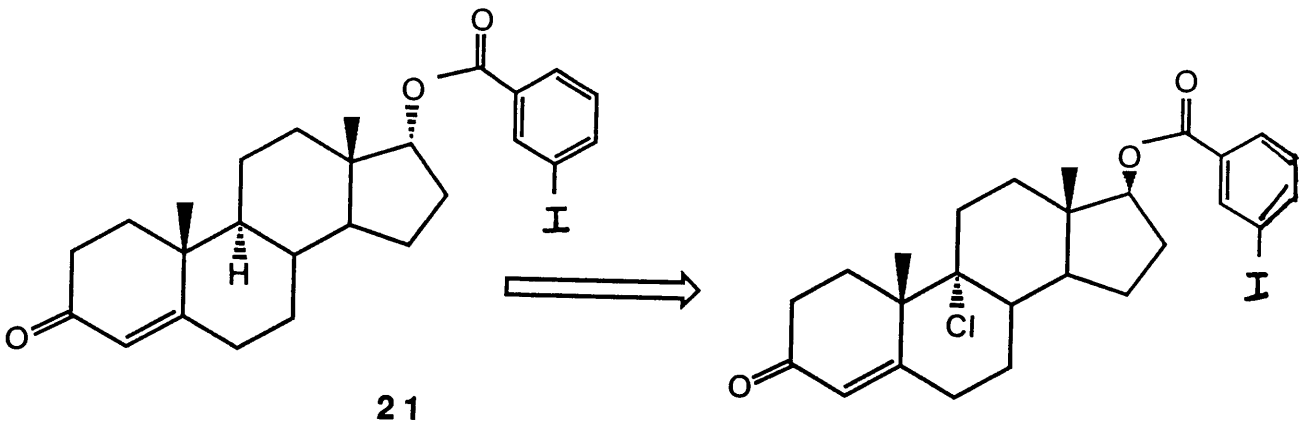


21

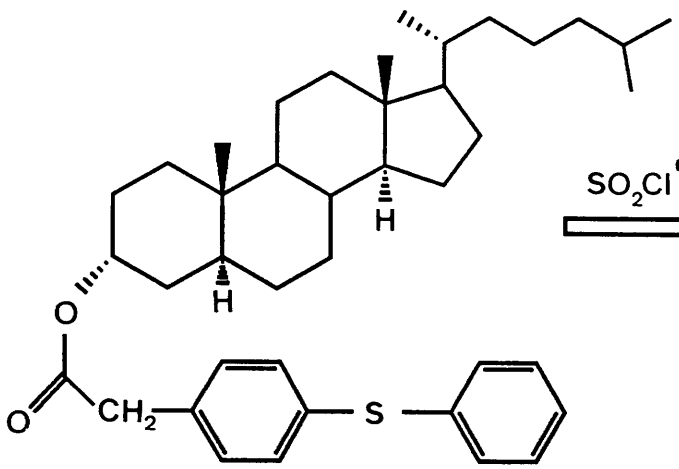
Other Templates

1) Sulphur Heterocycles as Templates[19,20]

In the course of exploring the radical relay mechanism, the Breslow group examined other atoms capable of bonding to halogen atoms, particularly sulphur. Thus, the diphenyl sulphide (22) was halogenated with $SO_2C\ell_2$ under free radical conditions in good yield. However, the sulphur atom is very easily oxidised and for this reason simple diaryl disulphides are not of practical value.

Catalytic multiple template-directed steroid chlorination was achieved when a single thiophene template was attached *via* silyl ether bonds to three substrate molecules[21] (23). The reaction with this system functionalises all three substrates as the template successively directs attack on each steroid nucleus.

The thioxanthone ring system [as in (24)] is a more stable rigid template which can be recognised as a diaryl sulphide in which the additional carbonyl group strongly deactivates the sulphur towards oxidation. Hence, in the radical relay chlorination, the Cℓ atom bound



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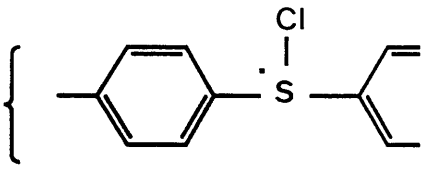

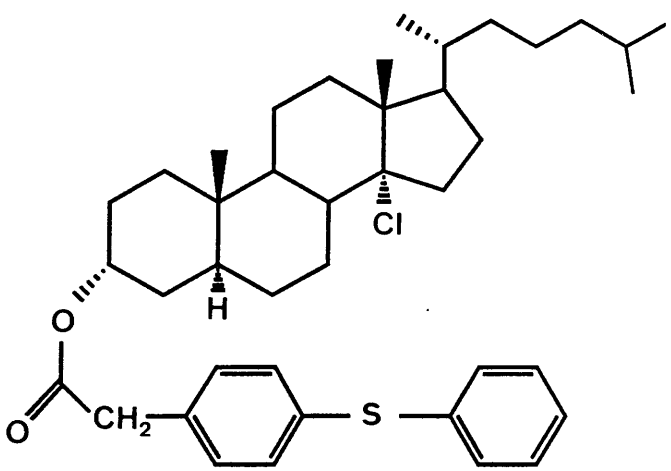

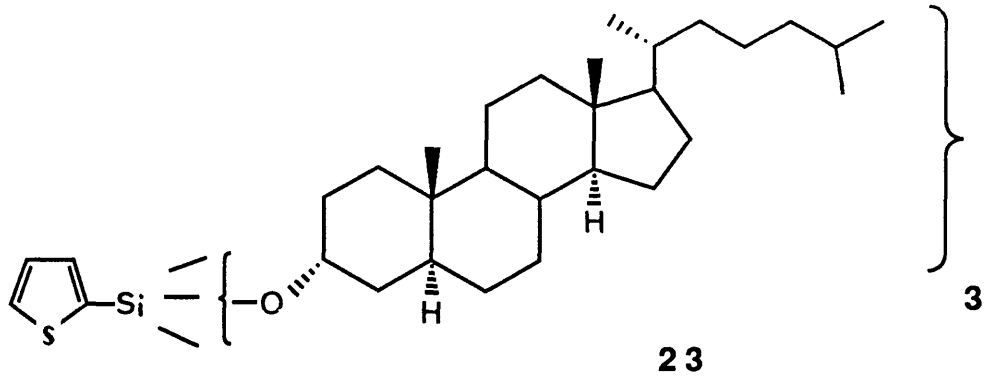

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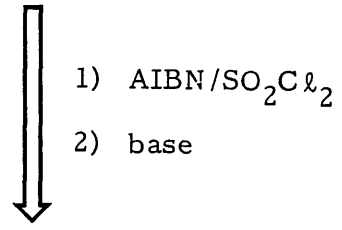
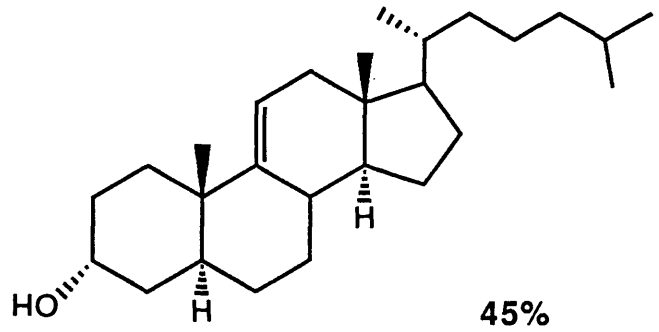
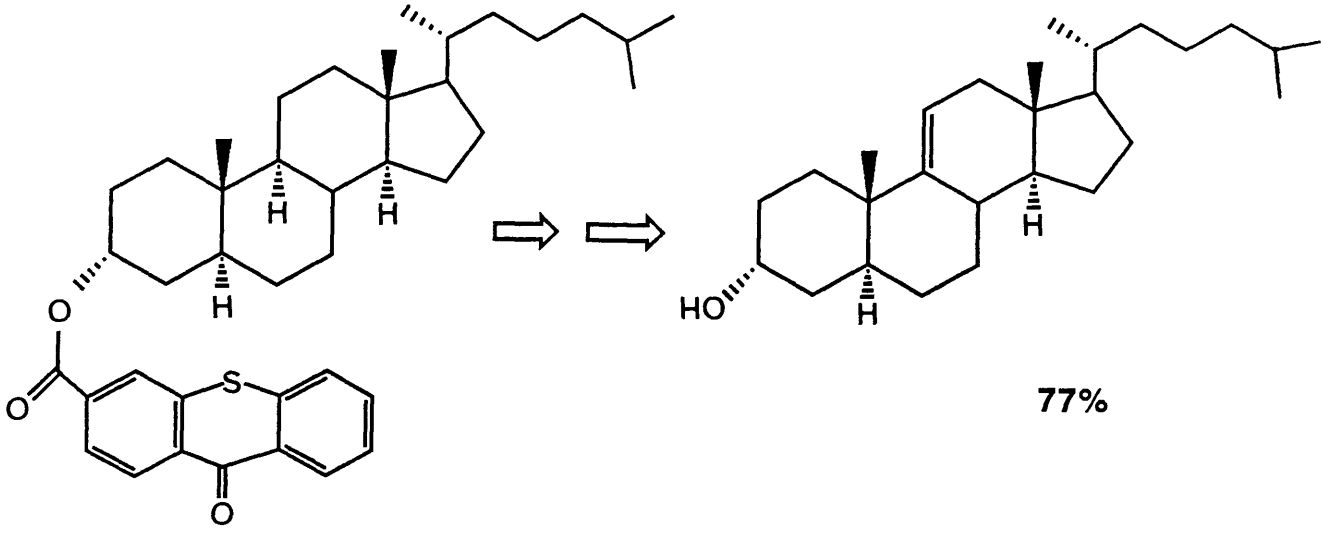




45%

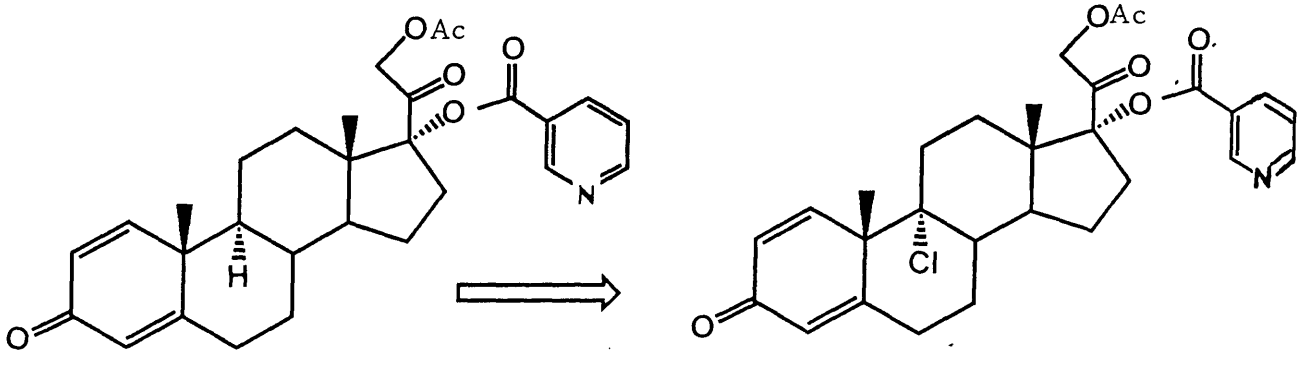
to the S-atom of the template can be delivered to a geometrically accessible hydrogen of the steroid. The resulting steroid carbon radical then reacts with $PhIC\ell_2$ to form a C-Cℓ bond and to regenerate PhI·Cℓ. Previous reports[22,23] of this system have been retracted[24] but, in a confirmatory study,[25] Breslow and Guo demonstrated by reproducible experiments that the thioxanthone is a good template for radical relay chlorinations.



24

2) Pyridine Templates

Breslow et al.[25] have shown that heterocycles, such as pyridine, can bind chlorine atoms and then deliver them with chemical and geometric control. It has also been shown that the Cℓ atom binds to the pyridine nitrogen with a weak 3-electron σ-bond.[27] These pyridine based templates are particularly promising because they show excellent geometric control in directing steroid chlorinations. Indeed, a practical route for dexamethasone has been devised, based on a nicotinic ester template.[28]



Radical relay chlorinations of straight-chain alcohols (such as dodecanol) as nicotinate esters have been studied.[29] The findings suggest that the pyridine captures a Cℓ atom and delivers it to an accessible hydrogen, although the flexibility of the chain causes a number of positions to be attacked. However, there was significant preference for attack at C-5. The same nicotinate template also directs chlorination at C-9 when attached to either C-3 or C-17 in steroids (Fig. 1). The attack point is again four carbons distant from the attachment point, as in the flexible chain system.

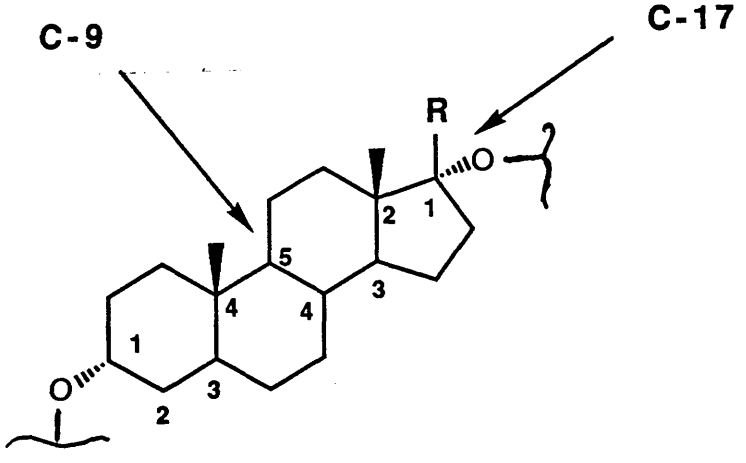
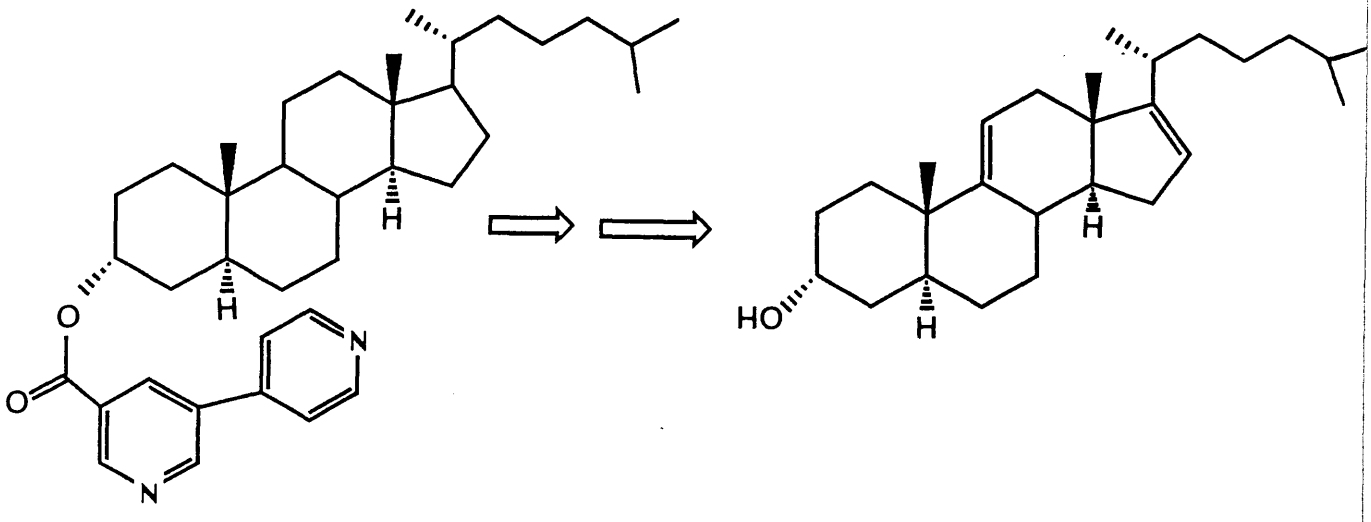


Fig. 1: Functionalising at C-9 using nicotinate ester attached at C-3 or C-17.

Bifunctional Templates - Double Functionalisation[30]

Functionalisation at both C-9 and C-17 would be useful in the practical synthesis of corticosteroids. Current fermentation methods for the degradation of sitosterol can produce compounds hydroxylated both at C-9 and in the side chain. Breslow and Guo have examined the possibility of achieving a similar conversion with a

bifunctional template covalently attached at C-3α, but designed to deliver chlorine atoms to both C-9 and C-17.



The bipyridinecarboxylic acid ester of 5α-cholestan-3α-ol was prepared and, under radical relay conditions, gave the 9,17-dichlorinated steroid in quantitative yield.

1.3 Gif System

The existence of enzymatic systems in nature that catalyse the monoxygenation of non-activated C-H bonds prompted Barton to develop a series of oxidants based upon the "Gif System" which would selectively functionalise saturated hydrocarbons under mild conditions.[31,32]

There are five variants of the Gif system, shown in Figure 2.

Gif III: Comprises Fe powder suspended in pyridine/acetic acid + oxygen or air.

Gif IV: Contains catalytic Fe species + Zn dust to provide electron input.

Gif-Orsay: Is the same as Gif IV, but with Zn replaced by the cathode of an electrochemical cell.

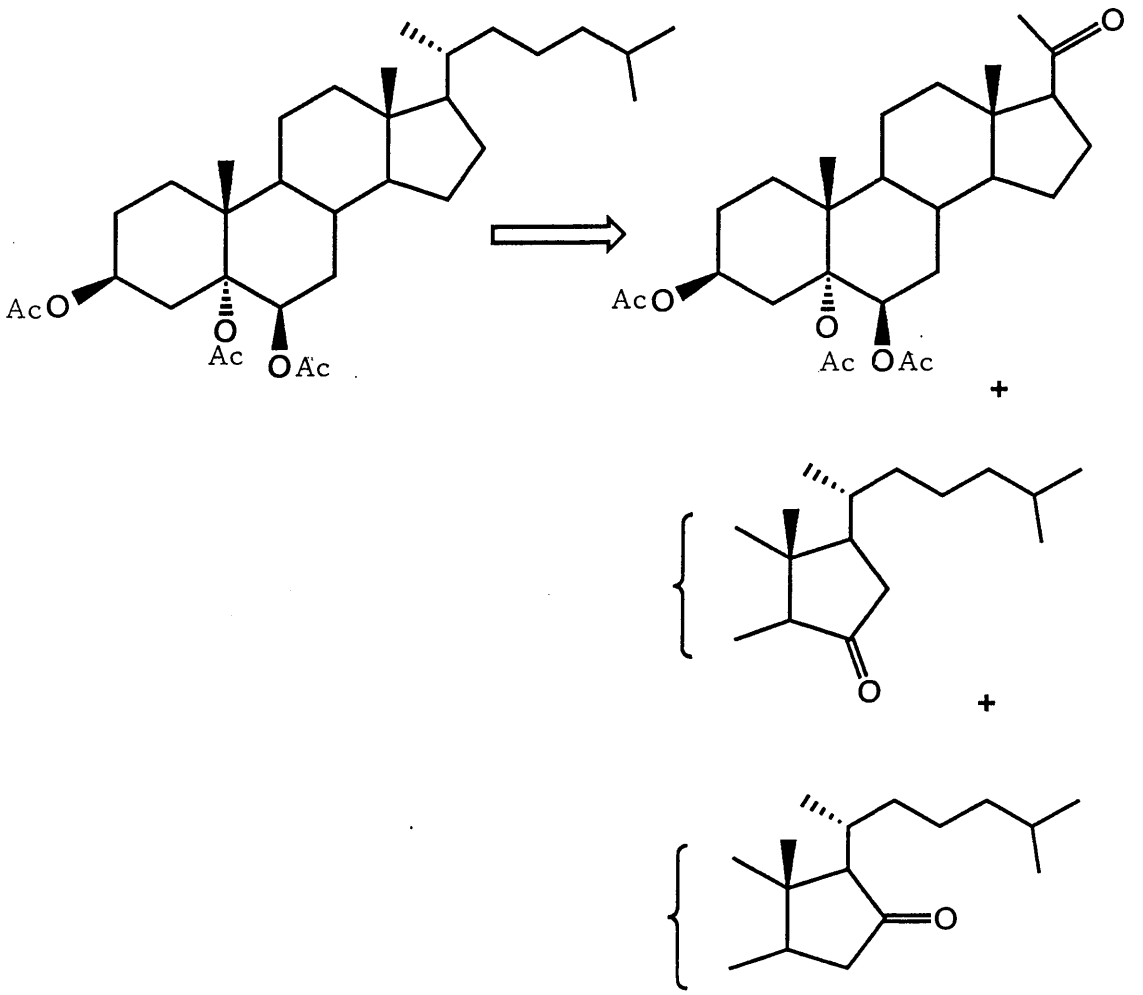
Go Agg^{I}: Uses pyridine/AcOH with stoichiometric Fe^{II} species + KO_2 under argon.

Go Agg^{II}: Uses pyridine/AcOH with catalytic Fe^{III} species + H_2O_2 under argon.

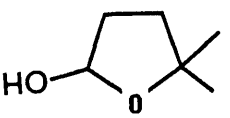
Fig. 2: Variants of the Gif system.

The Gif III system requires pyridine in the presence of Fe powder, oxygen and a carboxylic acid, and it was shown that this system had unusual oxidising power, i.e. there was selective attack at secondary, not tertiary positions. The selectivity of all Gif systems (sec. >> tert. ~ primary) can be explained by a combination of C-H bond strengths (1° > 2° > 3°) and steric resistance to insertion (3° > 2° > 1°). The balance normally favours the secondary positions; insertion into the tertiary position is seen only when the C-H bond is markedly exposed. Work has shown that the optimum temperature for reaction is ~30°C. Above 80°C there is no oxidation, and below -20°C the reaction is very slow. In the oxidation of adamantane by the Gif III system, the ratio of C^2/C^3, where C^2 is the total of oxidised

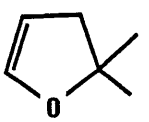
products at the secondary positions, and C^3 is the total of tertiary alcohol formed, giving a measure of the selectivity of the reaction. Thus, if adamantane is attacked non-selectively then $C^2/C^3 = 12/4 = 3$. However, under the Gif III system $C^2/C^3 = 3.7$ and for oxygen based radicals $C^2/C^3 = 0.15$. Therefore, the Gif system is selective for secondary positions. The Gif system has also been applied for steroids.[33] In the oxidation of 3β,5,6β-triacetoxy-5α-cholestane, the three major products were identified as the 20-ketopregnane (12%), together with the 15-ketone (7%), and 16-ketone (6%), retaining the cholestane nucleus.



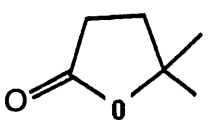
Comparison of the quantities of oxidised products obtained from a number of suitably functionalised cholestane derivatives showed that hydroxyl or carbonyl groups in ring A deactivate the ring towards oxidation, and also that the presence of enone systems in ring B deactivates all steroid rings, thus rendering the side chain susceptible to selective oxidation. However, conformational transmission effects could also contribute to this selectivity. Formation of the 20-keto-pregnane derivative as the major product in the Gif system oxidation implies loss of a C-6 fragment or other low molecular weight material. The volatile fractions (obtained by distillation of the crude reaction mixture) were examined by GC-MS. Three major compounds were detected (Fig. 3).



4-hydroxy-4-methylpentanal



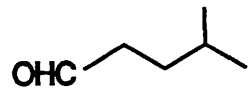
2,2-dimethyl-2,3-dihydrofuran



4,4-dimethyl-γ-butyrolactone

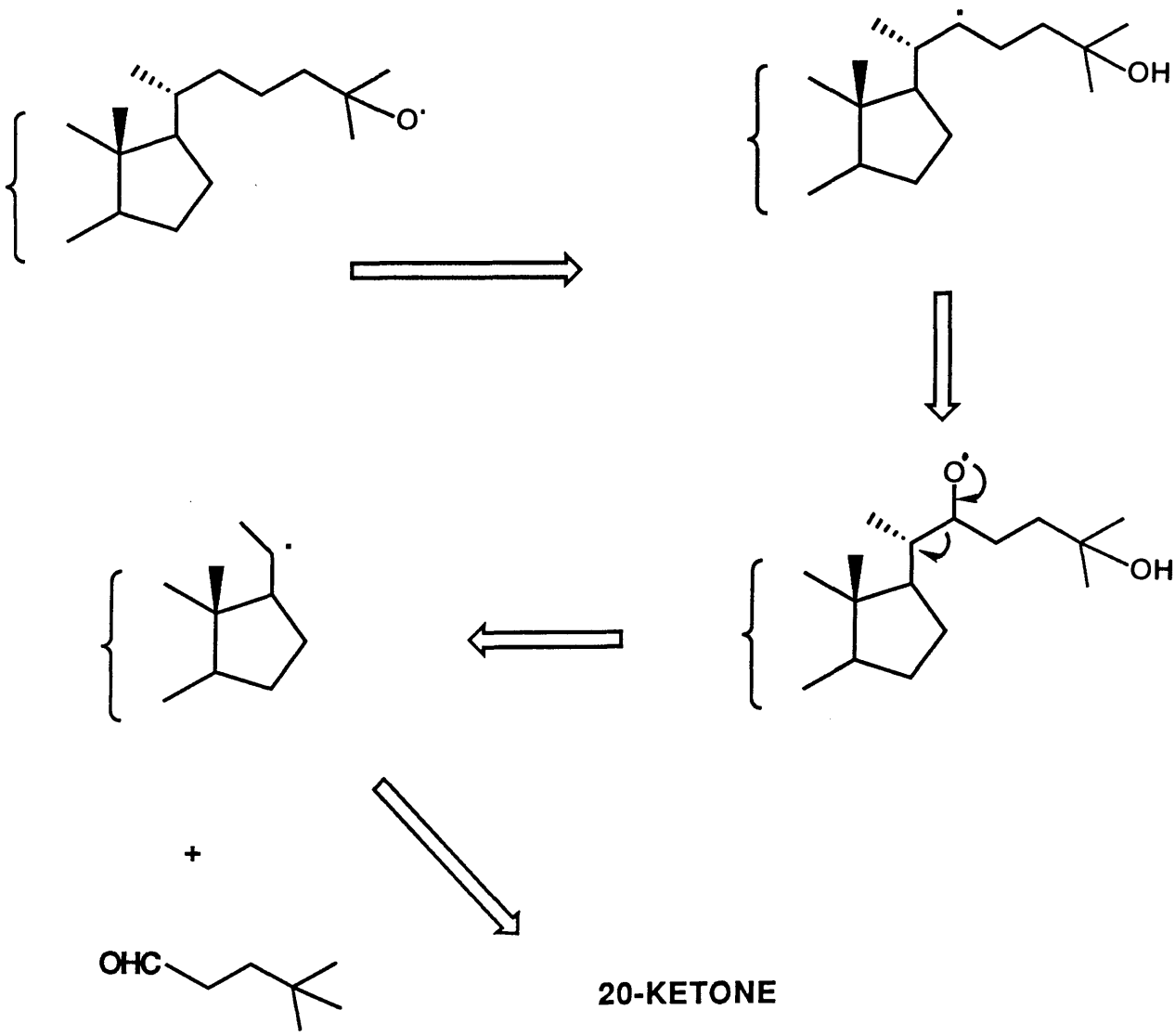
Fig. 3: Volatile products from Gif oxidation of 3β,5,6β-triacetoxy-cholestane.

The three products are all further oxidised forms of the expected 4-methylpentanal and since they all appear to contain an oxygen



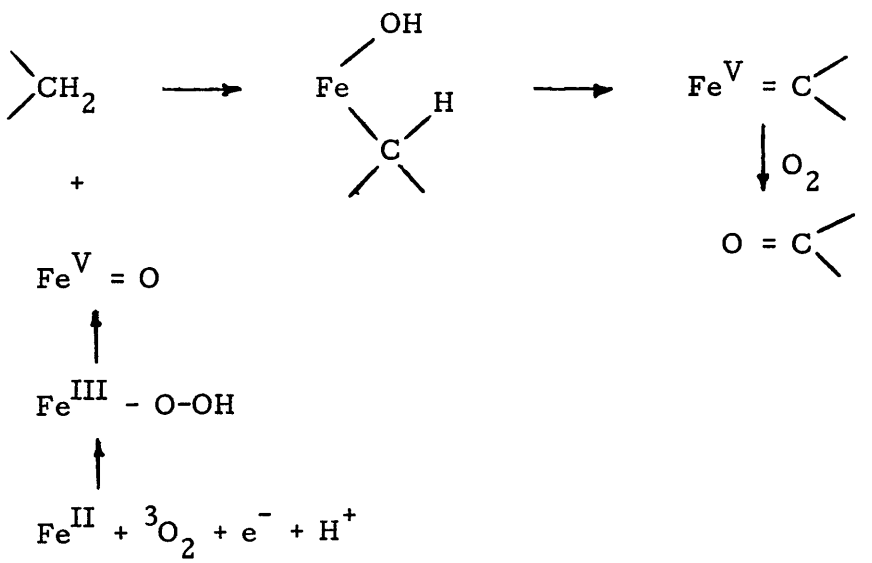
4-methylpentanal

function at C-25, the following mechanism has been postulated:



The 25-alkoxy radical is probably formed via a hydroperoxide. An intramolecular 1,5-hydrogen shift leads to the radical centred at C-22, and further radical chemistry leads to the C-20 radical which can be oxidised to the 20-ketone.

The Gif system is different from the cytochrome P-450/ $NADPH/O_2$ system (the enzymatic system responsible for alkane hydroxylation in living organisms) in that it gives ketones rather than alcohols and effects preferential attack of secondary positions. A mechanism has been proposed[32] to account for these facts. The formation of ketones can be explained by the postulation of an iron-carbene bond.



It is now thought that an Fe^V oxenoid species is involved in the oxidation[32] which is similar in valency to the iron in the cytochrome P-450/NADPH/O_2 system. First, there is formation of an Fe-C σ-bond (produced by insertion of $Fe^V = O$ into a C-H bond), this then evolves into a carbene which can be captured by O_2 to give the ketone.

The Gif-Orsay system is the electrochemical equivalent of the Gif IV system and is much more efficient at electron input (see Table 1).

Table 1: Oxidation of trans-decalins with the Gif IV and Gif-Orsay Systems.

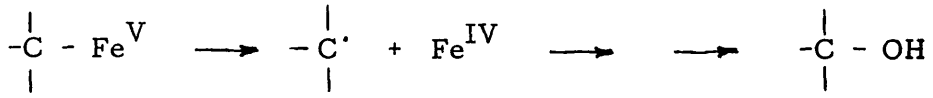
	% oxidation	C_2/C_3
Gif	16.4	16.3
Gif-Orsay	42	23

trans-Decalin showed little oxidation at tertiary positions, and mostly 1- and 2-decalones were produced. In both systems the oxidant has been shown to be superoxide and not H_2O_2. The non-oxidation of sulphur compounds (or primary and secondary alcohols) confirms the non-participation of hydroxyl radicals. Sawyer et al.[34] have shown that hydroxyl radicals react rapidly with pyridine. Therefore, the true explanation on the selectivity of the Gif systems may be dependent on hydroxyl radical suppression, so that the Fe^{V} oxenoid mechanism can occur without complication.

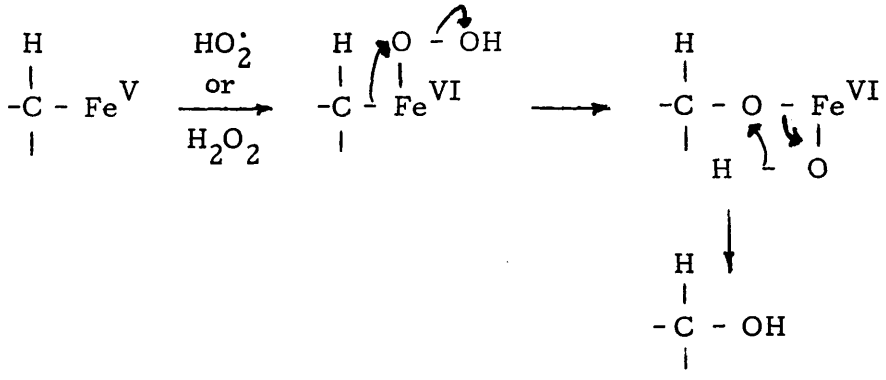
Reaction of cyclohexane with the usual mixture of pyridine/AcOH and Fe^{II} complex, containing KO_2 under argon (Go Agg^{I}) gave good selectivity[35] - only cyclohexanone was produced, with no cyclohexanol. However, experiments have shown that in oxidations using the Go Agg^{II} system the ratio of ketone/alcohol was 30/1, and when the Fe^{III} catalyst is reduced in quantity, significant amounts of cyclohexanol

are produced. It has been shown that the Fe^{III}/H_2O_2 ratio has a marked effect on the ratio of ketone to alcohol production in cyclohexane oxidation.

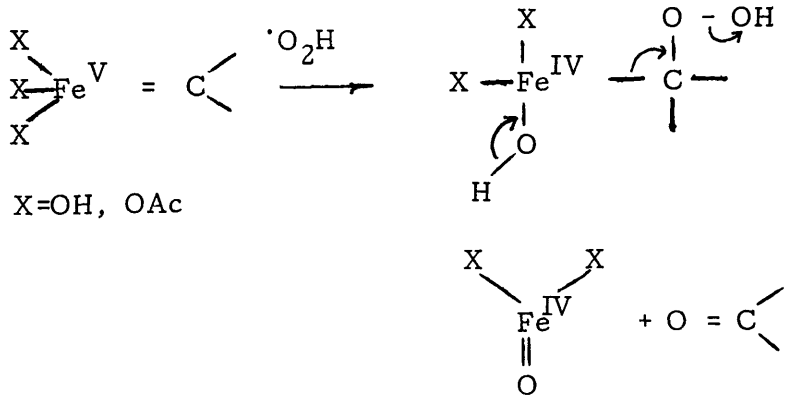
Various mechanisms have been postulated to account for these observations. Tertiary alcohols are formed mostly by fragmentation of the Fe-carbon σ-bond to give radicals



For secondary positions migration of the Fe-C σ-bond from iron onto oxygen occurs



Ketones are always the major product from these types of oxidations and the proposed $Fe^{V} = C$ is cleaved by superoxide (or H_2O_2) as shown.



Magnesium Monoperoxyphthalate

Hydroxylation of saturated hydrocarbons by magnesium monoperoxyphthalate (MMPP) catalysed by manganese porphyrins can be achieved in acceptable yields within 2-10 min.[36] MMPP is a very efficient oxygen donor for manganese porphyrin catalysed reactions. The reactions are normally performed at room temperature under phase transfer conditions with 0.5% of the catalyst plus a heterocyclic nitrogen base (usually 4-tert.-butylpyridine). With saturated hydrocarbons, alcohols and ketones are produced. There was no formation of oxygenated products when the reaction was repeated in the absence of the metal porphinate, while the N-base, although useful, was not essential for the reaction to take place. In these oxidations, there was a marked preference for oxidation at the tertiary C-H versus the secondary C-H bonds, and very little primary C-H oxidation. In the oxidation of adamantane, turnover rates (moles of products/mole of catalyst x min) up to 80 cycles/min can be obtained, giving 31% of adamantan-1-ol, 8% adamantan-2-ol, and 1% of adamantan-2-one.

1.4 CrO_3 Oxidations

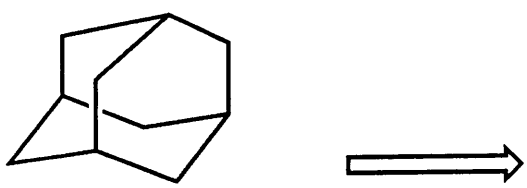
The selective oxidation of saturated hydrocarbons by inorganic oxidants is an important and often difficult procedure because the required vigorous conditions also promote second-stage oxidation: for example, tertiary alcohols may be dehydrated to olefins, which are then further attacked.

The chromium(VI) oxidation of unactivated carbon-hydrogen bonds is a rather selective process, strongly influenced by strain

factors. Oxidation of tertiary C-H bonds predominates over attack at CH_2, and methyl groups are essentially unaffected (the relative rates of oxidation of typical primary, secondary and tertiary hydrogens are 1:110:7000.[37] The reactions are generally performed using chromium (VI) oxide in suitable solvents.[38] When chromium(VI) oxide, which is a linear polymer $\{CrO_2O\}_n$, is dissolved in acetic anhydride, it undergoes depolymerisation and chromyl acetate is formed.[38]

$$(CrO_3)_n + n\ Ac_2O \rightleftharpoons n\ (AcO)_2\ CrO_2$$

Reaction of CrO_3 in $AcOH/Ac_2O$ with adamantane (25) gave mainly oxidation at the bridgehead.[37]



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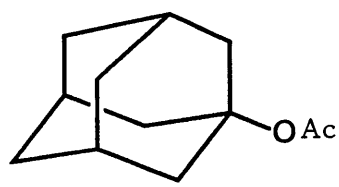

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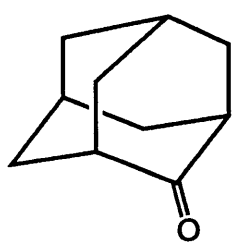

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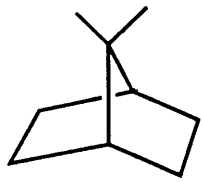
However, oxidation of norbornane (26) or bicyclo[2.2.1]heptane (27) gave no bridgehead products, yielding only ketones and some secondary alcohols.



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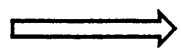

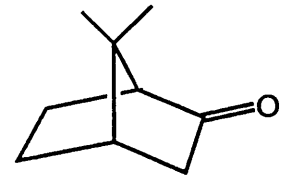

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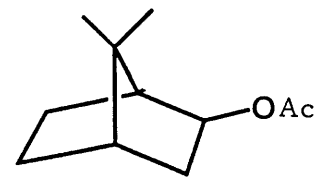
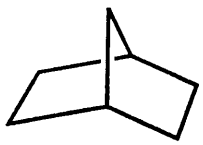


6%

The inhibition of bridgehead oxidation of small bicyclic systems such as bicyclo[2.2.1]heptane (27) is consistent with the accepted mechanism[39] of hydrocarbon oxidation by CrO_3. It is obvious that the cleavage of the C-H bond is the rate-determining step in the reaction, and this is confirmed by the reasonably large kinetic isotope effect observed in these oxidations. The initial step is believed to be hydrogen atom abstraction to give a caged radical pair as shown.

$$R_3C - H + Cr(VI) \longrightarrow R_3C^{\cdot} + Cr(V)$$

Collapse of this radical will give retention of configuration.[40] The radical can also undergo electron transfer to give carbonium ions from which certain products, e.g. those involving skeletal rearrangement, occasionally result. This mechanism is preferred over a direct insertion process (which would also give retention of configuration)



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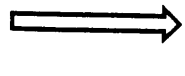

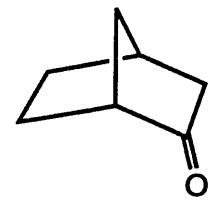

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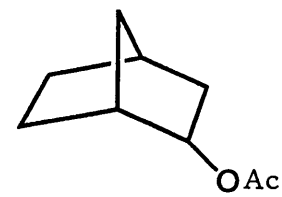

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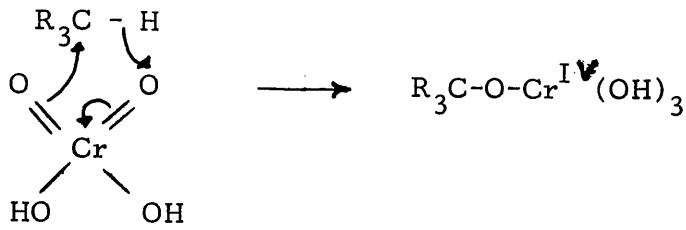
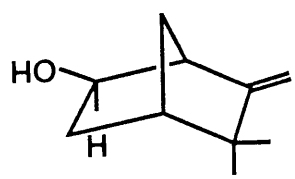


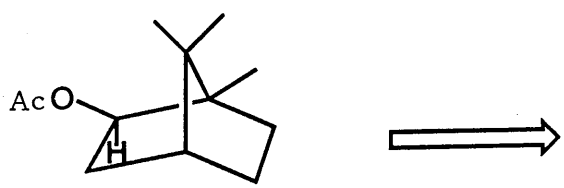
because in the insertion shown above, steric hindrance at the 5-valent activated complex should be apparent. However, exchanging the R groups with larger alkyl groups generally enhances, rather than reduces the reaction rate.[38]

The CrO_3 oxidation of hydrocarbons can be used synthetically, for example in a synthesis of the plant metabolite Nojigiku alcohol (28)[41] from Chrysanthemum japonense.



28

Highly selective remote oxidation of (-)-isobornyl acetate (29) with CrO_3 in $AcOH/Ac_2O$ gave a 4:1 mixture of 5-ketoisobornyl acetate (30) and its 6-keto-isomer (31) in 55% total yield.



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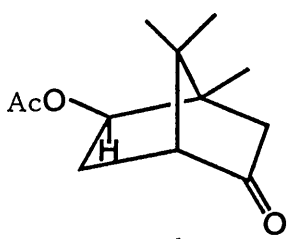

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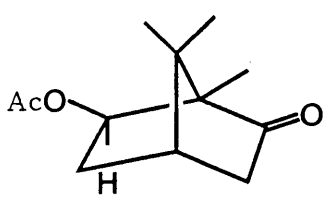
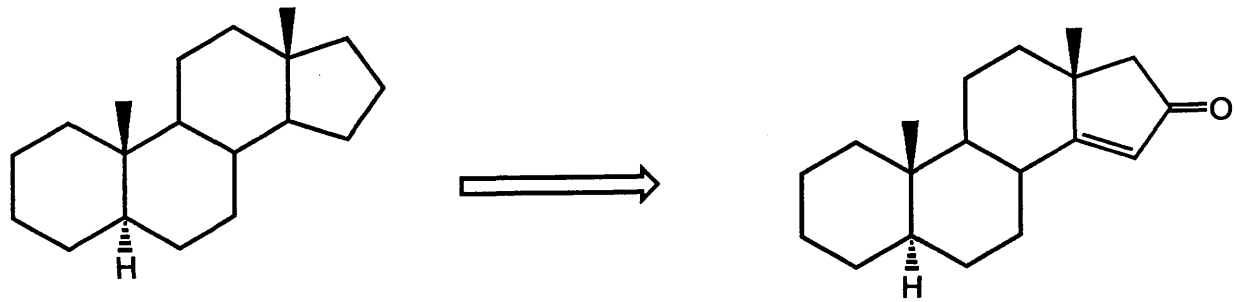


31

CrO_3 oxidation of non-activated C-H bonds in steroids has been shown to be a highly selective process in a few cases.[42] Oxidation of 5α-androstan-3β-yl acetate (33) and (3a) gave 5α-androst-14-en-16-one and 3β-acetoxy-5α-androst-14-en-16-one, respectively in excellent yield. The reaction conditions employed 5 molar equivalents of CrO_3 per steroid in $CH_2C\ell_2$/AcOH/Ac_2O with stirring at RT for 17h.



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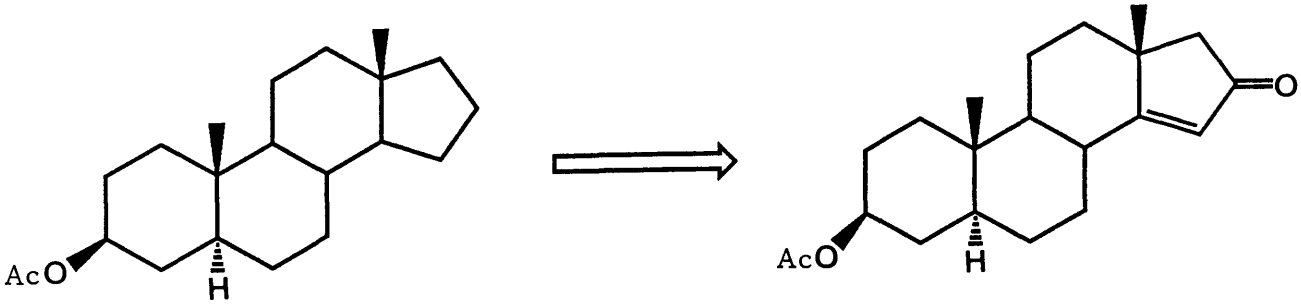


33

Section 2: Introduction

Functionalisation Over Short Distances

Many studies have been carried out on functionalisation of unactivated C-H bonds via an intramolecular abstraction of a hydrogen atom attached to a carbon atom by a reactive hetero-radical. The process usually demands a 6-membered cyclic transition state within the framework of a fairly rigid molecule. The diagram shown (Fig. 4)[43] reveals how to functionalise various angular methyl groups on a steroid with appropriately placed alkoxy radicals. However, the O-atoms shown can in principle be replaced by N-atoms.

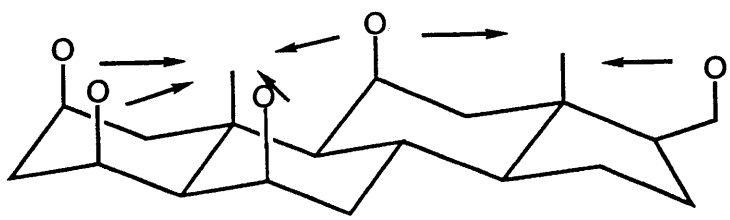
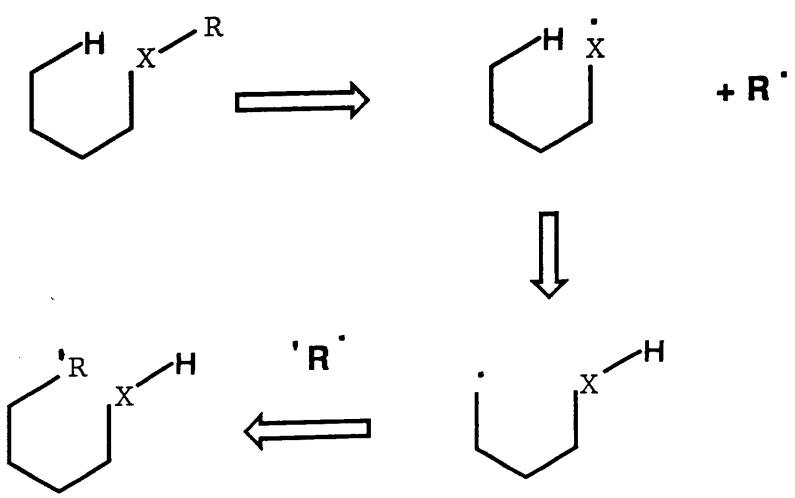


Fig. 4: Functionalisation of angular methyl groups.

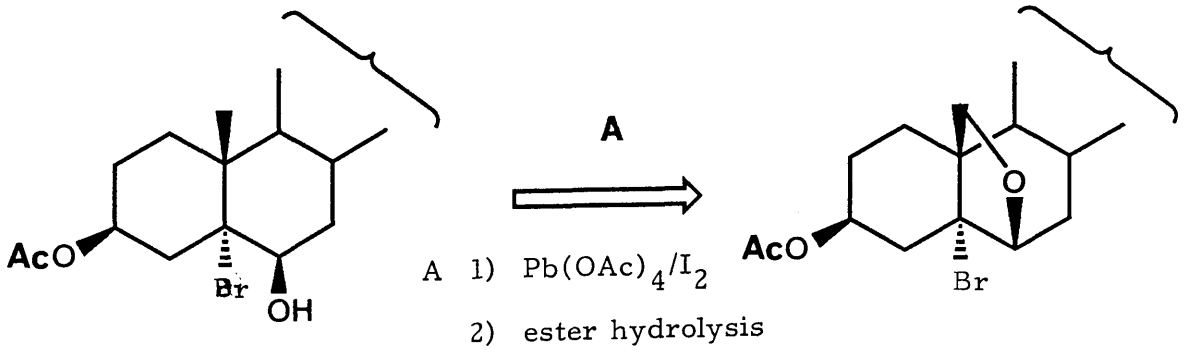
Formation of the Hetero-radical

The radical AX$^{\cdot}$ is formed by homolysis of the AX-R bond either thermally or photochemically

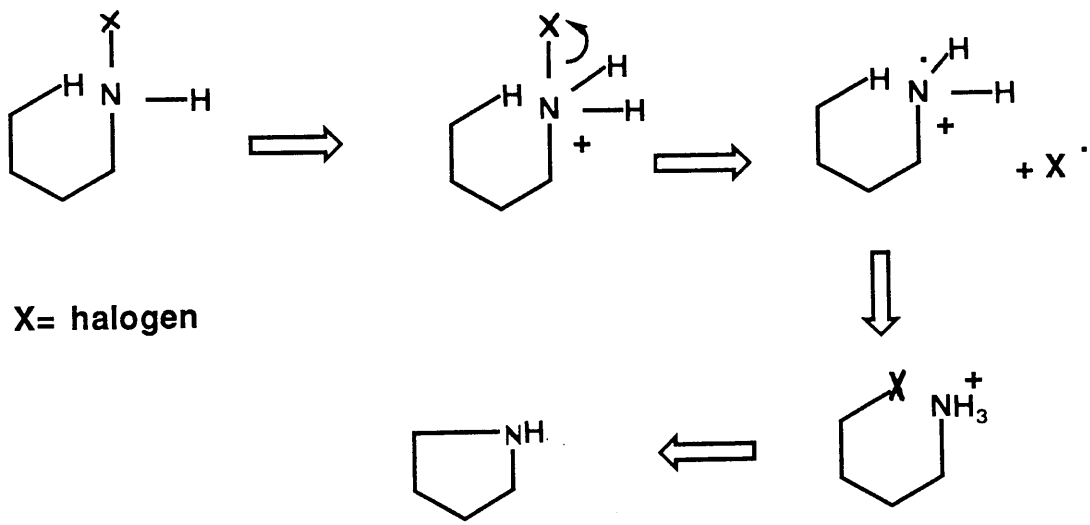


$Pb(OAc)_4$ can be used to generate the alkoxyl radical, although the Pb alkoxides have never been isolated and are usually generated *in situ*. The main disadvantages of using $Pb(OAc)_4$ to generate alkoxy radicals are: a large excess of the reagent is needed for completion; under thermal conditions secondary alcohol oxidation is predominant; acetylation can occur at allylic sites or α- to ketone groups; and epimerisation at one or two asymmetric centres can occur by a carbonyl-forming fragmentation process.

Alkoxy radicals can be generated from alkyl hypohalites, particularly from alkyl hypoiodites, although the latter type are usually prepared *in situ*, whereas the alkyl hypochlorites have been isolated. The alkyl hypoiodites are usually prepared from a heavy metal oxide or acetate *e.g.* (HgO, AgOAc, $Pb(OAc)_4$) by reaction with I_2 and alcohol. Among these, the $Pb(OAc)_4/I_2$ system is the one most often used to generate alkyl hypoiodites, and frequently appears to give the best yields.[43] For example, 5α-bromo-6β-alcohols were used in early work to functionalise the C-19 angular methyl group for the preparation of 19-nor-steroids from 19-substituted steroids.[43]

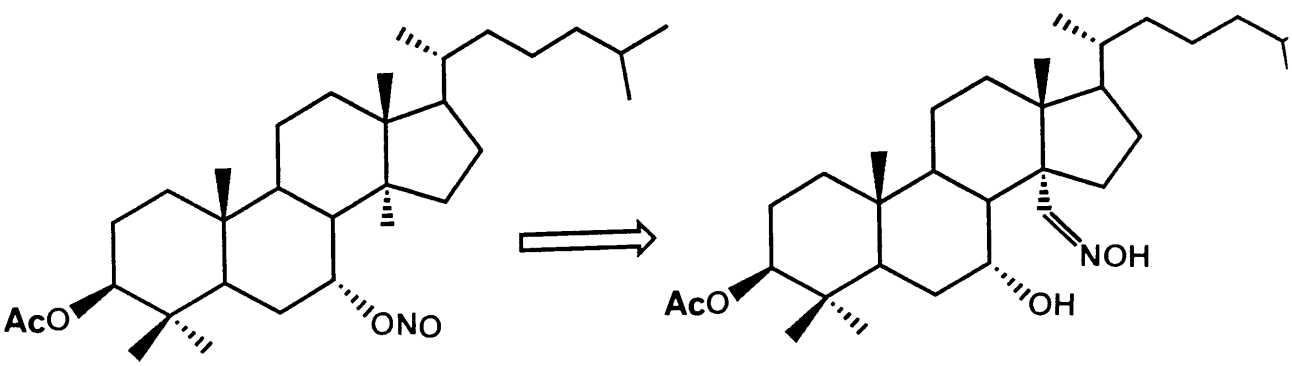


The Hofman-Löffler (N-chloroamine reaction) reaction has also been used to generate reactive hetero radicals. N-halogenated secondary amines can be irradiated under acid conditions to give an immonium radical intermediate as shown.



Subsequent hydrogen abstraction occurs in the γ-position which is then chlorinated in a chain-type reaction.

The Barton reaction[44,45] is a useful method for attacking unactivated sites via irradiation of solutions of steroidal alcohol nitrites.



60%

Studies on the photolysis of simple aliphatic and alicyclic nitrites have confirmed the need for a 6-membered transition state, therefore deciding the point of attack on the carbon chain. The photolysis of 3β-acetoxy-5α-lanostan-7α-yl nitrite gives an alkoxy radical which can attack the unactivated C-30 methyl group to form the 3β-acetoxy-30-hydroxyimino-lanostan-7α-ol in 60%[46] yield. Other important applications of the nitrite photolysis include the functionalisation of the C-18 and C-19 methyl groups with a nitrite ester attached at the 11β-position.

Photolysis of hydroxy compounds in the presence of iodobenzene diacetate [$PhI(OAc)_2$] and iodine leads to the generation of alkoxy radical derivatives which can then undergo hydrogen atom abstraction. This method has been developed by Suarez *et al.* as a continuation of their interest in intramolecular functionalisation reactions.[47]

$PhI(OAc)_2$ is a stable crystalline solid, and the reactions with $PhI(OAc)_2 + I_2$ proceed smoothly under mild conditions. $PhI(OAc)_2$ has the advantage over $Pb(OAc)_4$ in that only 1 mole equivalent is needed for complete reaction; moreover, yields are of the same order as, or better than, those with the $Pb(OAc)_4/I_2$ system (Scheme 5). The reaction mechanism is thought to involve hypoiodites and a possible pathway to these is shown:

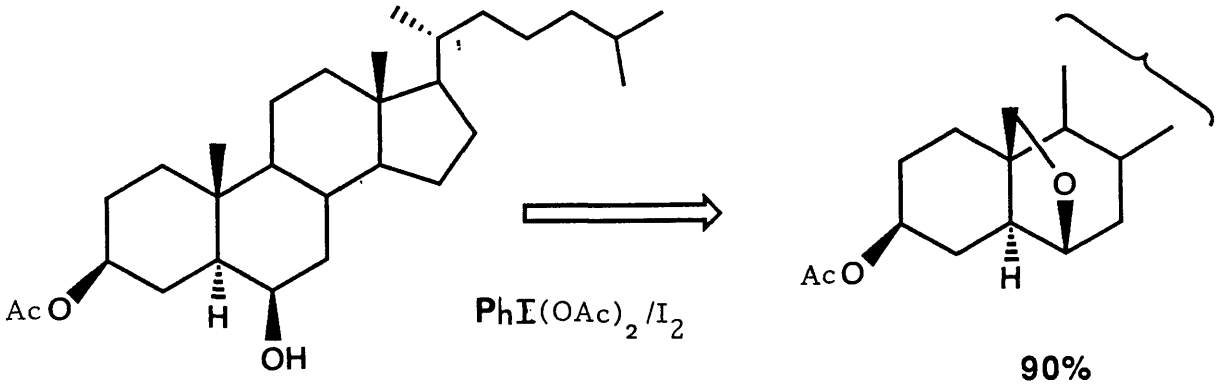
$$PhI(OAc)_2 \longrightarrow Ph\dot{I}OAc + \dot{O}Ac$$

$$I_2 + \dot{O}Ac \longrightarrow I^{\bullet} + IOAc$$

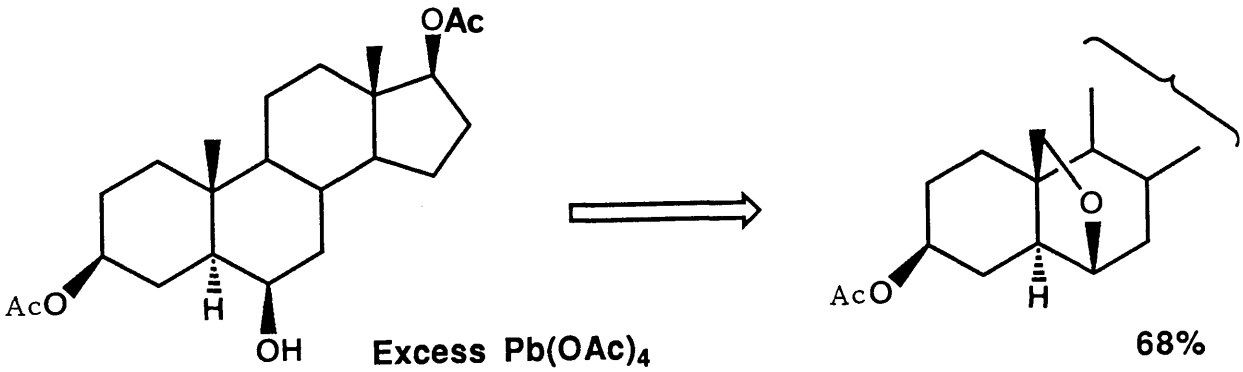
$$Ph\dot{I}OAc + .ROH \longrightarrow RO^{\bullet} + PhI + HOAc$$

$$RO^{\bullet} + I_2 \longrightarrow ROI + I^{\bullet}$$

SCHEME 5



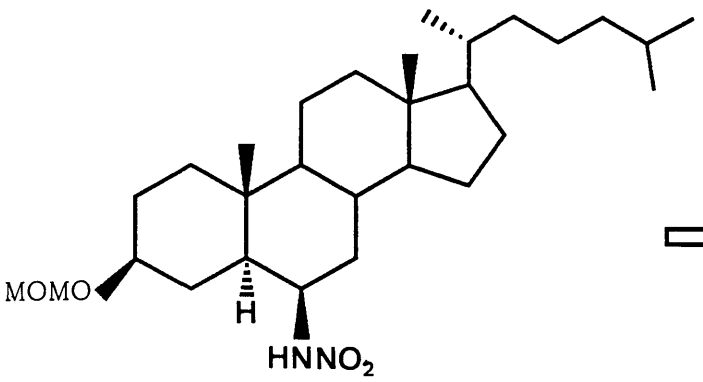
whereas $Pb(OAc)_4$ gave only a 68% yield, as shown:

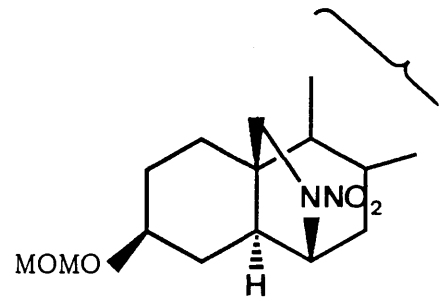


Generation of Neutral Nitrogen Radicals[48]

As mentioned earlier, the Hofmann-Löffler reaction, based on immonium radical intermediates, is of limited use in complex molecules, because of the highly acidic conditions needed. However, the $PhI(OAc)_2/I_2$ system is an excellent reagent for neutral nitrogen radical generation.

The steroidal 6β-N-nitro-amine was prepared, and on irradiation gave the pyrrolidine derivative as shown:[49]





$PhI(OAc)_2$ 1.1 mol

I_2 0.5 mol

77%

$Pb(OAc)_4$ 3 mol

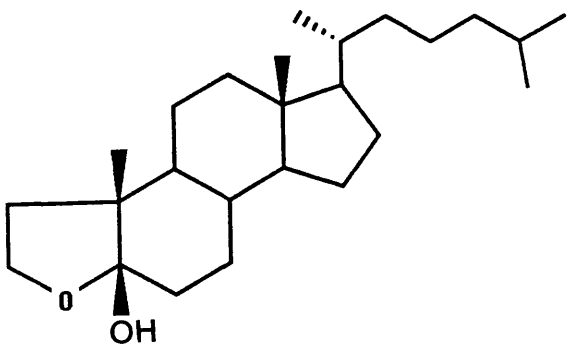
I_2 1 mol

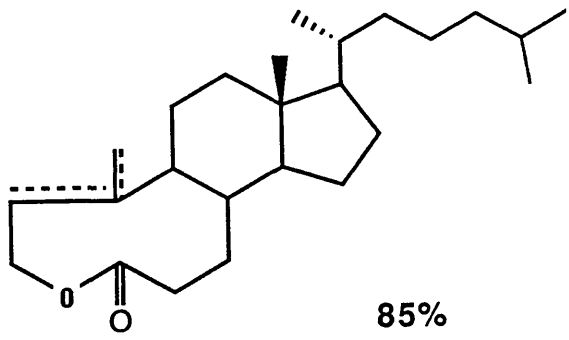
51%

N-iodonitroamines are thought to be generated in situ, then homolysis of the N-I bond would generate neutral N-radicals, which could undergo hydrogen atom abstraction. Intramolecular functionalisation of N-cyano radicals has also been achieved.[48,50]

Fragmentation of Alkoxy Radicals

The reaction of $PhI(OAc)_2/I_2$ with γ- and α-lactols can be used as an efficient method for the synthesis of medium-sized lactones.[51]



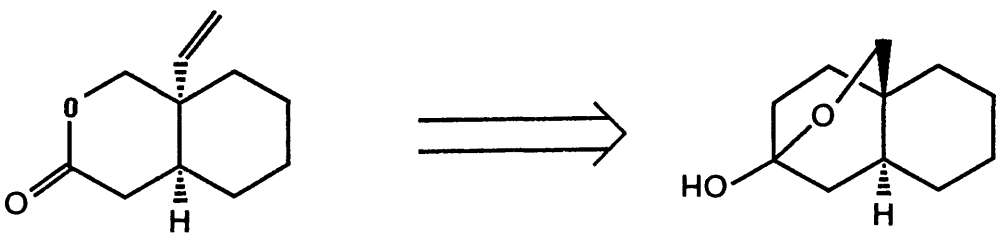


85%

1:1 ($\Delta^{10,19}$:$\Delta^{1,10}$)

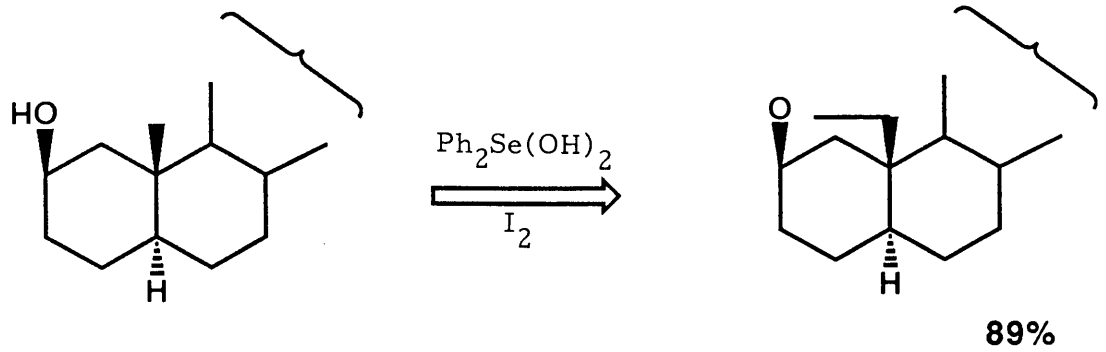
Fragmentation of the alkoxy radical generated in situ from the γ-lactol effected ring expansion to the olefinic 9-membered lactone. These fragmentations have led to a model study of the synthesis of ring A in vernolepin (a cytotoxic, antitumoral sesquiterpenoid of the elemane group). A retro-synthetic approach is shown in Scheme 6.

SCHEME 6



Recently the same group have reported[54] that tetravalent selenium compounds e.g. $Ph_2Se(OH)_2$, upon irradiation in the presence of I_2 and an alcohol, produce alkoxy radicals which could undergo hydrogen atom abstraction (Scheme 7) although no mechanistic detail was given.

SCHEME 7



89%

Ce(IV) is a very powerful single-electron oxidant [Ce(IV) + e ⟶ Ce(III) E° = 1.37 V) in 1M HNO_3. The most widely used Ce(IV) reagent for organic oxidation is diammonium hexakis-(nitrato-O-cerate) commonly known as ceric ammonium nitrate [CAN, $Ce(NH_4)_2(NO_3)_6$]. In these oxidations, cation radicals or free radicals are generated and normally these intermediates undergo rapid oxidation to afford neutral products by electron transfer or by ligand transfer:

$$R^{\cdot} + Ce(IV) \longrightarrow R^{+} + Ce(III)$$

or

$$R^{\cdot} + ONO_2\ Ce(IV) \longrightarrow R\text{-}ONO_2 + Ce(III)$$

Benzylic methyl and methylene groups can be converted to carbonyl functions by treatment with CAN in acidic media. The reaction normally stops at the mono-carbonyl stage. However, a second methyl group may undergo oxidation under more drastic conditions. For example, p-xylene can be oxidised to 4-methylbenzaldehyde.[55]

It is generally accepted that the side chain oxidation of alkylarenes by Ce(IV) occurs via a radical cation and not by direct hydrogen atom abstraction.

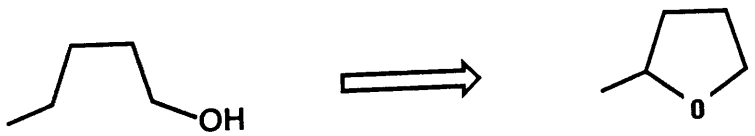
$$ArCH_3 + Ce(IV) \longrightarrow ArCH_3^{+\cdot} + Ce(III)$$

$$ArCH_3^{+\cdot} + B \longrightarrow ArCH_2^{\cdot} + BH^{+}$$

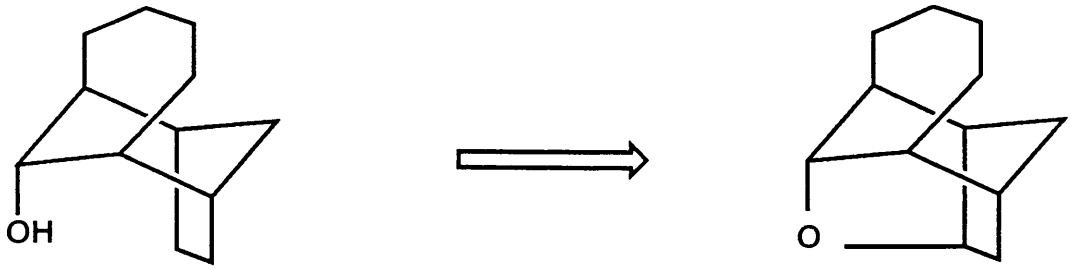
$$ArCH_2^{\cdot} + Ce(IV) \Longrightarrow \text{products} + Ce(III)$$

It is thought that the benzyl radical then undergoes a ligand transfer process, and not further oxidation to the benzylic cation as determined from the common ion effect.[56,57]

CAN can be used for the intramolecular oxidative cyclisation of alcohols to form, in most cases, ethers. Ceric ion oxidative cyclisation has been observed for n-pentanol, although in low yield.[58]

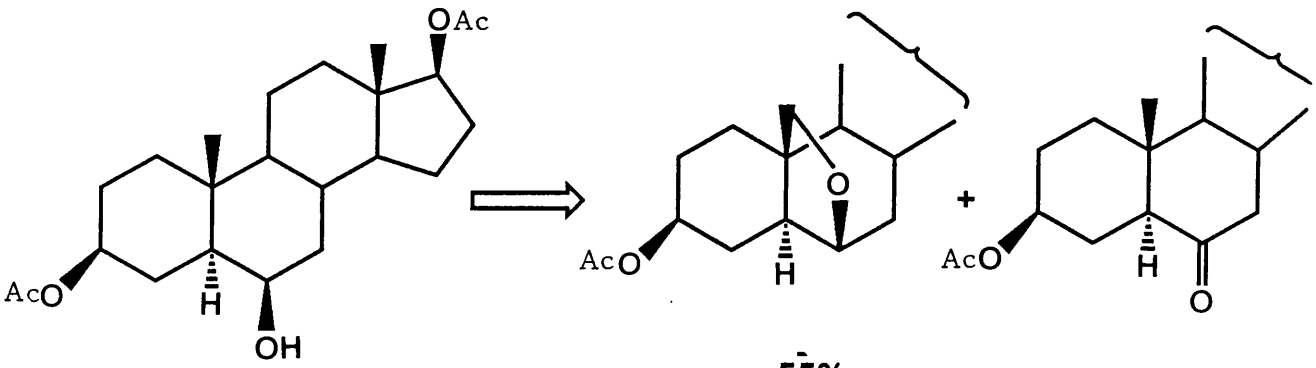


A higher yield was observed in the oxidation of the tricyclic alcohol as shown.[59]



85%

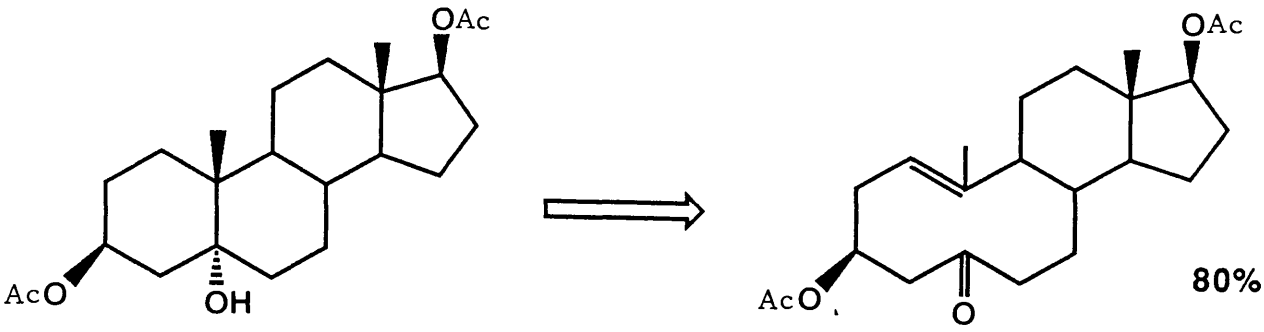
Balasubramanian and Robinson have shown that 6β-hydroxy-5α-steroids undergo smooth oxidative cyclisation to give the corresponding 6β,19-ether compounds in good yield.[60]



55%

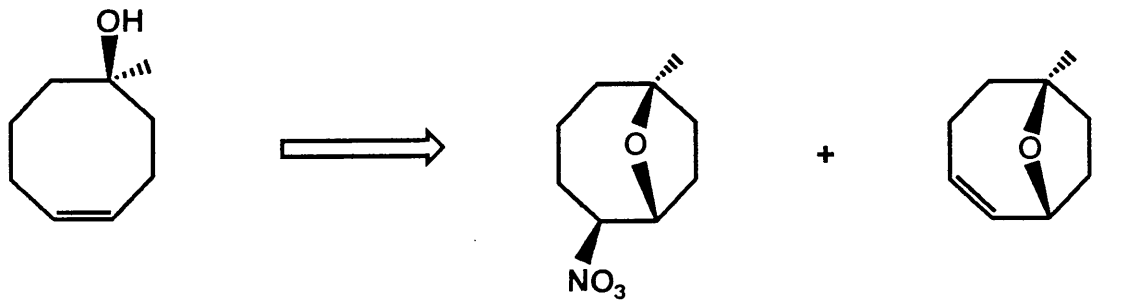
34%

The yield of the cyclic ether decreases with increase in the size of the substituent at C-5. These reactions are analogous to the transformations promoted by reagents such as $Pb(OAc)_4$, HgO/I_2 or $AgOAc/Br_2$.[61] The reactions are thought to occur by initially generating an alkoxy radical which can then undergo a 1,5-hydrogen shift (from a conformationally adjacent γ-carbon atom), then electron transfer regenerating the alkoxy radical, and finally radical coupling. CAN-induced oxidative fragmentation of tertiary alcohol (34) gave the secosteroid (35) in good yield.[60]

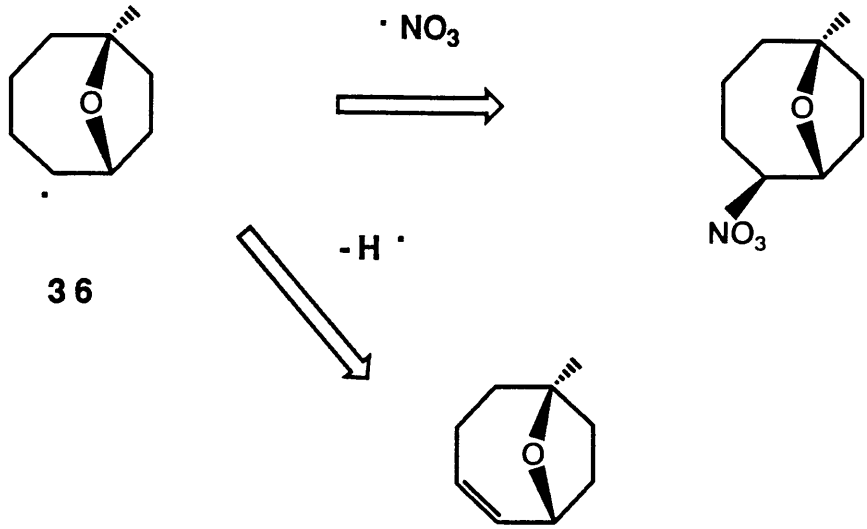


34 35

Regioselective and stereoselective oxidative cyclisation of cyclo-octenols has been achieved using CAN giving formal syn oxidative addition to the alkene.[62]



Radical cyclisation would give the secondary radical (36) which can then undergo ligand transfer to form the nitrate or lose an H-atom to form the alkene.



CAN has also been used to oxidise alkenes.[63] The products from the reaction are dependent on the solvent used, and the results can be explained by the addition of a nitrate radical to the double bond (see Section 3.2, page 63).

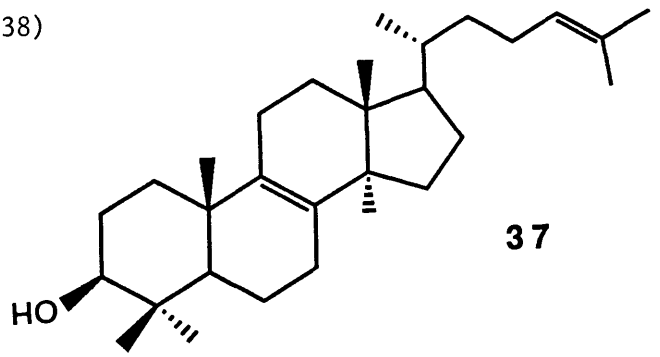
Section 3: Results - Short Range Functionalisation

3.1 Ceric Ammonium Nitrate Oxidation of 25-Hydroxy-5α-lanost-8-en-3β-yl Acetate

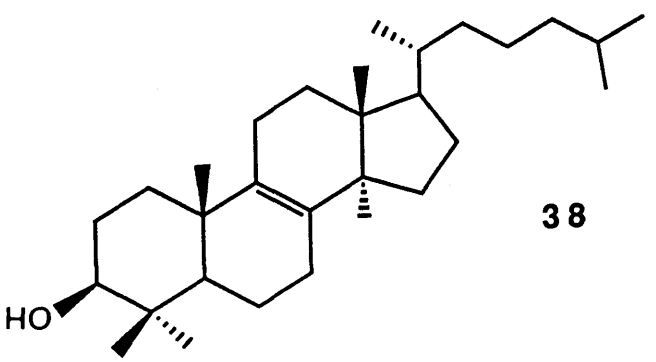
Ceric ammonium nitrate is a good oxidising agent *via* single electron transfer. 25-Hydroxy-5α-lanost-8-en-3β acetate is an interesting substrate for the reaction with CAN, since the proposed 25-oxygen radical (produced *via* single electron transfer) may be expected to undergo hydrogen atom abstraction by an intramolecular means, thus opening the possibilities for nuclear or side-chain functionalisation.

Preparation of 25-Hydroxy-5α-lanost-8-en-3β-yl Acetate[64]

Lanosterol (4,4,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol) (37) is an unsaponifiable or alcohol fraction of wool wax[65] and crude lanosterol contains about 40% of 24,25-dihydrolanosterol (5α-lanost-8-en-3β-ol) (38)



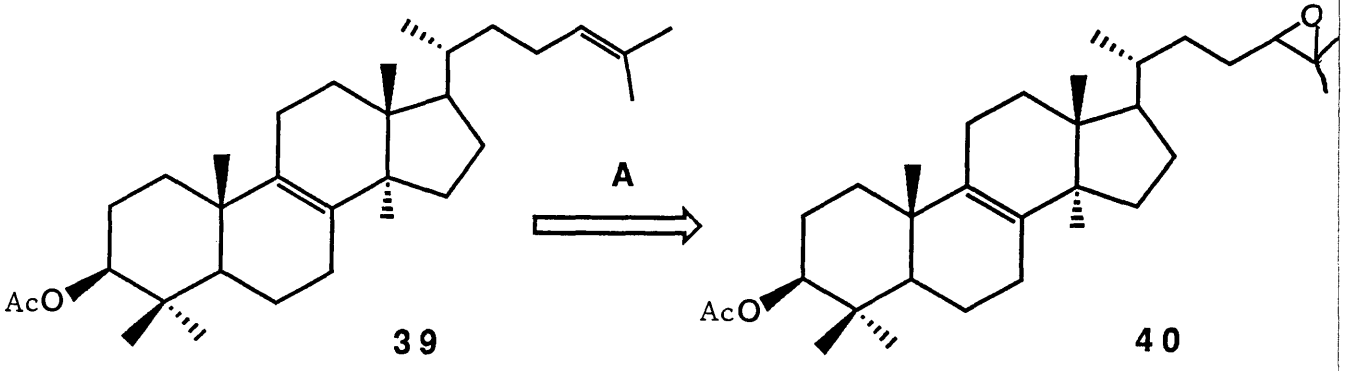
37



38

24,25-Dihydrolanosterol (38) can be separated from lanosterol (37) although in low yield,[66] by forming the 24(R,S)-25-dibromide, selective crystallisation of the dibromide and regenerating the Δ^{24}- double bond with Zn dust or NaI. It was proposed to separate the 24,25-dihydro-derivative at a later stage in the preparation. Crude lanosterol was acetylated using Ac_2O/pyridine to yield (39) plus the 24,25-dihydro-derivative (see Experimental section 3.1.1). 25-Hydroxylanost-8-en-3β-yl acetate has been prepared previously by Boar et al.[64] and it was proposed to follow their method, although there were some variations in procedure.

Monoepoxidation of Crude Lanosteryl Acetate

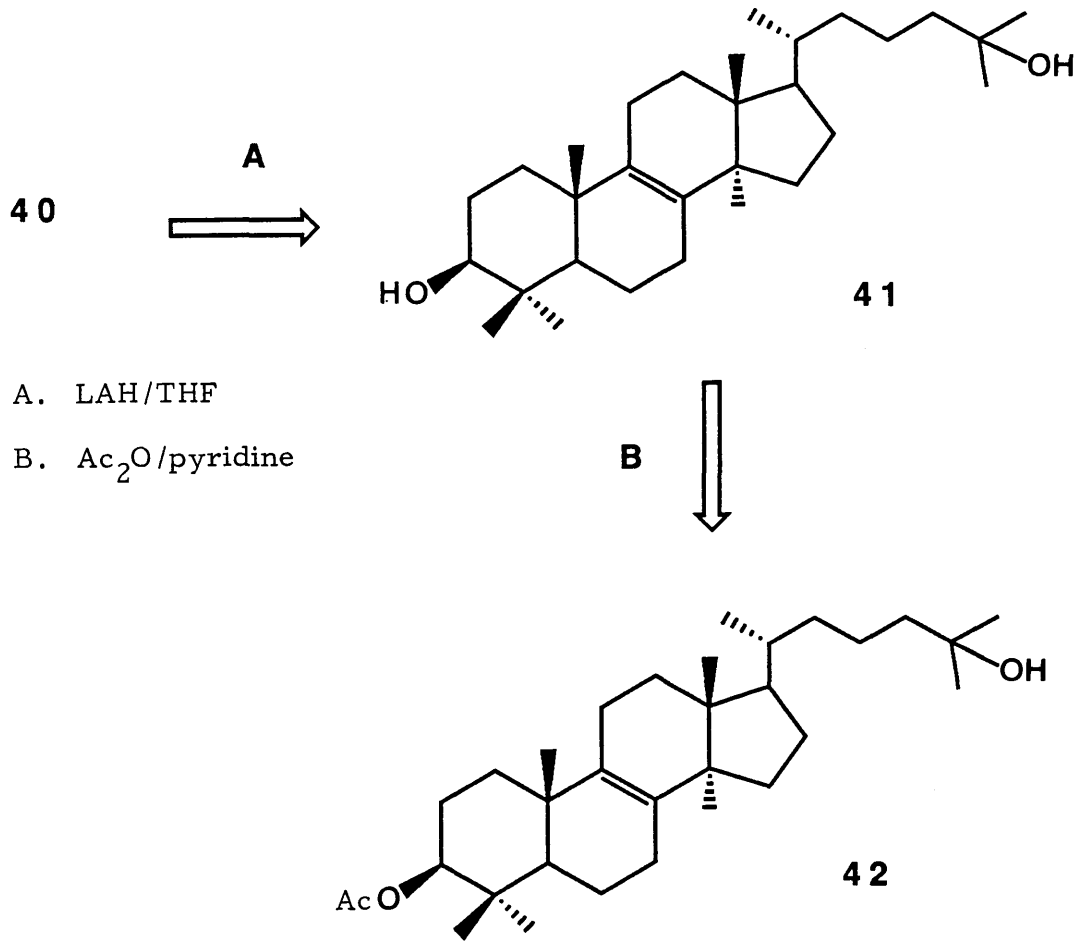


39 + 24,25-dihydro- 40

A 1.1 equiv. mcpba/-10°C

Specific monoepoxidation of the 24(25) double bond in crude lanosteryl acetate was achieved, giving a 1:1 mixture of diastereomeric epoxides (40), contaminated with 24,25-dihydrolanosteryl acetate. The 24,25-dihydrolanosteryl acetate was removed by column chroma-

tography. The two epoxides (40) were reduced using LAH in dry THF to give 25-hydroxylanost-8-en-3β-ol (41) as the main product. Monoacetylation gave the desired 25-hydroxylanost-8-en-3β-yl acetate (42). (See Experimental section 3.1.2).



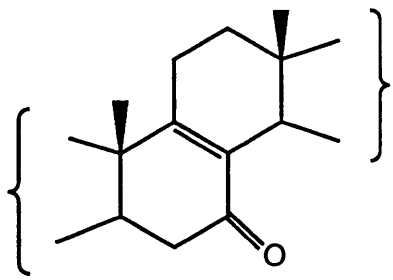
CAN Oxidation of 25-Hydroxylanost-8-en-3β-yl Acetate

Initial small-scale reactions were performed with monitoring with GLC/TLC. The method used was to dissolve the steroid in CH_3CN with heating to 80°C. Aqueous CAN was added dropwise and the reaction mixture left for 10-15 min (until the yellow colour had

disappeared. After this time, the reaction mixture was diluted with water then extracted with EtOAc[60] (see Experimental section 3.1.3).

Using 1.1 molar equivalents of CAN both GLC and TLC revealed no reaction. However, use of ca. 20 molar equivalents of CAN gave about 15 products (see Table 2) in varying proportions, with only a small amount of starting material remaining. Preparative TLC was used to remove three bands (see Table 3). The non-polar band, R_F 0.72 contained four major compounds, all of higher molecular weight than the starting material. The band at R_F 0.26 ($I^{OV-1}_{-270°}$ = 3870) contained a single compound, but no informative GC-MS analysis was obtained (even after derivatisation using BSTFA ($I^{OV-1}_{-270°}$ = 3930)). Few ions were produced for this compound, the most abundant ion being $[C_3H_6OTMS]^+$ at 131 amu, indicating the presence of the 25-hydroxy function. Other ions occurred at 660 or 661 and 364 amu. Work has been mainly focused on the polar (R_F 0.15) high molecular weight material. This component ($I^{OV-1}_{-270°}$ = 4100) was treated with BSTFA and subjected to GC-MS analysis which indicated the product to be nitrogenous: this was confirmed by direct-probe MS (see Table 4) showing $[M]^{+\cdot}$ at 629. Both results indicated the 25-hydroxy and the acetate group to be present. High resolution mass spectrometry (see Table 5) showed the molecular formula as $C_{37}H_{63}NO_5Si$. Thus, C_2HNO_2 has been added to 25-hydroxylanost-8-en-3β-yl acetate ($C_{35}H_{62}O_3Si$). It was concluded that the solvent (CH_3CN) had in part been incorporated into the steroid. Also, upon changing the reaction solvent to AcOH, GLC plus TLC analysis revealed none of this polar product, but still indicated a complex mixture of products. The IR spectrum of the

nitrogenous product (see Table 6(ii)) showed O-H stretching at 3610 cm^{-1} (w) and N-H stretching at 3400 cm^{-1} (w). In the carbonyl region, there was observed ν (C=O) (ester) at 1730 cm^{-1} (s), an absorption at 1685 cm^{-1} (s) which may be due to a carbonyl band of an enone or secondary amide (band I). An additional band at 1635 cm^{-1} (s) was observed which is possibly the amide II band. The UV spectrum indicated the presence of an enone $\lambda_{max.}$ EtOH = 252 nm, ε 11,800.

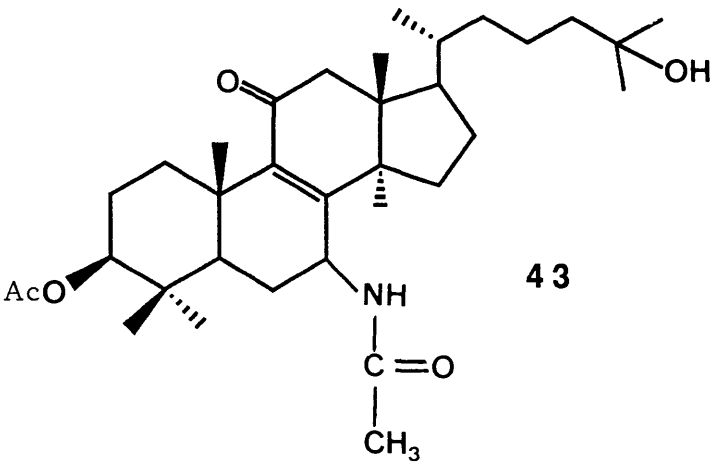


λ 253 nm, ε11,200[67]

25-Hydroxylanost-8-en-3β-yl acetate was subjected to 200 MHz ^{1}H and 25 MHz ^{13}C DEPT NMR study (see Table 7). Tentative assignments have been made for some signals. ^{13}C and ^{1}H NMR spectroscopy of the nitrogenous material was not very informative, due to the small amount of sample and the presence of impurities (see Table 8). However, the 200 MHz ^{1}H NMR spectrum revealed a NH proton doublet at 5.68 ppm (J = 10 Hz). The signal at 4.80 ppm (bd, J = 10 Hz) was thought to be the 7-H methine proton attached to an electronegative atom (N). The AB quartet system at 2.55 ppm (J = 19 Hz) suggested geminal coupling of methylene protons α- to a carbonyl group. An 11-keto group would account for this signal in which the C-12 methylene protons are geminally coupled.

Also, an additional CH_3CO- signal was consistent with an acetamido group (CH_3-$\overset{O}{\overset{\|}{C}}$-$\underset{H}{N}$-R). The 11-keto-acetamido structure shown (43)

was indicated by the spectroscopic evidence.



43

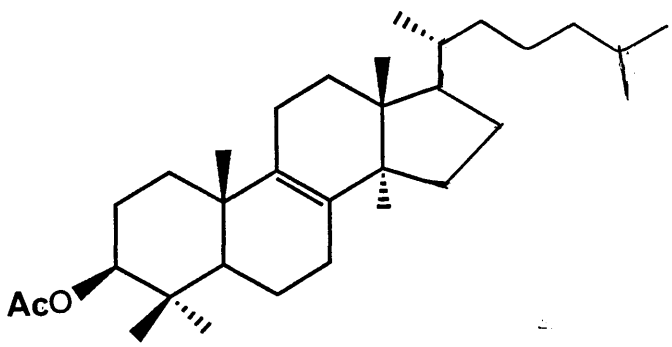
Products of base hydrolysis and acid extraction of the nitrogenous product were studied by GLC and TLC (see Experimental section 3.1.4). It was attempted to extract the liberated amine salt. However, only the organic extract contained products from the hydrolysis. Two compounds ($I^{OV-1}_{270°}$ = 3990, $I^{OV-1}_{270°}$ = 3640) were observed in the organic extract. The retention index of one, $I^{OV-1}_{270°}$ = 3990 was indicative of the hydrolysis of one acetate group, and on reacetylation this compound regenerated the original product. It therefore corresponds to a 3β-hydroxy analogue of the nitrogenous product. The component of $I^{OV-1}_{270°}$ = 3640 gave a product of $I^{OV-1}_{270°}$ = 3720 on reacetylation (for possible identities of components having $I^{OV-1}_{270°}$ = 3640 and $I^{OV-1}_{270°}$ = 3720, see Section on CAN oxidation of 5α-lanost-8-en-3β-yl acetate, page 62).

Due to difficulties in purification the CAN oxidation was attempted on a more convenient substrate i.e. 5α-lanost-8-en-3β-yl

acetate, and attention was temporarily diverted to elucidation of the allylic oxidation products, which appeared to have taken precedence over any significant oxidation in the steroid side chain.

3.2 Ceric Ammonium Nitrate Oxidation of 5α-lanost-8-en-3β-yl Acetate

The CAN oxidation of 5α-lanost-8-en-3β-yl acetate (44) was attempted, under the same reaction conditions as for 25-hydroxy-5α-lanost-8-en-3β-yl acetate, to ascertain if oxidation at the allylic positions is the main reaction pathway and to find out if a nitrogenous product would be formed from this simpler substrate.



Using a similar procedure to previous oxidations (see Experimental section 3.2.1) the crude reaction mixture was subjected to preparative TLC ($CHC\ell_3$:EtOAc 9:1 v/v). Two UV active bands were scraped off the plate (R_F 0.31, R_F 0.89). The more polar band (R_F 0.31) showed on GLC to be pure ($I^{OV-1}_{270°}$ = 3900), and GC-MS analysis (Table 9) indicated this compound to be nitrogenous in nature ($[M]^{+\cdot}$ = 541 amu). The fragmentation pattern suggested losses of 60 amu and 59 amu from the molecular ion which are consistent with losses of neutral molecules CH_3CO_2H and CH_3CONH_2, respectively.

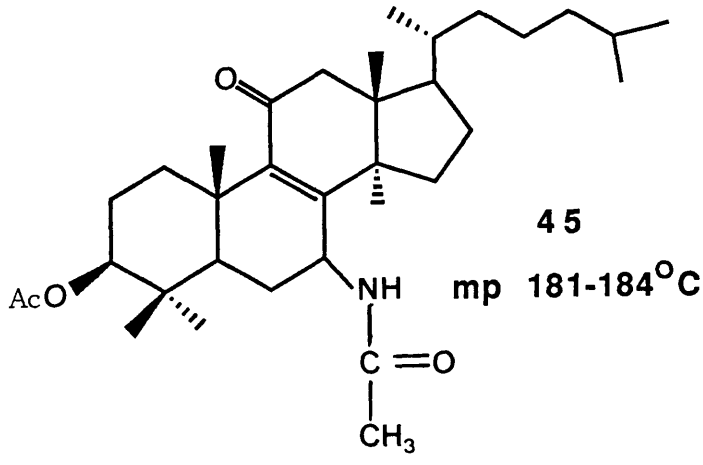
the loss of 59 amu being indicated by a metastable peak in the mass spectrum. The high resolution mass spectrum showed the molecular formula to be $C_{34}H_{55}NO_4$ (Table 10). The mass at 482 amu results from the loss of 59 amu from the molecular ion. This ion corresponds to molecular formula $C_{32}H_{50}O_3$, although it was not obtained on the Glasgow instrument. Two ions could have generated this mass (482 amu):

i) $C_{32}H_{50}O_3$ (calculated mass = 482.3759) which is the product from loss of $C_2H_5NO_2$ (59 amu) from the molecular ion ($C_{34}H_{55}NO_4$);

ii) $C_{31}{}^{13}CH_{51}NO_2$ (calculated mass = 482.3953) which is the product from loss of $C_2H_4O_2$ (60 amu) from the molecular ion containing one ^{13}C atom ($C_{33}{}^{13}CH_{55}NO_4$).

Therefore, a sample was sent to the S.E.R.C. MS Service (Swansea) with its higher resolving power instrument, to discriminate between masses (i) and (ii). Results indicated that both $C_{32}H_{50}O_3$ (6.23% intensity) and $C_{31}{}^{13}CH_{51}O_2N$ (17.56% intensity) existed (Table 11). Therefore the loss of 59 amu was indeed due to $C_2H_5NO_2$. The UV spectrum of the nitrogenous product indicated the presence of an enone system (λ_{max}^{EtOH} = 254 nm, ε = 8400). A larger quantity of the nitrogenous product was isolated by a combination of column chromatography and preparative TLC, and the IR spectrum (Table 12) showed a broad ν(N-H) band at 3392 cm^{-1} (w), an ester carbonyl band at 1733 cm^{-1} (s), an enone carbonyl band at 1684 cm^{-1} (s), and possible secondary amide stretching bands at 1715 cm^{-1} (m) (band I) and 1610 cm^{-1} (s) (band II). A 2D δH/δC NMR C.O.S.Y. experiment was

performed on the nitrogenous product (Table 13(ii)). The signal at 6.20 ppm (1H, d, J = 9 Hz) gave no 2D correlation. It was thought that this was due to an N-H proton. The signal at 4.78 ppm (1H, bd, J = 8 Hz) gave a 2D correlation to signal 48.253 ppm in the ^{13}C spectrum which corresponds to a CH in the DEPT analysis. In respect of the signal at 3.05 ppm (1H, bd with slight splitting, J = 14 Hz, J = 2 Hz), the 2D spectrum gave no $\delta C/\delta H$ observed correlation. It was thought that this signal was due to H-6, $C\underline{H}_2$. The signal at 2.49 ppm (2H, AB, J = 18 Hz) correlated to 51.915 ppm in the ^{13}C spectrum, which corresponds to a CH_2 in the DEPT analysis (H-12, CH_2). The signals at δ2.03 (3H, s) and δ1.99 (3H, s) correlated to carbon signals at 21.311 ppm and 23.338 ppm, respectively (both carbons were CH_3 adjacent to carbonyl groups). In accord with the NMR evidence, the 11-keto,7-acetamido structure (45) has been proposed.



45

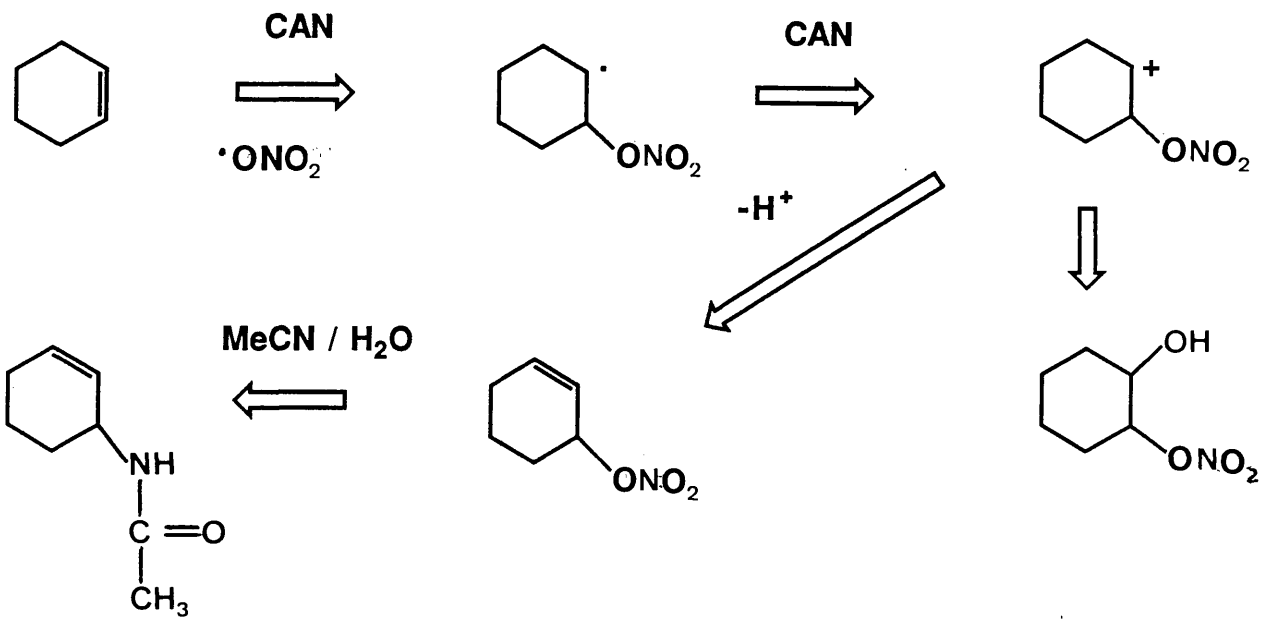
mp 181-184°C

Briguet et al.[63] have shown that the oxidation of cyclohexene with CAN in anhydrous DMSO leads to cyclohexene-3-nitrate, while in CH_3CN the 3-acetamide is formed and hydroxylated products

can be formed in the presence of water. The results have been explained in terms of the formation of an intermediate arising from the addition of the radical $NO_3^{\cdot}$ to the olefinic double bond (Scheme 8).

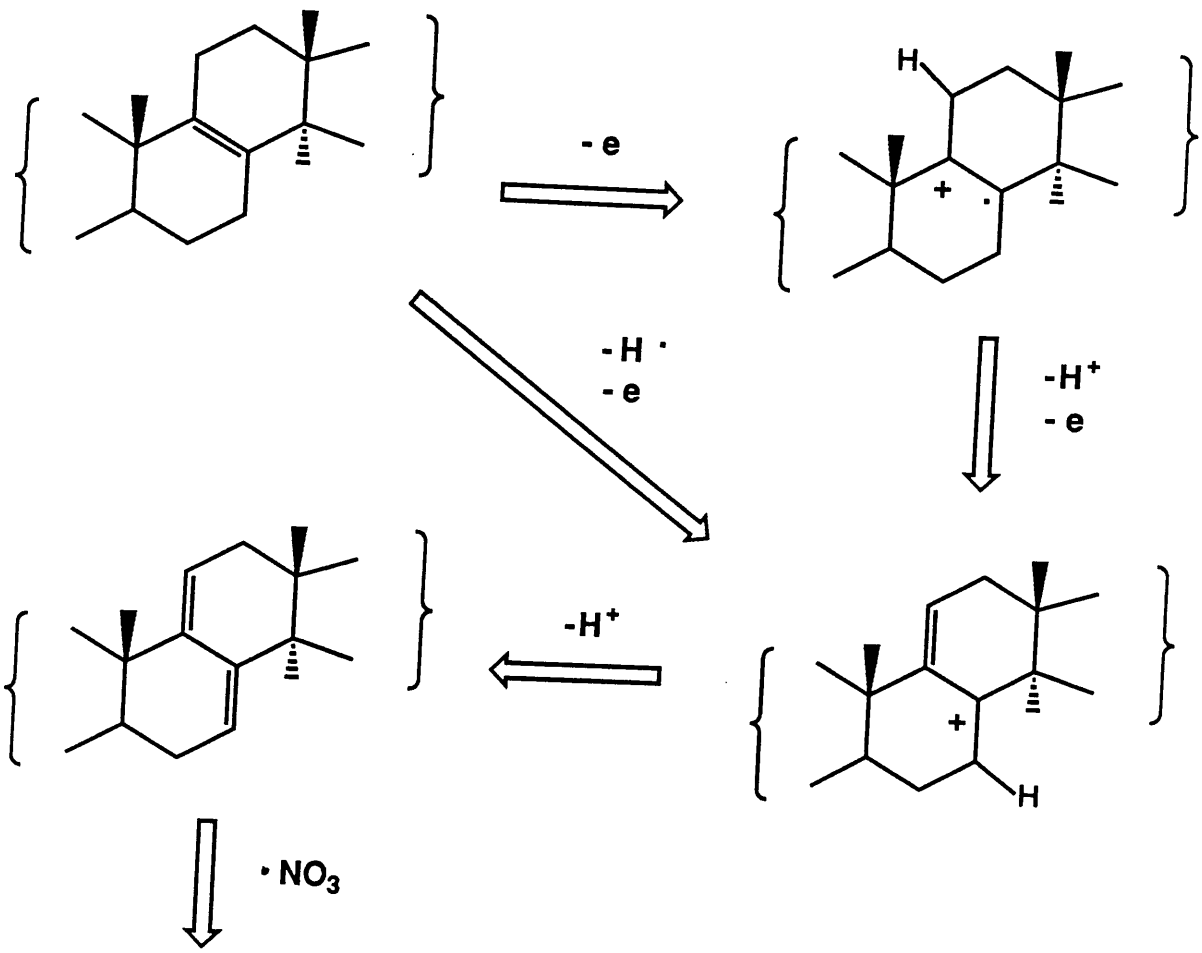
SCHEME 8

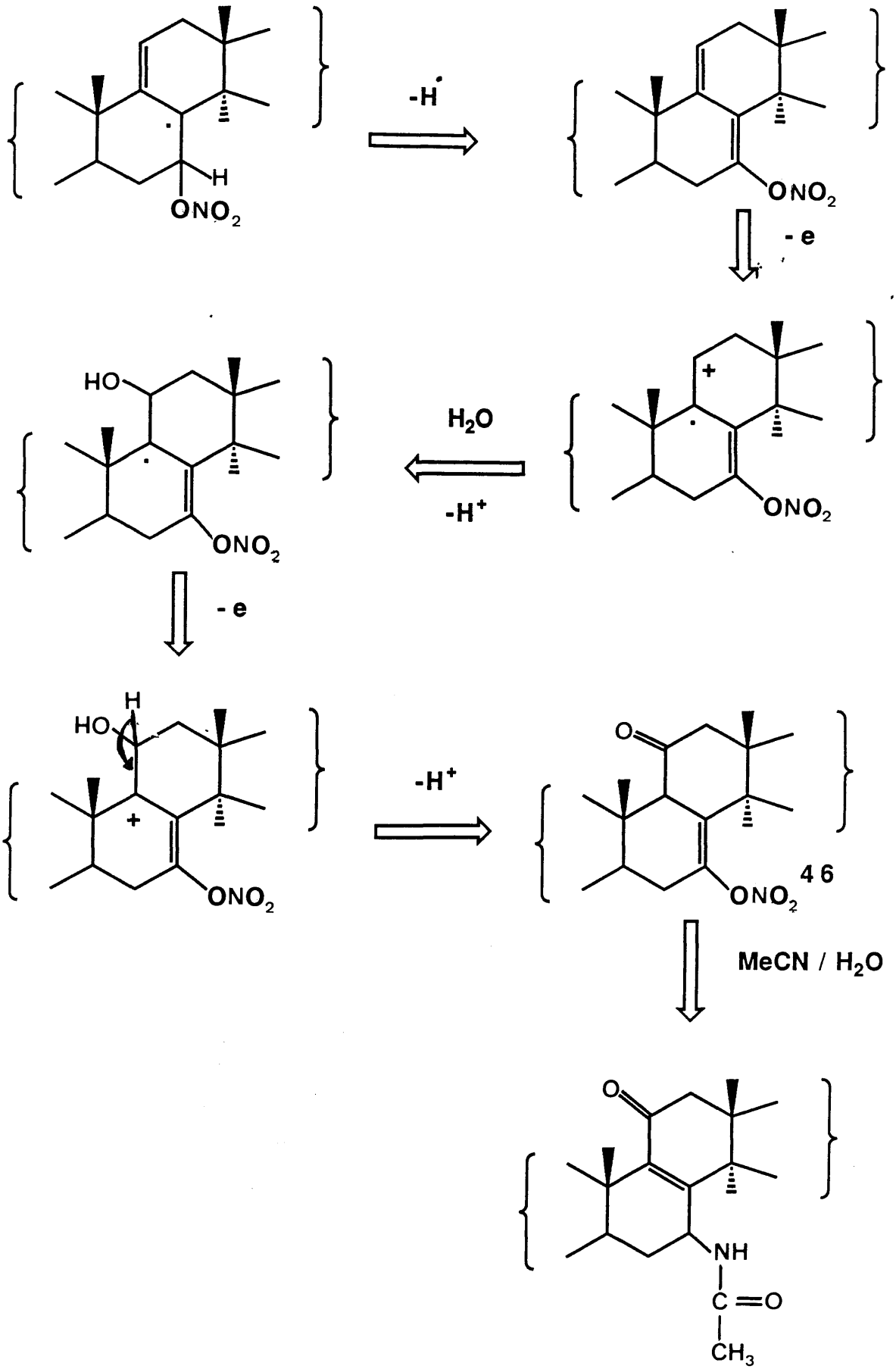
$$Ce(IV)\ NO_3^- \rightleftharpoons Ce(III) + {\cdot}NO_3$$



The nitrate radical is produced by ligand transfer oxidation, and can then add to the double bond. CAN oxidation of this nitrate radical would produce the carbocation, and loss of $H^{\oplus}$ would yield the allylic nitrate, which on solvolysis by CH_3CN and hydration would give the 3-acetamido-cyclohexene. In order to produce 45, it proposed that the nitrate radical adds to the diene system as shown (Scheme 9(i)).

SCHEME 9(i)




-H·
ONO2
H
- e
HO
H2O
-H+
- e
-H+
O
46
MeCN / H2O
NH
C=O
CH3

From the $\Delta^{8,9(11)}$-diene, there is a total of four electron oxidation steps to produce 46, the Δ^{7}-double bond can be brought into conjugation with the carbonyl group via the enol. Solvolysis of the 7-nitrate by CH_3CN, then hydration (Ritter reaction,[69] Scheme 9(ii)[70]) would yield 45.

SCHEME 9(ii)

$$PhCH_2OH + CH_2 = CHCN \xrightarrow{H_2SO_4}$$

$$PhCH_2\text{-}\underset{H}{N}\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH{=}CH_2$$

60%

Attempted N-acetylation of the nitrogenous compound (45) was unsuccessful even with heating for 24h with Ac_2O/pyridine/DMAP at 80°C.

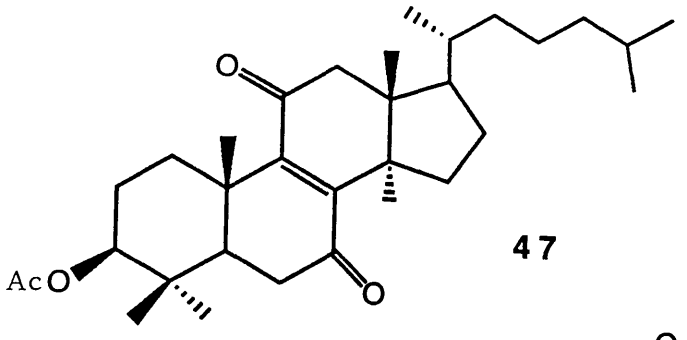
Acidic hydrolysis of the nitrogenous product (45) ($I^{OV-1}_{\pm\ 270°}$ = 3900) was attempted in order to liberate the amine which could then be extracted into the aqueous acidic phase. One hour's acid hydrolysis yielded a single component which was obtained in the organic extracts. Acetylation of this component ($I^{OV-1}_{\pm\ 270°}$ = 3830) gave back the starting material ($I^{OV-1}_{\pm\ 270°}$ = 3900). The ΔI = 70 is consistent with the hydrolysis of one acetate group to an alcohol. This was ascribed to hydrolysis of the 3β-acetate, which on reacetylation yields the original nitrogenous product. However, twenty-four hour acid hydrolysis

yielded two products occurring in the organic solvent (Table 14), with no amine being detected in the acidic extract (see Experimental section).

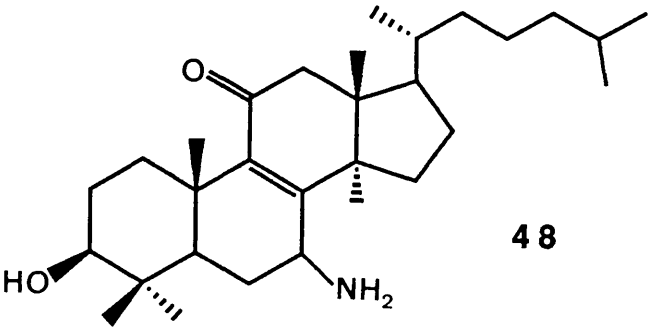
Table 14: 24h acid hydrolysis of nitrogenous products

$I^{OV-1}_{270°}$ Product of hydrolysis		$I^{OV-1}_{270°}$ Product after acetylation
3480	→	3530
3820	→	3900

Again, $I^{OV-1}_{170°}$ = 3820, corresponded to the alcohol formed by hydrolysis of the 3β-acetate group. The product with $I^{OV-1}_{270°}$ = 3530 has the I value identical to that of 7,11-diketo-5α-lanost-8-en-3β-yl acetate (47). GC-MS analysis (Table 15) of the hydrolysed material after acetylation, suggested that the compound with $I^{OV-1}_{270°}$ = 3530 was 47, with identical MS and GLC retention data to an authentic specimen. The other component with $I^{OV-1}_{270°}$ = 3900 was the original nitrogenous product (45). Thus acid hydrolysis of the nitrogenous product (45) led to simple hydrolysis of the 3-acetate and to a second non-nitrogenous product ($I^{OV-1}_{270°}$ = 3530 after acetylation), in which the expected amine (48) has been oxidised, presumably by air, to the diketone (47).

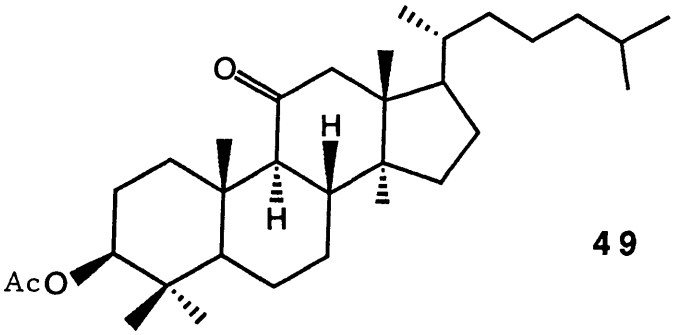


47



48

The CAN oxidation was attempted on a saturated lanostane derivative to find out if oxidation would take place in the absence of a double bond. The oxidation of 11-keto-5α-lanostan-3β-yl acetate (49) with 20 molar equivalents of CAN gave no oxidation products, yielding only starting material, as judged by TLC and GLC, and thus confirming that a double bond was essential for oxidation to occur in 5α-lanost-8-en-3β-yl acetate.



49

The other major UV-active band R_F 0.89 (from the CAN oxidation of 5α-lanost-8-en-3β-yl acetate) was subjected to GC-MS analysis. GLC showed there to be four components:

$I^{OV-1}_{-270°} = 3380$

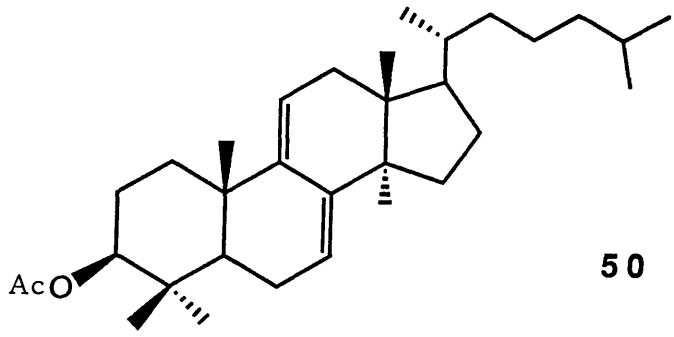
$I^{OV-1}_{-270°} = 3530$

(minor peaks in brackets)

$I^{OV-1}_{-270°} = (3610)$

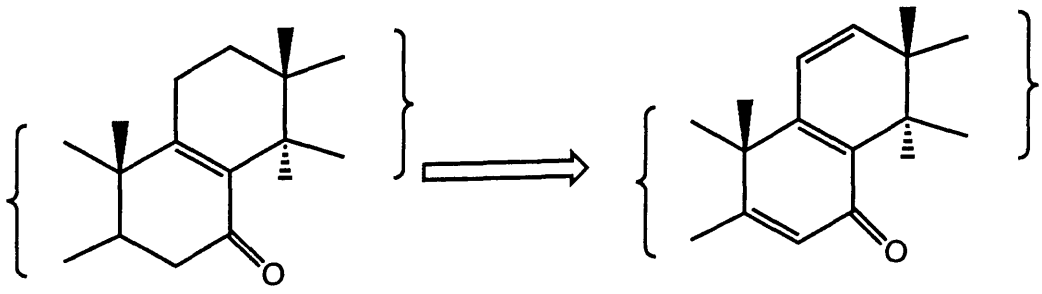
$I^{OV-1}_{-270°} = (3650)$

The mass spectrum corresponding to the peak of $I^{OV-1}_{-270°} = 3380$ (Table 16) revealed a molecular ion of 466 amu which corresponds to two additional double bonds with respect to the 5α-lanost-8-en-3β-yl acetate. This compound had a similar retention index to that of 5α-lanosta-7,9(11)-diene-3β-yl acetate (50).



50

In the CrO_3 oxidation of 5α-lanost-8-en-3β-yl acetate, 50 is produced[71] via oxidation-dehydrogenation. Furthermore, extensive SeO_2 oxidation of the Δ^8-7 one (51) yields the trienone (52) which

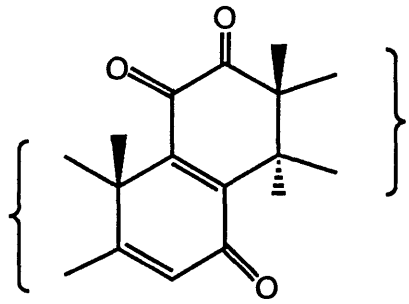


51 **52**

cannot be oxidised further in rings B and C without carbon-carbon bond cleavage. A 5,7,9(11)- or 6,8,9(11)-triene system may then account for this product from the CAN oxidation of 44.

The component of $I^{OV-1}_{270°}$ = 3530 was shown to be the enedione (47) by GC-MS (Table 17(ii)): the mass spectrum and retention index were the same as those of an authentic sample of 47 prepared using the method of Cavalla and McGhie.[72] This Δ^8-7,11-dione (47) can also be obtained by CrO_3 oxidation of the Δ^8-7 one (51) or the 7,9(11)-diene (50) system.[73]

On GC-MS analysis the peak of $I^{OV-1}_{270°}$ = 3610 yielded a molecular ion of 512 amu (Table 18) which may correspond to a triketone. The dienetrione (53) is known to be produced *via* the CrO_3 oxidation of trienone (52)[74]



53

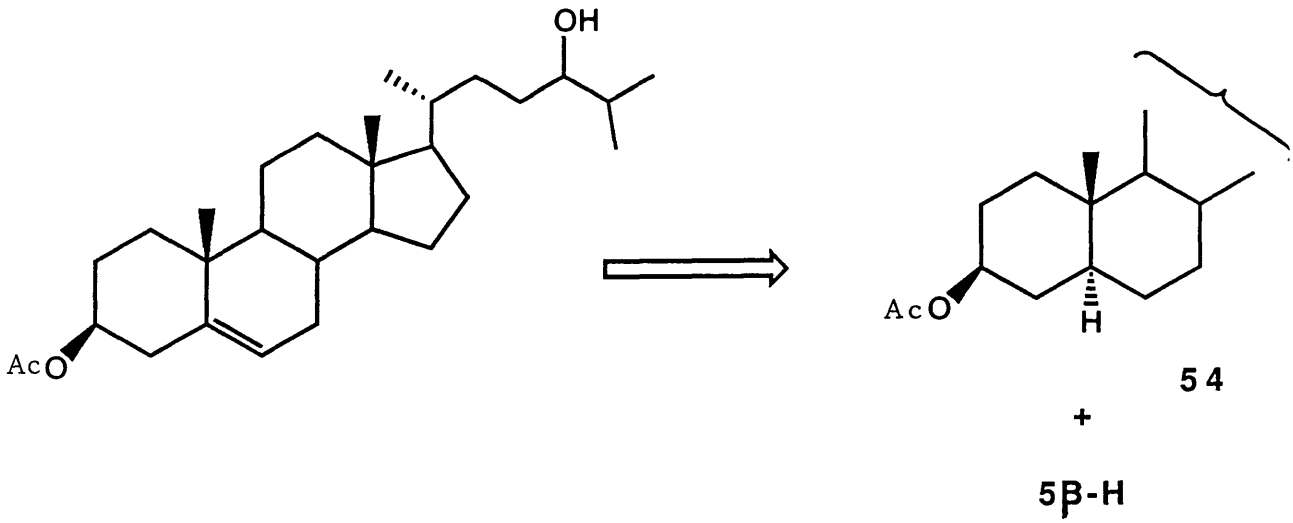
The CAN oxidation product may then be a Δ^8-7,11,12-triketone or Δ^8-6,7,11-triketone.

The peak of $I^{OV-1}_{270°}$ = 3650 gave a mixed mass spectrum on GC-MS analysis. This indicated the presence of a triketone of mass 512 amu (probably due to tailing of $I^{OV-1}_{270°}$ = 3610) and a component of apparent mass 500 amu.

3.3 Ceric Ammonium Nitrate Oxidation of 24(R,S)-hydroxy-5α-cholestan-3β-yl Acetate

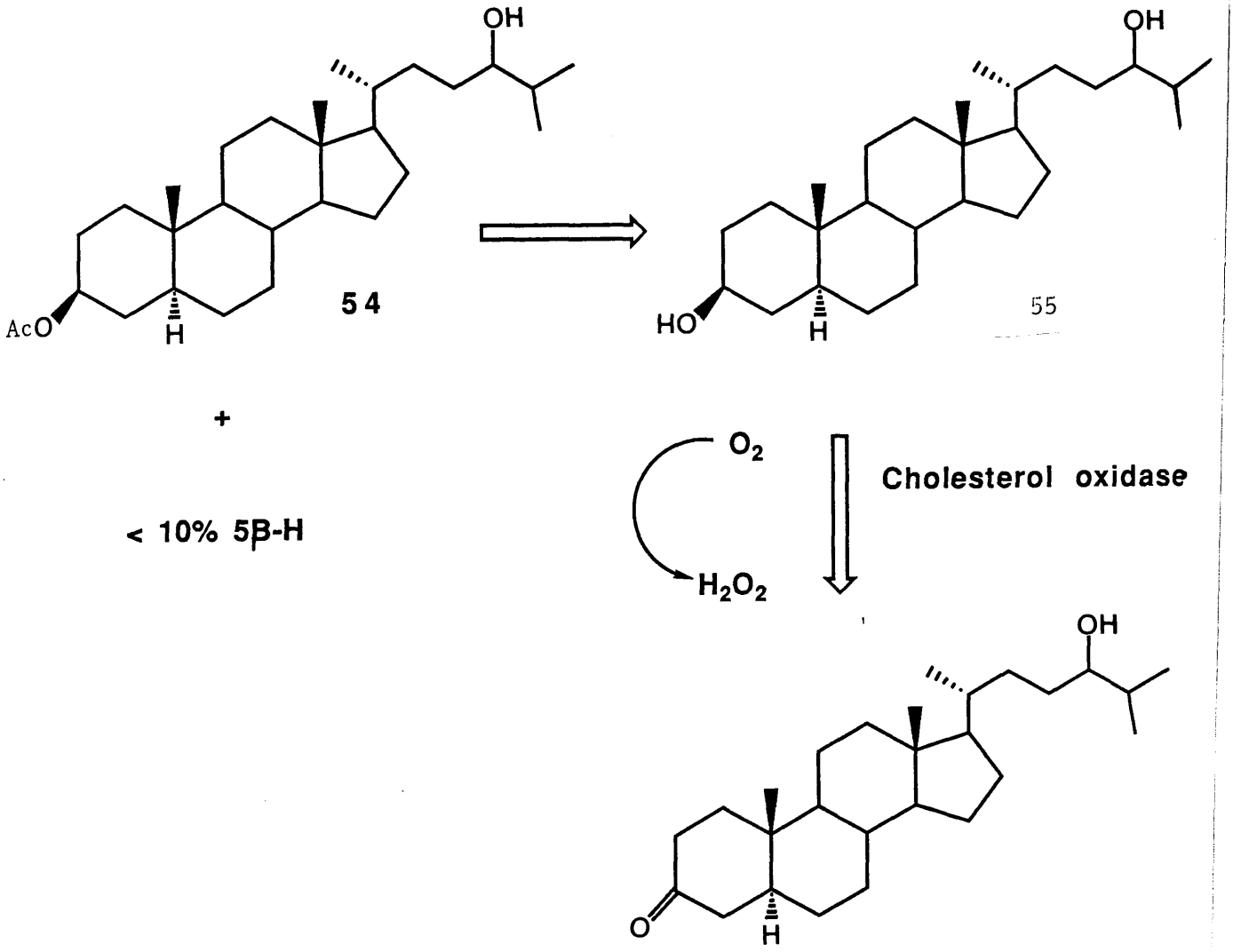
The CAN oxidation of 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate (54) was tried in order to attempt functionalisation of the steroid side chain or ring D by intramolecular hydrogen atom abstraction via the 24-alkoxy radical.

24(R,S)-Hydroxy-5α-cholestan-3β-yl acetate (54) was prepared from 24(R,S)-hydroxy-cholest-5-en-3β-yl acetate via catalytic hydrogenation[76] (see Experimental section 3.3.1).



To check that the hydrogenation was complete, the enzyme cholesterol oxidase was used. Δ^5 and 5α-H 3β-hydroxy steroids do not separate well on GLC, but the Δ^4- and 5α-H 3-keto derivatives formed by enzyme-catalysed oxidation are widely separated.[77] Accordingly, a small portion of the hydrogenated material was hydrolysed to the 3β, 24-diol (55) (Scheme 10).

SCHEME 10



Cholesterol oxidase is both regioselective and stereoselective in its mode of oxidation in that it will generally catalyse oxidation only at the 3-position of 3β-hydroxy Δ^5- or 5α-H substrates. The small amount of 5β-cholestane derivative produced in the hydrogenation will not be oxidised using cholesterol oxidase. After enzymic oxidation GLC showed

only the 3-keto-5α-steroid; it was concluded that the hydrogenation had been complete.

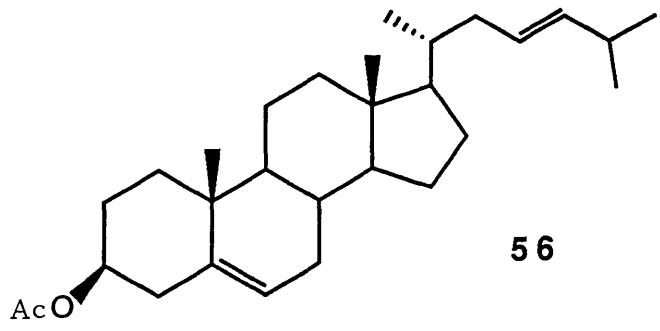
CAN oxidation of 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate (using 2 molar equivalents) gave two products as judged by TLC (see Experimental section 3.3.2). The major product was the starting material (R_F 0.69); also produced was a more non-polar component (R_F 0.83). GLC revealed two major components in the ratio 1.5:1.0:-

$I^{OV-1}_{\pm 260°}$ = 3480, (24(R,S)-hydroxy-5α-cholestan-3β-yl acetate)

$I^{OV-1}_{260°}$ = 3250

(3110)

The peak at $I^{OV-1}_{\pm 260°}$ = 3250 had a similar retention value to cholesta-5, 23-dien-3β-yl acetate ($I^{OV-1}_{\pm 260°}$ = 3240) (56) and was assumed to be a



56

dehydration product from the CAN oxidation.

Preparative TLC was used to isolate each component. The more polar band (R_F 0.69) gave $I^{OV-1}_{\pm 260°}$ = 3480, corresponding to 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate and, on acetylation, gave

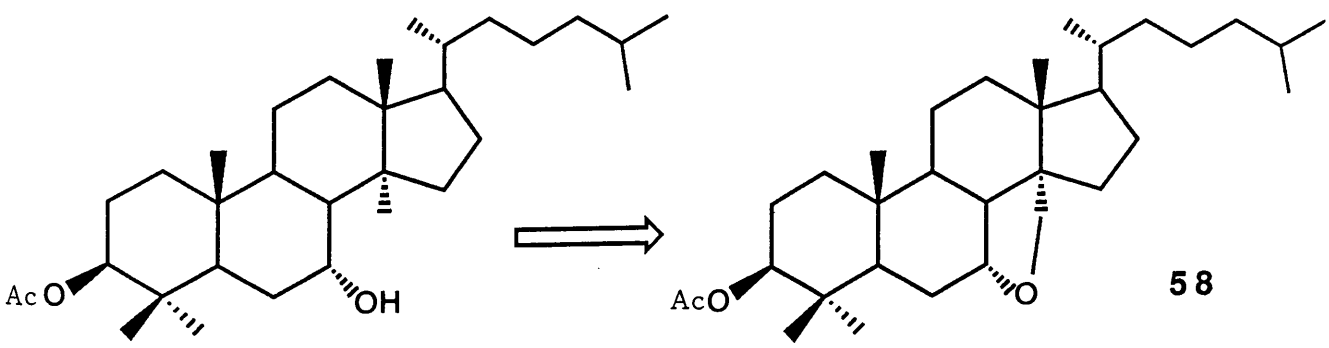
$I^{OV-1}_{\pm 270°}$ = 3540 (24(R,S)-acetoxy-5α-cholestan-3β-yl acetate) (see Table 19(i) and (ii)). The less polar band (R_F 0.83) contained two compounds as determined by GLC:

$$I^{OV-1}_{\pm 260°} = 3250$$
$$(3110)$$

This sample was subjected to GC-MS (Swansea) but no useful information was obtained - only low mass ions were observed (< 200 amu). It appeared that CAN oxidation of 54 produced compounds in which dehydration of the side chain had taken place.

3.4 Functionalisation of the C-30 Methyl Group in 7α-Hydroxy-5α-lanostan-3β-yl Acetate

The first report of functionalisation of the C-30 methyl group in 7α-hydroxy-5α-lanostan-3β-yl acetate (57) came from Fried et al.[78] in 1965. Using 7 molar equivalents of $Pb(OAc)_4$, under reflux in benzene, they obtained a 75% yield of the 7α,30-ether (58)[79,80]

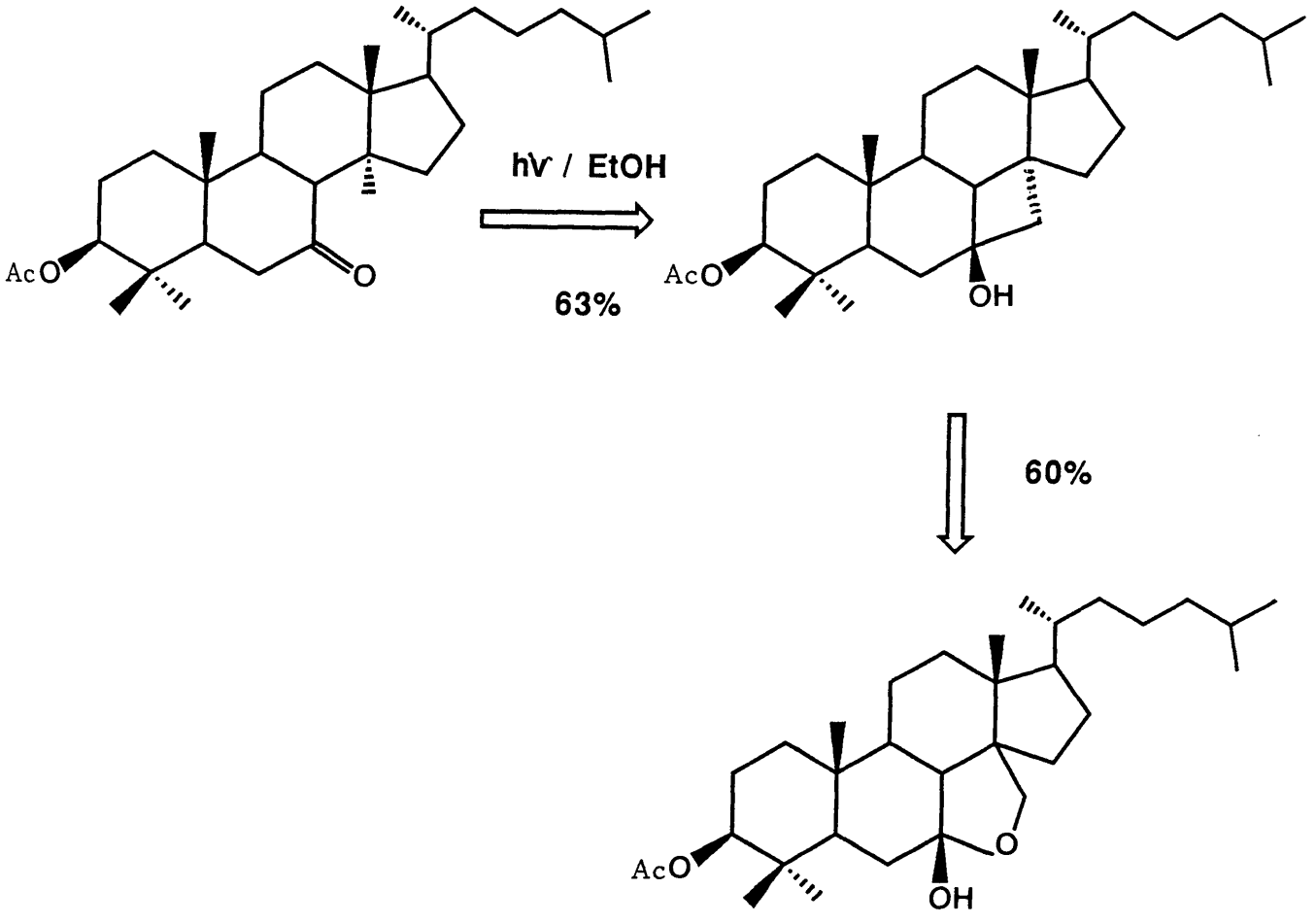


57 58

75%

The same group[81] also reported functionalisation of the C-30 methyl group *via* the 7-ketone. Photochemical excitation of the carbonyl group to its triplet state, followed by hydrogen atom abstraction and radical coupling gave the 7,32-cyclo-derivative (63% yield) which could be transformed to the hemi-acetal using $Pb(OAc)_4$ (Scheme 11) (60% yield).

SCHEME 11

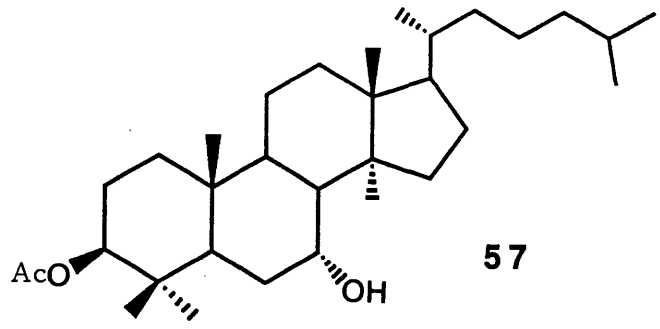


As already mentioned in Section 2, the Barton reaction has also been used to functionalise the C-30 position giving the 30-oxime in 60% yield[46] from 3β-acetoxy-5α-lanostan-7α-yl nitrite.

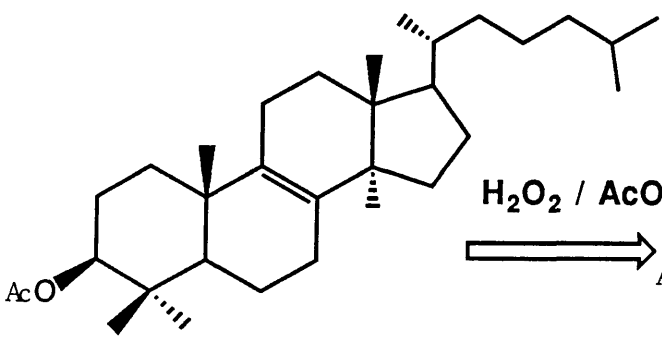
It was envisaged to functionalise the C-30 methyl group in an intramolecular manner using an alkoxy radical centred at the 7α-position: various reagents would be used to generate the alkoxy radical.

Preparation of 7α-Hydroxy-5α-lanostan-3β-yl Acetate

7α-Hydroxy-5α-lanostan-3β-yl acetate (57) was prepared from crude lanosterol. Catalytic hydrogenation[82,79] of crude lanosterol, then acetylation, yielded 5α-lanost-8-en-3β-yl acetate (44). Various literature methods were explored in the preparation of 57. The most convenient procedure used the method of Pinhey et al.[83] to oxidise 44 to 3β-acetoxy-5α-lanost-8-en-7 one (51) in 65% yield by the action of H_2O_2/AcOH/H_2SO_4 at R.T. (see Experimental section 3.4.1). The reaction probably proceeds through the 8α,9-epoxide (59) as shown in the next preparation. However, other methods were explored, such as the epoxidation of the Δ^8-double bond in 5α-lanost-8-en-3β-yl acetate (44) ($I^{OV-1}_{270°}$ = 3340) to give the 8α,9-epoxide (59) ($I^{OV-1}_{270°}$ = 3520) which on shaking with acid (concentrated HBr) undergoes cleavage (with loss of H_2O) giving the 7,9(11)-diene (50)[64] ($I^{OV-1}_{270°}$ = 3370) (see Experimental section 3.4.2).

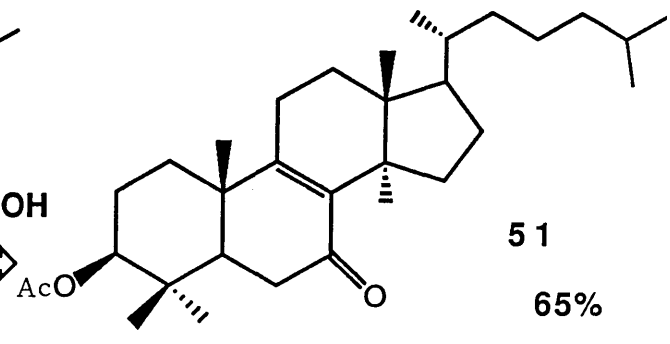


57



44

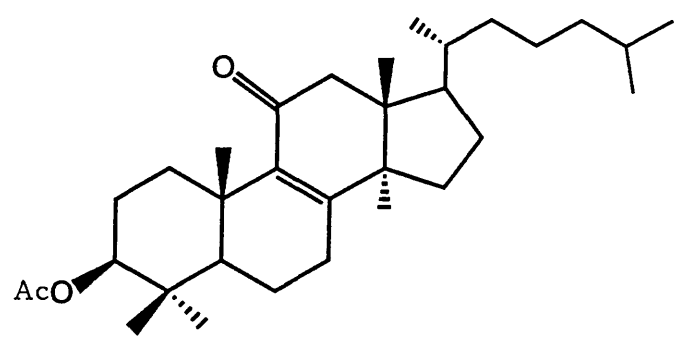
H_2O_2 / AcOH

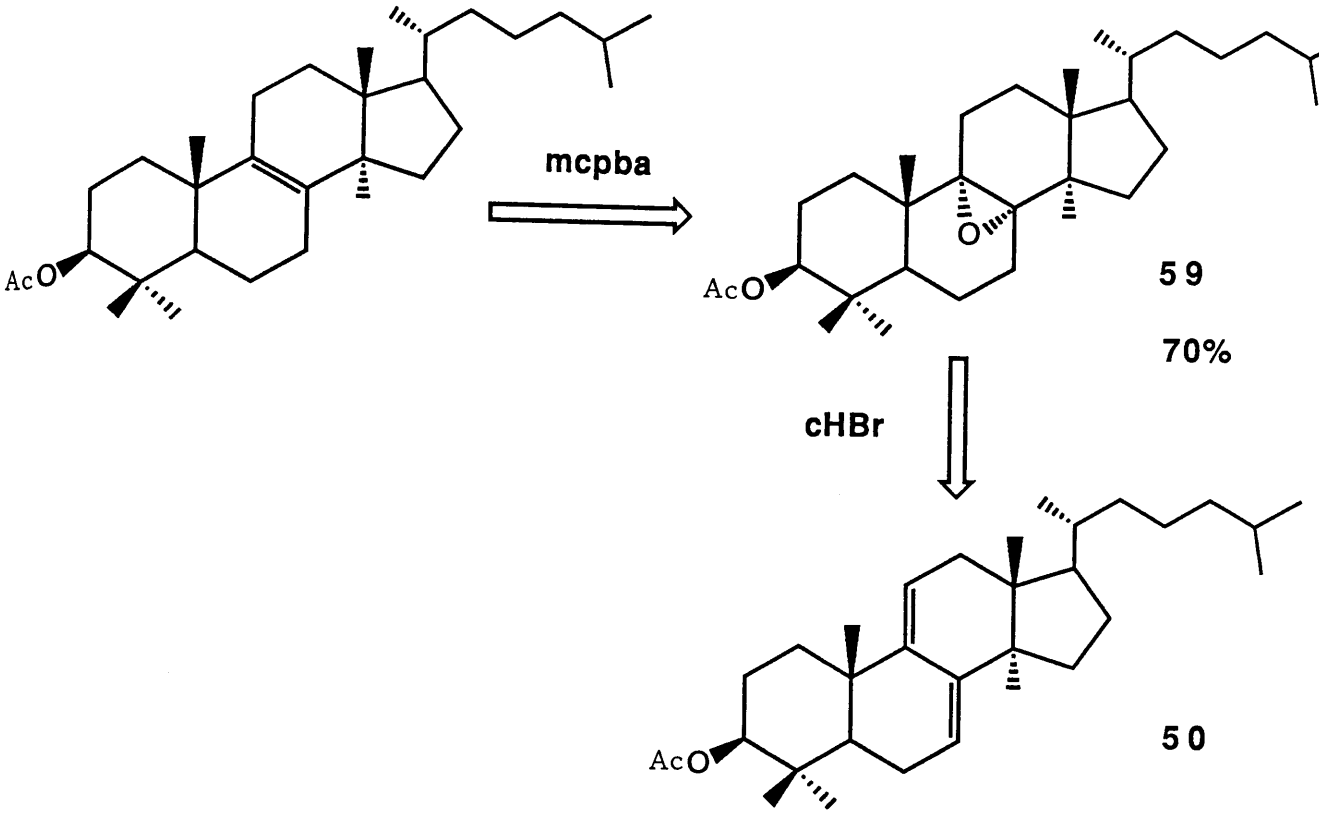


51

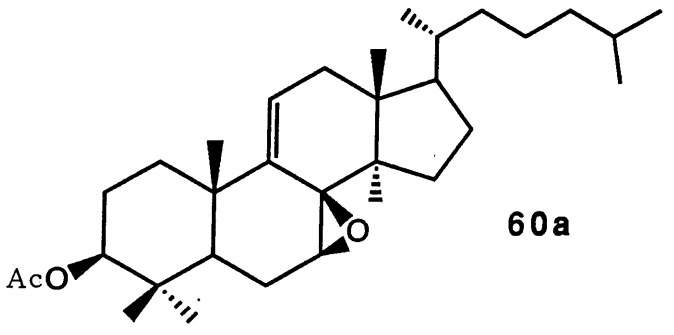
65%

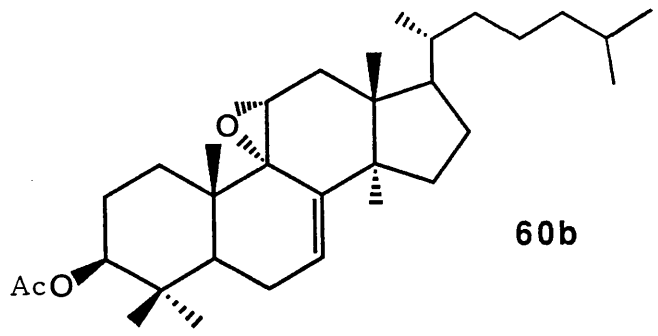
+



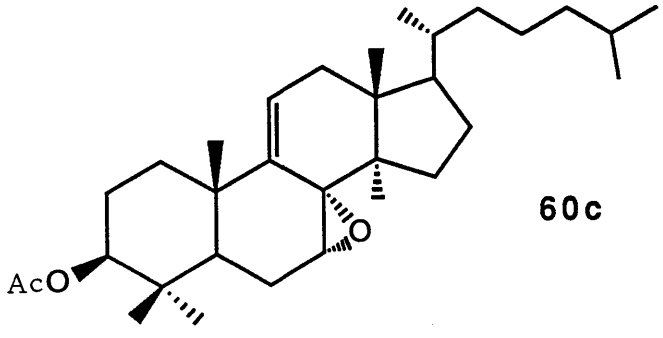


Epoxidation of the diene (50),[84,85,86] using 1.2 equiv. of mcpba gave two major spots on TLC (ether:light petroleum 60-80°C, 1:2 v/v) R_F 0.65 and R_F 0.52 in the ratio of 2:1, together with a very minor spot R_F 0.41. The major epoxides corresponded to the $\Delta^{9(11)}$-7β,8-epoxide (60a) R_F 0.65 and to the Δ^{7}-9α,11-epoxide (60b) R_F 0.52



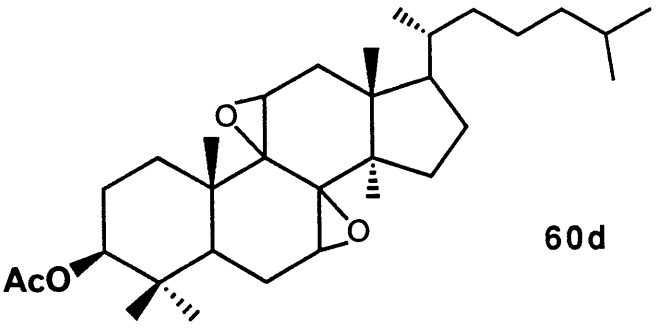


60b



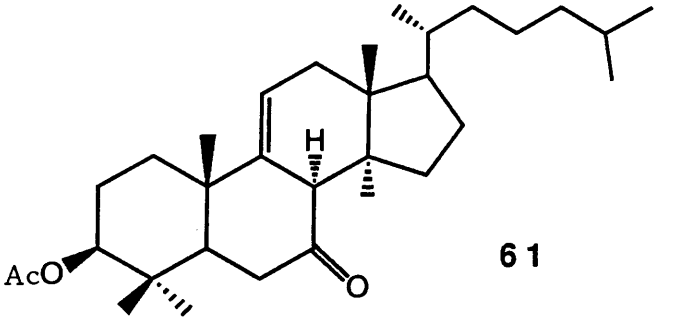
60c

Shoppee and Coll[85] have reported that treatment of 5α-lanosta-7,9(11)-dien-3β-yl acetate with 1 equiv. of mcpba yielded three products by silica TLC. Column chromatography on neutral alumina gave unchanged diene and epoxides 60a and 60b in equal amounts. The third most polar product was unstable under these chromatographic conditions, but was isolated by crystallisation of the crude epoxide mixture from ether, followed by preparative TLC. The product has been identified as $\Delta^{9(11)}$-7α,8-epoxide (60c) which can be converted, using BF_3, into the Δ^8-7 one (51). Scott et al.[84] have also reported on the epoxidation of 50. The major product isolated was epoxide 60a, together with a smaller amount of 60b. The most polar product from the reaction has been identified as a diepoxide (60d)



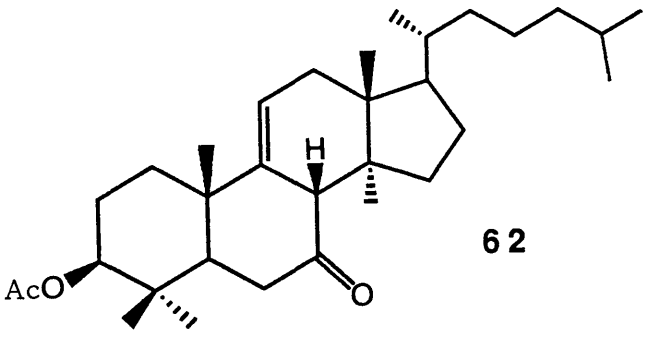
60d

After isolating the epoxide (60a) by flash column chromatography, its ^1H NMR spectrum was recorded.[71,84] However, after 3 days in solution in the NMR solvent ($CDC\ell_3$) the spectra changed, indicating isomerisation of (60) largely to the 8α-H 7-keto-derivative (61). This was mainly thought to be due to traces of acid in the NMR solvent.



61

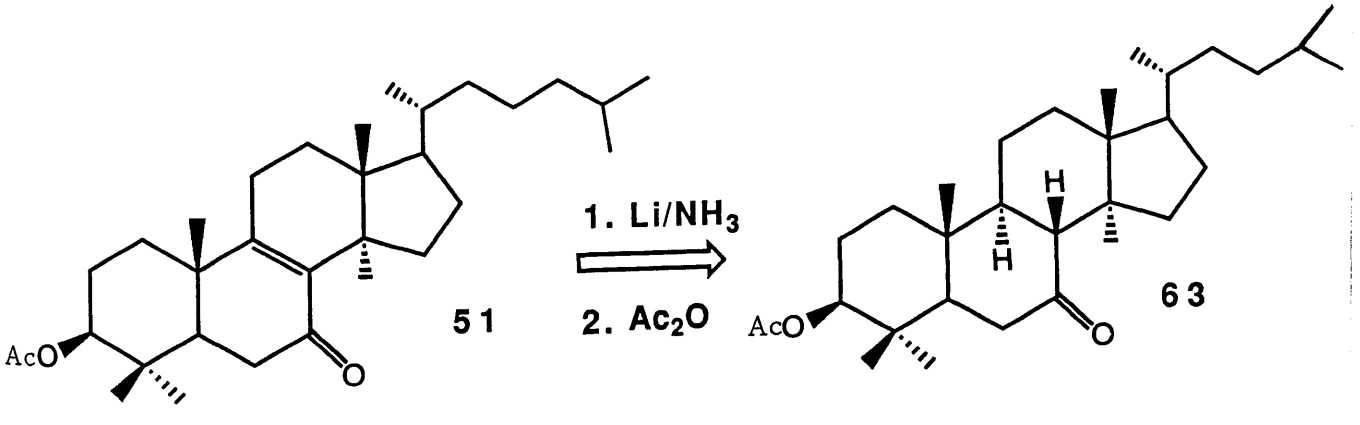
It has been shown by Scott et al.[84] that the epoxide (60a) when left on silica for 3h isomerises to the 8α-H-derivative (61). However, no significant isomerisation of the 8α-H 7-ketone (61) to the 8β-H 7-ketone occurs (62) on silica. Boar et al.[72] have reported that on neutral $A\ell_2O_3$ with AcOH the 8α-H derivative (61) is further isomerised to the 8β-H (62), together with the Δ^8-7-one (51) and that upon treatment under more vigorous conditions ($BF_3.Et_2O$) (61) is transformed into the conjugated ketone (51).



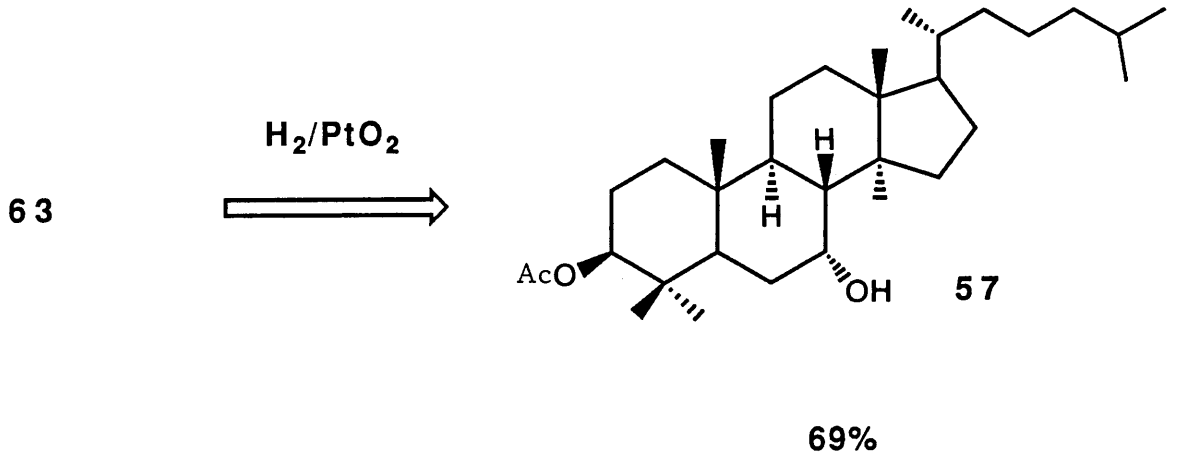
62

The mixture of epoxides was then treated with ptsa in AcOH under reflux for 3 min[87] to give the Δ^8-7-one ($I^{OV-1}_{\pm\ 270°}$ = 3570) (in 60% yield judged by GLC), contaminated with the Δ^8-11-one ($I^{OV-1}_{\pm\ 270°}$ = 3530). This method of preparing the Δ^8-7-one (51) was less attractive than the method developed by Pinhey et al.[83] because it involved more chemical steps.

3β-Acetoxy-5α-lanost-8-en-7-one, produced by the method of Pinhey et al.[83], was reduced with $Li/NH_{3(\ell)}$[87] in 86% yield (by GLC): the product on acetylation gave 7-keto-5α-lanostan-3β-yl acetate (63) ($I^{OV-1}_{\pm\ 270°}$ = 3600) (see Experimental section 3.4.4).

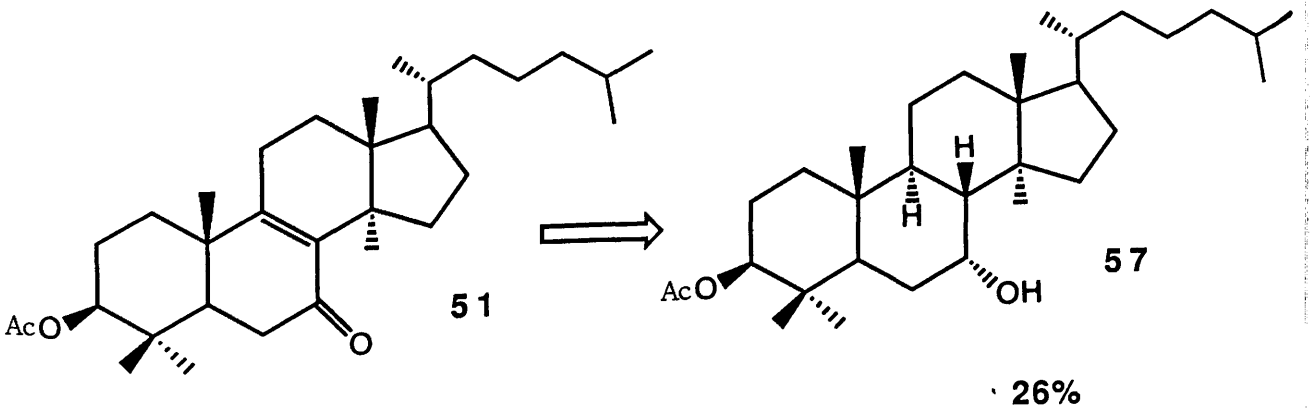


Methods were then sought to reduce the saturated 7-ketone (63) to the 7α-axial alcohol. Barton et al.[88,89] have shown that reduction (hydrogenation) of 7-keto-5α-lanostan-3β-yl acetate over Adams PtO_2 catalyst gave the 7α-alcohol in 69% yield. Chromatography of the mother liquors over grade O alumina gave 5α-lanost-7-en-3β-yl acetate and 7-keto-5α-lanostan-3β-yl acetate.



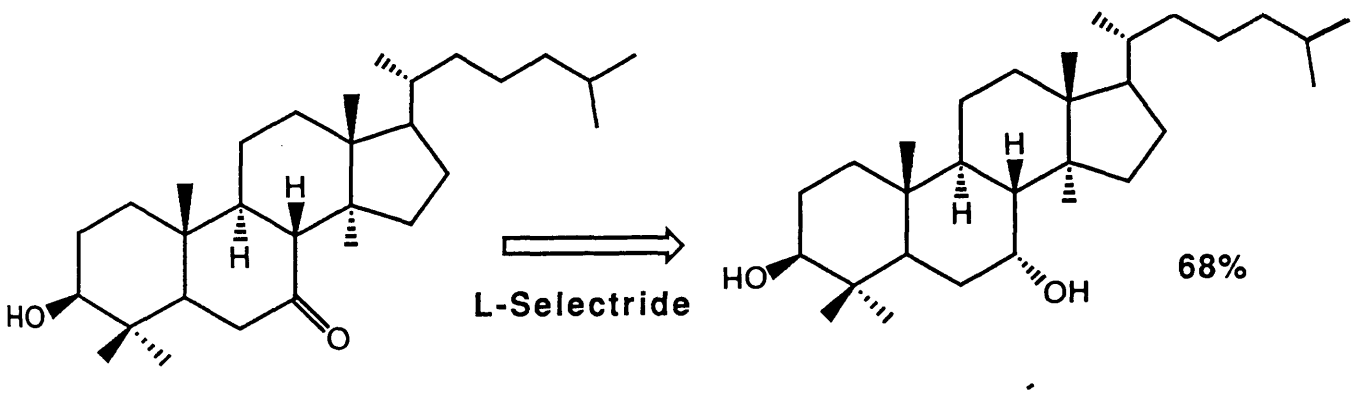
More recently, Morisaki et al.[90] have studied the stereoselectivity in reduction of steroidal 7-ketones. In their repetition of the reported hydrogenation of 7-keto-5α-lanostan-3β-yl acetate,[88,89] the crude product was saponified and treated with N-trimethylsilylimidazole (TMSIm) to give the TMS ethers. GC-MS of the crude reaction product after trimethylsilylation revealed it to be a mixture of 7α-ol and 7β-ol (as 3,7 di-TMS ethers) in the ratio 73:27, together with an unidentified component (probably a Δ^8-unsaturated 3,7-di-TMS ether $[M]^{+\cdot}$ = 588). The catalytic hydrogenation was repeated under 70 psi of H_2, using an Adams catalyst for 48h (see Experimental section 3.4.5). TLC of the products showed 4 spots (ether:pet. ether (60-80°C) 1:2 v/v) at R_F 0.82, probably 5α-lanost-7-en-3β-yl acetate,

R_F 0.55, corresponding to 7-keto-5α-lanostan-3β-yl acetate, R_F 0.31 and 0.27, corresponding respectively to 7β- and 7α-hydroxy-5α-lanostan-3β-yl acetate. Packed column GLC of the reaction mixture revealed a presumed 5α-lanosten-3β-yl acetate with $\underline{I}^{OV-1}_{270°}$ = 3355, but failed to separate the 7α- and 7β-alcohols (giving one peak $\underline{I}^{OV-1}_{270°}$ = 3640). The crude reaction mixture was subjected to preparative TLC (ether:pet. ether (60-80°C) 1:2 v/v). A band between R_F 0.10 and 0.40 was extracted and the material therefrom treated with TMSIm. Capillary GLC of the products revealed two peaks - $\underline{I}$ = 3586 and $\underline{I}$ = 3512 in the ratio 78:22 for 3β-OAc 7α-OTMS:3β-OAc 7β-OTMS, respectively (Table 20(i) and 20(ii)). This confirmed that 7α-hydroxy-5α-lanostan-3β-yl acetate is the major product from catalytic hydrogenation of 7-keto-5α-lanostan-3β-yl acetate. Morisaki et al.[90] also found that hydrogenation of 3β-hydroxy-5α-lanostan-7-one on Pt in EtOAc/AcOH gave the 7β-alcohol as the major product. Sato et al.[80] have shown that catalytic hydrogenation of 3β-acetoxy-5α-lanost-8-en-7-one, in the presence of PtO_2 in AcOH, gives the 7α-ol in 26% yield.



The other products from this reaction were separated by column chromatography, affording 5α-lanost-8-en-3β-yl acetate, 5α-lanost-7-en-3β-yl acetate, and 5α-lanosta-7,9(11)-dien-3β-yl acetate. Parish and Schroepfer[79] have also obtained 57 by reduction of a mixture of 7α,8α- and 8α,9α-epoxy-5α-lanostan-3β-ols, followed by selective acetylation. However, yields were low and the procedure proved troublesome.

Morisaki et al.[90] have shown that reduction of 3β-hydroxy-5α-lanostan-7-one with L-Selectride (lithium-tri-sec-butylborohydride) (Aldrich) selectively yields the 7α-axial alcohol (68% yield). With L-Selectride no reduction was observed when 7-keto-5α-lanostan-3β-yl acetate or 3β-acetoxy-5α-lanost-8-en-7-one was used.



The reduction of 3β-hydroxy-5α-lanostan-7-one with L-Selectride was effected initially on a small scale (see Experimental section 3.4.6). After acetylation, TLC (ether:light petroleum (60-80°C) 1:2 v/v) indicated 3 products: R_F 0.51, corresponding to 7-keto-5α-lanostan-3β-yl acetate, R_F 0.23, corresponding to 7α-hydroxy-5α-lanostan-3β-yl acetate, and a polar material streaking on the plate. Preparative TLC

was used to remove the 7α-hydroxy-5α-lanostan-3β-yl acetate. On GLC, one peak was observed at $\underline{I}^{OV-1}_{270°}$ = 3635 and on TMS ether formation gave $\underline{I}^{OV-1}_{270°}$ = 3620 (Table 20). Capillary GLC showed only one peak $\underline{I}$ = 3578 for 7α-hydroxy-5α-lanostan-3β-yl acetate and on TMS ether formation $\underline{I}$ = 3586 (Table 20(ii)). Therefore, as reported, L-Selectride yields only one stereoisomer. On scaling up the L-Selectride reduction of 7-keto-5α-lanostan-3β-yl acetate reactions, problems were encountered in that, after the reaction work-up, a waxy material contaminated the product. Washing with 1M NaOH failed to remove this waxy material. It was thought that this material was tri-sec-butylborane (R_3B) and/or di-sec-butylborinic acid (R_2BOH). Both materials can be oxidised with alkaline H_2O_2 to give the base-soluble $NaB(OH)_4$[91] (see Experimental section 3.4.7) (Scheme 12).

SCHEME 12

$$R_3B + 3H_2O_2 + NaOH \longrightarrow 3ROH + NaB(OH)_4$$

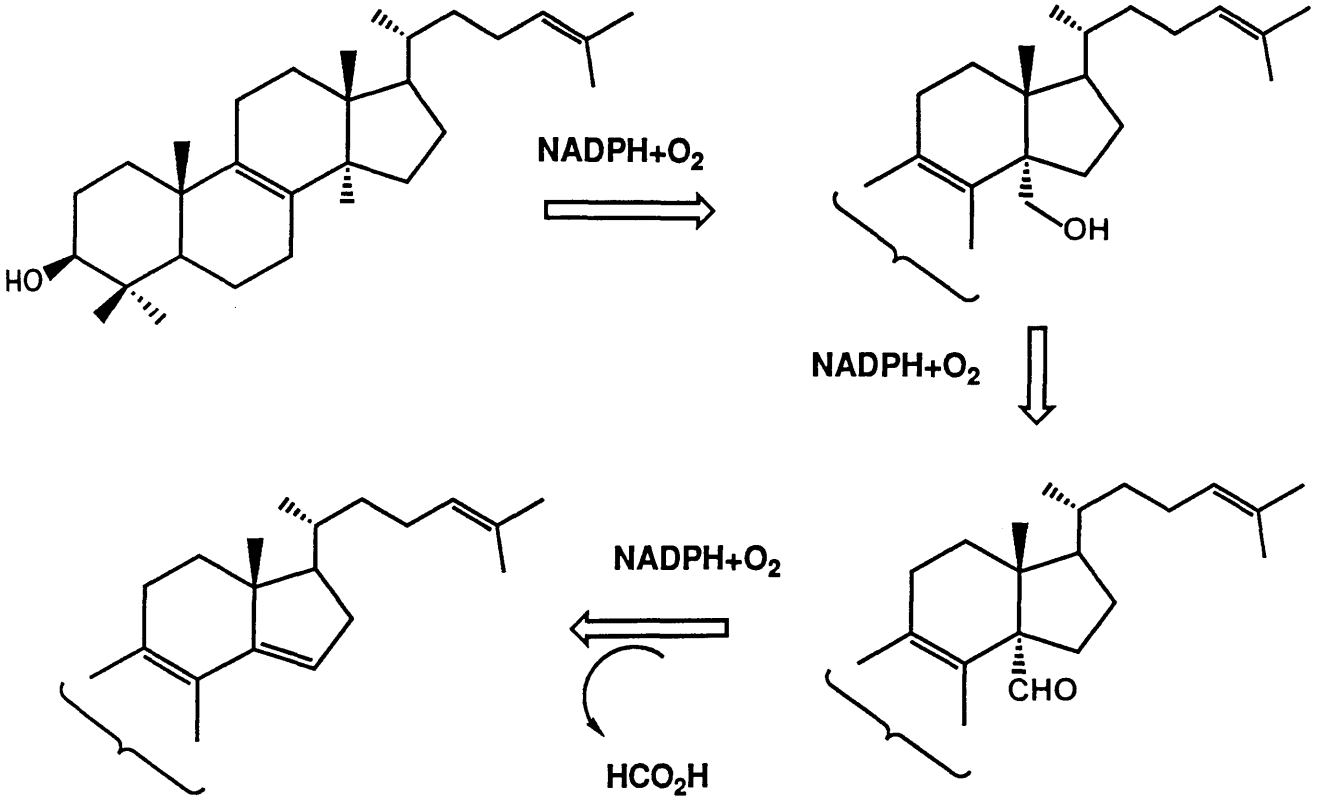
$$R_2BOH + 2H_2O_2 + NaOH \longrightarrow 2ROH + NaB(OH)_4$$

Biosynthesis

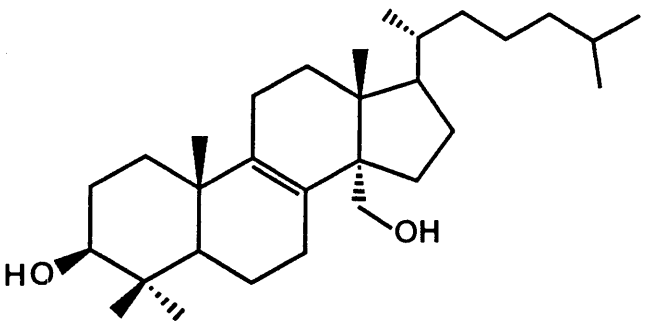
The key step in the biosynthesis of cholesterol and ergosterol from lanosterol is the removal of the C-14 methyl group (C-30) by the enzyme lanosterol 14α-methyl demethylase which is a cytochrome P-450 mono-oxygenase-containing enzyme.[92] In three O_2/NADPH-dependent steps, the 14α-methyl group is first hydroxylated and then oxidised

to the aldehyde. The nature of the third oxidation step, which results in the loss of the C-30 as formate and the formation of the 8,14-diene, is still unclear (Scheme 13)

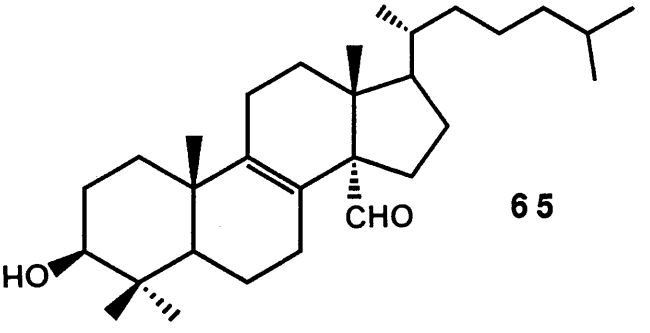
SCHEME 13



Lanosterol and 24,25-dihydrolanosterol are both efficiently converted to cholesterol. 30-Hydroxy-5α-lanost-8-en-3β-ol (64) (Δ^8-CH_2OH) and 3β-hydroxy-5α-lanost-8-en-30-ol (65) (Δ^8-CHO) have both been identified as metabolites.[92] However, 3β-hydroxy-5α-lanost-7-en-30-ol (66) (Δ^7-CHO), 5α-lanost-7-ene-3β,30-diol (67) (Δ^7-CH_2OH), and 5α-lanost-6-ene-3β,30-diol (68) (Δ^6-CH_2OH) were not metabolised to the 8,14-diene.



64



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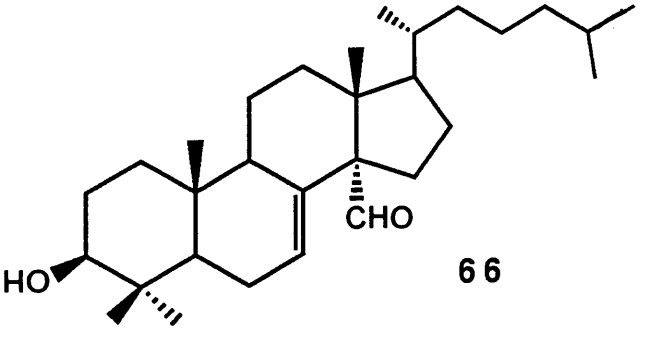

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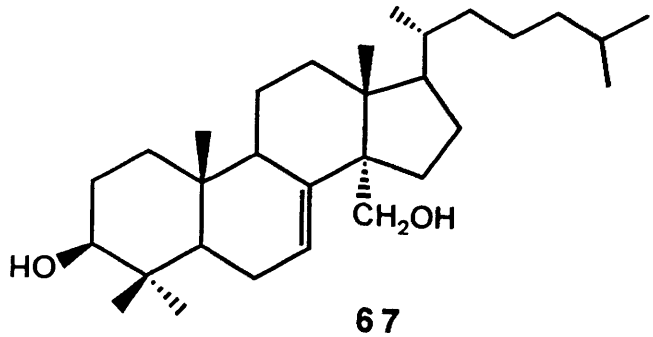
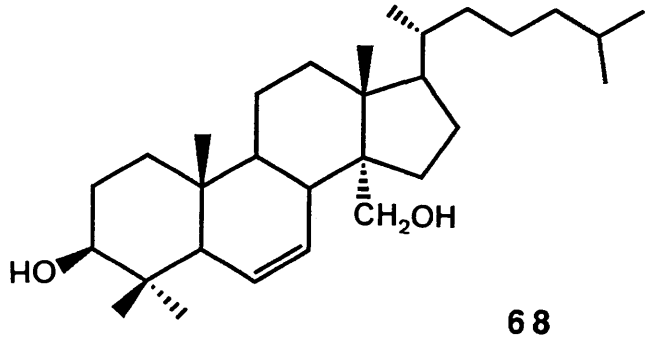


67

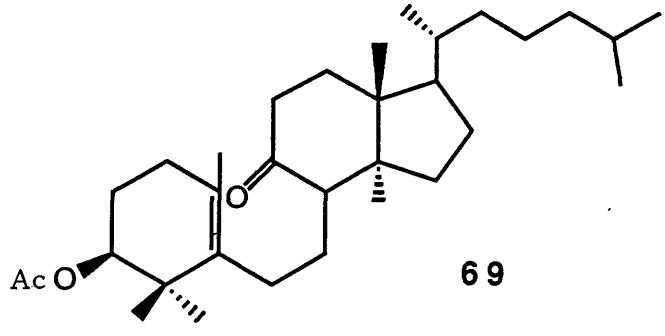


68

Thus there is a high degree of substrate specificity for the double bond position in the steroid nucleus.

Inhibitors of lanosterol 14α-methyl demethylase[93] are of use as possible cholesterol lowering agents and as antimycotics.[94] 24(S)-25-Epoxy-5α-lanost-8-en-3β-ol, 3β-hydroxy-5α-lanost-8-en-7-one, and 15-oxygenated lanosterol derivatives are all inhibitors of cholesterol biosynthesis from 24,25-dihydrolanosterol in vitro.[91] 30-Hydroxylated lanosterol derivatives have been shown to inhibit sterol biosynthesis in animal cell cultures,[92] and to regulate 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Therefore these naturally occurring oxygenated steroids may be important in the regulation of sterol biosynthesis.

The approach introduced by Fried et al.[65] for the functionalisation of the C-30 position was attempted. Both the 7α- and 9α-hydroxyl groups (1,3-diaxial to C-30) with O-C(30) distance of 2.5 to 2.6 Å are properly positioned for reaction to occur. However, 9α-hydroxy-5α-lanostan-3β-yl acetate gives fragmentation type product (69) resulting from the initial formation of the tertiary radical at C-10 by fission of the 9,10-bond.[95]



69

7α-Hydroxy-5α-lanostan-3β-yl acetate was treated with 7 equiv. of $Pb(OAc)_4$ under reflux in benzene overnight to give the 7α,30-ether (58) in 90% yield by GLC (see Experimental section 3.4.8).

No reaction occurred when a solution of 7α-hydroxy-5α-lanostan-3β-yl acetate containing I_2 in cyclohexane was irradiated: only the starting material was recovered unchanged. However, with $Pb(OAc)_4/I_2$ under irradiation conditions, a variety of products are formed and microanalysis (for iodine) has shown that none of the products contain iodine (see Experimental section 3.4.9). Monitoring the reaction by TLC revealed that after 45 min, none of the starting material (57) remained. TLC indicated ca. 7 spots (Table 20).

Table 20: TLC products from 7α-hydroxy-5α-lanostan-3β-yl acetate with $Pb(OAc)_4/I_2/hr$.

R_F	Proposed assignment
0.64	(major) unknown
0.58	(minor) 3β-OAc, 7-one
0.52	(major) 3β-OAc, 7α,30-ether
0.47	unknown
0.42	unknown
0.37	unknown
0.09	unknown

ether:light petroleum (1:2 v/v)

GLC of the crude products indicated a complex reaction mixture (Table 21)

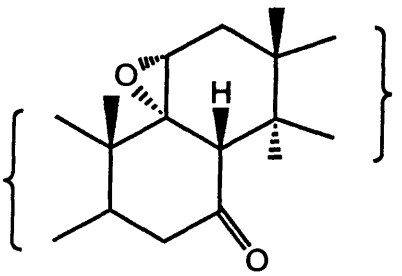
Table 21: GLC of products from 7α-hydroxy-5α-lanostan-3β-yl acetate reaction with $Pb(OAc)_4/I_2$

$I^{OV-1}_{270°}$	Proposed assignment
3390	broad peak
(3465)	broad peak
3560	broad peaks
3580	
3615	3β-OAc, 7α, 30-ether
(3660)	broad peak
3780	broad peak

A portion of the reaction mixture was subjected to preparative TLC (see Experimental section 3.4.9). The band removed at R_F 0.61 was slightly more non-polar than 7-keto-5α-lanostan-3β-yl acetate, and GLC showed two peaks, $\underline{I}^{OV-1}_{270°}$ = 3380 and $\underline{I}^{OV-1}_{270°}$ = 3560. The first peak, $\underline{I}^{OV-1}_{270°}$ = 3380, was similar in retention to 5α-lanosta-7,9(11)-dien-3β-yl acetate. The mass spectrum of R_F 0.61, containing two peaks by GLC, revealed the highest ion to be at $\underline{m/z}$ 500, and the IR spectrum gave two carbonyl absorptions at 1732 cm^{-1} and 1720 cm^{-1} corresponding to an ester carbonyl and an aldehyde or ketone carbonyl (saturated 6-ring) respectively.

GC-MS, under ammonia CI and EI conditions, was performed for this mixture. Under CI conditions, two peaks were observed: the first peak was thought to be $\underline{I}^{OV-1}_{270°}$ = 3380 (no precise retention data were obtained on GC-MS) and revealed the molecular ion to be 484, corresponding to loss of 4 hydrogens from the starting material (7α-hydroxy-5α-lanostan-3β-yl acetate, $\underline{m/z}$ 488) (Table 22). GC-MS (EI) confirmed this to be the molecular ion (Table 22). The retention index ($\underline{I}^{OV-1}_{270°}$ = 3380) on GLC was close (± 10) to that of 5α-lanosta-7,9(11)-dien-3β-yl acetate ($\underline{m/z}$ 468) (50). However, this type of structure was eliminated when GC-MS indicated an additional 16 amu ($\underline{m/z}$ 484) which may be due to epoxidation. The second peak, presumed to be $\underline{I}^{OV-1}_{270°}$ = 3560, revealed the molecular ion to be 500 amu under ammonia CI (Table 23). From the starting material (57) ($\underline{m/z}$ = 488), there must be loss of four hydrogens and addition of an oxygen atom to give $\underline{m/z}$ 500. Under EI conditions, the highest observed ion was at 454 amu, which is 46 amu (possibly CO + H_2O) lower than the molecular ion of 500 amu (Table 23).

After isolating a larger quantity (6.2 mg) of this mixture by column chromatography, the 200 MHz ^{1}H NMR spectrum (Table 24) revealed a 1H proton singlet at 8.05 ppm. This region is usually associated with a formate proton (-OCHO). A broad (1H, s) signal of 5.49 ppm indicated an olefinic proton. Another olefinic proton (1H, s) occurred at 5.02 ppm. The other signal of interest occurred at 3.65 ppm (1H, m). Therefore, possible structures for component $I^{OV-1}_{\pm 270°}$ = 3560 to fit the physical measurements would be (70) and (71)



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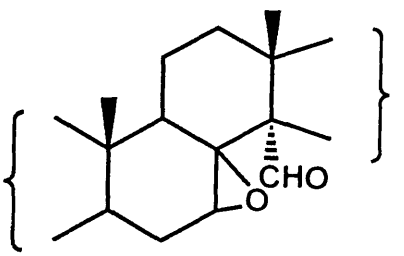
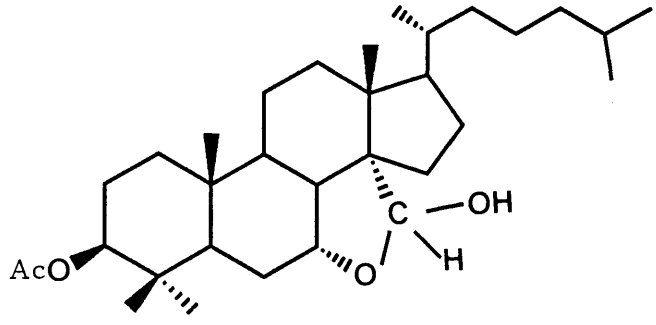


71

For the mixture, the signal in ^{1}H NMR at 8.05 is genuine in the reaction mixture, since it was present when the ^{1}H NMR spectrum of the whole reaction mixture was recorded.

Treatment of this mixture with $NaBH_4$ on a small scale (see Experimental section 3.4.10) gave two spots on TLC. The mixture had R_F 0.70 and with $NaBH_4$ reduction gave R_F 0.70 plus R_F 0.44 (ether:light petroleum, 1:2 v/v). GLC revealed incomplete reduction producing $I^{OV-1}_{\pm 270°}$ = 3540 (which is a shoulder) from $I^{OV-1}_{\pm 270°}$ = 3550 together with unchanged $I^{OV-1}_{\pm 270°}$ = 3380. The other bands removed from the preparative TLC plate were mixtures of compounds as judged by GLC.

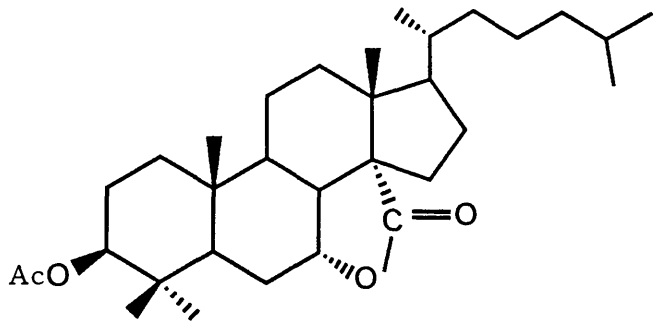
However, the band at R_F 0.09 gave $I^{OV-1}_{\underline{\ }270°}$ = 3770 on GLC and the mass spectrum showed the highest ion to be 484 amu (Table 25). It was thought that this product was the hemi-acetal (70A) and that the ion occurring at 484 amu was $[M^{+\cdot}\ (502)-H_2O]$.



70A

After the isolation of more of this material (70A) (2.4 mg) by column chromatography its IR spectrum showed a band at 3615 cm^{-1} (w) (free r(O-H)) indicating a non-hydrogen bonded O-H together with a band associated with the ester carbonyl (1732 cm^{-1} (s)) (Table 26). The ^1H NMR spectrum of (70A) was weak due to sample size (ca. 2 mg). However, it did show a signal at 5.45 ppm which has been assigned as the C-30 C<u>H</u> in hemi-acetal (70A) (Table 27). There was also a broad signal at 4.15 ppm assigned to the 7β-H.

Small-scale treatment of the hemi-acetal (R_F 0.11, ether:pet. ether 1:2 v/v) with PCC in $CH_2C\ell_2$ at RT for 1h 15 min produced a less polar spot on TLC (R_F 0.50) (see Experimental section 3.4.11). The IR spectrum of this material revealed two carbonyl absorptions at 1771 cm^{-1} and 1735 cm^{-1} assigned to a 5-ring lactone carbonyl and an ester carbonyl, respectively (Table 28). The MS of this suspected lactone (Table 29) (71A) had a molecular ion of 500 amu and the fragmentation pattern showed loss of 44 amu (CO_2) from the molecular ion.



7 1A

On repeating the $Pb(OAc)_4/I_2/hr$ reaction (under similar conditions as before on a slightly larger scale, 107 mg of the crude product was obtained and subjected to careful column chromatography (Table 30). The 7α,30-ether (58) could be isolated (fraction 10). Fraction 15 yielded material (1.3 mg) which gave two spots on TLC (R_F 0.59, R_F 0.54). GLC revealed two peaks: $I^{OV-1}_{\sim 270°}$ = 3615, corresponding to the 7α,30-ether (58), and the other having $I^{OV-1}_{\sim 270°}$ = 3770. IR of this 1:1 mixture had bands at 1770 cm^{-1} (m) and 1731 cm^{-1} (s) in the carbonyl region (Table 31), together with ether stretching. GLC retention data indicated that the more polar product in the mixture was lactone 71 and the IR confirmed a 5-ring lactone structure.

Reaction of 7α-Hydroxy-5α-lanostan-3β-yl Acetate with CAN

Treatment of 7α-hydroxy-5α-lanostan-3β-yl acetate (57) with 2.5 equiv. of aqueous CAN gave two products as judged by TLC and GLC (see Experimental section 3.4.12). The major product (R_F 0.50) corresponded to 7α,30-oxido-5α-lanostan-3β-yl acetate (58) and GLC gave $I^{OV-1}_{\sim 270°}$ = 3615 in 26% yield. The other minor product (R_F 0.06) gave $I^{OV-1}_{\sim 270°}$ = 3730 and comprised 4% yield as judged by GLC. About 69% of the starting material (57) was left unchanged.

Table 30. Column chromatography of crude products from the reaction of (57) with $Pb(OAc)_4/I_2/hr$

Selected fractions	Comments
7-9	combined (30 mg) spots:- R_F 0.73, 0.70, 0.66, 0.59
10	spots:- R_F 0.59 (11.6 mg) $I^{OV-1}_{\pm 270°}$ = 3615 (7α,30-ether)
15	spots:- R_F 0.59, 0.54 (1.3 mg) $I^{OV-1}_{\pm 270°}$ = 3615, $I^{OV-1}_{\pm 270°}$ = 3770
19-22	spots:- R_F 0.10 (5.5 mg) $I^{OV-1}_{\pm 270°}$ = 3770

Column chromatography was used to separate the products. Both the 7α,30-ether (58) and the 7α-ol (57) were isolated together with the unknown product having $I^{OV-1}_{\pm 270°}$ = 3730. The mass spectrum of this unknown component had the highest mass at 512 amu, but the fragmentation pattern suggested the molecular ion to be 502 amu (Table 32). The molecular weight of the product derived from 57 corresponded to the addition of an oxygen atom and loss of two hydrogens. The mass spectrum was different from that of the hemi-acetal (70).

Experimental Section - 3.1.1

Acetylation of crude lanosterol

A solution of crude lanosterol (21.88g) in dry redistilled pyridine (40 ml) and acetic anhydride (190 ml) was gently refluxed for 45 min. After this time, an aliquot was removed and checked by micro-TLC. The solvents were removed under reduced pressure and the material crystallised from Et_2O/MeOH to yield crude lanosteryl acetate (18.08g). GLC showed the ratio, lanosteryl acetate:24,25-dihydrolanosteryl acetate to be 60:40. 90 MHz ^1H NMR ($CDC\ell_3$): δ5.10 (1H, t, 24-olefinic H), 4.50 (2H, m, 3α-H), 2.0 (6H, s CH_3CO), 1.59 (3H, s, 26- or 27-CH_3), and 1.66 (3H, s, 26- or 27-CH_3).

Experimental Section - 3.1.2

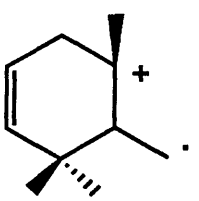
Epoxidation of crude lanosteryl acetate

Crude lanosteryl acetate (19.96g) was dissolved in $CHC\ell_3$) (60 ml). A mcpba solution (7.63g, 25.56 mmol, 1.7 molar equiv. with respect to lanosta-8,24-dien-3β-yl acetate) in $CHC\ell_3$ (100 ml) was added dropwise with stirring at -10°C. A white precipitate was noted after the total addition of the mcpba solution. The resulting mixture was left in the refrigerator overnight (~ 5°C). The solution was transferred to a separating funnel, washed twice with 1M NaOH solution then water. Extraction with $CHC\ell_3$ (see general experimental procedure) yielded a white solid (20.41g).

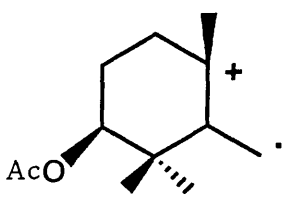
Mass spectral data for 8α,9-epoxy-5α-lanostan-3β-yl acetate (selected ions)

$C_{32}H_{54}O_2$ M = 486

m/z	Ion type	%
471	$[M^{+\cdot}-CH_3^{\cdot}]$	0.7
468	$[M^{+\cdot}-H_2O]$	15.6
453	$[M^{+\cdot}-H_2O-CH_3^{\cdot}]$	4.1
426	$[M^{+\cdot}-CH_3CO_2H]$	1.2
411	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	7.8
408	$[M^{+\cdot}-CH_3CO_2H-H_2O]$	4.0
393	$[M^{+\cdot}-CH_3CO_2H-H_2O-CH_3^{\cdot}]$	32.4
339		10.4
313	$[M^{+\cdot}$-side chain-$CH_3CO_2H]$	4.1
295	$[M^{+\cdot}$-side chain-$CH_3CO_2H-H_2O]$	2.7
291		27.6
196		2.0
136		59.1
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100



m/z 136



m/z 196

Infrared spectrometric data of 8α,9-epoxy-5α-lanostan-3β-yl acetate

(KBr disc)

Bands observed (cm^{-1})	Group
2950 (s)	ν(C-H)
1735 (s)	ν(C=O) ester
1465 (m)	(C-H) def.
1375 (s)	ν(C-H) $\underline{C}H_3\overset{O}{\overset{\|}{C}}$-
1260 (s)	ν(C-O)
900 (m)	ν(C-O-C)
800 (m)	ν(C-O-C)

200 MHz ^{1}H NMR of 8α,9-epoxy-5α-lanostan-3β-yl acetate[75]

δ 4.48 (1H, m, 3α-H), 2.01 (3H, s, $C\underline{H}_3CO_2$-), 1.12 (3H, s, C-19 $C\underline{H}_3$), 0.86 (3H, bs, C-30 $C\underline{H}_3$), 0.82 (3H, s, C-29 $C\underline{H}_3$), 0.80 (3H, s, C-28 $C\underline{H}_3$), 0.74 (3H, s, C-18 $C\underline{H}_3$).

Flash column chromatography with successive elution by toluene:light petroleum (60-80°C) (1:1), toluene, and finally $CHC\ell_3$, provided good separations. Under these conditions, 24,25-dihydrolanosteryl acetate is eluted first using toluene:light petroleum until the desired weight is obtained, then toluene and $CHC\ell_3$ elution give 24-(R,S)-25-epoxylanosteryl acetate. 90 MHz ^{1}H NMR ($CDC\ell_3$): δ4.50 (1H, m, 3α-H), 2.70 (1H, t, 24-H), 2.04 (3H, s, $C\underline{H}_3CO$), 1.31 (3H, s, 26- or 27-$C\underline{H}_3$), 1.28 (3H, s, 26- or 27-$C\underline{H}_3$), 1.01 (3H, s), 0.88 (9H, s), 0.69 (3H, s).

Reduction of 24(R,S)-25-epoxylanosteryl acetate

24(R,S)-25-epoxylanosteryl acetate (8.81g, 18.2 mmol) was dissolved in dry THF (370 ml). Lithium aluminium hydride (9.31g, 2.73 mmol) was added then the mixture refluxed for 12h. After this time the excess LAH was quenched with EtOAc, then water. The $A\ell(OH)_3$ was filtered off and washed with hot EtOAc. A sample was taken for GC and GC-MS analysis. The product was crystallised from EtOAc, m.p. 162-165°C (lit., 184-186°C).[68]

GC-MS analysis of products from the reduction of 24(R,S)-25-epoxylanosteryl acetate

A sample of the product (~ 500 μg) was treated with BSTFA (30 μℓ) at 80°C for 30 min to give products with $\underline{I}^{OV-1}_{270°}$ = 3540 (minor) and $\underline{I}^{OV-1}_{270°}$ = 3550 (major).

Scan 1 (minor peak) $I^{OV-1}_{270°} = 3540$

m/z	Ion type	%
588	$[M]^{+\cdot}$	34
573	$[M^{+\cdot}-CH_3^{\cdot}]$	15
498	$[M^{+\cdot}$-TMSOH]	20
483	$[M^{+\cdot}$-TMSOH-$CH_3^{\cdot}]$	44
393	$[M^{+\cdot}$-2 x TMSOH-$CH_3^{\cdot}]$	100
309		15
297	$[M^{+\cdot}$-SC-TMSOH]	15
229		21
213		24

Thought to be 24(R,S)-hydroxylanosterol di-TMS ether.

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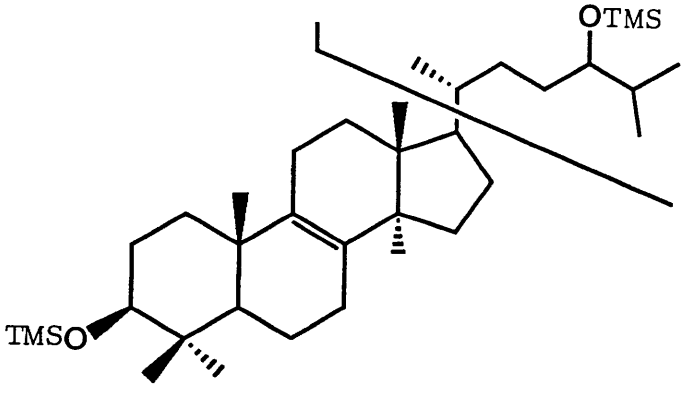
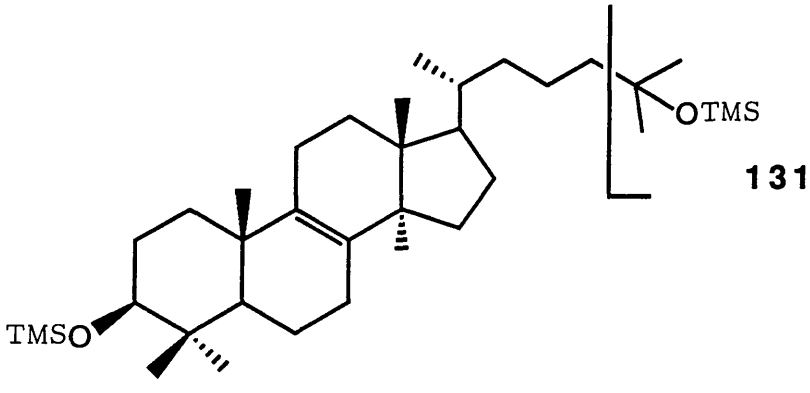


Scan 2 (major peak) $I^{OV-1}_{270°} = 3550$

m/z	Ion type	%
588	$[M]^{+\cdot}$	13
573	$[M^{+\cdot}-CH_3^{\cdot}]$	9
498	$[M^{+\cdot}$-TMSOH]	34
483	$[M^{+\cdot}$-TMSOH-$\dot{C}H_3$]	41
427		6
393	$[M^{+\cdot}$-2 x TMSOH-$\dot{C}H_3$]	100
350		3
337	[427-TMSOH]	26
309		5
297	$[M^{+\cdot}$-TMSOH]	4
283		6
272		7
253		5
229		10
215		10
201		7
199		4
187		12
174		10
161		9
149		7
135		20
131	$[C_3H_6OTMS]^+$	47

Metastable transitions

Observed	Calculated
585.5 M^+	$\frac{573^2}{588}$ = 558.38
469.0 M^+	$\frac{483^2}{498}$ = 468.45
407.0 M^+	$\frac{483^2}{573}$ = 407.13



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ΔI = 260 for 25-hydroxylanosterol di-TMS ether/lanost-8-en-3β-ol TMS ether (see Table 9).

25-Hydroxylanosterol (100 MHz) ^1H NMR (pyridine): δ3.08 (1H, m, 3α-H), 1.13 (6H, s, $(CH_3)_2C(OH)$, 0.93 (3H, s), 0.89 (3H, s), 0.78 (3H, s), 0.74 (3H, s), 0.60 (3H, s).

Monoacetylation of 25-hydroxylanosterol

25-Hydroxylanosterol (1.851g, 4.16 mmol) was dissolved in dry redistilled pyridine (100 ml) and acetic anhydride (200 ml). The mixture was heated at 85°C for 30 min. The solvents were removed

under reduced pressure and the material recrystallised from MeOH/ Et_2O, m.p. 151-155°C (lit., 167-168°C, Sublimes)[65]. The purity was checked by TLC and GLC (see Table 9).

Experimental Section - 3.1.3

CAN oxidation of 25-hydroxy-5α-lanost-8-en-3β-yl acetate

25-Hydroxy-5α-lanost-8-en-3β-yl acetate (93.6 mg, 0.19 mmol) was dissolved in CH_3CN (30 ml) and heated to 80°C. CAN (2.10g, 3.83 mmol) in water (4 ml) was added dropwise. On addition of the first few drops, decolorisation was noted. The reaction mixture was heated at 80°C for 15 min, then water (8 ml) was added and the reaction mixture extracted with $CH_2C\ell_2$. The extracts were dried over anhydrous $MgSO_4$ and the solvent removed to yield a brown oil (~ 100 mg).

Experimental Section - 3.1.4

Attempted base hydrolysis and acid extraction of the nitrogenous product $I^{OV-1}_{\pm 270°} = 4100$

The nitrogenous product (600 μg) in EtOH (0.5 ml) was treated with 10% NaOH(aq) (0.4 ml) for 45 min at 65°C. After this time, the EtOH was removed under a N_2 stream, the solution was neutralised with 2M HCℓ. Chloroform extraction gave the organic extract. The acidic aqueous extract was neutralised and extracted. However, GLC analysis revealed only minor amounts in that extract. The organic extract contained two compounds, $I^{OV-1}_{\pm 270°} = 3999$ and $I^{OV-1}_{\pm 270°} = 3640$, and both were more polar (by TLC) than the nitrogenous product ($I^{OV-1}_{\pm 270°} = 4100$).

GLC analysis or organic extract

$$I^{OV-1}_{\overline{-}270°} = 3990 \xrightarrow{Ac_2O} I^{OV-1}_{\overline{-}270°} = 4100$$

$$I^{OV-1}_{\overline{-}270°} = 3640 \xrightarrow{Ac_2O} I^{OV-1}_{\overline{-}270°} = 3720$$

Experimental Section - 3.2.1

CAN oxidation of 5α-lanost-8-en-3β-yl acetate

5α-Lanost-8-en-3β-yl acetate (103 mg, 0.219 mmol) was dissolved in CH_3CN (33 ml). CAN (2.55g, 4.65 mmol) was dissolved in water (2.3 ml) and added dropwise to the steroid solution with stirring. The mixture was then heated at 80°C for ca. 15 min, then diluted with water and extracted with EtOAc. The extracts were dried and the solvent removed under reduced pressure to yield a brown oil (97 mg).

GLC of products from the CAN oxidation of 5α-lanost-8-en-3β-yl acetate

$I^{OV-1}_{\overline{-}270°}$ = 3380 (minor peaks in brackets)

= 3530

= (3610)

= (3650)

= 3900

Preparative TLC

ca. 5 mg of the above reaction mixture was applied to a 20 x 20 cm, 0.25 mm TLC plate. Chromatography was effected with $CHC\ell_3$:EtOAc (9:1 v/v). Two UV-active bands were scraped off the plate and extracted (R_F 0.31, R_F 0.89).

Experimental Section - 3.2.2

Acid hydrolysis of the nitrogenous product (45)

The nitrogenous product (45) (1.1 mg) in absolute EtOH (1 ml) was heated with 1.1M HCℓ (2 ml) for 24h at 80°C. After this time, the EtOH was removed under a N_2 stream, the reaction mixture extracted with EtOAc, these extracts being further washed with aqueous acid. The combined acidic extracts were neutralised with NaOH(aq) and then re-extracted with EtOAc. However, no material was found in this extract. The organic extracts were dried over anhydrous Na_2SO_4 and solvent removed under a N_2 stream to yield a white solid (~ 800 μg).

Experimental Section - 3.3.1

Catalytic hydrogenation of 24(R,S)-hydroxy-cholest-5-en-3β-yl acetate[76]

To a solution of 24(R,S)-hydroxy-cholest-5-en-3β-yl acetate (205.7 mg, 0.46 mmol) in EtOAc (12 ml), 5% Pt on charcoal was added (632 mg), then the system was charged with excess

hydrogen. The reaction mixture was left stirring for 2 days at room temperature. After this time, the reaction mixture was filtered through a Celite 535 column to remove the Pt/C catalyst.

Acid hydrolysis

1 mg of the above reaction mixture in EtOH (0.9 ml) was treated with 2M HCℓ(aq) (0.5 ml) for 2h at 80°C. After this time, the solvents were removed under a N_2 stream, then water was added and the mixture extracted with EtOAc. The extracts were dried over anhydrous $MgSO_4$ and evaporated to yield 24(R,S)-hydroxycholestan-3β-ol (700 μg).

Cholesterol oxidase oxidation of 24(R,S)-hydroxy-cholestan-3β-ol

24(R,S)-Hydroxycholestan-3β-ol (100 μg) in isopropanol (100 μℓ) was diluted with phosphate buffer pH = 7 (1 ml) in a B-10 4" tube, and cholesterol oxidase (from Brevibacterium) (50 μℓ, 1 mg/ml) in phosphate buffer was added. The tube was stoppered and incubated at 37°C, with occasional stirring for 2h. After this time the mixture was extracted with EtOAc. The extracts were dried over anhydrous Na_2SO_4 and solvent removed to yield ~100 μg of material.

Experimental Section - 3.3.2

CAN oxidation of 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate

24(R,S)-Hydroxy-5α-cholestan-3β-yl acetate (159 mg, 0.35 mmol) was dissolved in CH_3CN (8 ml). An aqueous solution of CAN (0.73 ml of 0.97M) (2 mol equiv.) was added with stirring at 80°C for

20 min. After this time, the resulting reaction mixture was diluted with water and extracted with $CHC\ell_3$. The extracts were washed with water, then dried over anhydrous $MgSO_4$. On removal of the solvent, a white sticky solid resulted (163 mg).

Preparative TLC of products from CAN oxidation of 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate

The crude reaction mixture (1.1 mg) in EtOAc (150 μℓ) was applied to a 20 x 20 cm TLC plate. Chromatography was effected in toluene:EtOAc (3:1 v/v). After spraying to detect components on a portion of the plate, two bands were scraped off. These bands were extracted, then taken up in EtOAc (500 μℓ) for GLC analysis.

ca. 400 μg

Experimental Section - 3.4.1

Catalytic hydrogenation of crude lanosterol

Crude lanosterol (19.78g) in AcOH (200 ml) and EtOAc (100 ml), containing 5% Pt on charcoal (1g) was hydrogenated (65 psi) for 8h at 80°C. After this time, the reaction mixture was cooled, whereupon it solidified. After redissolving in Et_2O, the reaction mixture was filtered through a Celite column to remove the catalyst. GLC showed that the hydrogenation was complete and also revealed about 9% of 5α-lanost-8-en-3β-yl acetate had been produced.

Oxidation of 5α-lanost-8--en-3β-yl acetate with H_2O_2/AcOH/H_2SO_4[83]

5α-Lanost-8-en-3β-yl acetate (9.64g, 20.5 mmol) in glacial AcOH (700 ml) was cooled to 0-5°C. An ice-cold solution of concentrated H_2SO_4 (6 ml) in AcOH (10 ml) was added slowly with stirring immediately followed by a solution of 30% H_2O_2 (50 ml) in AcOH (10 ml). The reaction mixture was then left to stir at RT overnight. After this time an aliquot was removed and checked by GLC. GLC analysis revealed a 65% yield of 3β-acetoxy-5α-lanost-8-en-7-one ($\underline{I}^{OV-1}_{270°}$ = 3570) together with 3β-acetoxy-5α-lanost-8-en-11-one (23%) ($\underline{I}^{OV-1}_{270°}$ = 3530) and minor amounts of starting material. The reaction mixture was extracted with Et_2O, then the extracts dried and the solvents removed under reduced pressure to yield a white solid (10.11g).

Crystallisation from MeOH, m.p. 146-148°C (Lit. 150-151°C)[83] gave 3β-acetoxy-5α-lanost-8-en-7-one (5.16g). 200 MHz ^{1}H NMR ($CDC\ell_3$):[72] δ4.50 (1H, d of d, J = 10 Hz and 4.5 Hz, 3α-H), 2.41 (2H, m, C-6, $C\underline{H}_2$), 2.30 (2H, m, C-11, $C\underline{H}_2$), 2.04 (3H, s, 3β, $C\underline{H}_3\overset{O}{\overset{\|}{C}}$-O), 1.17 (3H, s, C-19, $C\underline{H}_3$), 0.94 (3H, s, C-29, $C\underline{H}_3$), 0.63 (3H, s, C-18, CH_3). UV $\lambda^{EtOH}_{max.}$ = 255 nm (ε = 9800).

The mother liquors were subjected to preparative TLC [ether:light petroleum, 60-80°C (1:2 v/v)] from which 3β-acetoxy-5α-lanost-8-en-11-one could be isolated as an oily solid (R_F = 0.64) [cf. 3β-acetoxy-5α-lanost-8-en-7-one (R_F = 0.53)]. The Δ^8-11-one could also be isolated by column chromatography. However, the remaining Δ^8-7-one in the mother liquors, when eluted, was contaminated with the Δ^8-11-ketone.

Infrared data of 3β-acetoxy-5α-lanost-8-en-7-one[87]

Bands observed (cm^{-1})	Group	Literature (cm^{-1})
2950-2920 (s)	ν(C-H)	
1738 (s)	ν(C=O) ester	1735
1649 (s)	ν(C=O) enone	1650
1580 (m)	ν(C=C)	1580
1468 (m) 1455 (m)	(C-H) def.	
1242 (s)	νC-O)	1242

Mass spectral data for 3β-acetoxy-5α-lanost-8-en-7-one (selected ions)

m/z	Ion type	%
484	$[M]^{+\cdot}$	28.4
469	$[M^{+\cdot}-CH_3^{\cdot}]$	100
441	$[M^{+\cdot}-43]$	0.4
424	$[M^{+\cdot}-CH_3CO_2H]$	1.8
409	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	3.3
371	$[M^{+\cdot}$-side chain (113)]	7.4
330	$[M^{+\cdot}$-ring D (154)]	1.4
311	$[M^{+}-CH_3CO_2H$-side chain]	1.6

200 MHz ^{1}H NMR of 3β-acetoxy-5α-lanost-8-en-11-one

($CDC\ell_3$), δ 4.52 (1H, m, 3α-H), 2.58 (2H, ABq, J = 23 Hz, C-12, $C\underline{H}_2$), 2.40 (2H, m, C-7, $C\underline{H}_2$), 2.04 (3H, s, $C\underline{H}_3CO_2$-), 1.13 (3H, s), 1.10 (3H, s), 0.88 (6H, s), 0.86 (3H, s), 0.83 (3H, s), 0.80 (3H, s).

Mass spectral data for 3β-acetoxy-5α-lanost-8-en-11-one

m/z	Ion type	%
484	$[M]^{+\cdot}$	2.4
424	$[M^{+\cdot}-CH_3CO_2H]$	1.7
409	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	0.8
332	$[M^{+\cdot}$-ring D-2H$^{\cdot}$]	1.9
277		3.0
257	$[M^{+\cdot}-CH_3CO_2H$-ring D-2H$^{\cdot}-CH_3]$	0.2
149		5.2
43	$[C_3H_7]^+$ or $[C_2H_3O]^+$	100

Infrared spectrometric data for 3β-acetoxy-5α-lanost-8-en-11-one[83]

Bands observed (cm^{-1})	Group	Literature[83]
2900 (s) 2940 (s) 2860 (s)	ν(C-H)	
1735 (s)	ν(C=O) ester	1736
1658 (s)	ν(C=O) enone	1656
1589 (m)	ν(C=C) enone	1583
1490 (m) 1470 (m)	ν(C-H) def.	
1375 (s) 1367 (s)	ν(C-H) of $CH_3\overset{O}{\overset{\|}{C}}-O$	
1246 (s)	ν(C-O)	1243

Experimental Section - 3.4.2

Epoxidation of 5α-lanost-8-en-3β-yl acetate[64]

To a stirred solution of 5α-lanost-8-en-3β-yl acetate (432 mg, 0.91 mmol) in $CH_2C\ell_2$ (10 ml) was added $NaHCO_3$ (100 mg) and mcpba (433 mg, 2.51 mmol) in $CH_2C\ell_2$ (10 ml). The reaction mixture was left to stir overnight at RT. After this time, an aliquot was removed and checked by micro TLC which indicated for completion of the reaction. 1M NaOH (20 ml) was then added and the mixture extracted with $CH_2C\ell_2$. The extracts were dried and the solvent removed under reduced pressure to yield a white solid which crystallised from MeOH (containing 5 drops of pyridine) (300 mg), m.p. 144-146°C (lit., 142°C).[64]

Preparation of 5α-lanosta-7,9(11)-dien-3β-yl acetate from 8α,9-epoxy-5α-lanostan-3β-yl acetate

8α,9-Epoxy-5α-lanostan-3β-yl acetate (7 mg) in $CH_2C\ell_2$ (1 ml) was shaken with 48% HBr (0.15 ml) for 30 min. After this time, water (10 ml) was added and the mixture extracted with $CH_2C\ell_2$, the extracts were dried and solvent removed under reduced pressure to yield a white solid (6.4 mg). Both GLC and TLC showed the material to be practically pure $I^{OV-1}_{270°}$ = 3370, R_F = 0.83 [Et_2O:pet. ether, 60-80°C (1:2 v/v)], m.p. 163-165°C (Lit. 168-169°C).[64]

Mass spectral data for 5α-lanosta-7,9(11)-dien-3β-yl acetate (selected ions)

$C_{32}H_{54}O_2$ M = 468

m/z	Ion type	%
468	$[M^{+\cdot}]$	65.2
453	$[M^{+\cdot}-CH_3^{\cdot}]$	10.6
408	$[M^{+\cdot}-CH_3CO_2H]$	10.7
393	$[M^{+\cdot}-CH_3CO_2-CH_3^{\cdot}]$	18.6
355	$[M^{+\cdot}$-side chain]	5.6
340	$[M^{+\cdot}$-side chain-$CH_3^{\cdot}]$	1.1
313	$[M^{+\cdot}$-ring D-$H^{\cdot}]$	19.2
295	$[M^{+\cdot}$-side chain-$CH_3CO_2H]$	9.9
253	$[M^{+\cdot}$-ring D-CH_3CO_2H-$H^{\cdot}]$	37.5
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

Infrared spectrometric data for 5α-lanosta-7,9(11)-dien-3β-yl acetate

Band observed (cm^{-1})	Group
2960-2930 (s)	ν(C-H)
1735 (s)	ν(C=O) ester
1465 (m)	(C-H) def.
1390 (m)	ν(C-H) CH_3CO-
1370 (m)	
1245 (s)	(C-O)

EtOH

UV data[64] 251 nm (ε 10,800)

243 nm (ε 16,100)

235 nm (ε 13,600)

200 MHz ^1H NMR of 5α-lanosta-7,9(11)-dien-3β-yl acetate[75]

δ5.44 (1H, m, C-7 C=C<u>H</u>), 5.31 (1H, d, J=5Hz, C-11 C=CH), 4.50 (1H, d of d, J=10 Hz and 5 Hz, 3α-H), 2.04 (3H, s, $C\underline{H}_3CO_2-$), 0.99 (3H, s, C-19 CH_3), 0.94 (3H, s, C-29 $C\underline{H}_3$), 0.87 (6H, bs, C-28 $C\underline{H}_3$ + C-21 $C\underline{H}_3$), 0.86 (3H, bs, C-30 CH_3), 0.84 (3H, s, C-27 CH_3), 0.83 (3H, s, C-26 CH_3), 0.54 (3H, s, C-18 CH_3).

Experimental Section - 3.4.3

Epoxidation of 5α-lanosta-7,9(11)-dien-3β-yl acetate[84,85,86]

To a solution of 5α-lanosta-7,9(11)-dien-3β-yl acetate (88.3 mg, 0.18 mmol) in $CH_2C\ell_2$ (5 ml) was added $NaHCO_3$ (100 mg) and mcpba (39 mg, 0.22 mmol) in 10 ml of $CH_2C\ell_2$. The reaction mixture was stirred at RT overnight. After this time an aliquot was removed and checked by TLC. Two major spots were observed at R_F 0.65 and 0.52 [Et_2O:pet. ether, 60-80°C (1:2 v/v)] with no starting material (R_F 0.80). The reaction mixture was then washed with 1M NaOH, then extracted with $CH_2C\ell_2$. The extracts were dried and on removal of solvent yielded an oil (64 mg). The crude material (38 mg) was subjected to flash column chromatography using toluene:light petroleum. 60-80°C (4:1 v/v). Fractions 2-5 gave a single spot on TLC (wt. = 22 mg) and 200 MHz ^1H NMR showed this to be 7β,8-epoxy-5α-lanost-9(11)-en-3β-yl acetate:[71,84] ($CDC\ell_3$): δ5.67 (1H, t, J_{AX} = 3.9 Hz, AX_2, C-11, C=CH), 4.43 (1H, m, 3α-H), 3.05 (1H, d of d, J = 5.9 Hz and 1 Hz, 7α-H), 2.18 (2H, d, J_{AX} = 3.8 Hz, C-12, CH_2), 2.04 (3H, s, $C\underline{H}_3CO_2$-).

After 3 days in the NMR solvent ($CDC\ell_3$), the ^1H NMR spectrum changed, revealing a 2:1 mixture of 3β-acetoxy-5α,8α-lanost-9(11)-en-7-one to 7β,8-epoxy-5α-lanost-9(11)-en-3β-yl acetate, respectively. 200 MHz ^1H NMR of 3β-acetoxy-5α,8α-lanost-9(11)-en-7-one,[71,84] ($CDC\ell_3$): δ5.49 (1H, m, C-11, C=C$\underline{H}$), 4.50 (1H, m, 3α-H), 3.14 (1H, m, 8α-H), 3.07-2.88 (1H, bm, 15β-H), 2.06 (3H, s, $C\underline{H}_3CO_2$-). Further elution from the column (Fraction 7) gave (11.9 mg) a 3:1 mixture of 9α,11-epoxy-5α-lanost-7-en-3β-yl acetate and

Mass spectral data for 7β,8-epoxy-5α-lanost-9(11)-en-3β-yl acetate

(selected ions)

$C_{32}H_{52}O_3$ (484)

m/z	Ion type	%
484	$[M]^{+\cdot}$	30.5
469	$[M^{+\cdot}-CH_3^{\cdot}]$	35.5
466	$[M^{+\cdot}-H_2O]$	1.1
455	$[M^{+\cdot}-CHO]$	11.0
424	$[M^{+\cdot}-CH_3CO_2H]$	7.6
409	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	0.9
406	$[M^{+\cdot}-CH_3CO_2H-H_2O]$	0.7
391	$[M^{+\cdot}-CH_3CO_2H-H_2O-CH_3^{\cdot}]$	4.5
371	$[M^{+\cdot}$-side chain]	6.4
330	$[M^{+\cdot}$-ring D]	1.6
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

7β,8-epoxy-5α-lanost-9(11)-en-3β-yl acetate as judged by ^{1}H NMR. 200 MHz ^{1}H NMR of 9α,11-epoxy-5α-lanost-7-en-3β-yl acetate,[71,84] δ5.72 (1H, t, J = 3.9 Hz, C-7, C=C<u>H</u>), 4.50 (1H, m, 3α-H), 3.20 (1H, d, J = 5.6 Hz, 11β-H), 2.02 (3H, s, C<u>H</u>$_3$CO$_2$-).

Experimental Section - 3.4.4

Reduction of 3β-acetoxy-5α-lanost-8-en-7-one[87]

To a solution of 3β-acetoxy-5α-lanost-8-en-7-one (1.17g, 2.41 mmol) in dry THF (10 ml) and $NH_{3(\ell)}$ (<u>ca</u>. 30 ml) was added Li metal (~ 300 mg). The reaction mixture was stirred at -50°C for 2h. After this time, MeOH was added to destroy the excess Li and the reaction was warmed to RT in order to distil off the NH_3. The crude reaction mixture was dissolved in ether/water, then extracted. The extracts were dried and solvent removed to yield a white solid (1.00g). The crude material was acetylated in dry pyridine (10 ml) and Ac_2O (20 ml) for 30 min at 60°C. Crystallisation from MeOH gave 7-keto-5α-lanostan-3β-yl acetate (0.81g) contaminated with 3β-acetoxy-5α-lanost-8-en-7-one, m.p. 145-149°C (Lit. 172°C).[89]

Experimental Section - 3.4.5

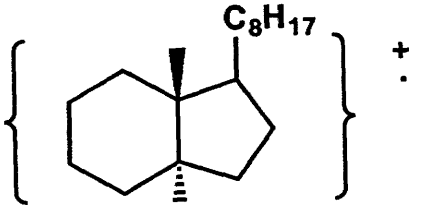
Catalytic hydrogenation of 7-keto-5α-lanostan-3β-yl acetate[88,89,90]

7-keto-5α-Lanostan-3β-yl acetate (1.14g, 2.34 mmol) was dissolved in EtOAc (50 ml) and AcOH (50 ml). PtO_2 type D (123 mg) was added and the vessel charged with H_2 (70 psi). The reaction mixture was shaken for 48h, then filtered through a Celite 235 column to remove the black Pt metal.

Mass spectral data for 7-keto-5α-lanostan-3β-yl acetate (selected ions)

$C_{32}H_{54}O_3$ (486)

m/z	Ion type	%
486	$[M]^{+\cdot}$	35.1
471	$[M^{+\cdot}-CH_3^{\cdot}]$	11.1
426	$[M^{+\cdot}-CH_3CO_2H]$	3.3
373	[$M^{+\cdot}$-side chain]	10.9
332	[$M^{+\cdot}$-ring D]	8.8
264	$[M^{+\cdot}-222]$	51.1
164	$[M^{+\cdot}-322]$	18.5
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100



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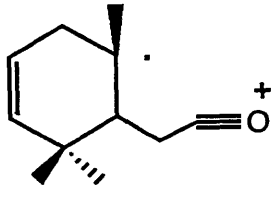


m/z 164

Infrared spectrometric data for 7-keto-5α-lanostan-3β-yl acetate ($CC\ell_4$)

Band observed (cm^{-1})	Group
2960 (s)	ν(C-H)
1742 (s)	ν(C=O) ester
1708 (m)	ν(C=O) ketone
1468 (m)	(C-H) def.
1374 (m)	ν(C-H) $C\underline{H}_3CO_2^-$
1240 (s)	ν(C-O)

200 MHz ^1H NMR for 7-keto-5α-lanostan-3β-yl acetate

($CDC\ell_3$): δ4.48 (1H, m, 3α-H), 2.27 (3H, m, 8βH and C-7, $C\underline{H}_2$), 2.03 (3H, s, $C\underline{H}_3CO_2$, 1.08 (3H, s), 0.88 (6H, s), 0.86 (3H, s), 0.82 (3H, s), 0.81 (3H, s).

Experimental Section - 3.4.6

L-Selectride reduction of 7-keto-5α-lanostan-3β-yl acetate[90]

To a solution of 3β-hydroxy-5α-lanostan-7-one (4.3 mg, 0.009 mmol) in THF (1 ml) was added 1M L-Selectride (150 μℓ) in THF. The reaction was stirred at RT for 4h. After this time, the solvent was removed under an N_2 stream, then the mixture extracted with EtOAc. The extracts were dried and, on removal of the solvent, yielded a gummy solid (7.2 mg); ca. 3 mg of the material was acetylated using Ac_2O (2 ml)/pyridine (1 ml) at 60°C for 10 min.

Experimental Section - 3.4.7

Scale-up of L-Selectride reduction

To a solution of 3β-hydroxy-5α-lanostan-7-one (0.92g, 2.07 mmol) in dry THF (15 ml) was added 1M L-Selectride (8 ml) in THF. The reaction mixture was stirred overnight at RT. After this time, the reaction was slowly quenched with water. NaOH (0.73g) was added, then water (15 ml). 30% H_2O_2 (10 ml) was added slowly dropwise (exothermic reaction!). After complete addition of H_2O_2 the mixture was refluxed for 4h. Any precipitate was redissolved by addition of THF. The THF was removed under reduced pressure then the mixture extracted with EtOAc. The extracts were dried and, on removal of the solvent, yielded a white solid. This material was acetylated using Ac_2O (4 ml) and pyridine (20 ml) with stirring at 20°C for 5½h: the reaction was monitored by TLC. On removal of the solvent, a white solid resulted (0.951g). TLC [ether:light petroleum, 60-80°C (1:1 v/v)] revealed a major spot at R_F 0.40

Infrared spectrometric data for 7α-hydroxy-5α-lanostan-3β-yl acetate (containing acetone) - (KBr disc)

Band observed (cm^{-1})	Group
3545 (m) sharp	ν(O-H) enol of lactone
3420 (m) broad	ν(O-H)
2940 (s)	ν(C-H)
2870 (s)	
1735 (s)	ν(C=O) ester
1710 (s)	ν(C=O) acetone
1462 (m)	(C-H) def.
1384 (m)	
1242 (s)	ν(C-O)

Acetone was difficult to remove even under vacuum overnight at 45°C.

Mass spectral data for 7α-hydroxy-5α-lanostan-3β-yl acetate (selected ions)

$C_{32}H_{56}O_3$ (488)

m/z	Ion type	%
488	$[M]^{+\cdot}$	0.4
470	$[M^{+\cdot}-H_2O]$	1.2
455	$[M^{+\cdot}-H_2O-CH_3^{\cdot}]$	1.3
413	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	0.2
410	$[M^{+\cdot}-CH_3CO_2H-H_2O]$	0.6
395	$[M^{+\cdot}-CH_3CO_2H-H_2O-CH_3^{\cdot}]$	1.9
315	$[M^{+\cdot}-CH_3CO_2H$-side chain]	2.1
297	$[M^{+\cdot}-CH_3CO_2H-H_2O$-side chain]	1.0
256	$[M^{+\cdot}-CH_3CO_2H-H_2O$-ring D]	1.1
255	$[M^{+\cdot}-CH_3CO_2H-H_2O$-ring D-H]	4.2
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

(7α-hydroxy-5α-lanostan-3β-yl acetate), R_F 0.67 (7-keto-5α-lanostan-3β-yl acetate) and minor spot at R_F 0.09 (5α-lanostane-3β,7α-diol). The crude material was subjected to column chromatography using ether: light petroleum, 60-80°C (1:1 v/v). Fractions 5 and 6 (226 mg) contained pure (as judged by TLC and GLC) 7α-hydroxy-5α-lanostan-3β-yl acetate which was recrystallised from acetone/H_2O, m.p. 203-207°C (Lit. 209-211°C, 205-206°C, 209.0-210.5°C, and 212°C).[79] 200 MHz ^{1}H NMR ($CDC\ell_3$), δ 4.51 (1H, d of d, J = 10 Hz and 4 Hz, 3α-H), 4.05 (1H, bs, 7β-H), 2.03 (3H, s, $C\underline{H}_3CO_2$-), 1.06 (3H, s, C-30), 0.93 (3H, s, C-19), 0.72 (3H, s, C-18).

Experimental Section - 3.4.8

Reaction of 7α-hydroxy-5α-lanostan-3β-yl acetate with $Pb(OAc)_4$

7α-Hydroxy-5α-lanostan-3β-yl acetate (72 mg, 0.14 mmol) was dissolved in benzene (50 ml). $Pb(OAc)_4$ (0.468g, 1.05 mmol) was added and the mixture refluxed for 24h. A 20% solution of KI (10 ml) was then added to give a yellow solid which dissolved on addition of saturated $Na_2S_2O_3$ solution. The reaction mixture was then extracted with EtOAc, the extracts dried and solvent removed to yield a white solid (65 mg). TLC indicated one product [ether:light petroleum 60-80°C (1:2 v/v)]. R_F 0.53, crystallisation from acetone/water gave 7α,30-epoxy-5α-lanostan-3β-yl acetate (33 mg), m.p. 192-195°C (Lit. 201-203°C, 195-197°C, 181-183°C, and 202-204°C).[79] 200 MHz ^{1}H NMR ($CDC\ell_3$): δ 4.47 (1H, d of d, J = 10 Hz and 5 Hz, 3α-H), 4.15 (1H, m, 7β-H), 3.96 (1H, d, J = 7.8 Hz, C-3=, C$\underline{H}$), 3.32 (1H, d, J = 7.5 Hz, C-30, C$\underline{H}$), 2.03 (3H, s, $C\underline{H}_3CO_2$-).

Infrared spectrometric data for 3β-acetoxy-5α-lanostan-7α 30 ether

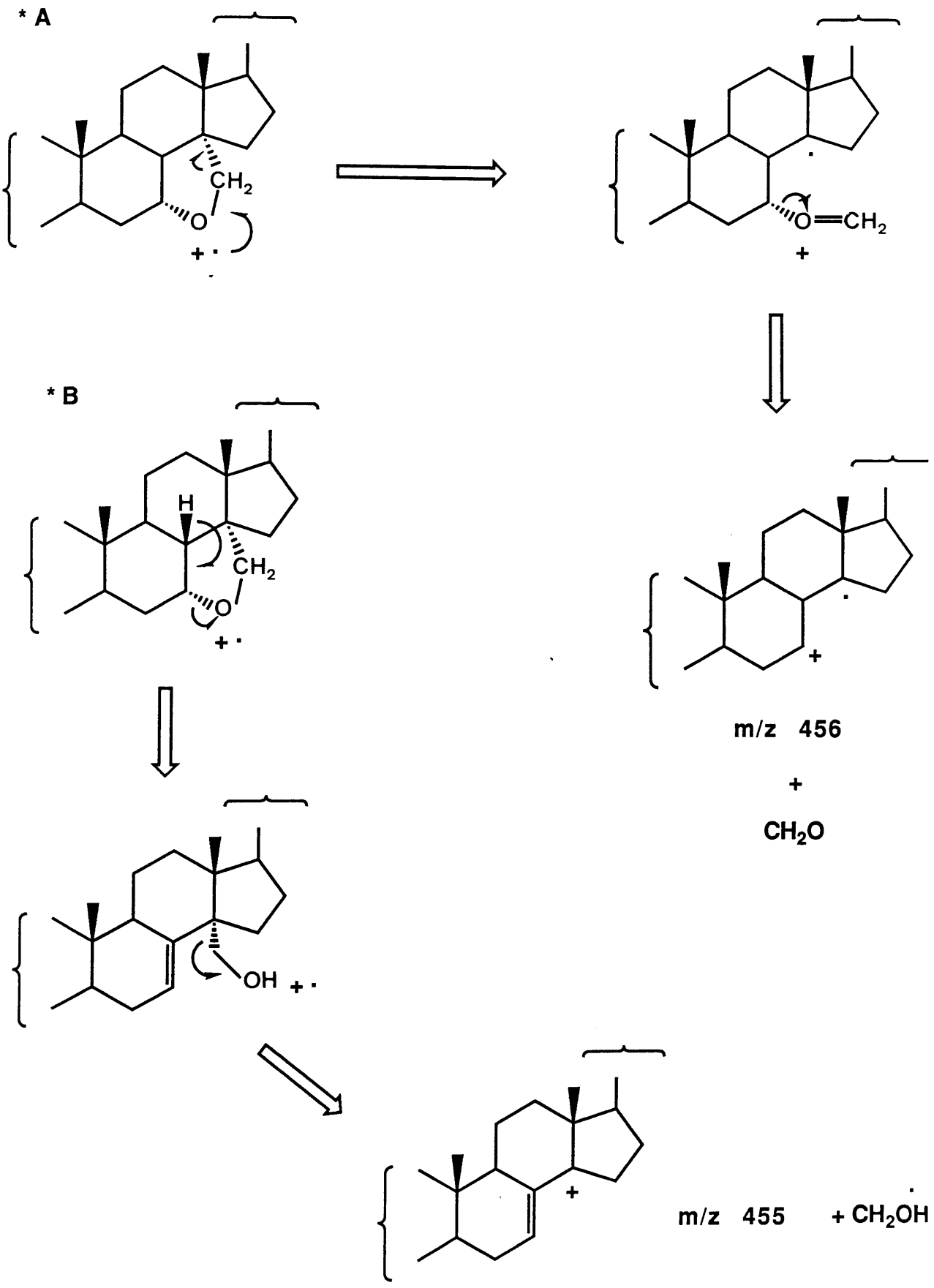
(1% $CC\ell_4$ solution)

Band observed (cm^{-1})	Group
2955 (s)	ν(C-H)
2870 (s)	
1732 (s)	ν(C=O) ester
1469 (m) 1460	(C-H) def.
1378 (m) 1370	O CH_3C-O (C-H)
1244 (s)	ν(C-O)
1041 (m)	ν(C-O-C)
935 (m) 922	

Mass spectral data for 3β-acetoxy-5α-lanostan-7α,30 ether

$C_{32}H_{43}O_3$ (486)

m/z	Ion type	%
486	$[M]^{+\cdot}$	0.9
471	$[M^{+\cdot}-\dot{C}H_3]$	1.2
*A 456	$[M^{+\cdot}-CH_2O]$	47.8
*B 455	$[M^{+\cdot}-CH_2OH^{\cdot}]$	65.2
441	$[M^{+\cdot}-CH_3^{\cdot}-H_2O]$	4.9
396	$[M^{+\cdot}-CH_3CO_2H-CH_2O]$	16.8
395	$[M^{+\cdot}-CH_3CO_2H-CH_2OH^{\cdot}]$	35.9
381	$[M^{+\cdot}-CH_3CO_2H-CH_2O-\dot{C}H_3]$	8.7
373	$[M^{+\cdot}$-side chain]	3.0
343	$[M^{+\cdot}$-side chain-CH_2O]	18.4
341	$[M^{+\cdot}$-side chain-32]	38.2
331	$[M^{+\cdot}$-side chain-42]	2.3
283	$[M^{+\cdot}-CH_3CO_2H-CH_2O$-side chain]	6.8
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100


* A
CH2
O
+ ·
O=CH2
+
* B
H
CH2
O
+ ·
+
m/z 456
+
CH2O
OH + ·
+
m/z 455
+ CH2OH

Experimental Section - 3.4.9

Reaction of 7α-hydroxy-5α-lanostan-3β-yl acetate with $Pb(OAc)_4/I_2/hr$

7α-Hydroxy-5α-lanostan-3β-yl acetate (56 mg, 0.11 mmol) was dissolved in cyclohexane (15 ml) and $CH_2C\ell_2$ (3 ml). $Pb(OAc)_4$ (0.64g, 1.44 mmol) and I_2 (64 mg, 0.25 mmol) was added. The mixture was then irradiated using a 300W tungsten filament lamp and warmed/cooled to maintain gentle reflux for ca. 1h. 20% KI (20 ml) was added, resulting in a yellow precipitate which dissolved on addition of saturated $Na_2S_2O_3$. The mixture was then extracted with EtOAc, the extracts being dried and solvent removed to yield an oil (76 mg).

Preparative TLC

1 mg of the crude product was chromatographed on a 5 x 20 cm TLC plate using ether:light petroleum (1:2 v/v). Bands were extracted at R_F 0.61.

Experimental Section - 3.4.10

$NaBH_4$ reduction of components $I^{OV-1}_{270°} = 3380$ and $I^{OV-1}_{270°} = 3560$

To a solution of the above component (4.0 mg) in MeOH (1 ml) and NaOAc (ca. 5 mg) was added $NaBH_4$ (8 mg, 0.21 mmol). Effervescence was noted. The reaction mixture was then stirred for 30 min at RT. After this time, the solvent was removed under a N_2 stream and water (0.5 ml) added. The reaction mixture was then extracted with EtOAc, the extracts dried and solvent removed to yield an oil (4.4 mg).

Experimental Section - 3.4.11

Treatment of hemi-acetal (70) with PCC

To a solution of hemi-acetal (70) (1.3 mg, 0.001 mmol) in $CH_2C\ell_2$ (1 ml) was added PCC (6.2 mg, 0.02 mmol) with stirring at RT for 1h 15 min. Water (1 ml) was added and the excess PCC reduced with $Na_2S_2O_5$ solution. The reaction mixture was extracted with $CH_2C\ell_2$, the extracts dried and solvent removed to give the product (0.9 mg).

Experimental Section - 3.4.12

Reaction of 7α-hydroxy-5α-lanostan-3β-yl acetate with CAN

To a solution of 7α-hydroxy-5α-lanostan-3β-yl acetate (40.0 mg, 0.081 mmol) in CH_3CN (25 ml) was added CAN (112 mg, 0.20 mmol) in water (0.5 ml). The reaction mixture was heated at 80°C for 30 min (until decolorised). Water (5 ml) was added then the CH_3CN removed under pressure. The mixture was extracted with EtOAc, the extracts were dried, and solvent was removed to yield a sticky white solid (35.4 mg).

Column chromatography: ca. 30 mg were used [light petroleum:ether (2:1 v/v)].

Fraction 2-4 yielded 7α,30-ether (58) (6.3 mg)

Fraction 7-12 yielded 7α-ol (57) (14.2 mg)

Fraction 19 yielded unknown, R_F 0.06, I = 3730 (200 μg).

Table 2. GLC analysis of CAN oxidation of 25-hydroxy-5α-lanost-8-en-3β-yl acetate

$I^{OV-1}_{-270°}$
(3510)
(3500)
3600
3700
3730
3850
3870
(3910)
(3940)
(3980)
4100

Minor peaks in brackets

Table 3. GLC and TLC analyses of products from the CAN oxidation of 25-hydroxy-5α-lanost-8-en-3β-yl acetate

	TLC (R_F)		
	R_F 0.72	R_F 0.26	R_F 0.15
GLC $I^{OV-1}_{\pm 270°}$	3620	3870	4100
	3700	-	-
	3740	-	-
	3770	-	-

TLC Solvent System - 3:1 $CHC\ell_3$:EtOAc (v/v)

Table 4. Mass Spectrum of component of R_F 0.15, $I^{OV-1}_{\pm 270°}$ = 4100 (selected ions)

m/z	Ion type	%
629	$[M]^{+\cdot}$	14
614	$[M^{+\cdot}-CH_3^{\cdot}]$	11
571	$[M^{+\cdot}-CH_3^{\cdot}-43]$	4
556	$[571-CH_3^{\cdot}]$	5
539	$[M^{+\cdot}$-TMSOH]	100
524	$[M^{+\cdot}$-TMSOH-$CH_3^{\cdot}]$	3
496	$[M^{+\cdot}$-AcOH-$CH_3^{\cdot}$-58]	5
479	$[M^{+\cdot}$-TMSOH-AcOH]	5
436	[479-43]	5.1
131	$[C_3H_6OTMS]^+$	95.4

Table 5. High Resolution Mass Spectral data for component R_F 0.15, $I^{OV-1}_{270°}$ = 4100 (selected ions)

	Formula	%	Observed mass
$[M]^{+\cdot}$ (^{13}C)	$C_{36}{}^{13}CH_{63}NO_5Si$	4.71	630.4486
$[M]^{+\cdot}$	$C_{37}H_{63}NO_5Si$	5.69	629.4261
M(^{13}C)-15	$C_{35}{}^{13}CH_{60}NO_5Si$	4.35	615.4261
M-15	$C_{36}H_{60}NO_5Si$	5.74	614.4248
M(^{13}C)-90	$C_{33}{}^{13}CH_{55}NO_4$	26.52	540.4008
M-90	$C_{34}H_{53}NO_4$	69.6	539.3989
M-60-15-58	$C_{32}H_{52}O_2Si$	11.2	496.3751
M-90-60	$C_{32}H_{49}NO_2$	20.2	479.3754
M-90-60-43	$C_{30}H_{46}NO$	16.03	436.3564
	$C_{29}H_{41}O$	31.10	405.3149
M-90-60-15	$C_{31}H_{46}NO_2$	8.24	464.3506

Table 6(i). Infrared spectrometric data of 25-hydroxy-5α-lanost-8-en-3β-yl acetate in $CC\ell_4$: cell pattern 0.5 cm, scan time 10 min.

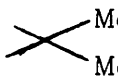
Observed (cm^{-1})	Group	Expected range
3610 (w)	ν(OH)	3650-3590 (v)
2940 (s)	$>CH_2$, CH_3-	2960-2850 (s)
2860 (s)	ν(C-H)	
1723 (s)	ν(C=O) ester	1750-1730 (s)
1365 (s)	$OCOCH_3$ CH def	1385-1365 (s)
1459 (m) } 1445 (m) }	$>CH_2$ } def $-CH_3$ }	1470-1430 (m)
1385 (m)		1380 (m) doublet
1240 (s)	ν(C-O)	1300-1050 (s)

Table 6(ii). Infrared spectrometric data of R_F 0.15, $I^{OV-1}_{270°}$ = 4100 (in $CC\ell_4$)

Observed (cm^{-1})	Group	Expected range
3610 (w)	ν(OH)	3650-3590 (w)
3440 (w)	ν(N-H)	3460-3400 (m)
2960 (s) 2930 (s) 2870 (s)	$>CH_2$ $-CH_3$ ν(C-H	2960-2850 (s)
1730 (s)	ν(C=O) ester	1750-1735 (s)
1685 (s)	ν(C=O) enone	
1635 (s)	ν(C=O) amide II	
1480 (m)	CH def	1470-1430 (m)
1370 (s)	$OCOCH_3$ CH def	1385-1365 (s)
1240	ν(C-O)	

200 MHz ^{1}H NMR of 25-hydroxy-5α-lanost-8-en-3β-yl acetate

($CDC\ell_3$): δ 4.485 (1H, d of d, 10 Hz, 5 Hz, 3α-H), 2.03, (3H, s, $CH_3\overset{O}{\overset{\|}{C}}O$), 1.19 (6H, s, $(CH_3)_2C(OH)$), 0.98 (3H, s, CH_3), 0.86 (6H, s, 2 x CH_3), 0.67 (3H, s, CH_3).

Table 7. 25 MHz ^{13}C NMR D.E.P.T. for 25-hydroxy-5α-lanost-8-en-3β-yl acetate ($CDC\ell_3$)

$C_{32}H_{54}O_3$ contains $9xCH_3$, $11xCH_2$, 4xCH, $8x\text{-}\overset{|}{\underset{|}{C}}\text{-}$

observed: $9xCH_3$, $11xCH_2$, 4xCH, $8x\text{-}\overset{|}{\underset{|}{C}}\text{-}$

Observed (ppm)	Group
170.985	C=O acetate
134.455	C-8
134.233	C-9
80.908	CH, C-3
71.063	C, C-25
50.486	2xCH { C-17 + C-5
49.784	C, C-14
44.458	C, C-13
44.399	CH_2, C-24
37.778	C, C-4
36.876	C, C-10
36.715	CH_2, C-22
36.457	CH, C-20
35.250	CH_2, C-1
30.954	CH_2, C-12

Observed (ppm)	Group
30.793	CH_2, C-15
29.312	CH_3, C-26 or C-27
29.202	CH_3, C-26 or C-27
28.220	CH_2, C-16
27.894	CH_3, C-28
26.363	CH_2, C-7
24.241	CH_3, C-30
24.156	CH_2, C-2
21.314	CH_3, Ac-CH_3
21.112	CH_2, C-23
20.978	CH_2, C-11
19.167	CH_3, C-19
18.665	CH_3, C-21
18.104	CH_2, C-6
16.515	CH_3, C-29
15.743	CH_3, C-18

Table 8. ^{1}H NMR of nitrogenous product

200 MHz ^{1}H NMR ($CDC\ell_3$): δ5.68 (1H, d, J=10 Hz, NH), 4.80 (1H, bd, J=10 Hz, H-7), 4.56 (1H, m, 3α-H), 3.65 (1H impurity), 3.10 (1H, bd, J=15 Hz, H-6), 2.55 (AB quartet, J=19 Hz, 12-CH_2), 2.05 (3H, s, $C\underline{H}_3\overset{O}{\overset{\|}{C}}$), 2.03 (3H, s, $C\underline{H}_3\overset{O}{\overset{\|}{C}}$), 2.00 (3H, s, $C\underline{H}_3\overset{O}{\overset{\|}{C}}$), (one $CH_3\overset{O}{\overset{\|}{C}}$-O is an impurity).

Table 9. GLC Analysis Results

Compound	Retention Index ($I^{OV-1}_{270°}$)
5α-lanosta-8,24-dien-3β-ol	3300
5α-lanosta-8,24-dien-3β-ol TMS ether	3315
5α-lanosta-8,24-dien-3β-yl acetate	3400
5α-lanost-8-en-3β-ol	3270
5α-lanost-8-en-3β-ol TMS ether	3290
5α-lanost-8-en-3β-yl acetate	3360
24(R,S)-25-epoxy-5α-lanost-8-en-3β-yl acetate	3530
5α-lanost-8-ene-3β,25-diol	3460
5α-lanost-8-ene-3β,25-diol di-TMS ether	3550
25-hydroxy-5α-lanost-8-en-3β-yl acetate	3550
5α-lanost-8-ene-3β,25-diol diacetate	3660
5α-lanostane-3β,7β-diol	3570
5α-3β,7β,11β-triol lanostane 3,7-diacetate	3890
7-keto-5α-lanostan-3β-ol	3530
11-keto-5α-lanostan-3β-yl acetate	3600
5α-lanost-7-en-3-one	3300
5α-lanost-9(11)-en-3β-yl acetate	3410
5α-lanosta-7,9(11),24-trien-3β-yl acetate	3370
5α-lanosta-7,9(11)-dien-3β-yl acetate	3380
7,11-diketo-5α-lanost-8-en-3β-yl acetate	3545

Column: 1% OV-1 at 270°C, N_2 = 30 ml/min.

ΔI values

3β-ol ⟶ 3β-OAc	lanosterol	+ 100
	dihydrolanosterol	+ 90
	25-hydroxylanosterol	+ 90
$\Delta^{24(25)}$ ⟶ epoxides of lanosteryl acetate		+ 130
3β-ol ⟶ 3β-OTMS	lanosterol	+ 15
	dihydrolanosterol	+ 20
3β-OTMS, Δ^8 ⟶ 3β-OTMS, Δ^8-25-OTMS		+ 260

Table 9(i). HPLC Analysis Results

Column: : 5 cm analytical C-18 ODS

Solvent system : MeOH at 0.5 ml/min

Detector : UV at 240 nm, 0.05 attenuation

Samples were 10 mg/ml in 0.8 ml MeOH + 0.2 ml hexane.

Compound	Retention time (min)
5α-lanosta-8,24-dien-3β-ol	3.3
5α-lanosta-8,24-dien-3β-yl acetate	5.0
5α-lanost-8-en-3β-ol	3.9
5α-lanost-8-en-3β-yl acetate	6.0
24(R,S)-25-epoxy-5α-lanost-8-en-3β-yl acetate	2 peaks broad 2.8
25-hydroxy-5α-lanost-8-en-3β-ol	1.5
25-hydroxy-5α-lanost-8-en-3β-yl acetate	2.1
"nitrogenous product"	0.9

Table 9(ii) GC-MS analysis of $I^{OV-1}_{270°}$ = 3900, R_F 0.31, oxidation product (22 eV) - selected ions.

m/z	Ion type	%
451	$[M]^{+\cdot}$	15
426	$[M^{+\cdot}-CH_3^{\cdot}]$	3
482	$[M^{+\cdot}-CH_3CONH_2]$	21
481	$[M^{+\cdot}-CH_3CO_2H]$	38
466	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	9
438	$[M^{+\cdot}-CH_3CO_2H-43]$	6
422	$[M^{+\cdot}-CH_3CO_2H-CH_3CO]$	23
407	$[M^{+\cdot}-CH_3CO_2H-CH_3CONH_2-CH_3^{\cdot}]$	100

Metastable transition

541 amu ⟶ 482 amu

M^* calculated 429.43

M^* observed 428.90

Table 10. High resolution mass analysis of $I^{OV-1}_{270°} = 3900$

Formula	m/z	%	Ion type
$C_{34}H_{55}NO_4$	541.4128	16.50	$[M]^{+\cdot}$
$(C_{30}H_{50})$	482.3893	21.52	
$C_{32}H_{31}NO_2$	481.3911	36.69	$[M^{+\cdot}-60]$
$C_{31}H_{48}NO_2$	466.3671	7.05	$[M^{+\cdot}-60-15]$
$C_{30}H_{46}O$	422.3449	21.89	$[M^{+\cdot}-60-59]$
$C_{29}H_{43}O$	407.3310	100	$[M^{+\cdot}-60-59-15]$

Calculated $C_{32}H_{51}NO_2$ at 481.3919

$\left\{ \begin{array}{ll} C_{31}{}^{13}CH_{51}NO_2 & \text{at } 482.3953 \\ C_{32}H_{50}O_3 & \text{at } \underline{482.3759} \end{array} \right.$

$\Delta M \quad \underline{0.0193}$

$\therefore$ Resolving power $\frac{M}{\Delta M} \approx 25{,}000$

Table 11. High resolution MS

Formula	m/z	%
$C_{31}{}^{13}CH_{51}NO_2$	482.3925	17.56
$C_{32}H_{50}O_6$	482.3784	6.23

Table 12. Infrared spectrometric data for nitrogenous products

Observed (cm^{-1})	Group
3392 (b)	ν(N-H)
2960 (s)	ν(C-H)
2936 (s)	
2870 (s)	
1733 (s)	ν(C=O) ester
1715 (m)	ν(C=O) amide (I)
1684 (s)	ν(C=O) enone
1610 (s)	ν(C=O) amide (II)
1521 (m)	
1465 (m)	CH def.
1375 (m)	
1245 (s)	ν(C-O)

Table 13(i). ^{13}C DEPT NMR of 5α-lanost-8-en-3β-yl acetate (in $CDC\ell_3$)

Observed (ppm)	Group	Observed (ppm)	Group
170.954	Ac, CO	22.527	C-26
134.504	C-8	20.991	C-11
134.230	C-9	19.164	C-19
80.917	C-3	18.704	C-21
50.496	C-5 + C-17	18.118	C-6
49.793	C-14	16.512	C-29
44.441	C-13	15.739	C-18
39.508	C-24	21.292	Ac-CH_3
37.790	C-4		
36.688	C-10		
36.464	C-20 & C-22		
35.267	C-1		
30.969	C-12		
30.808	C-15		
28.198	C-16		
27.980	C-25		
27.695	C-28		
26.377	C-7		
24.238	C-30		
24.166	C-2		
24.095	C-23		
22.811	C-27		

Table 13(ii). 2D $\delta^{1}H/\delta^{13}C$ COSY NMR of nitrogenous product

δ6.20 ppm (1H, d, J = 9 Hz, NH) no correlation, δ4.78 ppm (1H, bd, J = 8 Hz, H-7) correlated to 48.253 ppm (CH), δ4.50 ppm (1H, bt, J = 8 Hz, 3α-H) correlated to 80.225 ppm (CH), δ3.05 ppm (1H, bd, J = 14 Hz and 2 Hz, H-6) no correlation, δ2.49 (2H, AB, J = 18 Hz, H-12) correlated to 51.915 ppm (CH_2), δ2.03 (3H, s, CH_3CO-) correlated to 21.311 ppm (CH_3), δ1.99 (3H, s, CH_3CO-) correlated to 23.338 ppm (CH_3).

Table 13(ii). 50 MHz ^{13}C DEPT NMR of nitrogenous product

$C_{34}H_{55}NO_4$ contains 10 x CH_3, 9 x CH_2, 6 x CH, 9 x C.

Found: 10 x CH_3, 9 x CH_2, 6 x CH, 9 x C.

Signal (ppm)	Group	Signal (ppm)	Group
200.71	C=O, C-11	34.36	CH_2
171.17	C=O, AcO	30.27	CH_2
168.92	C=O, AcN	28.42	CH_3
159.94	>C=C, C-8	28.10	CH_3
142.49	>C=C, C-9	27.97	CH
80.22	CH, C-3	27.14	CH_2
51.91	CH_2, C-12	25.60	CH_2
50.90	C	24.15	CH_2
50.25	CH	24.05	CH_2
48.25	CH, C7	23.33	CH_3, CH_3CO-
47.77	CH	22.78	CH_3
47.23	C	22.48	CH_3
39.37	CH_2	21.31	CH_3, CH_3CO-
38.81	C	18.34	CH_3
37.53	C	17.77	CH_3
36.18	CH	16.91	CH_3
36.12	CH_2	16.85	CH_3

Table 15. GC-MS analysis of product from acid hydrolysis of nitrogenous product after acetylation, $I^{OV-1}_{270°}$ = 3530 (selected ions)

m/z	Ion type	%
498	$[M]^{+\cdot}$	100
483	$[M^{+\cdot}-15]$	6
481		5
438	$[M^{+\cdot}-60]$	24
423		6
422		7
407		14
385	$[M^{+\cdot}$-side chain (113)]	7
345		5
331		6
318		16
302	$[M^{+\cdot}-196]$	26
292		27
270		5
255		5
189		4
163		3
149		4
136		7
121		10

Table 16. GC-MS analysis of component of $I^{OV-1}_{270°} = 3380$ from CAN oxidation of 5α-lanost-8-en-3β-yl acetate

m/z	Ion type	%
466	$[M]^{+\cdot}$	12
423	$[M^{+\cdot}-43]$	6
406	$[M^{+\cdot}-CH_3CO_2H]$	100
391	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	31
313		9
293	$[M^{+\cdot}$-side chain-$CH_3CO_2H]$	3
285		4
279		11
263		4
251	$[M^{+\cdot}$-ring D-$CH_3CO_2H-H^{\cdot}]$	11
237	$[M^{+\cdot}$-ring D-$CH_3CO_2H-CH_3^{\cdot}]$	10
225		14
209		6
195		5
183		12
169		4
148		2
135		1
123		2

Table 17(i). GC-MS analysis of 7,11-diketo-5α-lanost-8-en-3β-yl acetate

m/z	Ion type	%
498	$[M]^{+\cdot}$	100
483	$[M^{+\cdot}-CH_3^{\cdot}]$	5
470	$[M^{+\cdot}-CO]$	3
456	$[M^{+\cdot}-CH_2C{=}O]$	3
438	$[M^{+\cdot}-CH_3CO_2H]$	25
423		4
410		3
395		2
391		2
385	$[M^{+\cdot}$-side chain (113)]	6
372		2
357		3
345		4
331		5
318		13
302	$[M^{+\cdot}-196]$	24
292		18

Table 17(ii). GC-MS analysis of component $I^{OV-1}_{270°} = 3530$

m/z	Ion type	%
498	$[M]^{+\cdot}$	100
483	$[M^{+\cdot}-CH_3^{\cdot}]$	7
470	$[M^{+\cdot}-CO]$	4
456		3
438	$[M^{+\cdot}-CH_3CO_2H]$	24
423		2
420	$[M^{+\cdot}-CH_3CO_2H-H_2O]$	3
410	$[M^{+\cdot}-CH_3CO_2H-CO]$	3
391		3
385	$[M^{+\cdot}$-side chain (113)]	7
372		2
357	$[M^{+\cdot}$-side chain-CO]	3
345		5
331		7
318		14
302	$[M^{+\cdot}-196]$	28
270		7
255		9
243		2
227		2
215		2
203		5

Table 17(ii) (contd.)

m/z	Ion type	%
189		3
173		3
163		4
147		3
136	196-60	6
121	196-60-15	7

Table 18. GC-MS analysis of component $I^{OV-1}_{270°} = 3610$

m/z	Ion type	%
512	$[M^{+\cdot}]$	100
498		8
484	$[M^{+\cdot}-CO]$	9
469		5
452	$[M^{+\cdot}-CH_3CO_2H]$	4
436		3
422		4
407		6
399	$[M^{+\cdot}$-side chain (113)]	21
391		3
385		3
371	$[M^{+\cdot}$-side chain-CO]	4
355		5
343	$[M^{+\cdot}$-ring D-$CH_3^{\cdot}$]	5
332		8
317		7
303		6
295		5
261		3
255		5
243		3
227		5

Table 18 (contd.)

m/z	Ion type	%
203		9
187		6
181		7
161		6
147		4
135		3
123		7
111		5

Table 19(i). GLC data at 260°C, 1% OV-1

Compound	$I^{OV-1}_{260°}$
24(R,S)-hydroxycholest-5-en-3β-yl acetate	3500
24-ketocholest-5-en-3β-yl acetate	3300
24(R,S)-hydroxy-5α-cholestan-3β-yl acetate	3480
24(R,S)-hydroxy-5β-cholestan-3β-yl acetate	3430
24(R,S)-hydroxy-5α-cholestan-3β-ol	3360
24(R,S)-hydroxy-5β-cholestan-3β-ol	3330
24(R,S)-hydroxy-5α-cholestan-3-one	3370
24(R,S)-hydroxy-5α-cholestan-3-one methyl oxime	3410
cholesta-5,24-dien-3β-yl acetate	3270
cholesta-5,23-dien-3β-yl acetate	3240
24(R,S)-acetoxycholest-5-en-3β-yl acetate	3540

Table 19(ii). Mass spectral data for 24(R,S)-hydroxy-5α-cholestan-3β-yl acetate (selected ions)

m/z	Ion type	%
446	$[M^{+\cdot}]$	0.8
428	$[M^{+\cdot}-H_2O]$	7.3
386	$[M^{+\cdot}-CH_3CO_2H]$	7.8
371	$[M^{+\cdot}-CH_3CO_2H-CH_3^{\cdot}]$	2.8
359	$[M^{+\cdot}-C_5H_{11}O^{\cdot}]$	3.5
345	$[M^{+\cdot}-C_6H_{13}O^{\cdot}]$	0.1
290	$[M^{+\cdot}-C_{10}H_{20}O^{\cdot}]$	1.3
276	$[M^{+\cdot}$ - ring D]	3.0
230	$[M^{+\cdot}-CH_3CO_2H-C_{10}H_{20}O^{\cdot}]$	5.3
216	$[M^{+\cdot}-CH_3CO_2H$-ring D]	8.0
215	$[M^{+\cdot}-CH_3CO_2H-C_{10}H_{20}O-CH_3]$	16.5
201	$[M^{+\cdot}-CH_3CO_2H$-ring D$-CH_3]$	5.2
73	$[C_4H_9O]^+$	26.6
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

Table 20(i). GLC data

Column: 6 ft 1% OV-1 at 270°C; N_2 carrier gas at 40 ml/min

Compound	$I^{OV-1}_{270°C}$
5α-lanost-8-en-3β-yl acetate	3340
5α-lanost-8-en-3β-ol TMS ether	3290
5α-lanosta-8,24-dien-3β-ol TMS ether	3315
5α-lanosta-7,9(11)-dien-3β-yl acetate	3370
8α,9-epoxy-5α-lanostan-3β-yl acetate	3520
3β-hydroxy-5α-lanostan-7-one	3520
7-keto-5α-lanostan-3β-yl acetate	3600
7-keto-5α-lanostan-3β-ol TMS ether	3550
3β-acetoxy-5α-lanost-8-en-7-one	3570
3β-acetoxy-5α-lanost-8-en-11-one	3530
3β-acetoxy-5α-lanost-8-ene-7,11-dione	3530
7α-hydroxy-5α-lanostan-3β-yl acetate	3640
5α-lanostane-3β,7α-diol	3560
3β-acetoxy-5α-lanostan-7α-ol TMS ether	3620
5α-lanostan-3β,7α-diol di-TMS ether	3535
5α-lanostane-3β,7α-diol diacetate	3630
5α-lanostane-3β,7β-diol	3555
5α-lanostane-3β,7β-diol di-TMS ether	3490
3β-acetoxy-5α-lanostan-7β-ol TMS ether	3550
5α-lanostane-3β,7β-diol diacetate	3660
7α,30-oxido-5α-lanostan-3β-yl acetate	3615

ΔI 3β-OH ⟶ 3β-OAc

(in 7-keto) = +80

(in 7α OH) = +80

Table 20(ii). Capillary GLC data

Compound	I
7-keto-5α-lanostan-3β-yl acetate	3536
7α-hydroxy-5α-lanostan-3β-yl acetate	3570
3β-acetoxy-5α-lanostan-7α-ol TMS ether	3586
3β-acetoxy-5α-lanostan-7β-ol TMS ether	3512
7α-hydroxy-5α-lanostan-3β-yl acetate/ 7β-hydroxy-5α-lanostan-3β-yl acetate	one peak 3576
7α,30-oxido-5α-lanostan-3β-yl acetate	3544

Column: Cp Sil 5cb, 25mx0.32mm ID 0.11 μm film thickness

Carrier gas: He at 3 ml/min

Program:
- Initial value = 80°C
- Initial time = 2 min
- Level 1 Prgm rate = 30°C/min
- Final value = 230°C
- Final time = 1 min
- Level 2 Prgm rate = 1°C/min
- Final value = 280°C
- Final time = 20 min

Table 22. GC-MS CI (NH_3) and EI for component $I^{OV-1}_{270°} = 3380$ (R_f 0.61)

CI (ammonia) gave:

m/z	Ion type	%
502.5	$[M + NH_4^+]$	7
485.5	[M + H]	100
467.4	[M + H]-18	19
425.4	[M + H]-60	58
407.4	[M + H]-60-18	48

EI gave:

m/z	Ion type	%
484	$[M]^{+\cdot}$	4
454	$[M^{+\cdot}-30]$	2
371	$[M^{+\cdot}$-side chain]	13
353	$[M^{+\cdot}$-side chain-H_2O]	2
342		6
311	$[M^{+\cdot}-CH_3CO_2H$-side chain]	25
293	$[M^{+\cdot}-CH_3CO_2H-H_2O$-side chain]	18
281	$[M^{+\cdot}-CH_2O-CH_3CO_2H$-side chain]	12
259		2
145		42
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

Table 23. GC-MS CI (NH_3) and EI for component $I^{OV-1}_{270°} = 3560$

CI (ammonia) gave:

m/z	Ion type	%
518.5	$[M + NH_4^+]$	32
455.4	[M + H-46]	100
395.4	[M + H-46-AcOH]	73
341.3	[M-46-side chain]	5

EI gave:

m/z	Ion type	%
454	$[M^{+\cdot}-46]$	7
439	$[M^{+\cdot}-46-15]$	9
387	$[M^{+\cdot}$-side chain]	4
369	$[M^{+\cdot}$-side chain-H_2O]	4
341	$[M^{+\cdot}-341$-side chain]	50
281	$[M^{+\cdot}-46$-side chain-AcOH]	15
145		71
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

Table 24. 200 MHz ^{1}H NMR for components of R_f 0.61, $I^{OV-1}_{270°}$ = 3560, $I^{OV-1}_{270°}$ = 3380

($CDC\ell_3$): δ8.05 (1H, s, unknown), 5.49 (1H, bs, olefinic H), 5.33 (0.5H, m, unknown), 5.02 (1H, bs, olefinic H), 4.50 (2H, d of d, 2 x 3α-H), 4.24 (0.5H, bm, unknown), 3.65 (1H, s, unknown), 2.25 (3H, m).

Table 25. Mass spectral data on hemiacetal (70) (selected ions)

$C_{32}H_{54}O_4$ (502)

m/z	Ion type	%
484	$[M^{+\cdot}-H_2O]$	0.4
469	$[M^{+\cdot}-H_2O-CH_3^{\cdot}]$	0.2
456	$[M^{+\cdot}-46]$ $[M^{+\cdot}-CH_2O_2]$	14.6
442	$[M^{+\cdot}-AcOH]$	1.1
441	$[M^{+\cdot}-46-CH_3^{\cdot}]$	3.3
396	$[M^{+\cdot}-AcOH-46]$	3.2
381	$[M^{+\cdot}-AcOH-46-CH_3^{\cdot}]$	6.2
343	$[M^{+\cdot}-46$-side chain]	2.1
283	$[M^{+\cdot}-46-AcOH$-side chain]	4.3
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

Table 26. Infrared spectrometric data for hemiacetal (70)

Band observed (cm^{-1})	Group
3615 (w) sharp	free ν(O-H)
2955 (s)	ν(C-H)
2870 (s)	
1732 (s)	ν(C=O) ester
1468 (m)	(C-H) def.
1370 (m)	
1248 (s)	ν(C-O)

Table 27. ^{1}H NMR data for hemiacetal (70)

($CDC\ell_3$): δ5.45 (0.2H, bs), 4.50 (2H, m, 3α-H), 4.15 (1H, bm), 2.03 (6H, s, $C\underline{H}_3CO_2^-$).

Table 28. Infrared spectrometric data for lactone (71)

Band observed (cm^{-1})	Group
2958 (s) 2875 (s)	ν (C-H)
1771 (m)	ν(C=O) lactone
1735 (s)	ν (C=O) ester
1460 (m) 1370 (m)	(C-H) def.
1245 (s)	ν (C-O)

Table 29. Mass spectral data for lactone (71)

m/z	Ion type	%
500	$[M]^{+\cdot}$	1.4
485	$[M^{+\cdot}-CH_3^{\cdot}]$	1.9
472	$[M^{+\cdot}-CO]$	1.9
456	$[M^{+\cdot}-CO_2]$	7.7
441	$[M^{+\cdot}-CO_2-CH_3^{\cdot}]$	2.7
440	$[M^{+\cdot}-AcOH]$	2.3
425	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	2.4
396	$[M^{+\cdot}-AcOH-CO_2]$	2.6
381	$[M^{+\cdot}-AcOH-CO_2-CH_3^{\cdot}]$	4.8
327	$[M^{+\cdot}$-side chain-AcOH]	4.6
283	$[M^{+\cdot}$-side chain-AcOH-$CO_2]$	4.2
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

Table 31. Infrared spectral data for components $I^{OV-1}_{-270°} = 3615$ and $I^{OV-1}_{-270°} = 3770$

Band observed (cm^{-1})	Group
2955 (s) 2870 (s)	ν(C-H)
1770 (m)	ν(C=O) lactone
1731 (s)	ν(C=O) ester
1375 (m) 1368 (m)	(C-H) def.
1245 (s)	ν(C-O)
1098 (m) 1031 (m)	ν(C-O-C)

Table 32. Mass spectral data for component $I^{OV-1}_{270°} = 3730$

m/z	Ion type	%
512		0.4
503		0.2
502	$[M]^{+\cdot}$	0.9
484	$[M^{+\cdot}-H_2O]$	1.7
456	$[M^{+\cdot}-46]$	3.5
442	$[M^{+\cdot}-AcOH]$	3.3
441	$[M^{+\cdot}-46-CH_3^{\cdot}]$	0.9
427	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	0.3
396	$[M^{+\cdot}-46-AcOH]$	2.0
381	$[M^{+\cdot}-46-AcOH-CH_3^{\cdot}]$	1.7
343	$[M^{+\cdot}-46$-side chain]	1.3
283	$[M^{+\cdot}-46-AcOH$-side chain]	2.3
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	100

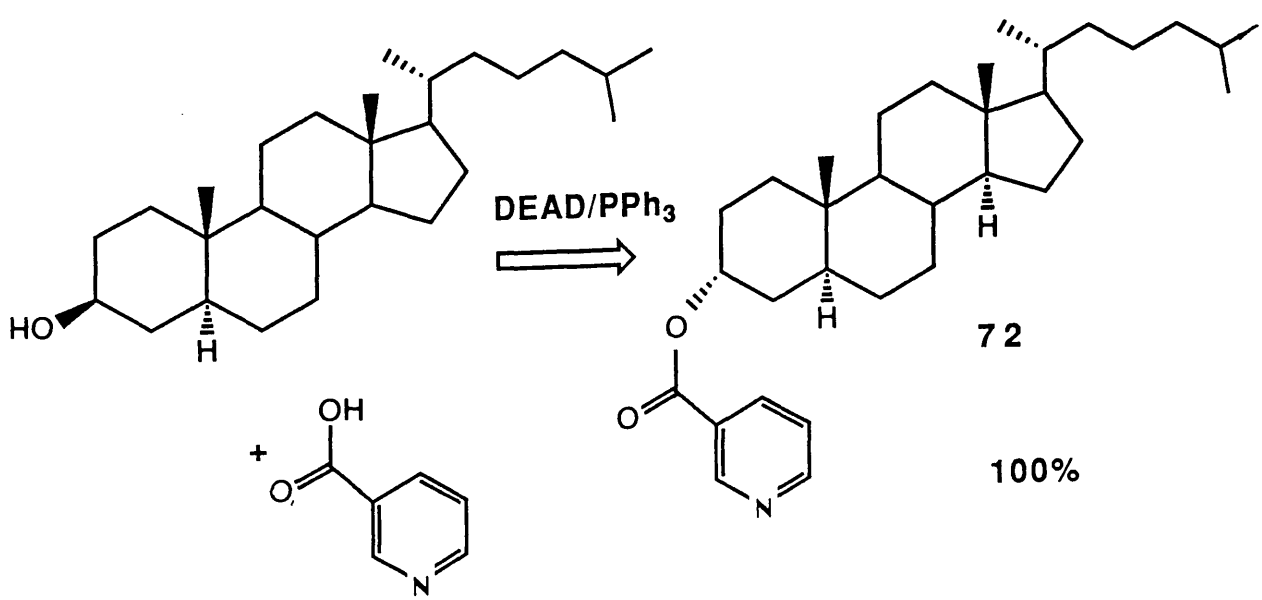
Section 4: Results - Long Range Functionalisation

4.1 Radical Relay Chlorinations of 3-Pyridylmethyl-3α-acetoxy-5β-cholan-24-oate

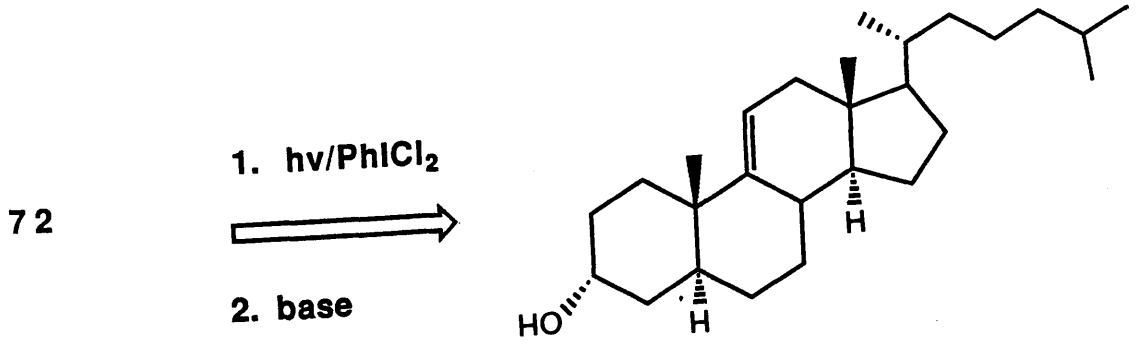
Introduction

Photochemical Methods to Achieve Functionalisation

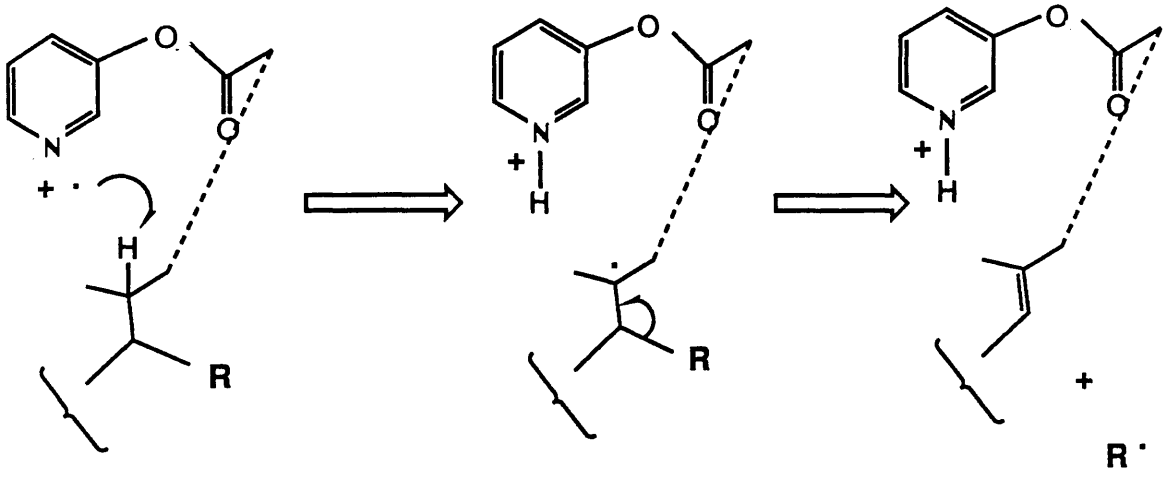
Recent work in this field has mainly come from the Breslow group in particular, using attached pyridine ester templates to selectively direct steroid chlorinations (see Section 1.2). The most useful application of the template method so far is the chlorination at C-9 directed by the template attached at C-17.[28] In a repetition of Breslow's work,[26] 5α-cholestan-3α-yl nicotinate (72) was prepared by the Mitsunobu reaction[96] on 5α-cholestan-3β-ol (100% yield) (see Experimental section 4.1.1). Other methods were attempted to prepare 72 from 5α-cholestan-3α-ol by condensation with nicotinic acid *via* nicotinyl chloride.[97] However, the reaction did not go to completion and isolation of the desired product (72) was difficult due to the formation of tarry material.



On irradiation of a solution of 72 containing $PhIC\ell_2$ (see Experimental section 4.1.2), a slightly more polar material was produced which was presumed to be the 9α-chloro-derivative of 72. Saponification and dehydrochlorination yielded 5α-cholest-9(11)-en-3α-ol, whose ^1H NMR was similar to that published.[6] Breslow et al. have reported that the 14α-chloro derivative of 72 was also produced and on saponification and dehydrochlorination yielded 5α-cholest-14-en-3α-ol (3% yield) which contaminated the major product. In our work no vinyl H resonance (δ5.10) of 5α-cholest-14-en-3α-ol was observed in the ^1H NMR of the product, only two low intensity broad signals at δ5.43 and 5.38, which are probably due to products formed via double chlorination.

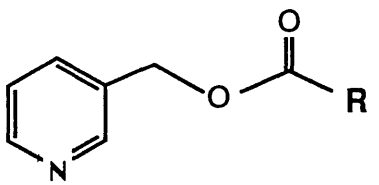


SCHEME 14

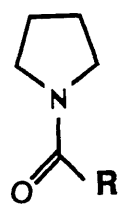


esters

3-Pyridylmethyl (73) of long-chain branched and unsaturated fatty acids have been used to determine the position of the branch point and the double bond position by mass spectrometry.[98] Electron bombardment of these derivatives produces a radical cation (molecular ion) which can undergo hydrogen atom abstraction then radical-induced cleavage (Scheme 14). Because the fatty acid picolinyl derivative contains nitrogen, it is very easy to interpret the fragmentation pattern (for the nitrogen containing fragments $[A]^{\oplus\cdot}$, these yield even numbered ions). These 3-picolinyl derivatives are superior to previously used acyl pyrrolidides (74), both in ease of preparation and in the abundance of structurally diagnostic ions.

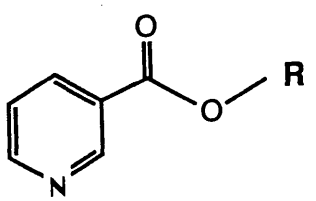


73



74

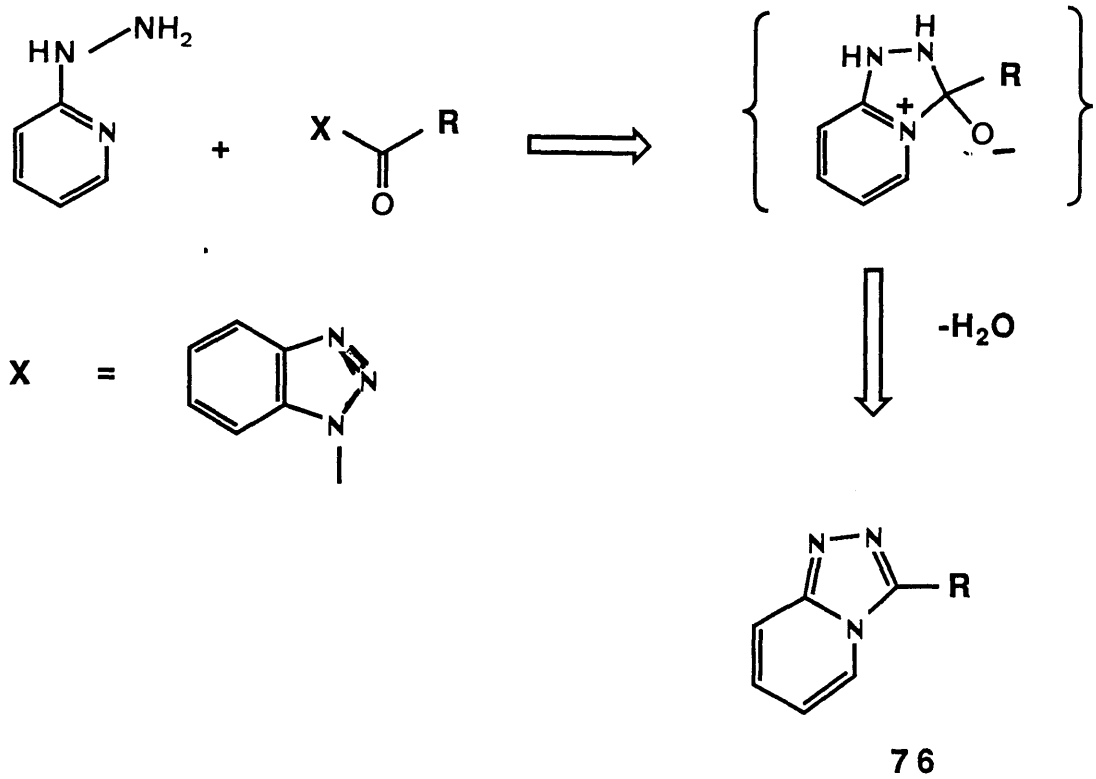
Vetter and Meister have introduced nicotinates (75) for similar structural studies on long-chain fatty alcohols.



75

Triazolopyridine (76) derivatives of fatty acids[101] have been found to be superior to pyrrolidides and slightly superior to 3-picolinyl esters with regard to the specific fragmentation pattern. The triazolo-pyridine derivative is prepared by reacting the activated fatty acid with 2-hydrazinopyridine followed by cyclisation. These derivatives are

thus more difficult to prepare than the corresponding acyl pyrrolidides.



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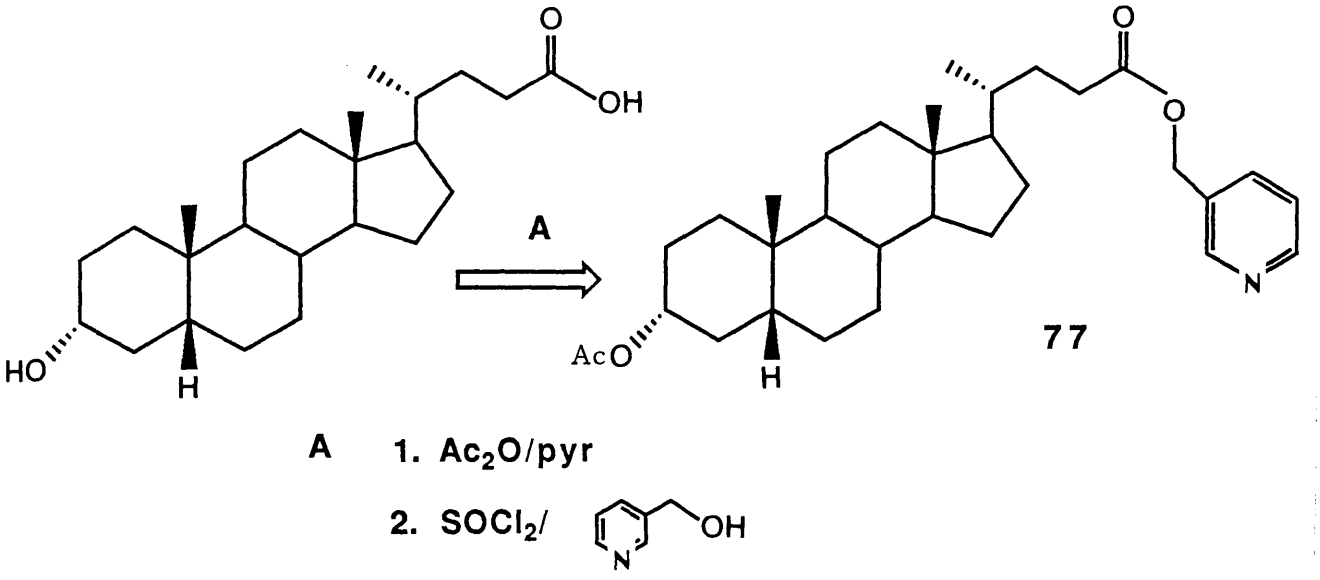
It was proposed to attempt radical relay chlorination on the 3-pyridyl-methyl ester of 3α-acetoxy-5β-cholan-24-oic acid (77). In this case, a steroidal acid is used and the template is the alcohol portion, 3-pyridylmethanol. Models suggest that the chlorine (attached to the nitrogen of the pyridine ring) could be delivered to both the steroidal side chain and to ring D. 3-Pyridylmethyl 3α-acetoxy-5β-cholan-24-oate (77) was prepared from litocholic acid (3α-hydroxy-5β-cholan-24-oic acid) by acetylation to form the 3α-acetate, followed by reaction with $SOC\ell_2$ to give the acid chloride which was condensed with 3-pyridylcarbinol to give the ester (77) (see Experimental section 4.1.3).



The reaction of 77 ($I^{OV-1}_{270°}$ = 4020) with $PhICl_2$ under irradiating conditions (see Experimental section 4.1.4) was monitored by GLC (TLC failed to separate the products from the starting material). Under irradiation for 45 min, a major product was formed ($I^{OV-1}_{270°}$ = 3880) in a 1:1 mixture with the starting material. It was thought that this was a chloro-derivative of 77 which completely eliminated HCl on GLC. After 45 min reaction time, more products were produced as judged by GLC. Figure 5, scan B, shows the 'total ion chromatogram' recorded by GC-MS under CI conditions. Traces C and D represent selected ion current chromatograms for m/z 508 and 510, respectively. Components $I^{OV-1}_{270°}$ = 3880 and $I^{OV-1}_{270°}$ = 4020 are represented by peaks observed in the region of scan numbers 747 and 929, respectively. GC-MS under CI conditions (Figure 6) of component $I^{OV-1}_{270°}$ = 3880 (scan 747) gave the [M+1] ion at 508, indicating a double bond to be present. The other major product (Figure 7) I = 4020 (scan 929) was identified as starting material having [M+1] at 510. A minor

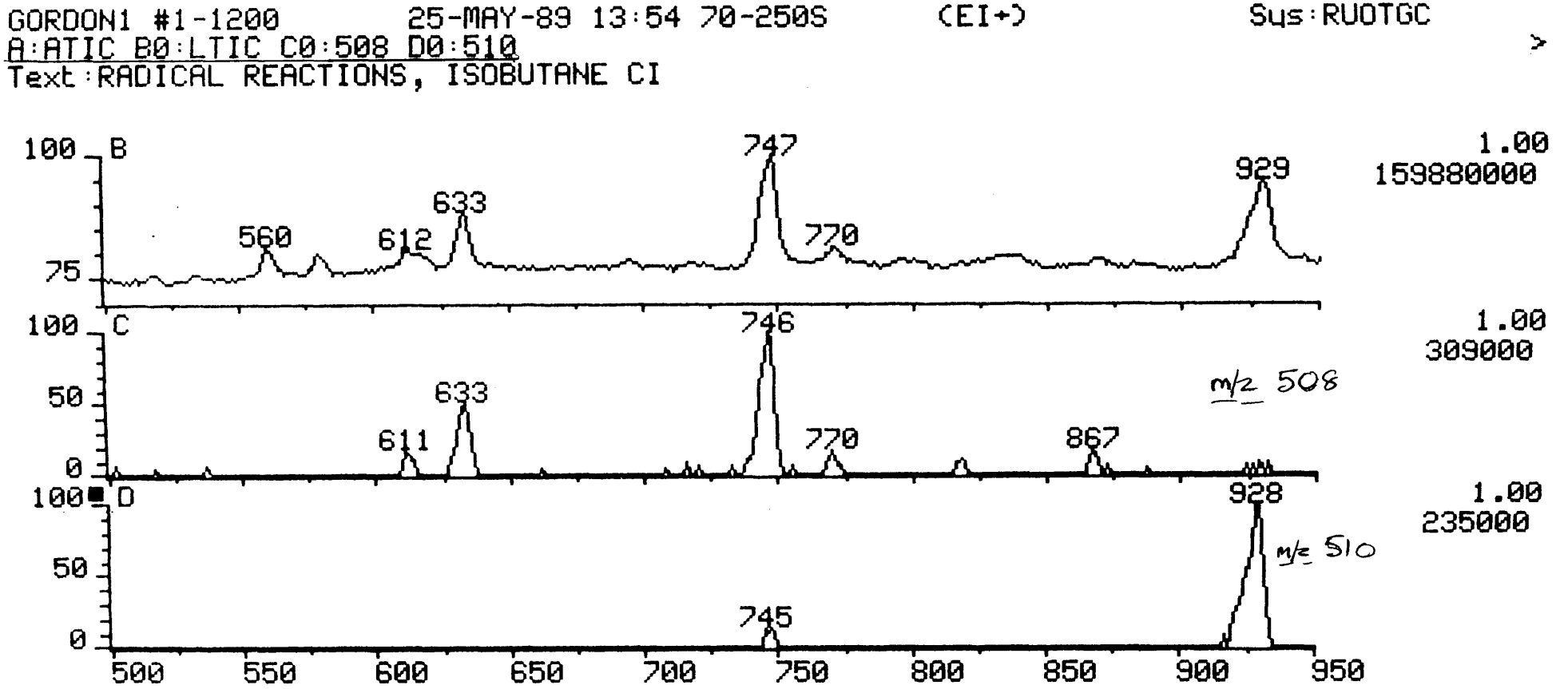
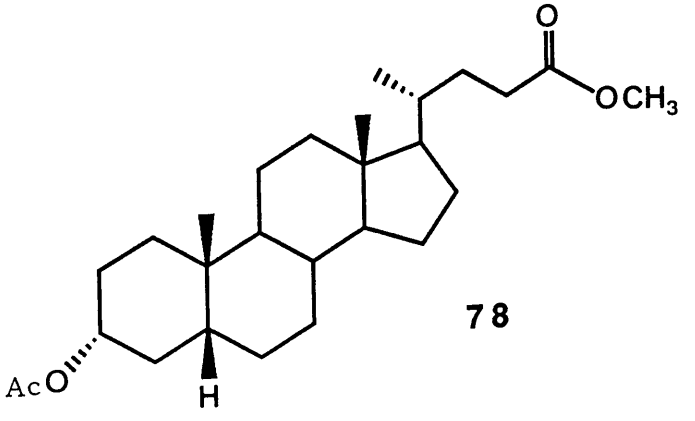


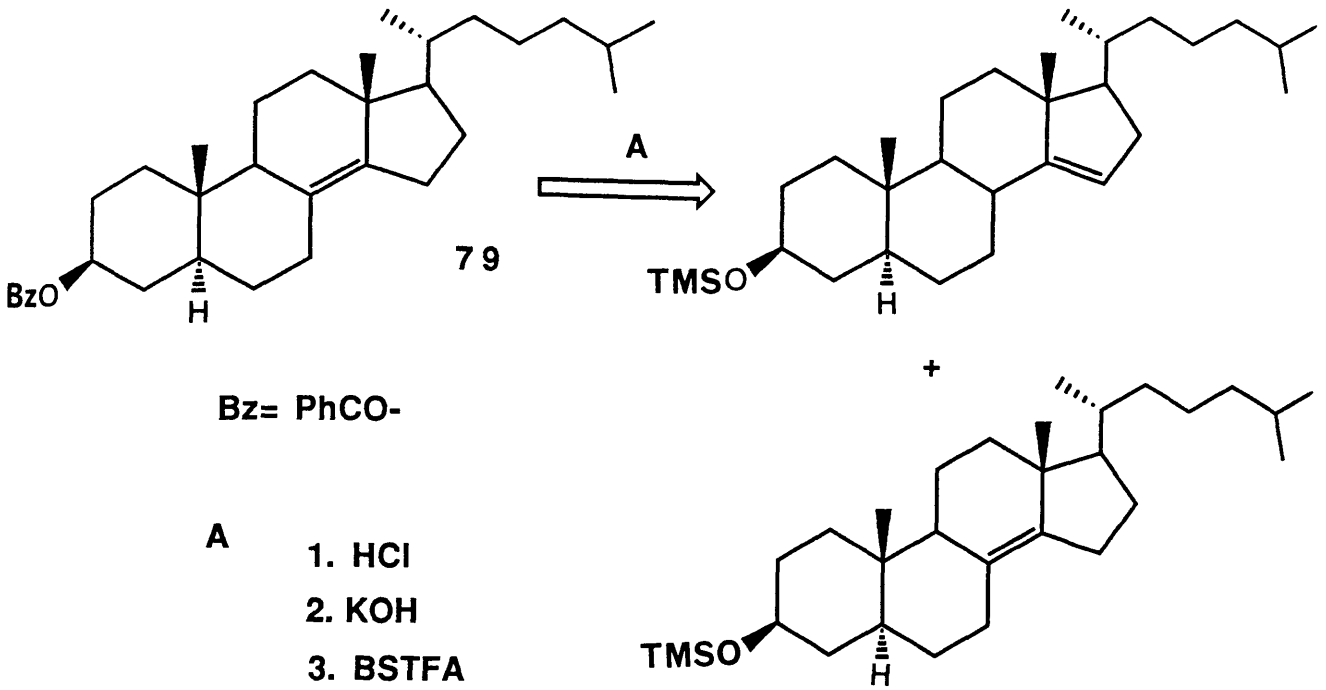
Figure 5. GC-MS TIC under CI conditions.

product was also observed to contain a double bond with [M+1] at 508 (Figure 8, scan 633). Component with $\underline{I}^{OV-1}_{270°}$ = 3880 under EI GC-MS conditions (Table 55, scan 510, Figure 9) was shown to contain a double bond, with molecular ion at $\underline{m/z}$ 507. A strong ion was observed at $\underline{m/z}$ 255, corresponding to a fragment $[C_{19}H_{27}]^+$ in which two double bonds occur in the steroid nucleus (one double bond originating from loss of AcOH). Component of $\underline{I}^{OV-1}_{270°}$ = 4020 (Table 56, scan 574, Figure 10) corresponded to the starting material 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate. However, the direct probe mass spectrum of this 1:1 mixture (Table 57) revealed an ion at 543 amu, thought to be due to the monochloro-derivative. The reaction mixture was saponified and dehydrochlorinated using KOH, then methylated (HCℓ/MeOH or CH_2N_2) and finally acetylated to give products with lower retention values on GLC. The products had retention index values of $\underline{I}^{OV-1}_{260°}$ = 3105, $\underline{I}^{OV-1}_{260°}$ = 3190 (which were both suspected of containing double bonds) and $\underline{I}^{OV-1}_{270°}$ = 3230 (which was identical to methyl 3α-acetoxy-5β-cholan-24-oate (78) ($\underline{I}^{OV-1}_{270°}$ = 3230) corresponding to unreacted starting material.



78

Capillary GLC showed that the peak at $I^{OV-1}_{\underline{\ }260°}$ = 3190 was made up of two components and GC-MS confirmed that components of the peaks at $I^{OV-1}_{\underline{\ }260°}$ = 3105 (Table 58, Figure 11) and $I^{OV-1}_{\underline{\ }260°}$ = 3190 (Table 59, Figure 12) were nuclear olefinic steroids - both having base peak at m/z 255, corresponding to a $[C_{19}H_{27}]^+$ fragment. However, the fragmentation pattern did not allow the position of the double bond to be determined. Table 60 and Figure 13 show the mass spectrum of component $I^{OV-1}_{\underline{\ }260°}$ = 3230 identified as 78. Various methods have been used to find double bond positions and most rely on fixing the double bond by epoxidation or hydroxylation and subsequent conversion of these products into derivatives such as TMS ethers, cyclic alkane boronates or acetonides, which are suitable for study by GC-MS. Diol formation followed by trimethylsilylation is particularly attractive, as the resulting spectra are usually dominated by the ions from α-cleavages, yielding the required structural information. It was proposed to use OsO_4 to form the 1,2-diol of the unknown olefinic products. GC-MS of these derivatives could possibly have indicated the double bond position via the fragmentation patterns. Models suggested that chlorination in 77 would be most likely to be directed to the C-14 position and therefore subsequent dehydrochlorination would yield a mixture of $\Delta^{8(14)}$ and Δ^{14}-double bond isomers. With this in mind, it was proposed to compare the reaction of the unknown methyl 3α-acetoxy-5β-cholan-24-oate with that of a similar double bond system. Cholest-8(14)-en-3β-yl benzoate (79) was treated with dry HCℓ for 2h.[102] A portion of the reaction mixture was hydrolysed and the TMS ether formed (see Experimental section 4.1.5). Capillary GLC indicated a 2.8:1.0 mixture in favour of 5α-cholest-14-en-3β-ol TMS ether.

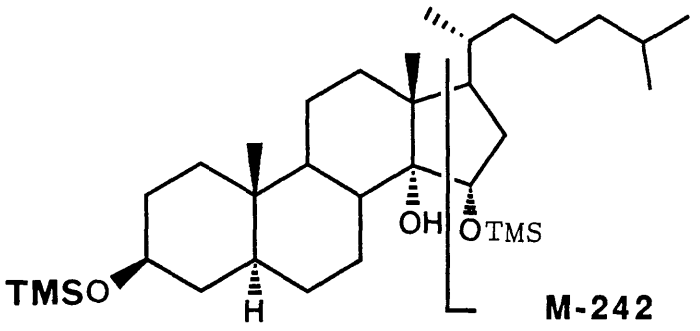


Bz= PhCO-

A
1. HCl
2. KOH
3. BSTFA

Both pure 5α-cholest-8(14)-en-3β-ol and the 2.8:1.0 mixture of 5α-cholest-14-en-3β-yl benzoate:5α-cholest-8(14)-en-3β-yl benzoate were treated with OsO_4 in pyridine for 3 days.[103] The benzoates were removed by base hydrolysis and all the products treated with BSTFA. GLC indicated that little reaction had occurred with 5α-cholest-8(14)-en-3β-ol. Capillary GLC also showed this; however the Δ^{14}:$\Delta^{8(14)}$ mixture yielded two major peaks, i.e. unreacted 5α-cholest-8(14)-en-3β-ol TMS ether and an unknown component resulting from complete reaction of the Δ^{14}-double bond in 5α-cholest-14-en-3β-yl benzoate. GC-MS[104] confirmed this.

Table 61 and Figure 14 show the mass spectrum of the unreacted 5α-cholest-8(14)-en-3β-ol TMS ether after treatment of the $\Delta^{14}/\Delta^{8(14)}$ mixture with OsO_4. However, the Δ^{14}-cholestene derivative yielded a diol mono-TMS ether (80) and the fragmentation pattern (Table 62, Figure 15) showed the expected cleavage through the diol system.

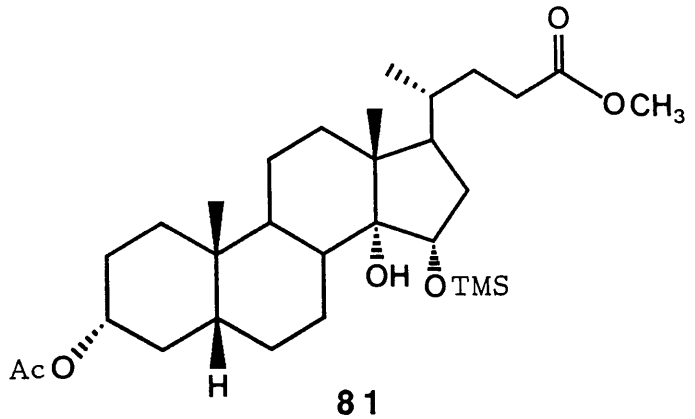


80

Table 63 and Figure 16 show the mass spectrum of 5α-cholestan-3β-ol TMS ether which was an impurity in the original reaction mixture.

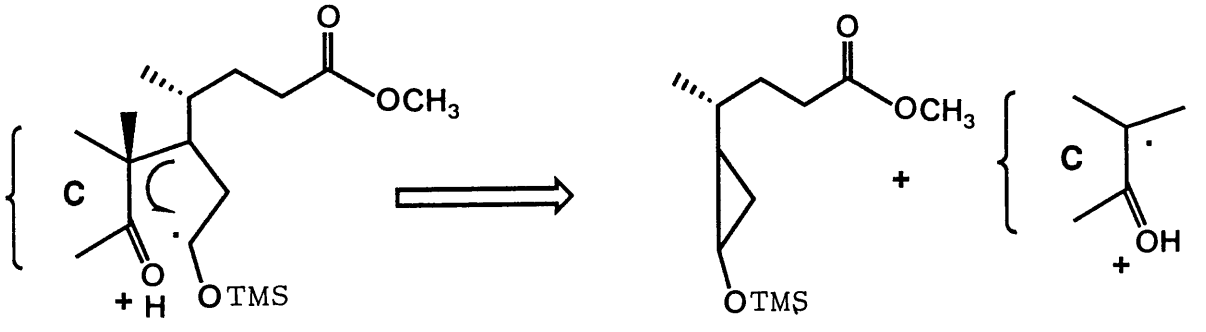
The reaction of the unknown methyl 3α-acetoxy-5β-cholen-24-oate with OsO_4 was studied by GC-MS. The major product was unfunctionalised starting material i.e. methyl 3α-acetoxy-5β-cholan-24-oate (78) (Table 64, Figure 17). The other product (Table 65, Figure 18) gave $[M]^{+\cdot}$ at m/z 536 and corresponded to the parent triol 3-acetate mono-TMS ether ($C_{30}H_{52}O_6Si$) (81). The mass spectrum revealed cleavage through the diol system to give ions m/z 292 and m/z 243, consistent with the Δ^{14} position of the original double bond (Scheme 15).

GC-MS also confirmed that the double bond isomer with $I^{OV-1}_{260°} = 3105$ (Table 66, Figure 19) gave no reaction with OsO_4. [5α-Cholest-8(14)-en-3β-yl benzoate also showed no reaction with OsO_4]. It thus appears that the unreactive double bond in the cholenoate was located at the $\Delta^{8(14)}$-position. The calculated retention index for methyl 3α-acetoxy-5β-chol-8(14)-en-24-oate was found to be $I^{OV-1}_{260°} = 3195$ (Table 67). Sjövall and Eneroth[105] reported the relative retention of

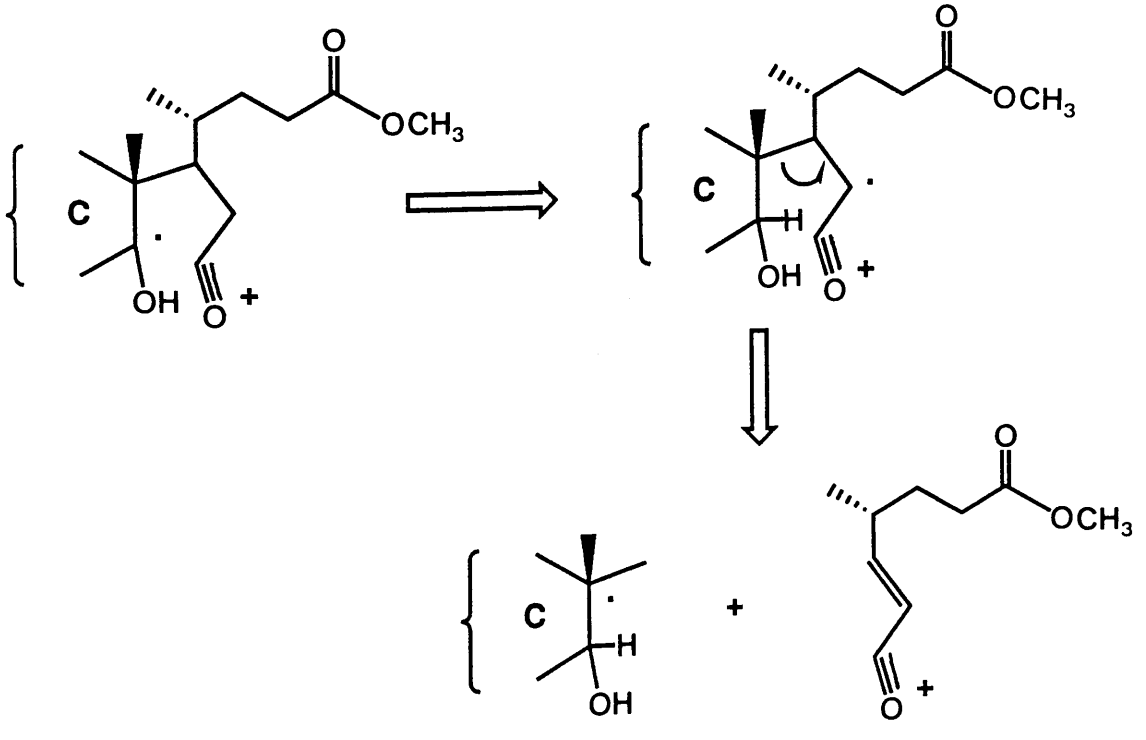


81

SCHEME 15



m/z 292



m/z 243

methyl 3α,12α-di-trifluoroacetoxy-5β-chol-8(14)-en-24-oate as 0.86 compared to methyl 3α,12α-di-trifluoroacetoxy-5β-cholan-24-oate.

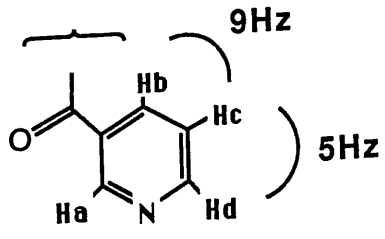
The mixture of chlorinated/unsubstituted steroids was isolated by column chromatography. Numerous TLC systems failed to achieve any further separation. The ^{1}H NMR spectrum[106] of the mixture gave broad signals for the aromatic protons (Table 60a) and showed two acetate ($C\underline{H}_3CO$-) signals in the ratio 1.5:1.0, indicating two compounds present. However, the ^{13}C NMR (DEPT) spectrum (Table 68b) showed four signals for the C-24 carbonyl and three signals for the acetate carbonyl group, indicating a mixture of more than two compounds. Similarly, there were three unresolved signals for the C-3 carbon position. The spectrum did show two quaternary carbons at 93.5 ppm and 93.3 ppm in the ratio of 3.5:1.0, respectively, which could be due to a carbon of a C-Cℓ bond.

Therefore, functionalisation was achieved in 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate (77) under Breslow's conditions, even though there was limited evidence for chlorinated steroid compounds. However, through saponification and dehydrochlorination of the products from this reaction, GC-MS has shown that a Δ^{14} double bond has been introduced. The synthesis of the Δ^{14} derivative of compound 78 would start from methyl 3α-acetoxy-12α-hydroxy-5β-chol-8(14)-en-24-oate (available from α-apocholic acid - supplier Aldrich). Oxidation of the 12α-axial alcohol to yield the 12-ketone which on Clemmensen or Haung-Minlon reduction would give the $\Delta^{8(14)}$ steroid that could be partly isomerised to the Δ^{14}-steroid as in the case of 5α-cholest-8(14)-en-3β-yl esters.

Experimental Section - 4.1.1

Preparation of 5α-cholestan-3α-yl nicotinate

To a solution of 5α-cholestan-3β-ol (1.138g, 2.93 mmol) in dry THF (60 ml) was added Ph_3P (1.15g, 4.38 mmol) and nicotinic acid (0.547g, 4.44 mmol). Diethyl azodicarboxylate (DEAD) (1.44 ml, 9.15 mmol) in dry THF (2 ml) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred at RT for 20h. An aliquot was removed and both GLC and TLC revealed that the reaction had gone to completion. ($I^{OV-1}_{260°}$ = 3870 for 5α-cholestan-3α-yl-nicotinate). The solvent was removed under reduced pressure. Excess DEAD was removed by vacuum distillation (110°C/0.5 mm Hg). The crude reaction mixture was subjected to column chromatography to remove Ph_3PO (solvent system $CHC\ell_3$EtOAc 3:1 v/v). 5α-Cholestan-3α-yl nicotinate was eluted from the column and a first crop crystallised from MeOH (0.609g) m.p. 105-107°C (lit., not recorded). 200 MHz ^1H NMR ($CDC\ell_3$), δ9.28 (1H, bs, Ha), 8.78 (1H, d, J = 5 Hz, Hd), 8.30 (1H, dofd, J = 9 Hz and 5 Hz, Hb), 7.40 (1H, dofd, J = 9 Hz and 5 Hz, Hc), 5.32 (1H, bs, 3β-H), 0.83 (3H, s), 0.64 (3H, s).



Mass spectral data for 5α-cholestan-3α-yl nicotinate

$C_{33}H_{51}NO_2$ (493)

m/z	Ion type	%
493	$[M]^{+\cdot}$	32.1
478	$[M^{+\cdot}-CH_3^{\cdot}]$	1.4
380	$[M^{+\cdot}-SC]$	0.7
370	$[M^{+\cdot}$-pyr $CO_2H]$	10.6
355	$[M^{+\cdot}$-pyr $CO_2H-CH_3^{\cdot}]$	11.0
316	$[M^{+\cdot}$-pyr CO_2H-ring A(rDA)]	3.1
257	$[C_{16}H_{23}]^+$	22.8
124	[pyr $HCO_2H]^+$	100

Experimental Section - 4.1.2

Reaction of 5α-cholestan-3α-yl nicotinate with $PhIC\ell_2/h\nu$

To a solution of 5α-cholestan-3α-yl nicotinate (110.4 mg, 0.22 mmol) in deoxygenated $CH_2C\ell_2$ (20 ml). $PhIC\ell_2$ (250 mg, 0.90 mmol) in $CH_2C\ell_2$ (2 ml) was added with stirring. The solution was irradiated using a 300W tungsten filament lamp for 1h. After this time, an aliquot was removed, and GLC showed complete conversion, with no evidence of starting material [$I^{OV-1}_{260°}$ = 3870]. Only two peaks were observed: $I^{OV-1}_{260°}$ = 3810, thought to be 5α-cholest-9(11)-en-3α-yl-nicotinate, and an unknown component $I^{OV-1}_{260°}$ = 3685. The solvent was removed under reduced pressure, and THF (20 ml) and MeOH (1 ml) were added. 10% KOH (2 ml) in MeOH was added, and the mixture refluxed for 2½h. The solvents were removed under reduced pressure, and the reaction mixture was extracted with EtOAc. The extracts were dried and the solvent removed to yield a yellow solid (54 mg). Crystallisation gave a first crop (8 mg) of impure (cf. NMR data) 5α-cholest-9(11)-en-3α-ol, m.p. 134-137°C (lit., 164-167°C); second crop (2.6 mg) and third crop (2.0 mg). 200 MHz ^1H NMR of 5α-cholest-9(11)-en-3α-ol ($CDC\ell_3$) δ5.43 (0.25 H, impurity), 5.38 (0.25 H, impurity), 5.28 (1H, d, J = 5 Hz, C-11, CH), 4.05 (1H, m, 3β-H). $I^{OV-1}_{260°}$ = 3075 (cf. $I^{OV-1}_{260°}$ = 3100 for 5α-cholestan-3α-ol).

The mass spectrum revealed incomplete dehydrochlorination of the 9α-chloro-derivative and indicated the presence of a diene ($M^{+\cdot}$ = 384) probably formed via a double chlorination. This would therefore account for the low m.p. of crude 5α-cholest-9(11)-en-3α-ol).

Mass spectral data for crude 5α-cholest-9(11)-en-3α-ol

m/z	m/z	Ion type	%
422		$[M^{+\cdot}-C\ell^{37}]$	1.7
	420	$[M^{+\cdot}-C\ell^{35}]$	4.9
405		$[M^{+\cdot}-C\ell^{35}-CH_3^{\cdot}]$	1.8
	403	$[M^{+\cdot}-C\ell^{35}-H_2O]$	1.6
386		$[M]^{+\cdot}$	58.9
	384	$[M]^{+\cdot}$(diene)	20.8
371		$[386-CH_3^{\cdot}]$	20.7
	369	$[384-CH_3^{\cdot}]$	16.0
368		$[386-H_2O]$	6.0
	366	$[384-H_2O]$	3.9
353		$[386-H_2O-CH_3^{\cdot}]$	39.1
	351	$[384-H_2O-CH_3^{\cdot}]$	10.4

Experimental Section - 4.1.3

Preparation of 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate

3α-Hydroxy-5β-cholan-24-oic acid (lithocholic acid) (11.15g, 29.65 mmol) was dissolved in dry redistilled pyridine (25 ml) and acetic anhydride (50 ml). The reaction mixture was heated under gentle reflux for 1h. The solvents were removed under reduced pressure to yield a brown oil. Without any further purification at this stage, the crude material was dissolved in MeCN (200 ml), $SOCl_2$ (20 ml, 276.4 mmol) was added dropwise, and the reaction mixture refluxed for 45 min. The solvents were removed under reduced pressure, and the acid chloride was ~~dried~~ with CH_3CN then the residual CH_3CN removed under reduced pressure. CH_3CN (200 ml) was added together with 3-pyridylcarbinol (5 ml, 51 mmol). The reaction mixture was heated under reflux for 20 min. The solvents were removed under reduced pressure to yield a dark oil. This was dissolved in Et_2O and filtered through a short silica column. The product eventually crystallised, after some difficulty, from MeOH, m.p. 96-99°C. Micro-analysis (C.H.N.); Found: C, 75.42; H, 9.19; N, 2.54. $C_{32}H_{47}NO_4$ requires C, 75.40; H, 9.29; N, 2.75%. Yield 20%

Mass spectrum of 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate

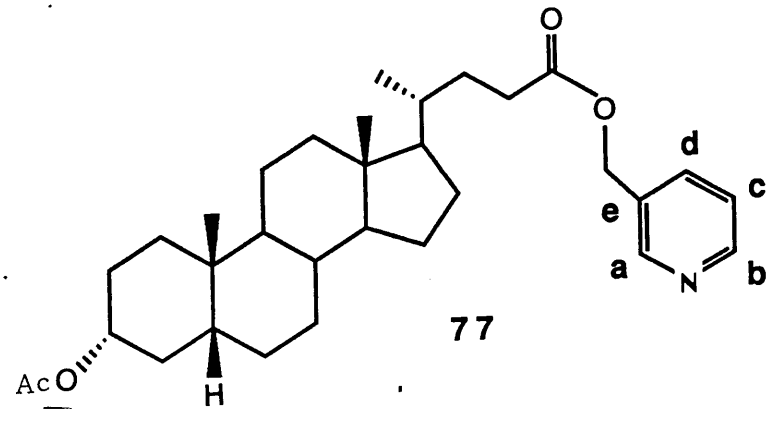
m/z	Ion type	%
509	$[M]^{+\cdot}$	5.7
494	$[M^{+\cdot}-CH_3^{\cdot}]$	4.1
449	$[M^{+\cdot}-AcOH]$	4.2
434	$[M^{+\cdot}-AcOH-CH_4^{\cdot}]$	7.3
358	$[M^{+\cdot}-pyr\ CH_2\dot{O}CO]$	19.3
220		10.1
215	$[C_{16}H_{23}]^{+}$	15.1
164	$\overset{+}{C}H_2CH_2CO_2CH_2$ pyr	18.1
109		12.6
108	$[pyr\ CH_2O]^{+}$	17.6
107		14.4
92	$\overset{+}{C}H_2$ pyr	81.2
91		23.4
43	$[CH_3CO]^{+}$	100

D.E.P.T. ^{13}C NMR of 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate[105]

$C_{32}H_{47}$ contains 11 x CH, 12 x CH_2, 4 x CH_3 and 5 x -C-

Signal (ppm)	Group
173.39	C=O, C-24
170.53	C=O, acetate
149.36	C(a)
149.23	C(b)
136.12	C(d)
131.77	C(e)
123.41	C(c)
74.28	CH, C-3
63.36	CH_2-OAr
56.36	CH, C-14
55.84	CH, C-17
42.62	C, C-13
41.77	CH, C-5
40.28	CH, C-9
40.02	CH_2
35.66	CH, C-8
35.20	CH, C-20
34.92	CH_2
34.47	C, C-10
32.13	CH_2
31.04	CH_2

Signal (ppm)	Group
30.82	CH_2
28.09	CH_2
26.91	CH_2
26.52	CH_2
26.21	CH_2
24.06	CH_2
23.24	CH_3, C-19
21.39	CH_3, acetate
20.72	CH_2
18.14	CH_3, C-21
11.93	CH_3, C-18



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Infrared spectrometric data for 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate

($CHC\ell_3$ solution)

Band observed (cm^{-1})	Group
2945 (s)	ν(C-H)
2870 (s)	
1729 (s)	ν(C=O) ester
1598 (w)	aromatic
1580 (w)	
1468 (m)	(C-H) def.
1450 (m)	
1382 (m)	
1364 (m)	
1254 (s)	ν(C-O)
1029 (m)	

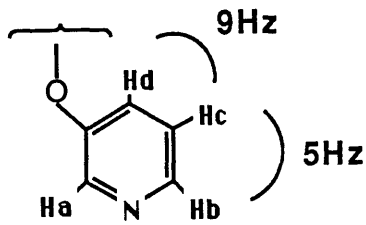
UV (EtOH) λ_{max} 264 nm ε_{max} 1630

λ_{max} 259 nm ε_{max} 2200

λ_{max} 254 nm ε_{max} 2030

200 MHz ^{1}H NMR of 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate

($CDC\ell_3$) δ8.60 (1H, bs, Ha), 8.55 (1H, bd, J = 5 Hz, Hb), 7.70, (1H, bd, J = 9 Hz, Hd), 7.30 (1H, bd of d, J = 9 Hz and 5Hz, Hc), 5.10 (2H, s, O-CH_2-Ar), 4.70 (1H, m, 3β-H), 2.32 (3H, m), 2.02 (3H, s, $C\underline{H}_3CO_2$-), 0.89 (3H, s, C-19, $C\underline{H}_3$), 0.59 (3H, s, C-18, $C\underline{H}_3$).



Experimental Section - 4.1.4

3-Pyridylmethyl 3α-acetoxy-5β-cholan-24-oate with $PhIC\ell_2$/hν

To a solution of 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate (216 mg, 0.42 mmol) in deoxygenated $CH_2C\ell_2$ (40 ml) was added $PhIC\ell_2$ (408 mg, 1.48 mmol) in $CH_2C\ell_2$ (4 ml). The solution was irradiated at 20-25°C for 45 min. The solvent was removed under reduced pressure to yield a brown oil (332 mg). GLC gave $\underline{I}^{OV-1}_{270°}$ = 4020 (3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate) and $\underline{I}^{OV-1}_{270°}$ = 3880 (unknown). 1:1 ratio by GLC

Saponification/dehydrochlorination of products from Breslow reactions

The crude oil (200 mg) in 1,4-dioxan (20 ml) was treated with 10% KOH (3 ml) in MeOH under reflux for 1h. Solvents were removed under reduced pressure, then the mixture was neutralised. The mixture was extracted with EtOAc, the extracts were dried and solvent removed. Without purification, the material was methylated either by HCℓ/MeOH or by CH_2N_2, then acetylated using Ac_2O/pyridine.

Experimental Section - 4.1.5

Isomerisation of 5α-cholest-8(14)-en-3β-yl benzoate

5α-Cholest-8(14)-en-3β-yl benzoate (9 mg) in $CHCℓ_3$ (3 ml) was treated with excess dry HCℓ for 2h. After this time the solvent was removed under a N_2 stream and the mixture dissolved in Et_2O and neutralised with $NaHCO_3$ (aq). The mixture was then extracted with Et_2O. A portion of the material (200 μg) in EtOH (0.4 ml) was treated with 10% KOH (0.15 ml) in MeOH with heating at 80°C for 2h. The solvents were removed under reduced pressure and the mixture extracted with EtOAc. The extracts were dried, and evaporated to dryness, and a portion of this material was treated with BSTFA for 30 min with heating at 80°C. Capillary GLC indicated a 2.8:1.0 mixture in favour of 5α-cholest-14-en-3β-yl TMS ether.

Treatment of 5α-cholest-14-en-3β-yl benzoate/5α-cholest-8(14)-en-3β-yl benzoate with OsO_4

To a solution of the mixture of benzoates (8.6 mg) in pyridine (0.5 ml) was added OsO_4 (5 ml, 2.5 mg/ml) in pyridine (3 equiv.). The reaction mixture was stirred at RT for 3 days. The osmate esters were reduced using saturated $Na_2S_2O_5$ solution to give a black solution. The pyridine was removed under reduced pressure and the mixture extracted with EtOAc. The extracts were dried and on removal of the solvent gave an oil (9.4 mg). A portion of this material (200 μg) in EtOH (0.2 ml) was treated with 10% KOH (0.1 ml) in MeOH at 80°C for 1h. After work-up, the crude material was treated with BSTFA at 80°C for 30 min.

A similar procedure was used for the reaction of 5α-cholest-8(14)-en-3β-ol with OsO_4.

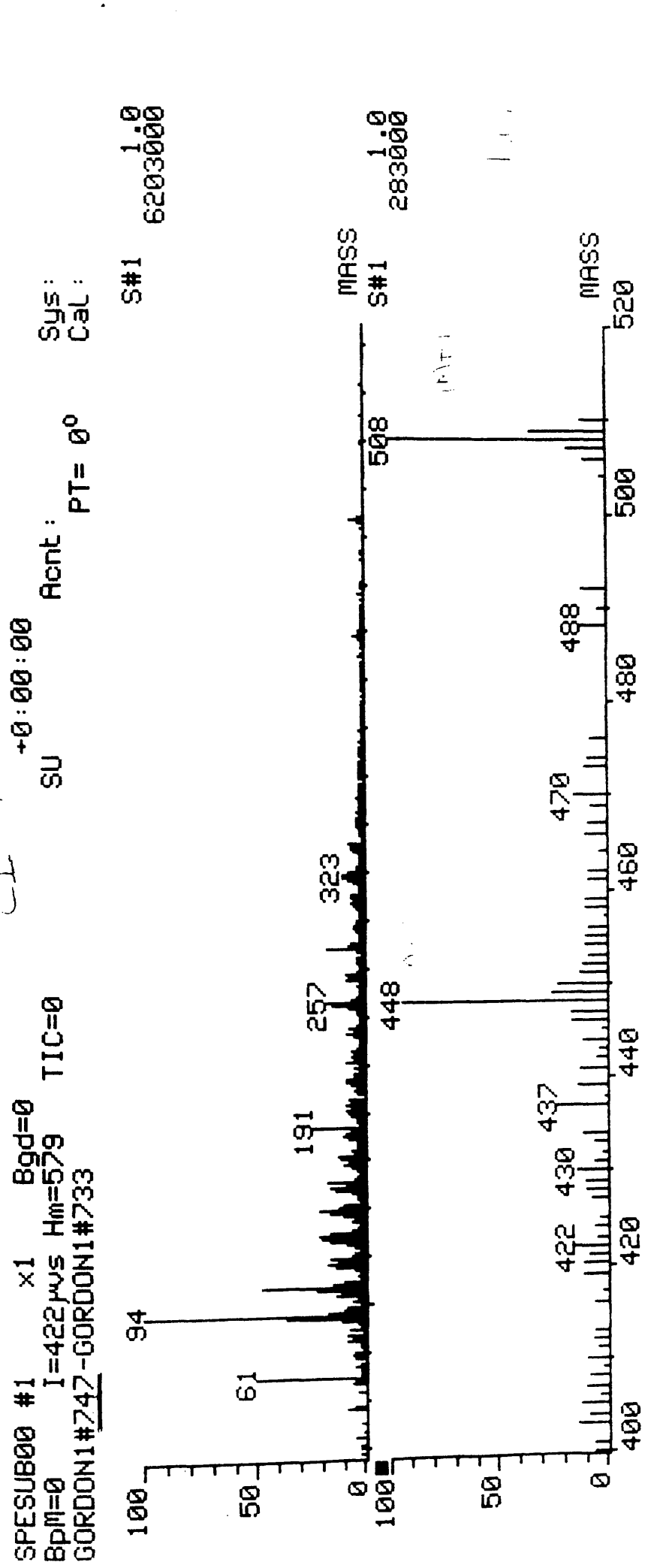


Figure 6. GC-MS (CI) of component $I^{OV-1}_{270°} = 3880$

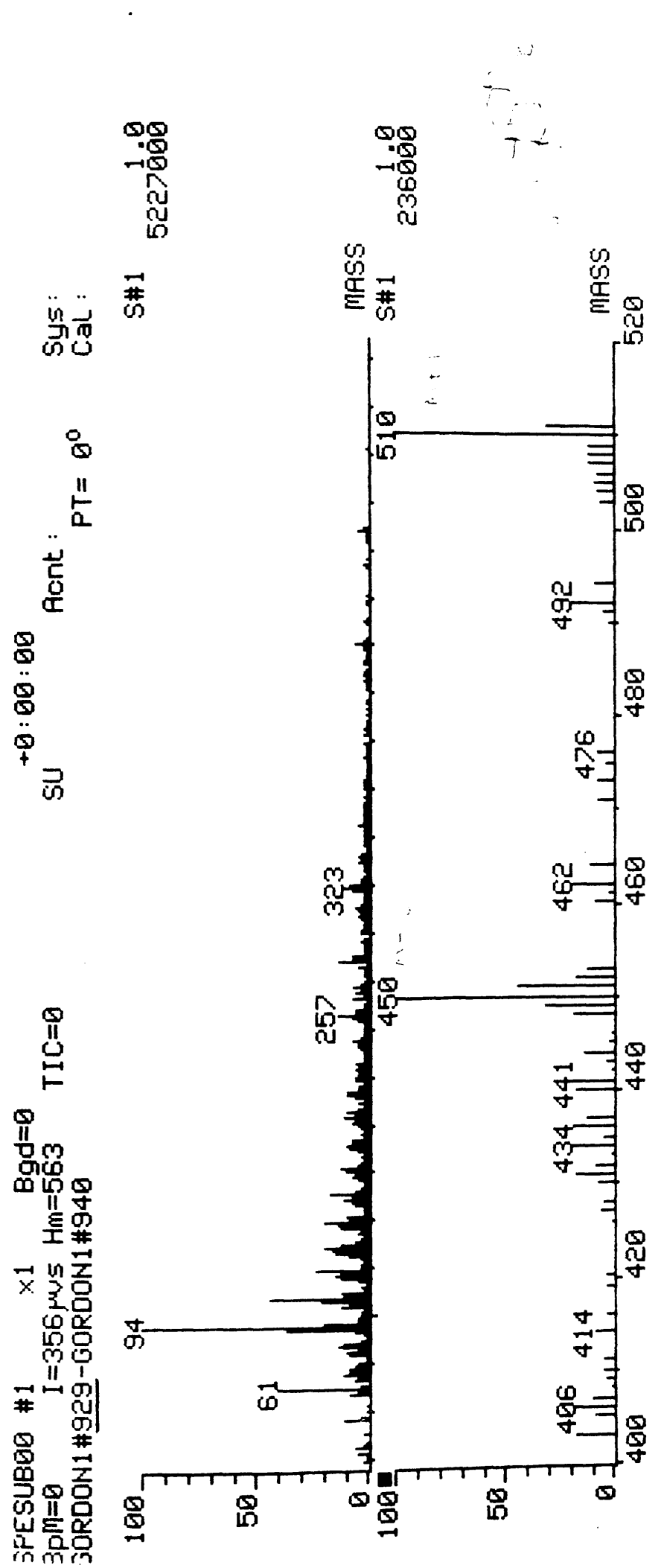


Figure 7. GC-MS (CI) of component $I^{OV-1}_{270°} = 4020$.

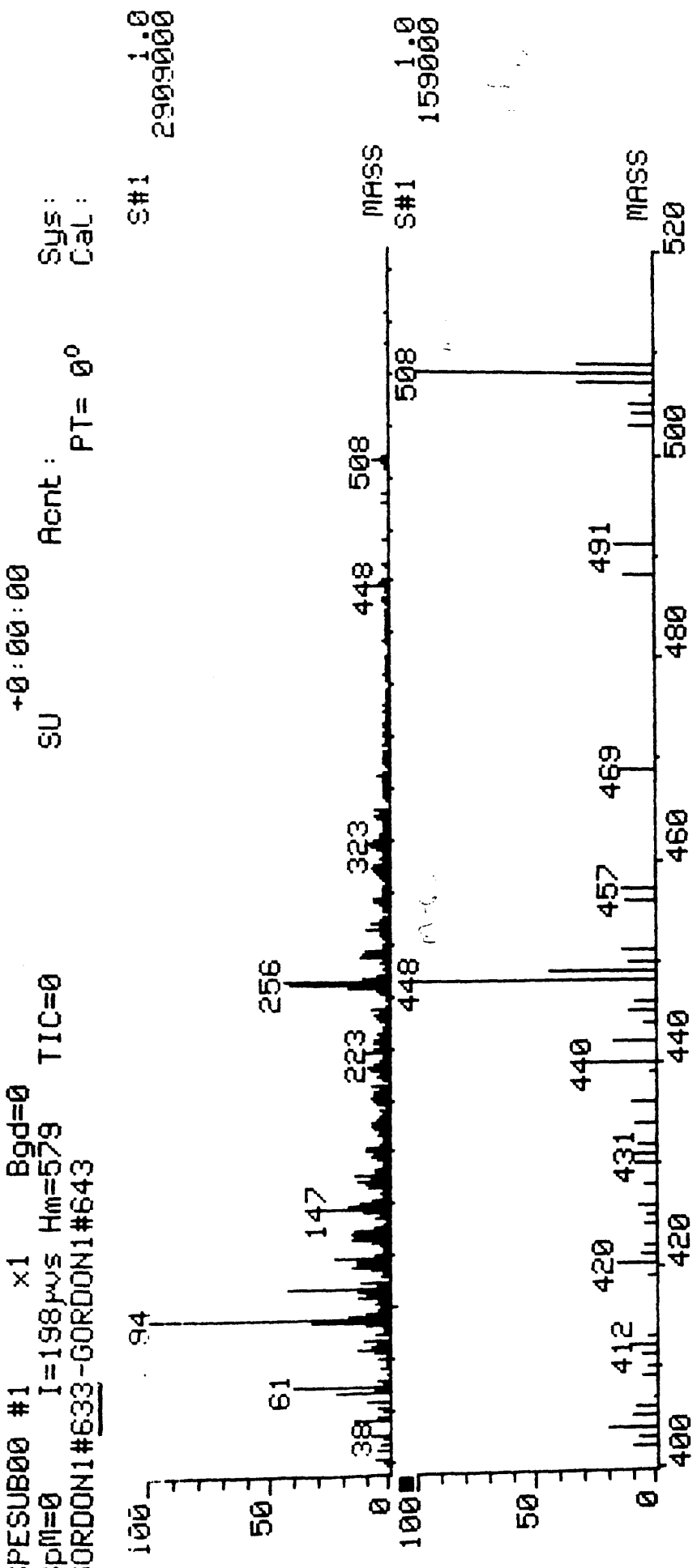
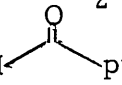


Figure 8. GC-MS (CI) of minor component scan 633.

Table 55. GC-MS (EI) of component $I^{OV-1}_{270°}$ = 3880 (scan 510)

m/z	Ion type	%
507	$[M]^{+\cdot}$	90
492	$[M^{+\cdot}-CH_3^{\cdot}]$	19
447	$[M^{+\cdot}-AcOH]$	10
432	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	69
355	$[M^{+\cdot}-AcOH-pyr\ CH_2^{\cdot}]$	29
284	$[M^{+\cdot}-114(rDA\ ring\ A)-pyr\ CH_2OH]$	13
255	$[M^{+\cdot}-AcOH-side\text{-}chain]$	47
165	$[^{\cdot}CH_2CH_2\overset{O}{\overset{\|}{C}}-O-CH_2-pyr\ H^+]$	16
164	$[CH_2{=}CH\overset{O}{\overset{\|}{C}}-O-CH_2-pyr\ H^+]$	15
151	$[\dot{C}H_2-C(=O)-O-CH_2-pyr\ H^+]$	100
107		87
93	$[^{\cdot}CH_2pyr\ H^+]$	94
92	$[CH_2\text{-}pyr]^+$	97

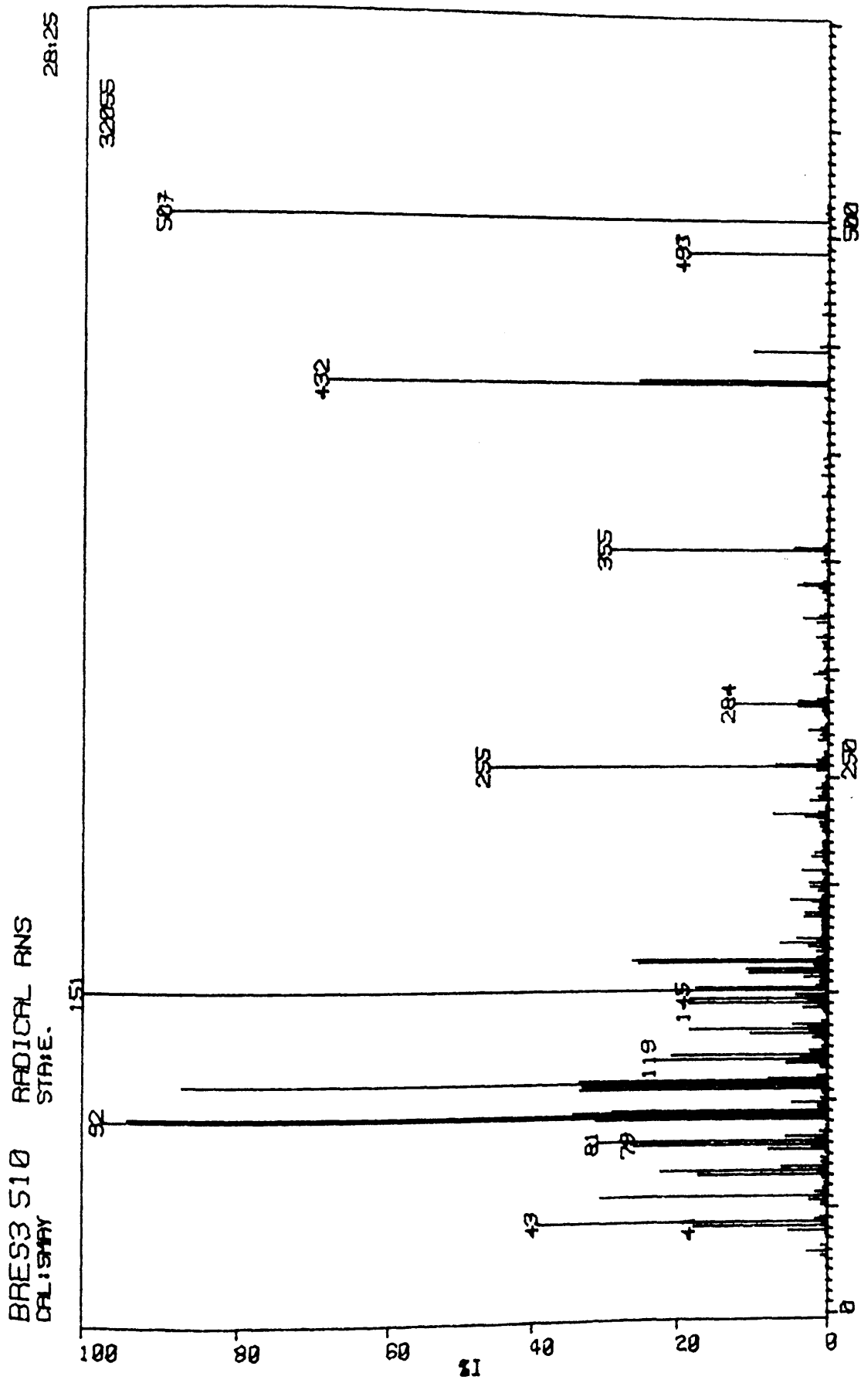
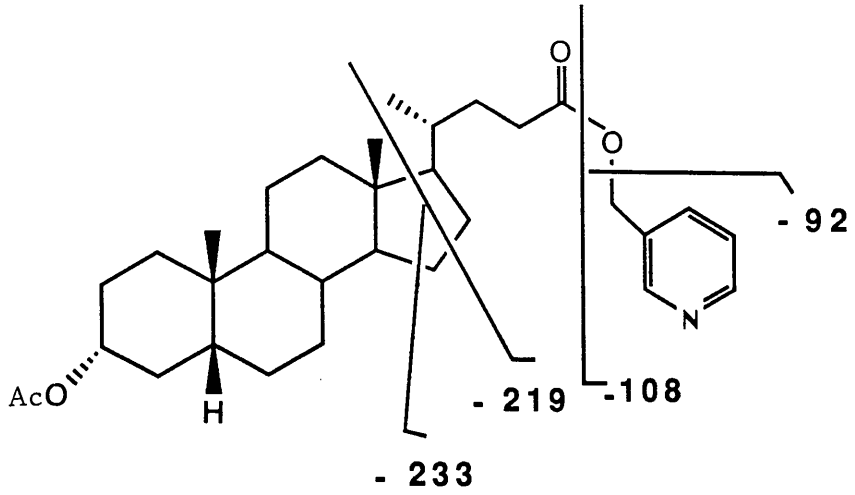


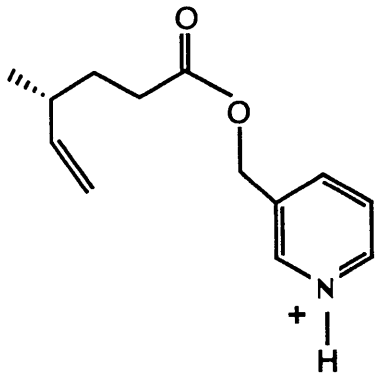
Figure 9. GC-MS (EI) of component $I^{OV-1}_{270°} = 3880$ (scan 510).

Table 56. GC-MS (EI) of component $I^{OV-1}_{270^\circ} = 4020$ (scan 574)

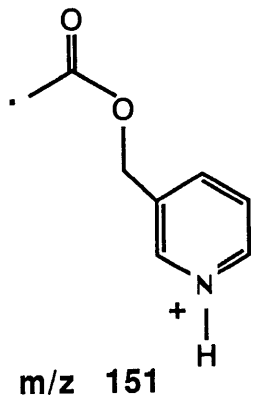
Identified as 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate

m/z	Ion type	%
509	$[M]^{+\cdot}$	25
494	$[M^{+\cdot}-CH_3^{\cdot}]$	6
449	$[M^{+\cdot}-AcOH]$	26
434	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	27
429		21
355		30
341	$[M^{+\cdot}-AcOH-108]$	5
290	$[M^{+\cdot}-219]$	17
281		25
234		38
230	$[M^{+\cdot}-AcOH-219]$	6
220	$[C_{13}H_{18}NO_2]^+$	90
215	$[C_{16}H_{23}]^+$	20
193	(side chain + H)$^+$	7
164	$C_{20}/_{22}$ cleavage, $\overset{+}{C}H_2CH_2CO_2CH_2pyr$	100
151	$[\dot{C}H_2CO_2CH_2pyr\overset{+}{H}]$	3
147	$[C_{11}H_{15}]^+$	49
121	$[C_9H_{13}]^+$	18
107	$[pyr\text{-}C(=O)H]^{+\cdot}$	80
93	$[\dot{C}H_2pyr\overset{+}{H}]$	96
92	$\overset{+}{C}H_3pyr$	97





m/z 220



m/z 151

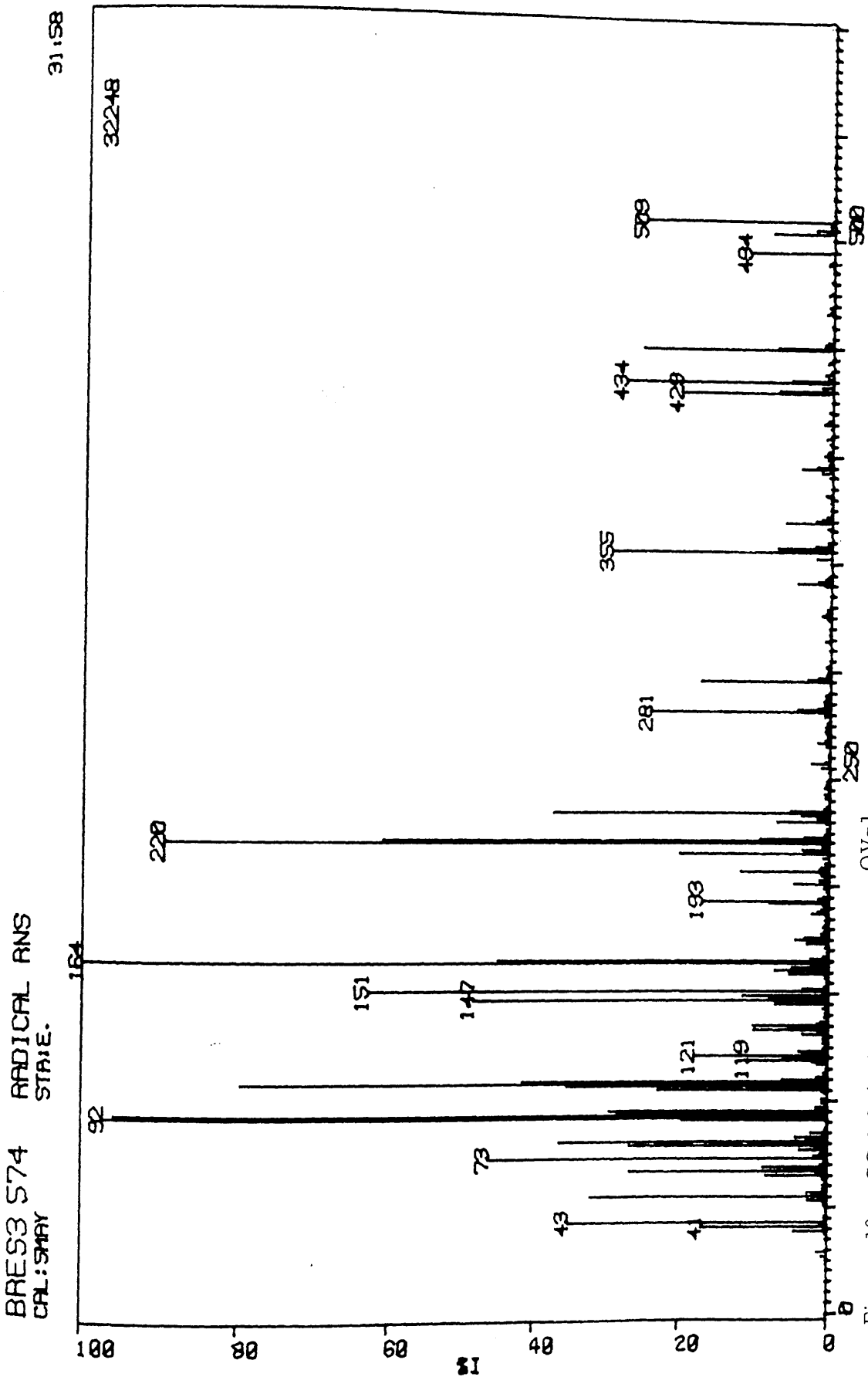


Figure 10. GC-MS (EI) of component $I^{OV-1}_{270°}$ = 4020 (scan 574).

Identified as 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate.

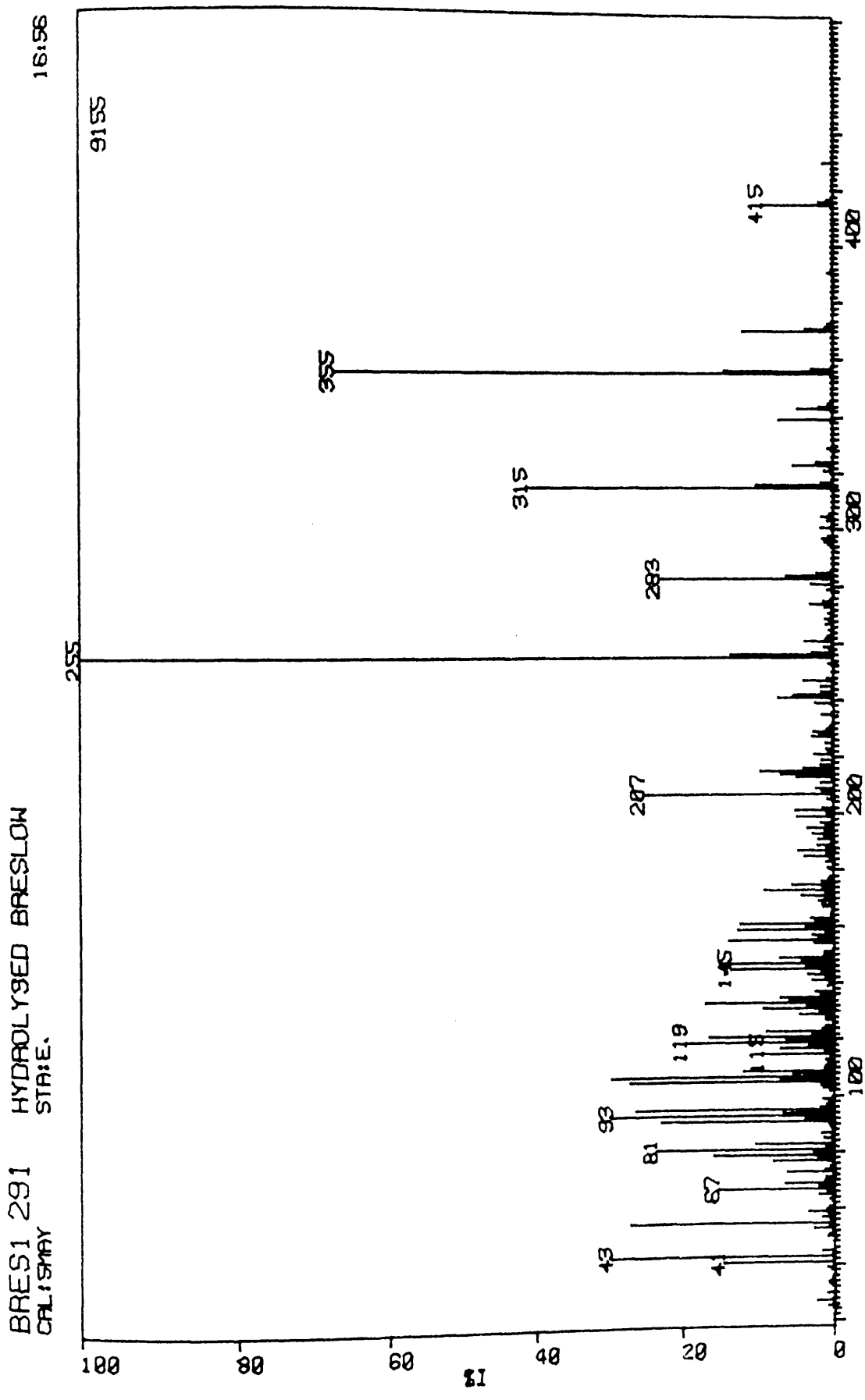


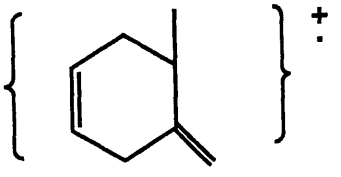
Figure 11. GC-MS of component $I^{OV-1}_{-260°}$ = 3105 (scan 291).

Table 57. Mixed mass spectrum of components from Breslow reaction on 3-pyridylmethyl 3α-acetoxy-5β-cholan-24-oate

m/z	Ion type	%
543	$[M]^{+\cdot}C\ell^{35}$	0.6
509	$[M]^{+\cdot}$	3.4
507	$[M^{+\cdot}-HC\ell]$	14.2
494	$[M^{+\cdot}-CH_3^{\cdot}]$	1.8
492	$[M^{+\cdot}C\ell^{35}-HC\ell-{}^{\cdot}CH_3]$	2.6
449	$[M^{+\cdot}-AcOH]$	6.7
447	$[M^{+\cdot}C\ell^{35}-HC\ell-AcOH]$	5.8
434	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	5.1
432	$[M^{+\cdot}C\ell^{35}-HC\ell-AcOH-CH_3^{\cdot}]$	13.0
355		6.3
341	$[M^{+\cdot}-AcOH-108]$	1.6
339	$[M^{+\cdot}C\ell^{35}-AcOH-108]$	2.3
315	$[M^{+\cdot}C\ell^{35}-HC\ell$-side chain]	2.7
255	$[C_{19}H_{27}]^+$	16.1
220	$[C_{13}H_8NO_2]^+$	15.1
215	$[C_{16}H_{23}]^+$	5.9
193	[side-chain + H]$^+$	6.1
161	(see Table 55)	41.5
151	$[CH_3CO_2CH_2\ pyr]^{+\cdot}$	57.4
147	$[C_{11}H_{15}]^+$	11.5
145		11.2

Table 57 (contd.)

m/z	Ion type	%
133		10.2
121	$[C_9H_{13}]^+$	10.9
119		13.9
109		20.2
108	(see assignment)	45.7
107		20.4
105		24.1
93		100



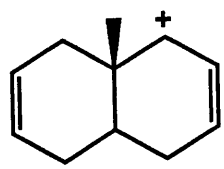
m/z 108

Table 58. GC-MS (EI) of component $I^{OV-1}_{\sim 260°}$ = 3105 (scan 291)

m/z	Ion type	%
430	$[M]^{+\cdot}$	2
415	$[M^{+\cdot}-CH_3^{\cdot}]$	11
370	$[M^{+\cdot}-AcOH]$	12
355	$[M^{+\cdot}-AcOH-\dot{C}H_3]$	68
341		5
339	$[M^{+\cdot}-AcOH-MeO^{\cdot}]$	7
323		5
315	$[M^{+\cdot}$-side-chain]	41
283	$[M^{+\cdot}$-side-chain-MeOH]	24
255	$[C_{16}H_{23}]^{+}$	100
207		26

Table 59. GC-MS (EI) of component $I^{OV-1}_{260°} = 3190$ (two peaks by capillary GLC) (scan 312)

m/z	Ion type	%
430	$[M]^{+\cdot}$	1
415	$[M^{+\cdot}-CH_3^{\cdot}]$	3
370	$[M^{+\cdot}-AcOH]$	13
355	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	15
343		1
339	$[M^{+\cdot}-AcOH-MeO^{\cdot}]$	4
315	$[M^{+\cdot}$-side-chain]	19
283	$[M^{+\cdot}$-side-chain-MeOH]	8
255	$[C_{19}H_{27}]^+$	100
215	$[C_{16}H_{23}]^+$	4
207		14
147	$[C_{11}H_{15}]^+$	14



m/z 147

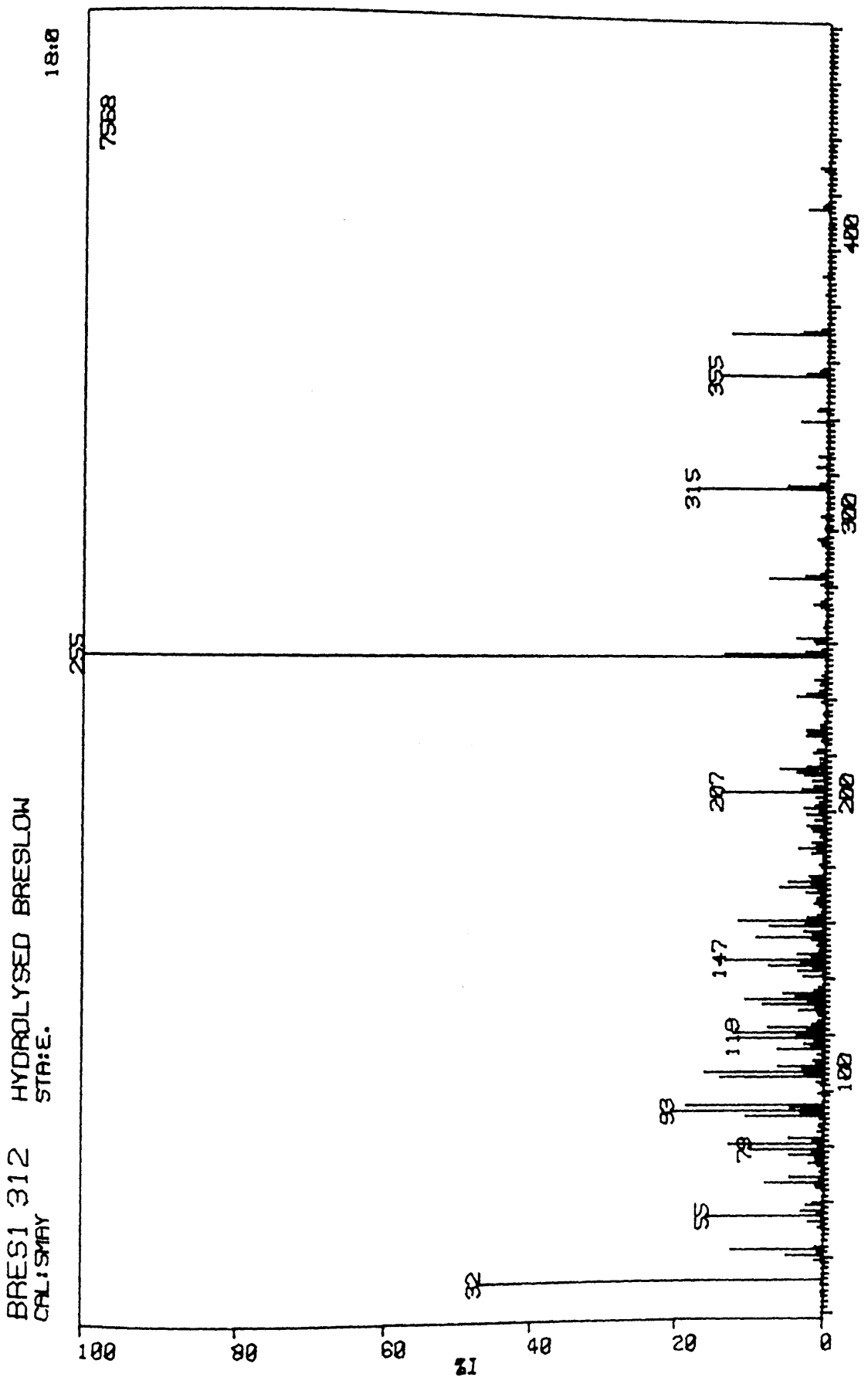


Figure 12. GC-MS (EI) of component $I^{OV-1}_{260°} = 3190$.

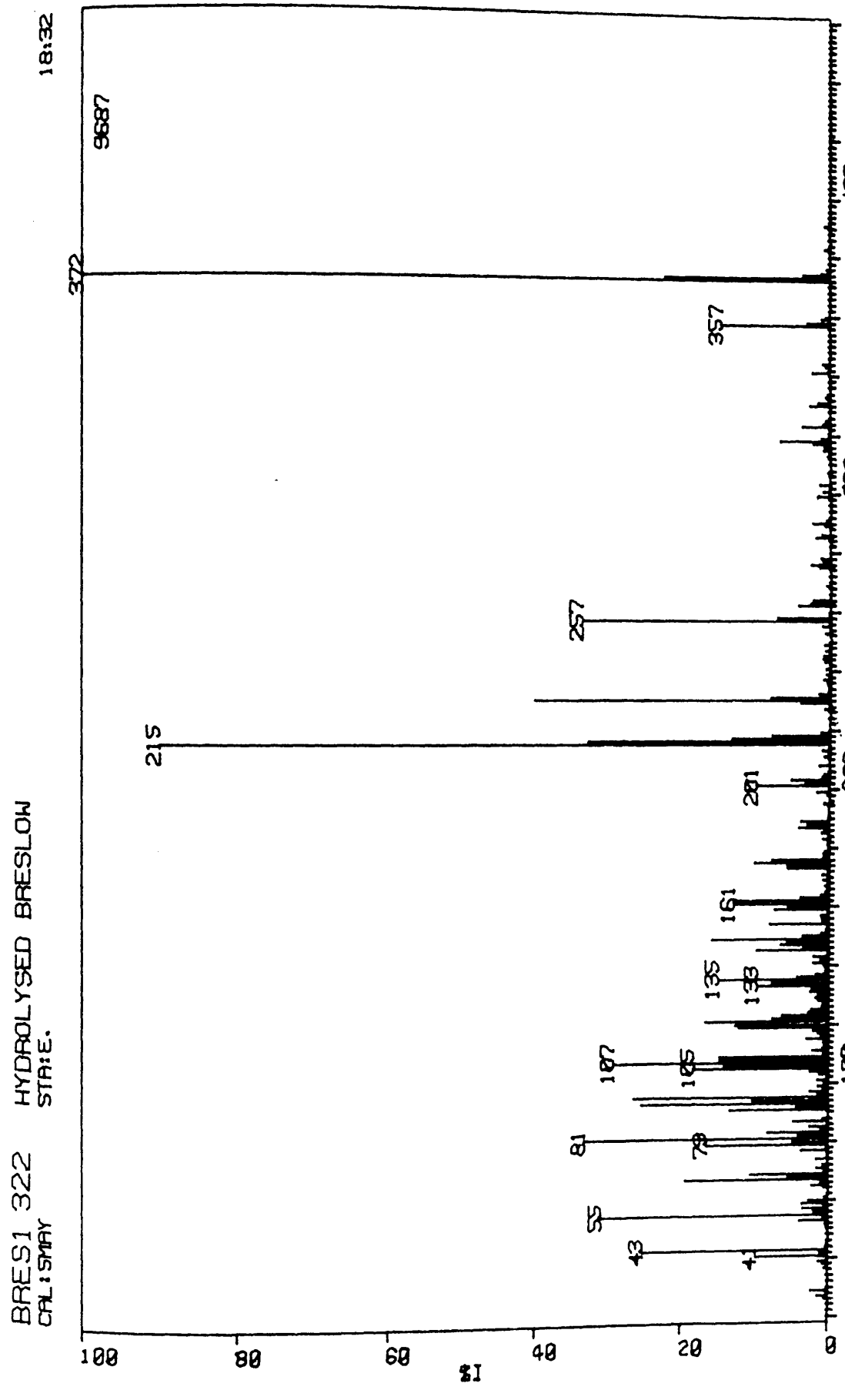
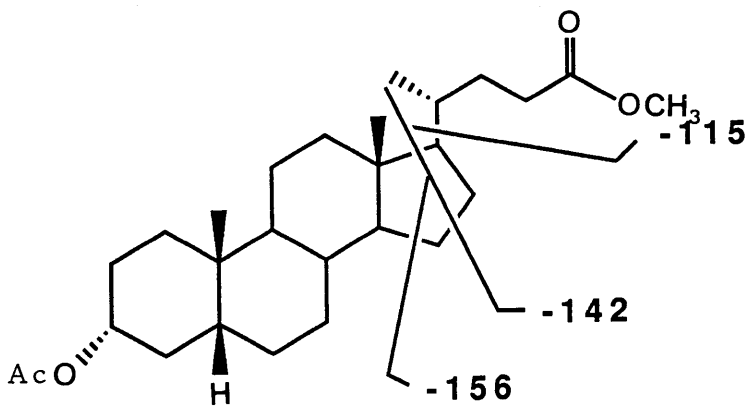


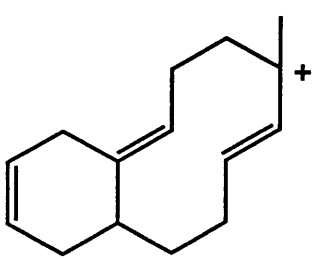
Figure 13. GC-MS (EI) of component $I^{OV-1}_{260°} = 3230$.
Methyl 3α-acetoxy-5β-cholan-24-oate.

Table 60. GC-MS (EI) of component $I^{OV-1}_{260°}$ = 3230 (scan 322)

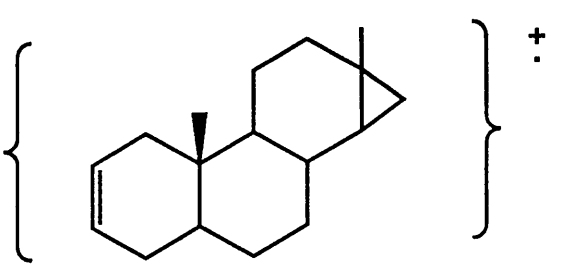
$[M]^{+\cdot}$ = 432, methyl 3α-acetoxy-5β-cholan-24-oate

m/z	Ion type	%
372	$[M^{+\cdot}$-AcOH]	100
357	$[M^{+\cdot}$-AcOH-$CH_3^{\cdot}$]	15
341	$[M^{+\cdot}$-AcOH-MeO$^{\cdot}$]	2
318	$[M^{+\cdot}$-AcOH-rDA ring A]	7
257	$[M^{+\cdot}$-AcOH-side-chain]	34
230	$[M^{+\cdot}$-AcOH-142]	40
215	$[C_{16}H_{23}]^+$	91
201	$[C_{15}H_{21}]^+$	10
107		30





m/z 201



m/z 230

Table 61. GC-MS of products from reaction of 5α-cholest-14-en-3β-ol TMS ether/5α-cholest-8(14)-en-3β-ol TMS ether with OsO_4

Scan 219 ≡ unreacted 5α-cholest-8(14)-en-3β-ol TMS ether

m/z	Ion type	%
458	$[M]^{+\cdot}$	100
443	$[M^{+\cdot}-CH_3]$	45
368	$[M^{+\cdot}-TMSOH]$	17
353	$[M^{+\cdot}-TMSOH-CH_3^{\cdot}]$	35
345	$[M^{+}$-side-chain]	10
255	$[M^{+\cdot}$-side-chain-TMSOH]	37
229	$[C_{17}H_25]^{+}$	58
213	$[C_{16}H_{21}]^{+}$	50
147	$[C_{11}H_{15}]^{+}$	50
107	$[C_8H_{11}]^{+}$	90

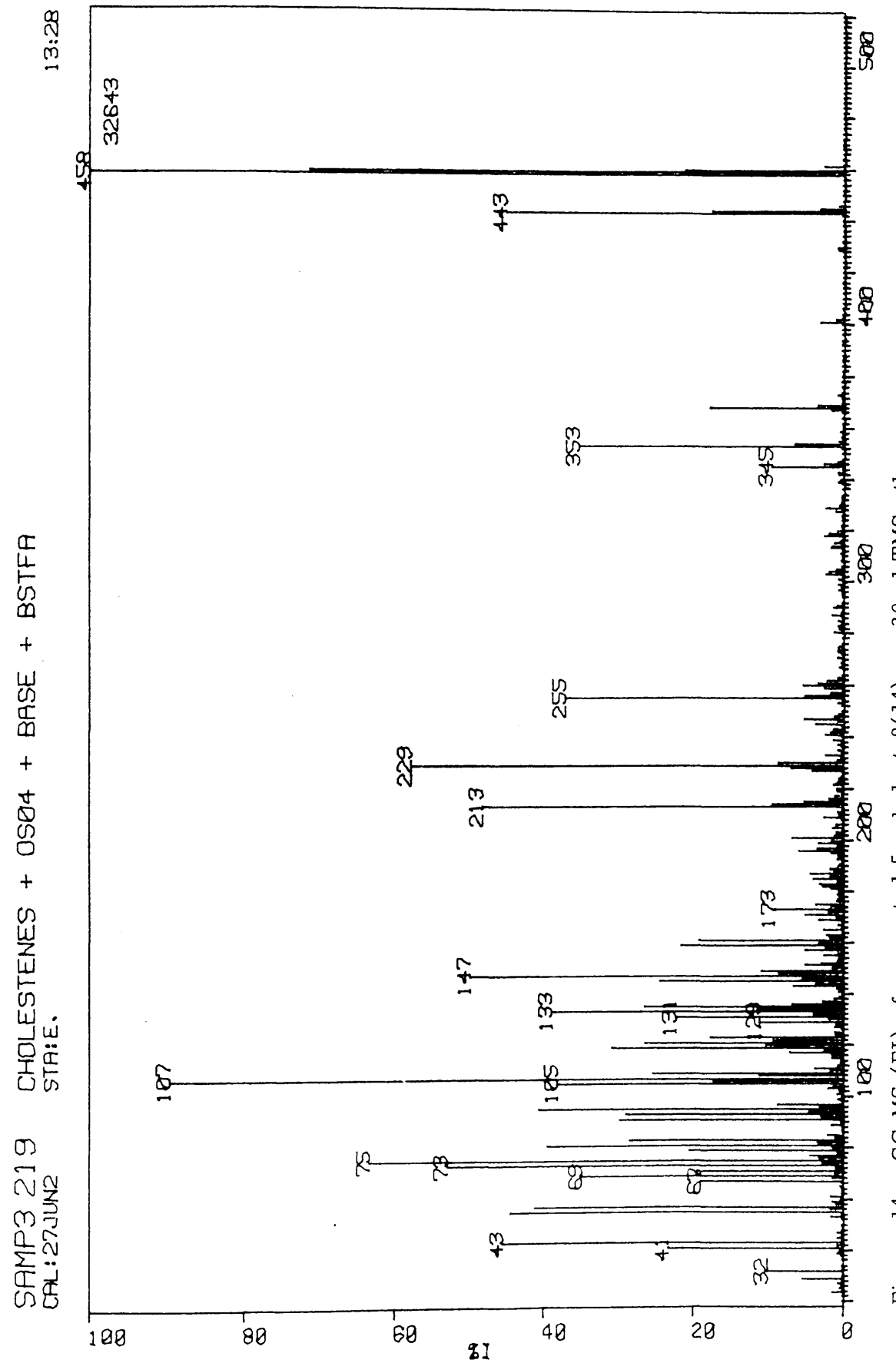


Figure 14. GC-MS (EI) of unreacted 5α-cholest-8(14)-en-3β-ol TMS ether.

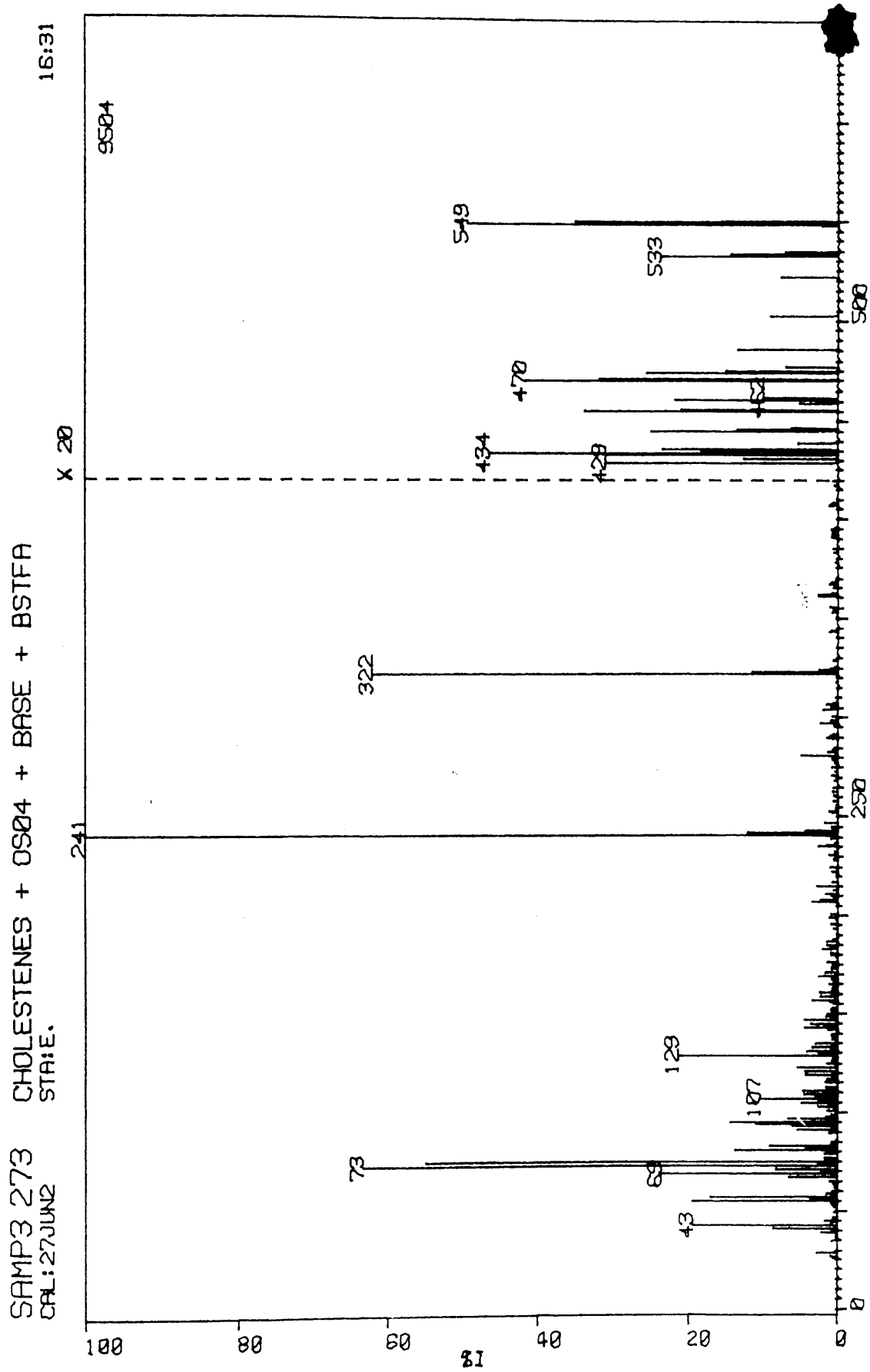
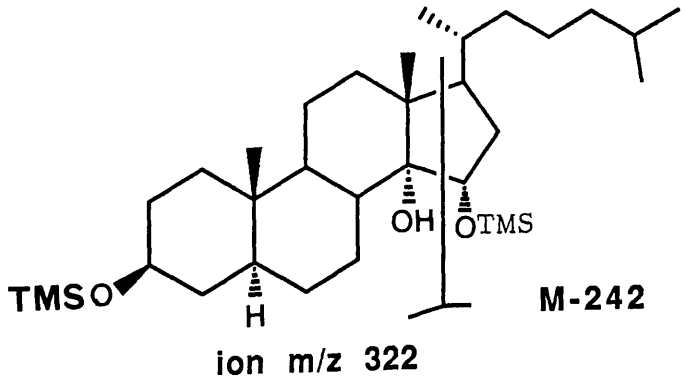


Figure 15. GC-MS of 5α-cholestan-3β,14α,15α-triol 3,15-di-TMS ether.

Table 62. GC-MS of 5α-cholestan-3β,14α,15α-triol-3,15-di-TMS ether (scan 273) $[M]^{+\cdot}$ = 564

m/z	Ion type	%
549	$[M^{+\cdot}-CH_3^{\cdot}]$	2
474	$[M^{+\cdot}$-TMSOH]	1
361	$[M^{+\cdot}$-TMSOH-side chain]	3
322	$[M^{+\cdot}-242]$	62
304	$[M^{+\cdot}-242-18]$	2
281	$[C_8H_{17}]^+$	5
241	$[M^{+\cdot}-323]$	100



ion m/z 322

Table 63. GC-MS of 5α-cholestan-3β-ol TMS ether + impurity (scan 222)

m/z	m/z	Ion type	%
460		$[M]^{+\cdot}$	55
	(456)	$[M_2]^{+\cdot}$	31
445		$[M_1{}^{+\cdot}\text{-}CH_2{}^{\cdot}]$	82
	(441)	$[M_2{}^{+\cdot}\text{-}CH_3{}^{\cdot}]$	4
403		$[M_1{}^{+\cdot}\text{-}C_4H_9{}^{\cdot}]$	11
370		$[M_1{}^{+\cdot}\text{-TMSOH}]$	28
355		$[M_1{}^{+\cdot}\text{-TMSOH-}CH_3{}^{\cdot}]$	43
	(351)	$[M_2{}^{+\cdot}\text{-TMSOH-}CH_3{}^{\cdot}]$	37
305		$[M_1{}^{+\cdot}\text{-ring D-H}^{\cdot}]$	40
215		$[C_{16}H_{23}]^{+}$	100

Table 64. GC-MS of component having $I^{OV-1}_{260°}$ = 3230 (methyl 3α-acetoxy-5β-cholan-24-oate

(scan 76) $[M]^{+\cdot}$ = 432

m/z	Ion type	%
372	$[M^{+\cdot}$-AcOH]	92
357	$[M^{+\cdot}$-AcOH-$\dot{C}H_3$]	10
341	$[M^{+\cdot}$-AcOH-$\dot{M}eO$]	3
318	$[M^{+\cdot}$-AcOH-rDA ring A]	6
257	$[M^{+\cdot}$-AcOH-side-chain]	41
230	$[M^{+\cdot}$-AcOH-112]	49
215	$[C_{16}H_{23}]^+$	100
201	$[C_{15}H_{21}]^+$	10
107	$[C_8H_{11}]^+$	51

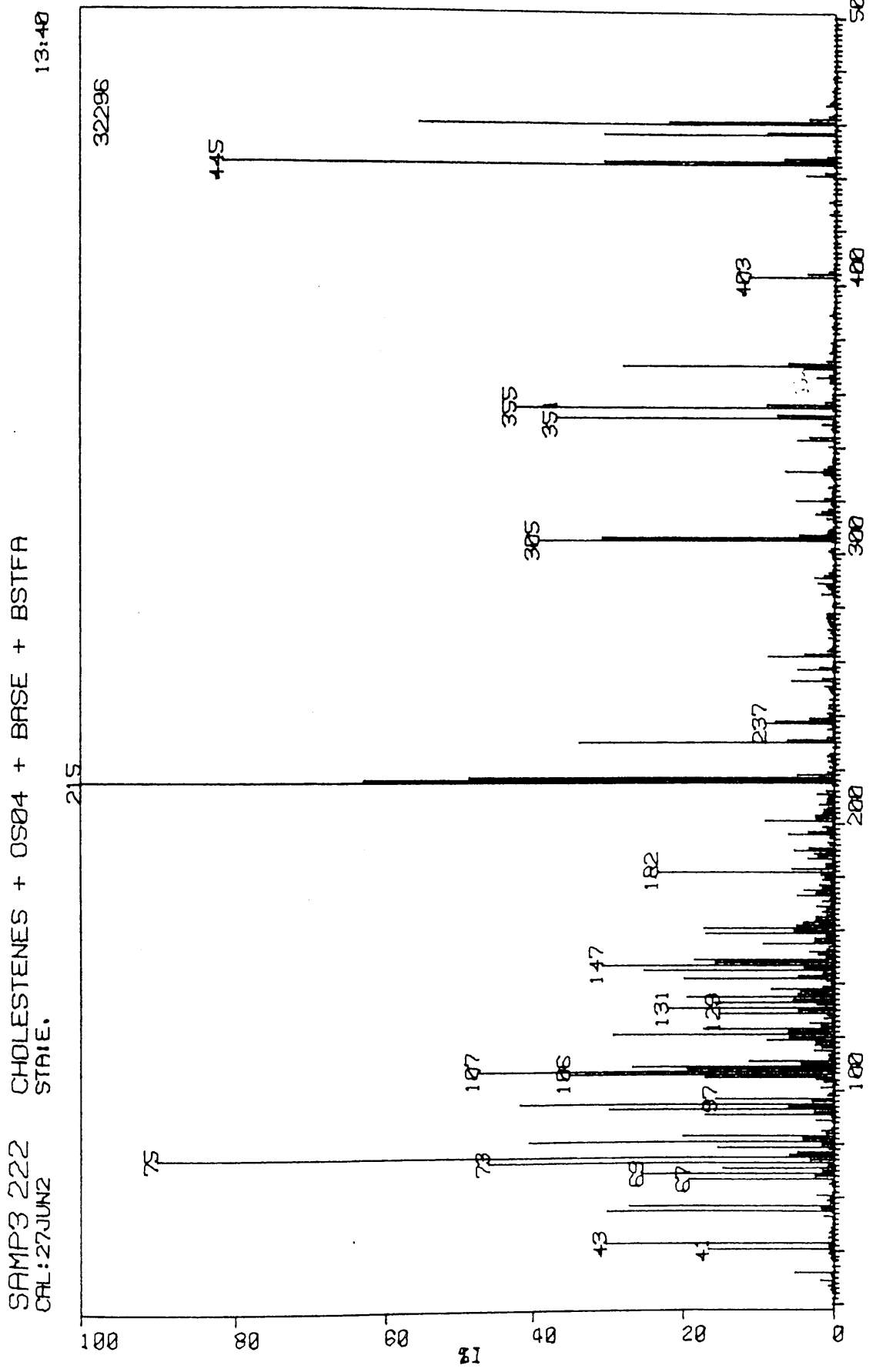


Figure 16. GC-MS of 5α-cholestan-3β-ol TMS ether + impurity.

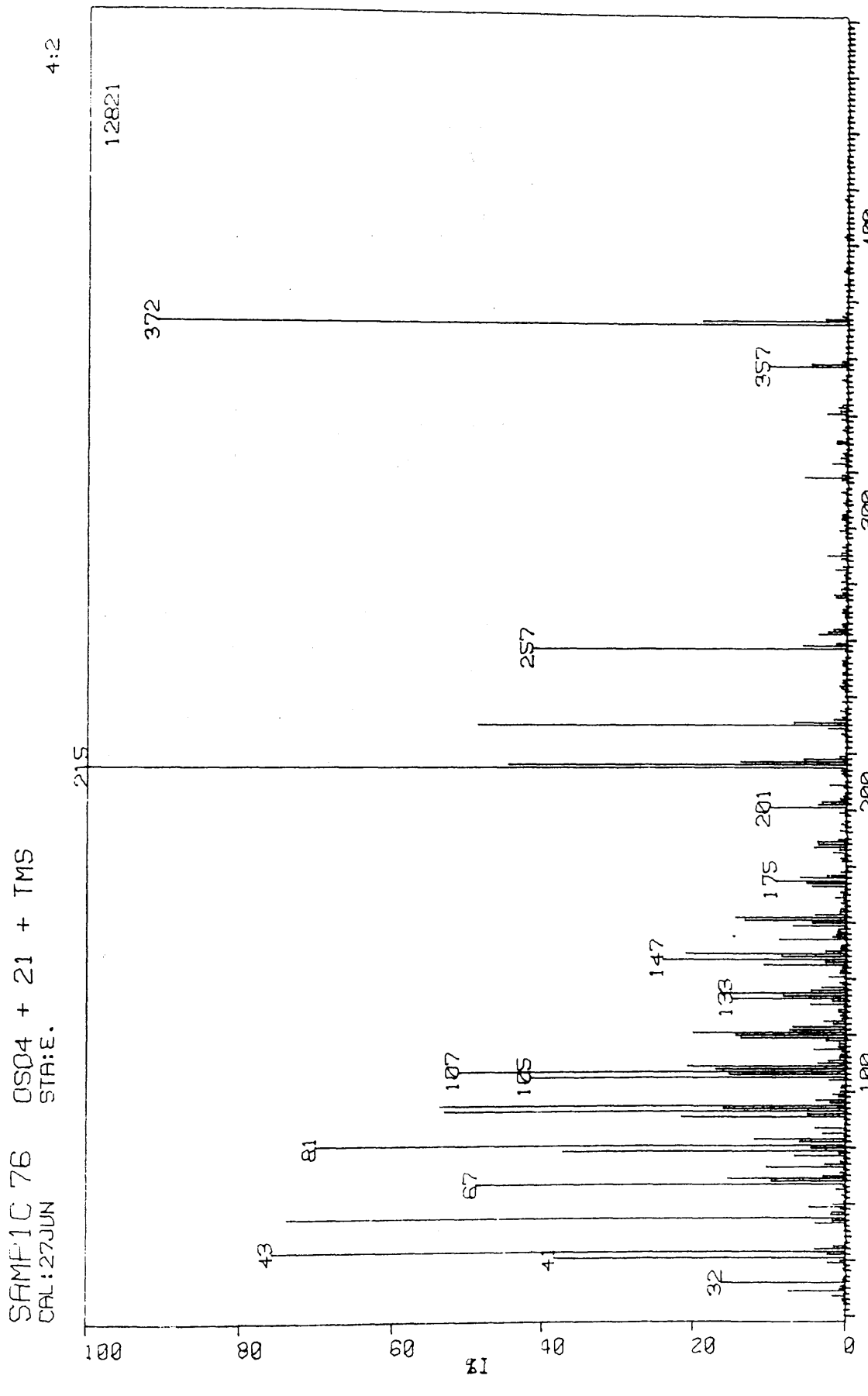
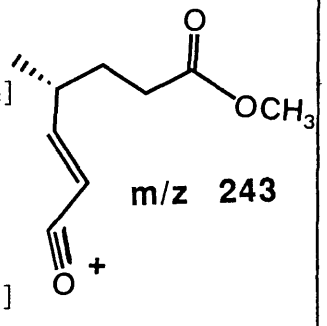
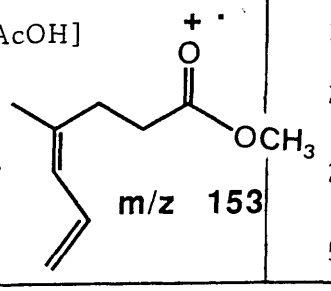


Figure 17. GC-MS of component $I^{OV-1}_{270°}$ = 3230 (methyl 3α-acetoxy-5β-cholan-24-oate).

Table 65. GC-MS of products from reaction of methyl 3α-acetoxy-5β-cholen-24-oate with OsO_4 + BSTFA (scan 116)

m/z	Ion type	%
536	$[M]^{+\cdot}$	0.2
518	$[M^{+\cdot}-H_2O]$	1.3
503	$[M^{+\cdot}-H_2O-CH_3^{\cdot}]$	1.0
487		0.7
476	$[M^{+\cdot}-AcOH]$	0.3
461	$[M^{+\cdot}-AcOH-Me^{\cdot}]$	0.4
446	$[M^{+\cdot}-TMSOH]$	3.8
431	$[M^{+\cdot}-TMSOH-CH_3^{\cdot}]$	1.3
428	$[M^{+\cdot}-TMSOH-H_2O]$	2.3
421	$[M^{+\cdot}$-side-chain]	0.2
403	$[M^{+\cdot}$-side-chain-H_2O]	4
368	$[M^{+\cdot}-TMSOH-H_2O-AcOH]$	6
366		4
331	$[M^{+\cdot}$-side-chain-TMSOH]	9
299		6
292	$[M^{+\cdot}-244]$  m/z 243	18
274		3
272		7
243	$[M^{+\cdot}-293]$	100
232	[292-AcOH]  m/z 153	30
154		27
153	$[C_9H_{13}O_2]^{+\cdot}$	29
121		52

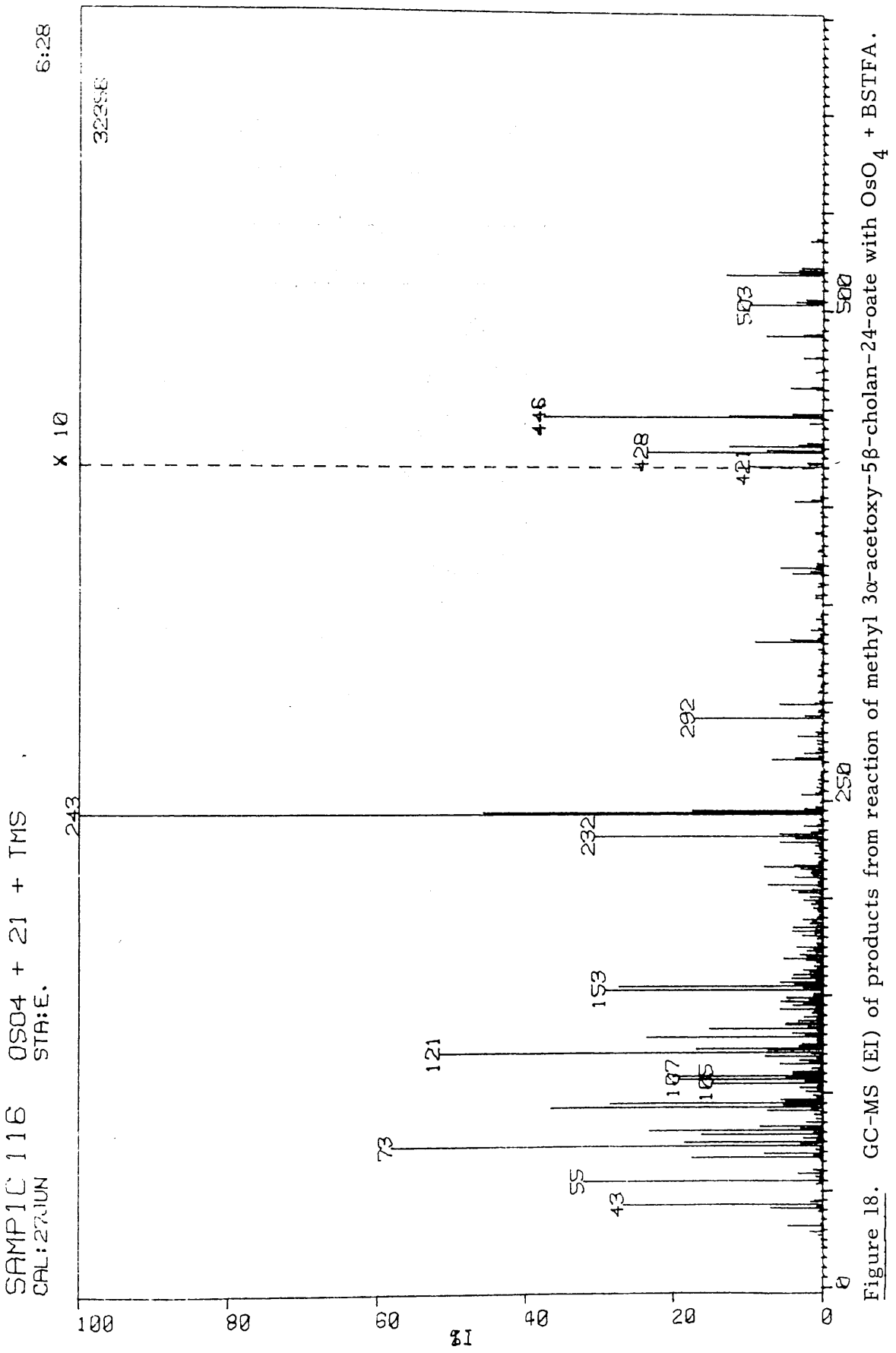


Figure 18. GC-MS (EI) of products from reaction of methyl 3α-acetoxy-5β-cholan-24-oate with OsO_4 + BSTFA.

Table 66. GC-MS (EI) of unknown methyl 3α-acetoxy-5β-cholen-24-oate after reaction with OsO_4 and BSTFA

Scan 52 = I^{OV-1}_{-260} = 3105 - probably methyl 3α-acetoxy-5β-chol-8(14)-en-24-oate

m/z	Ion type	%
430	$[M]^{+\cdot}$	11
415	$[M^{+\cdot}-CH_3^{\cdot}]$	11
370	$[M^{+\cdot}-AcOH]$	16
355	$[M^{+\cdot}-AcOH-\dot{C}H_3]$	69
315	$[M^{+\cdot}$-side-chain]	69
283	$[M^{+\cdot}$-side-chain-MeOH]	37
255	$[C_{19}H_{27}]^+$ $[M^{+\cdot}$-AcOH-side-chain]	100

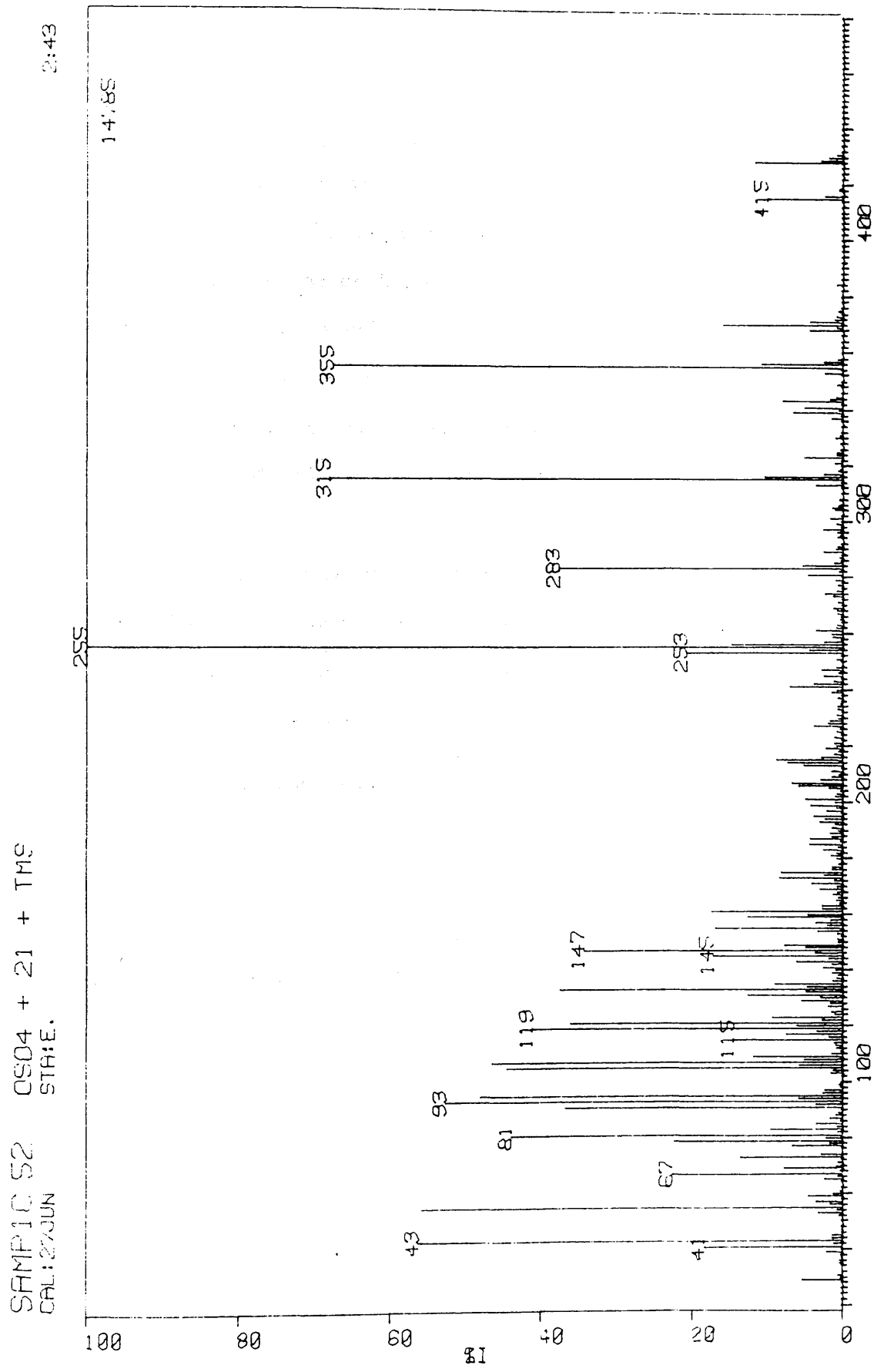


Figure 19. GC-MS of unknown methyl 3α-acetoxy-5β-cholen-24-oate after reaction with OsO_4 + BSTFA.

Table 67. GLC retention data

Compound	$I^{OV-1}_{\pm 260°}$
methyl 5β-cholan-24-oate	2890
methyl 3α-hydroxy-5β-cholan-24-oate	3125
methyl 3α-acetoxy-5β-cholan-24-oate	3230
methyl 3α,7α-dihydroxy-5β-cholan-24-oate	3325
methyl 3α-acetoxy,7α-hydroxy-5β-cholan-24-oate	3440
methyl 3α,7α-diacetoxy-5β-cholan-24-oate	3380
methyl 3α,12α-dihydroxy-5β-cholan-24-oate	3290
methyl 3α-acetoxy,12α-hydroxy-5β-cholan-24-oate	3245
methyl 3α,12α-diacetoxy-5β-cholan-24-oate	3330
methyl 3α,12α-dihydroxy-5β-chol-8(14)-en-24-oate	3280
methyl 3α,12α-diacetoxy-5β-chol-8(14)-en-24-oate	3290

Column: 6 ft 1% OV-1 at 260°

Carrier gas: N_2 at 30 ml/min.

ΔI

3H ⟶ 3α-OH + 235

3α-OH ⟶ 3α-OAc + 105

in presence of 7α-OH + 115

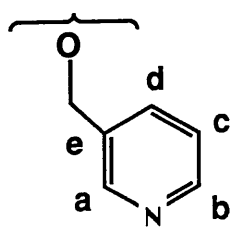
Table 68a. 200 MHz ^{1}H NMR of chlorinated/unsubstituted methyl 3α-acetoxy-5β-cholan-24-oate from Breslow reaction[106]

($CDC\ell_3$): δ8.60 (b, Ha + Hb), 7.66 (bd, J = 9 Hz, Hd), 7.28 (bm, Hc), 5.10 (bs, CH_2OAr), 4.69 (m, 3β-H), 1.997 (s, CH_3CO-), 1.992 (s, CH_3CO-).

Table 68b. ^{13}C DEPT NMR of chlorinated/unsubstituted methyl 3α-acetoxy-5β-cholan-24-oate

Group	Signal (ppm)
C-24	173.82
	173.49
	173.41
	173.29
C=O, acetate	170.69
	170.59
	170.55
C(a)+C(b)	3 peaks unresolved
	149.52
CH, C(d)	136.08
	135.99

Group	Signal (ppm)
-C-, C(e)	broad 131.61
-C-, C(c)	broad 123.44
-C-	93.55
-C-	93.37(weak)
CH, C-3	74.29
	74.17
	3 peaks unresolved



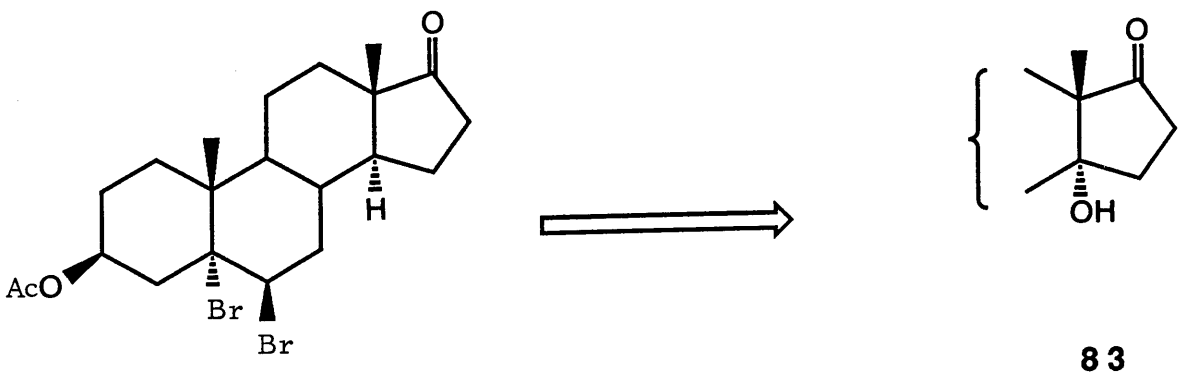
Section 5: Results - Long Range Functionalisation

5.1 CrO_3 Oxidation of 5α-Androstan-3β-yl Acetate

5.1(a) Previous Work

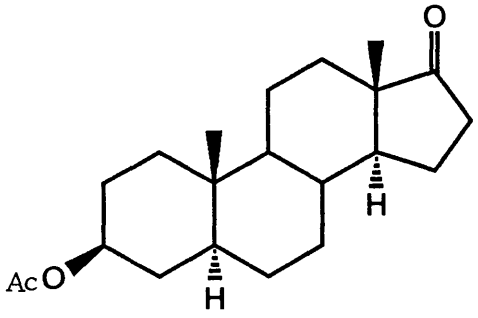
As reported by Linz and Schäfer,[42] the CrO_3 oxidation of 5α-androstan-3β-yl acetate yielded 3β-acetoxy-5α-androst-14-en-16-one in 68% yield. It was attempted to repeat this reaction on other substrates, such as 5α-cholestan-3β-yl acetate, in order to ascertain if some degree of similar selectivity could be achieved with a steroid containing a side chain.

St. André *et al.*[107] have reported that the CrO_3 oxidation of 3β-acetoxy-5α,6β-dibromoandrostan-17-one (82) gave a 25% yield of the 14α-hydroxy derivative (83).



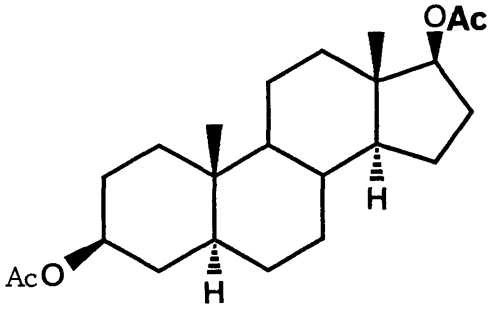
82 **83**

The same group[108] also reported that CrO_3 oxidation of 3β-acetoxy-5α-androstan-17-one (84) and 5α-androstane-3β,17β-diol diacetate (85) gave products which do not contain any oxygen at the C-14 position. However, products were identified as 86 and 87 respectively.



6-ketone + 5α-hydroxy

86

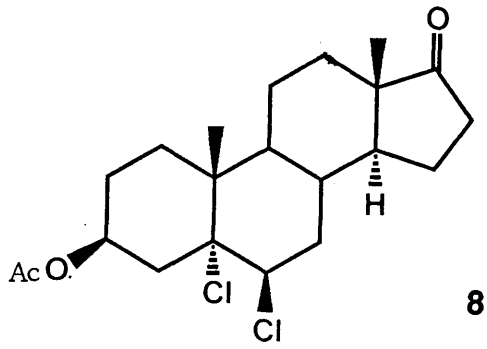


6-ketone + 5α-hydroxy

87

Sykes and Kelly[109] reported no oxidation when 3β-acetoxy-5α,6β-dichloroandrostan-17-one (88) was subjected to the CrO_3 oxidation procedure. Previous successful oxidations have always been carried out by treatment of the Δ^5-steroid with Br_2 in AcOH to protect the 5,6-position and then, without isolating the dibromide, to oxidise it using CrO_3. Thus, the oxidation has always been carried out in the presence of HBr. When a sample of the dichloroketone (88) was

oxidised with CrO_3 to which HBr was added, a 20% yield of the expected[109] 14α-hydroxy-17-ketone was obtained. Therefore, it is necessary to have HBr present in the reaction mixture in order to form the tertiary alcohol at C-14.



88

5.1(b) Present Work

Few experimental details were given by Linz and Schäfer[42] for the CrO_3 oxidation of 5α-androstan-3β-yl acetate. The research was monitored by us using GLC. Using 20 molar equivalents of powdered CrO_3 in $CH_2C\ell_2/Ac_2O/AcOH$ with stirring at RT. After 30 min, 60 min and 120 min, the yields of the Δ^{14}-16-one (89) were 8, 14 and 20%, respectively. The UV active enone (89) (λ232 nm) was isolated by preparative TLC (Table 69) (see Experimental section 5.1.1). Other unknown products were also produced in the CrO_3 oxidation. Linz and Schäfer[110] have reported data on an unknown 3β-acetoxy-5α-androstan-x-one, obtained in 7% yield. The IR spectrum of this compound contained two carbonyl bands at 1755 cm^{-1} and 1720 cm^{-1} (ester carbonyl). The absorption at 1755 cm^{-1} was probably due to a 5-ring ketone. The 300 MHz ^1H NMR revealed a one-proton quartet at 3.4 ppm and a one-proton singlet at 3.3 ppm.

Table 70. Capillary GLC of products from oxidation of 5α-androstan-3β-yl acetate with CrO_3 under Schäfer's conditions

I (Me oxime)	I	Compound
2363	2364	5α-androstan-3β-yl acetate ($C_{21}H_{34}O_2$)
2478	2479*	very similar (in I value) to 3β-acetoxy-5α-androstan-11-one ($C_{21}H_{32}O_3$)
(2498)	(2496)	very minor compounds - no peaks observed on GC-MS analysis
(2508)	(2510)	
(2591)	(2534)	
	(2550)	
2679 ← 91	2588*	3β-acetoxy-5α-androstan-16-one ($C_{21}H_{32}O_3$)
2689 ← 99		
2755 ← 115	2640*	$[M]^{+\cdot}$=346 (3β-acetoxy-diketone) ($C_{21}H_{30}O_4$)
2761 ← 121		
2738 ← 30	2706*	3β-acetoxy-5α-androst-14-en-16-one ($C_{21}H_{30}O_3$)
	-	$[M]^{+\cdot}$ = 376 ($C_{23}H_{36}O_4$)

* Major compounds

Table 71. Capillary GLC data

Compound	I	I (Me oxime)
3β-acetoxy-5α-androstan-17-one	2584	91 → 2655
3β-acetoxy-5α-androstan-11-one	2484	-
3β-acetoxy-5α-androstan-12-one	2589	8 → 2597
3β-acetoxy-5α-androstan-16-one	2586	91 → 2677; 99 → 2685
3β-acetoxy-5α-androstan-7-one	2540	46 → 2586
5α-androstan-15-one	2182* / 2206‡	48 → 2254
5α-androstan-17-one	2230	-
3β-acetoxy-5α-androstane-7,17-dione	2746	127 → 2873

* 14β-H ‡ 14α-H

ΔI 3β-H → 3β-OAc = 354

∴ Expected I value for 3β-acetoxy-5α-androstan-15-one = 2536*

" " " " " " " " " " = 2560‡

ΔI values for 3β-acetoxy-5α-androstan-x-one/3β-acetoxy-5α- androstane

ΔI	x
220	17
120	11
225	12
222	16
176	7
196	15 (14β-H)
172	15 (14α-H)
382	7,17-di

Column: CP-Sil 5CB, 25m x 0.31 ID, 0.13 μm

Carrier: He, 3 ml/min, 50:1 split, FID

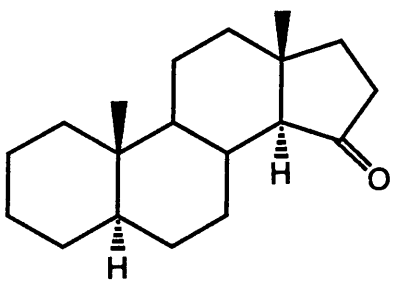
Initial value: 80°C

Initial time: 2 min

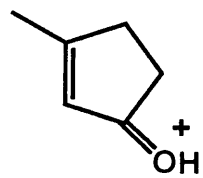
Level 1		Level 2	
Prog. rate	= 30°C/min	Prog. rate	= 2°C/min
Final value	= 170°C	Final value	= 260°C
Final time	= 1 min	Final time	= 15 min

After the experimental details of the CrO_3 oxidation became known to us[110] the reaction was repeated under Schäfer's conditions (see Experimental section 5.1.2). Capillary GLC revealed the Δ^{14}-16-one (89) to be formed in ca. 40% yield (Tables 70 and 71). The reaction mixture was subjected to GC-MS under CI and EI conditions.

Figure 20 shows the TIC for CI GC-MS. Scan 299 ($\underline{I}$ = 2479, had similar retention index to 3β-acetoxy-5α-androstan-11-one ($\underline{I}$ = 2484) and GC-MS (CI) revealed the [M+1] quasi-molecular ion to be 333 amu (Scan 378, Figure 22) thus corresponding to a monoketone. EI GC-MS (Table 73 and Figure 23) also confirmed this. However, the mass spectrum was completely different from the 3β-acetoxy-5α-androstan-11-one[111,112] standard and did not match any of the standards available to us (17-, 11-, 12-, 16-, 7-, or 15-ketones). Djerassi et al.[111] have recorded the mass spectrum of 5α-androstan-15-one (90) and shown the base peak to be 97 amu (91). Other strong ions observed were 151 amu (92) and 109 amu (93)

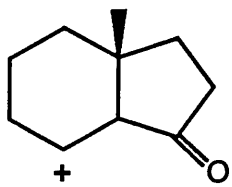


90



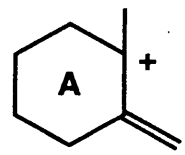
m/z 97

91



m/z 151

92



m/z 109

93

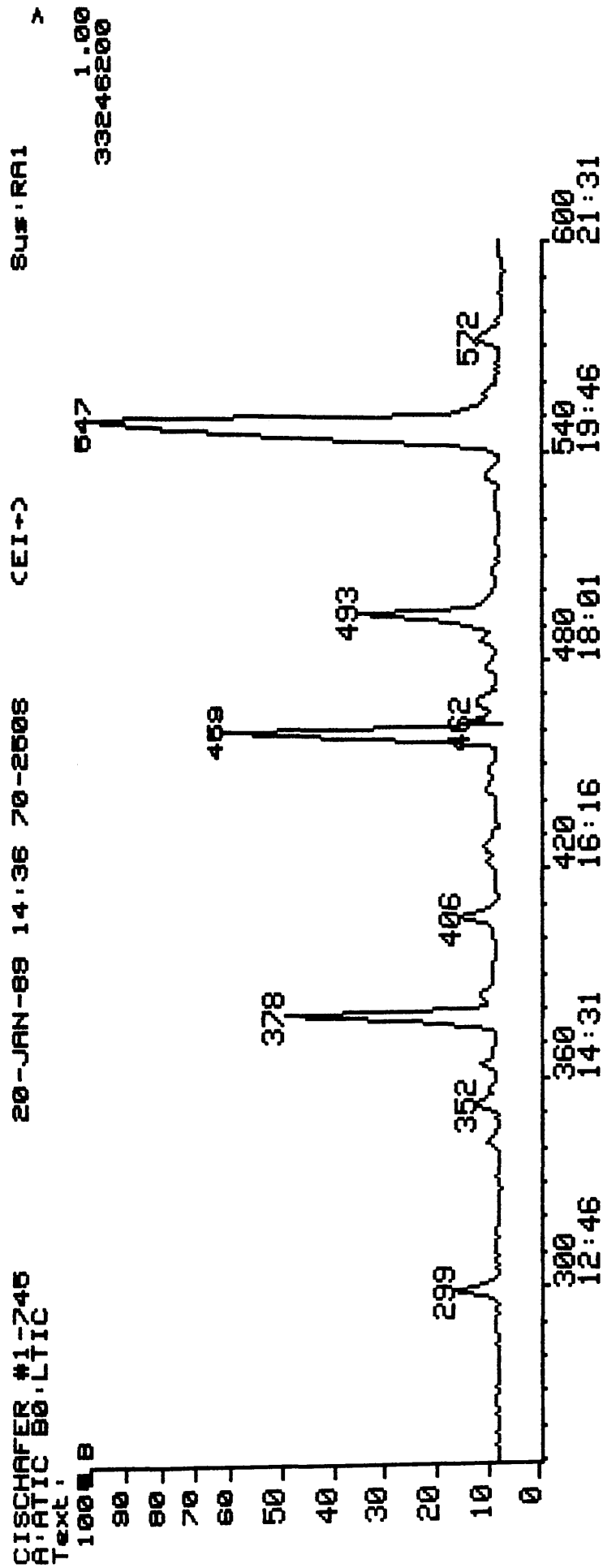


Figure 20. GC-MS TIc under CI conditions.

Although m/z 97 and 197 are strong for component I = 2479, no ion at 151 m/z was observed. Therefore, it seems that this 3β-acetoxy-5α-androstan-x-one is not the 15-ketone (14α-H or 14β-H), nor does the retention value fit for this compound. The retention value for this unknown ketone, I = 2479, suggests that the ketone is sterically hindered. The most hindered secondary position in the steroid nucleus is the 11-position.

Another minor unidentified 3β-acetoxy-5α-androstan-one was produced (Scan 406, Figure 24). Again the retention index, I = 2510, and mass spectrum (Table 74 and Figure 25) did not match with any of the known standard ketones (Figures 26 to 33 and Tables 75 to 82 in Appendix).

GC-MS (CI) scan 459 (Figure 34) was attributed to 3β-acetoxy-5α-androstan-16-one. Capillary GLC gave the retention index I = 2588 which was in good agreement with standard 3β-acetoxy-5α-androstan-16-one (I = 2586). The mass spectrum of I = 2588 (Table 83 and Figure 35) was also identical to that of the standard 16-ketone (Table 76 and Figure 27). This component of the reaction mixture was the second major compound produced in the CrO_3 oxidation of 5α-androstan-3β-yl acetate.

As mentioned earlier, Linz and Schäfer isolated an unidentified 3β-acetoxy-5α-androstanone in 4% yield (GLC yield 7%), but the mass spectrum reported[110] was completely different to that of our 3β-acetoxy-5α-androstan-16-one (Table 76 and Figure 27). However, there were some similarities in mass spectra between Linz and Schäfer's unknown ketone[110] and our ketone, I = 2479 (Table 76 and Figure 26).

GC-MS (CI) Scan 493 (Figure 36) gave the [M+1] ion at m/z 347, and EI GC-MS further agreed with this (Table 84 and Figure 37). It was thought that this component, I = 2640, was a diketone since on methoximation ΔI increased by 121 and 115 units (cf. ΔI for methoximation of 3β-acetoxy-5α-androstane-7,17-diene = 127). The fragmentation pattern of this diketone (Table 84 and Figure 37) revealed an ion at 274 amu [$M^{+\cdot}$-72] which may be due to cleavage through [ring D-24'], if both ketones were located in ring D. The ΔI for this ketone with respect to 5α-androstan-3β-yl acetate is 276 (cf. ΔI 3β-acetoxy-5α-androstan-7,17-diene = 382), suggesting that one of the ketone groups is sterically hindered, but not an 11-ketone as it gives a dimethoxime.

The major product from the CrO_3 oxidation was 3β-acetoxy-5α-androst-14-en-16-one (Scan 547) (I = 2706). This was identified by CI and EI mass spectra (Figures 38 and 39, Table 85).

The minor product (Scan 572) (Figure 40) on GC-MS (EI) gave a molecular ion, 378 amu (Table 86, Figure 41), which corresponds to the addition of 60 amu (CH_3CO_2H) to the starting material 5α-androstan-3β-yl acetate.

In summary, CrO_3 oxidation of 5α-androstan-3β-yl acetate produces, as already reported by Linz and Schäfer, 3β-acetoxy-5α-androst-14-en-16-one as the major product, together with 3β-acetoxy-5α-androstan-16-one and an, as yet, unidentified 3β-acetoxy-5α-androstanone. Major oxidation is being directed into ring D with formation of the Δ^{14}-16-one. Ring D in 5α-androstan-3β-yl acetate is slightly strained, and oxidation to a 16-ketone would relieve steric strain via opening bond angles from 109.5° to 120°. The C-16 position must be

more accessible to the oxidising species (chromyl acetate). The Gif system is also able to oxidise the C-15 and C-16 positions in steroids.[33] The CrO_3 oxidation introduced by Linz and Schäfer[42] is an important one-step procedure for the introduction of the Δ^{14}-16-ketone functionality into the saturated 5α-androstan-3β-yl acetate.

Wiberg[39] has suggested that the mechanism for the CrO_3 oxidation of hydrocarbons involves hydrogen atom abstraction from tertiary C-H positions as the first step. No 5α-androst-14-en-3β-yl acetate was detected in the reaction products. Therefore, a mechanism implying initial formation of the Δ^{14}-double bond which, on allylic oxidation, would give the Δ^{14}-16-ketone, is not thought likely to occur. Since the saturated 16-ketone was identified as a major product, it is likely that oxidation is directed into the C-16 methylene group. The mechanism by which this occurs is still unclear.

Experimental Section - 5.1.1

CrO_3 oxidation of 5α-androstan-3β-yl acetate

To a solution of 5α-androstan-3β-yl acetate (15.5 mg, 0.048 mmol) in $CH_2C\ell_2$ (0.35 ml), AcOH (0.12 ml) and Ac_2O (0.12 ml) was added powdered CrO_3 (94 mg, 0.94 mmol) with stirring at RT. Aliquots (1 mg) were removed at 30 min, 60 min and 120 min, and the reaction quenched with sodium metabisulphite solution, then extracted with EtOAc. The extracts were dried and filtered through Celite for GLC analysis.

Table 69. Mass spectrum of 3β-acetoxy-5α-androst-14-en-16-one

$(C_{21}H_3O_3)$ (330)

m/z	Ion type	%
330	$[M]^{+\cdot}$	8.0
315	$[M^{+\cdot}-CH_3^{\cdot}]$	1.4
302	$[M^{+\cdot}-CO]$	4.9
288	$[M^{+\cdot}-42]$	0.8
270	$[M^{+\cdot}-AcOH]$	36.0
255	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	13.7
216	$[M^{+\cdot}$-ring A]	5.3
149	$[C_{11}H_{17}]^+$	61.1
43	$[CH_3CO]^+$	100

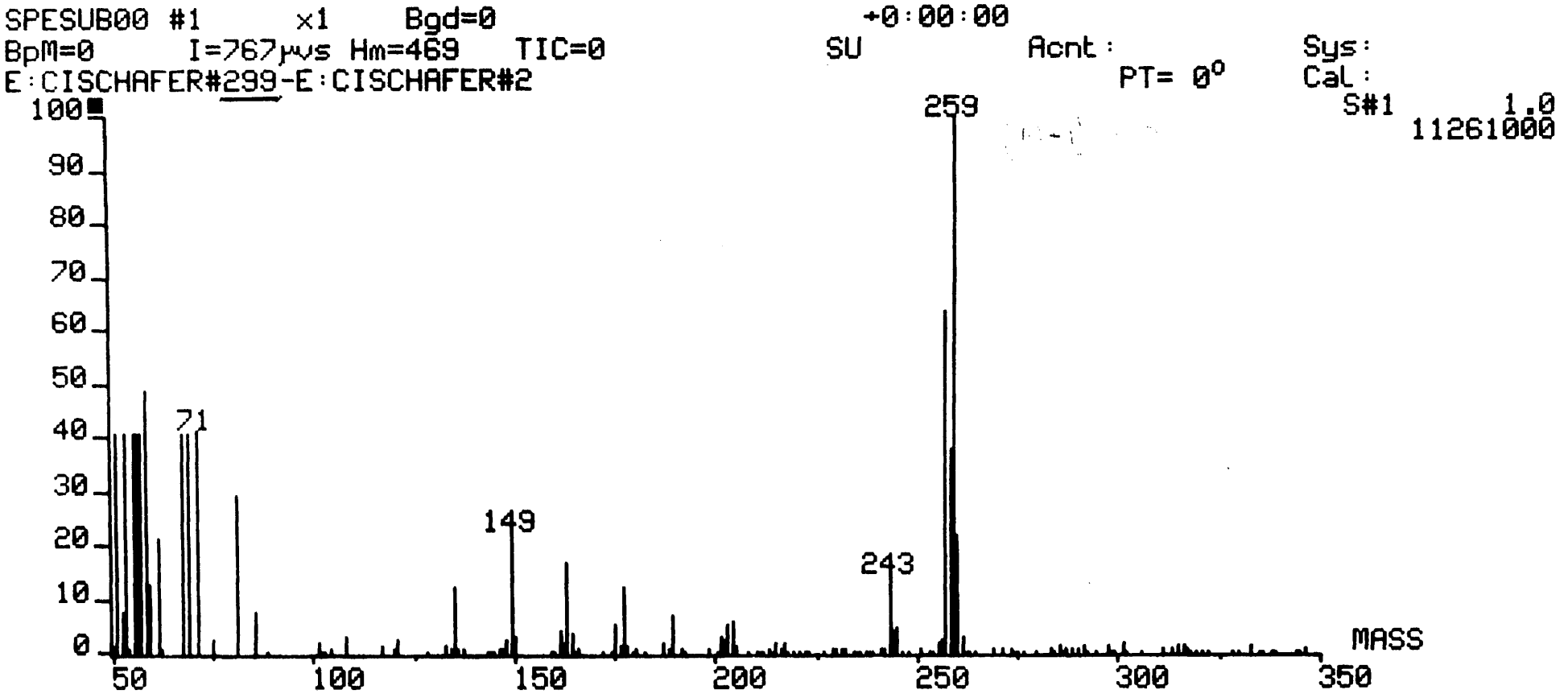


Figure 21(a). GC-MS (CI) of component I = 2364 (5α-androstan-3β-yl acetate).

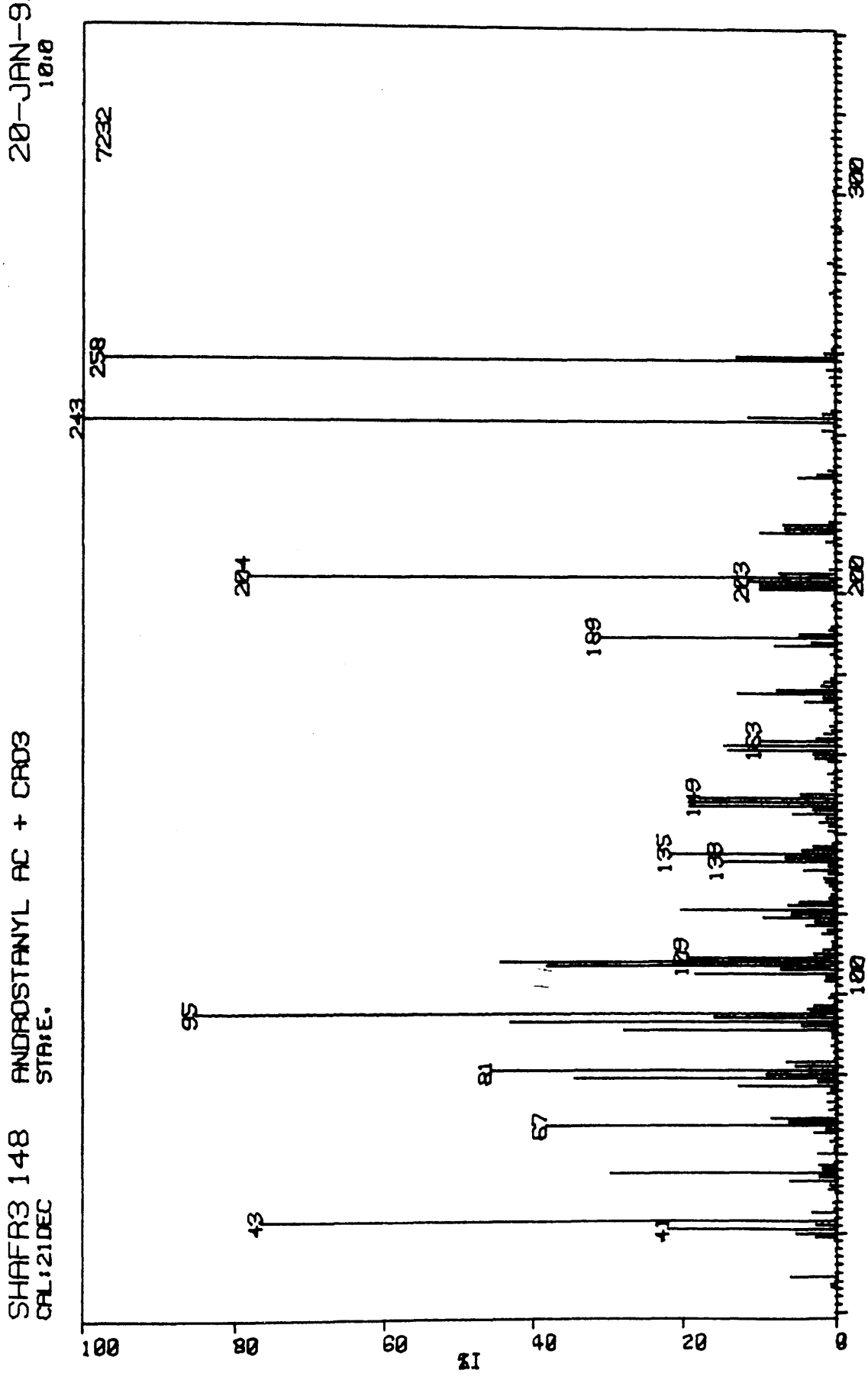


Figure 21(b). GC-MS (EI) of component I = 2364 (5α-androstan-3β-yl acetate).

Table 72. Mass spectral data for component I = 2364 (5α-androstan-3β-yl acetate) (scan 148)

m/z	Ion type	%
258	$[M^{+\cdot}-AcOH]$	97
243	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	100
204	$[M^{+\cdot}$-ring A]	80
189	$[M^{+\cdot}$-ring A-$CH_3^{\cdot}]$	32
95		87
43	$[CH_3CO]^+$	78

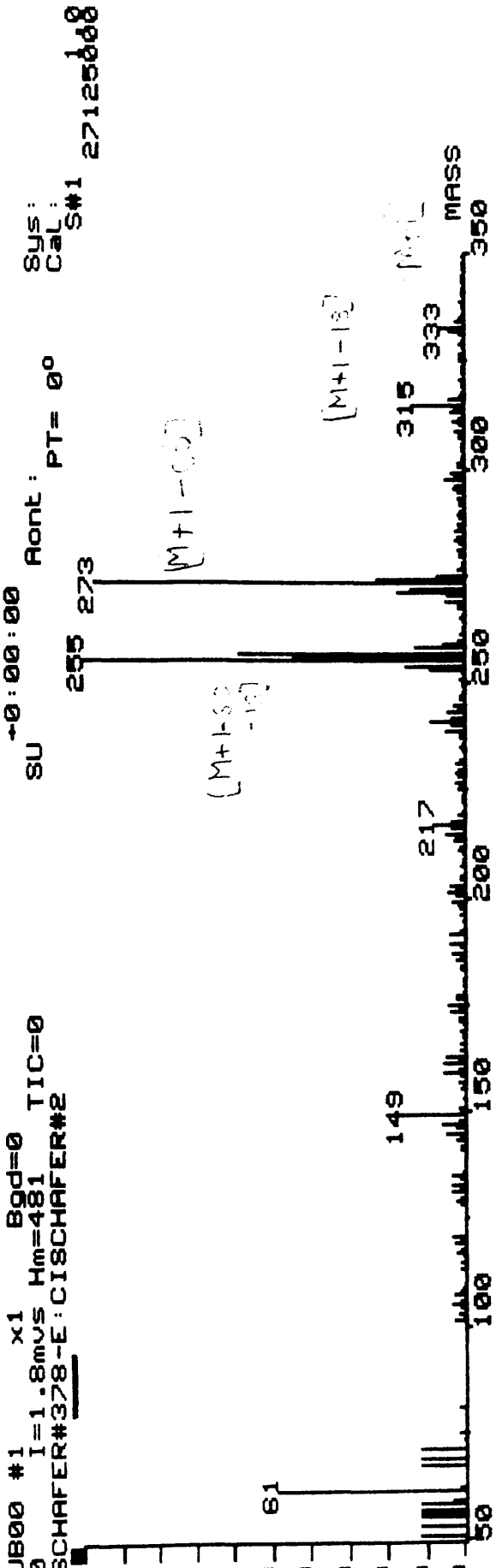


Figure 22. GC-MS (CI) of component I = 2479 (3β-acetoxy-5α-androstanone).

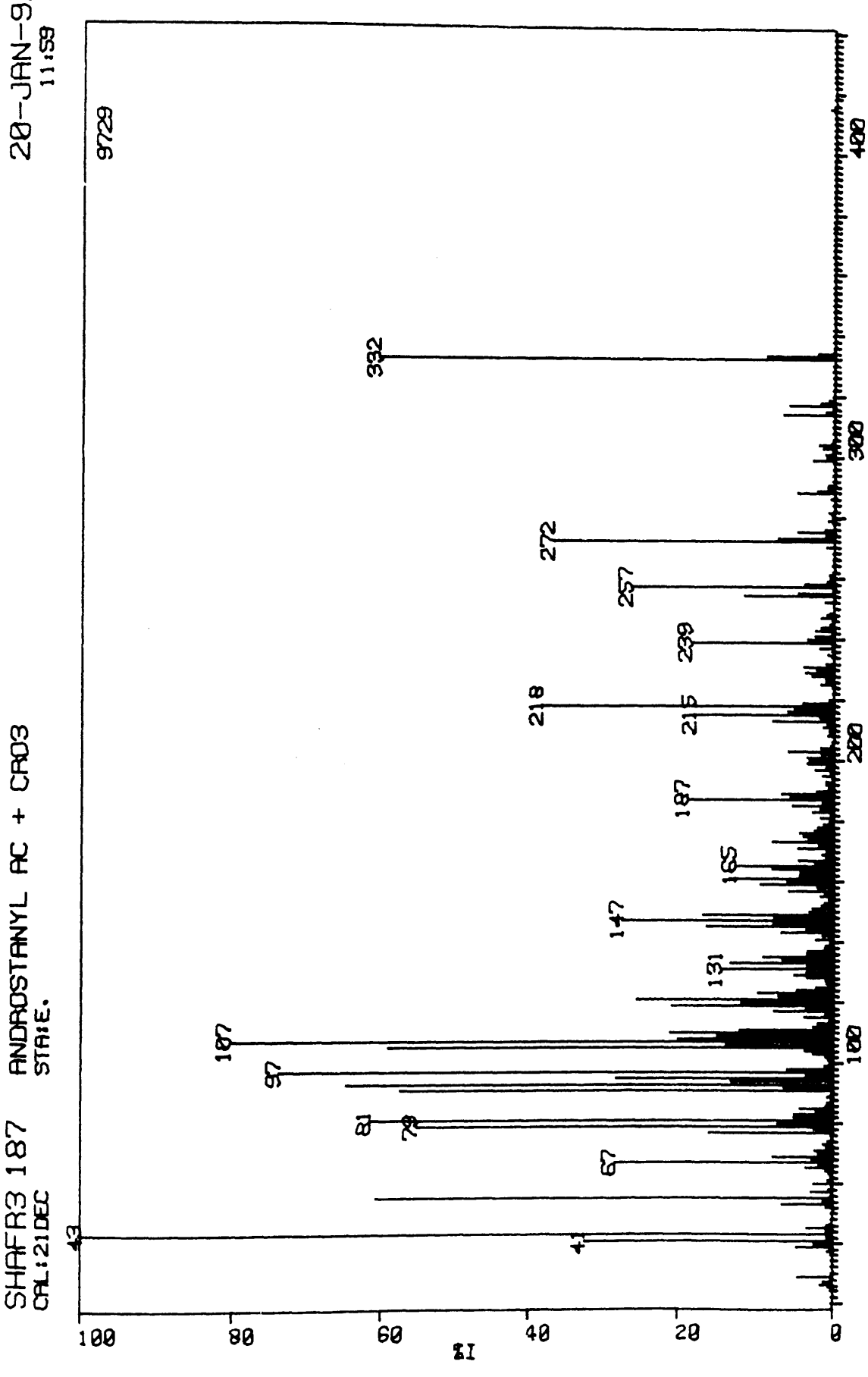
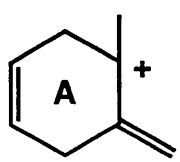


Figure 23. GC-MS (EI) of component I = 2479 (3β-acetoxy-5α-androstanone).

Table 73. Mass spectral data for component I = 2479; a 3β-acetoxy-5α-androstanone (scan 187)

m/z	Ion type	%
332	$[M]^{+\cdot}$	61
317	$[M^{+\cdot}-CH_3^{\cdot}]$	6
314	$[M^{+\cdot}-H_2O]$	7
288		5
272	$[M^{+\cdot}-AcOH]$	39
257	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	27
239		19
218	$[M^{+\cdot}$-ring A]	41
215	$[C_{16}H_{23}]^{\oplus}$	20
187		20
147		29
107	$[C_8H_{11}]^{+}$	81
97		75
95		66
91		59
43		100



m/z 107

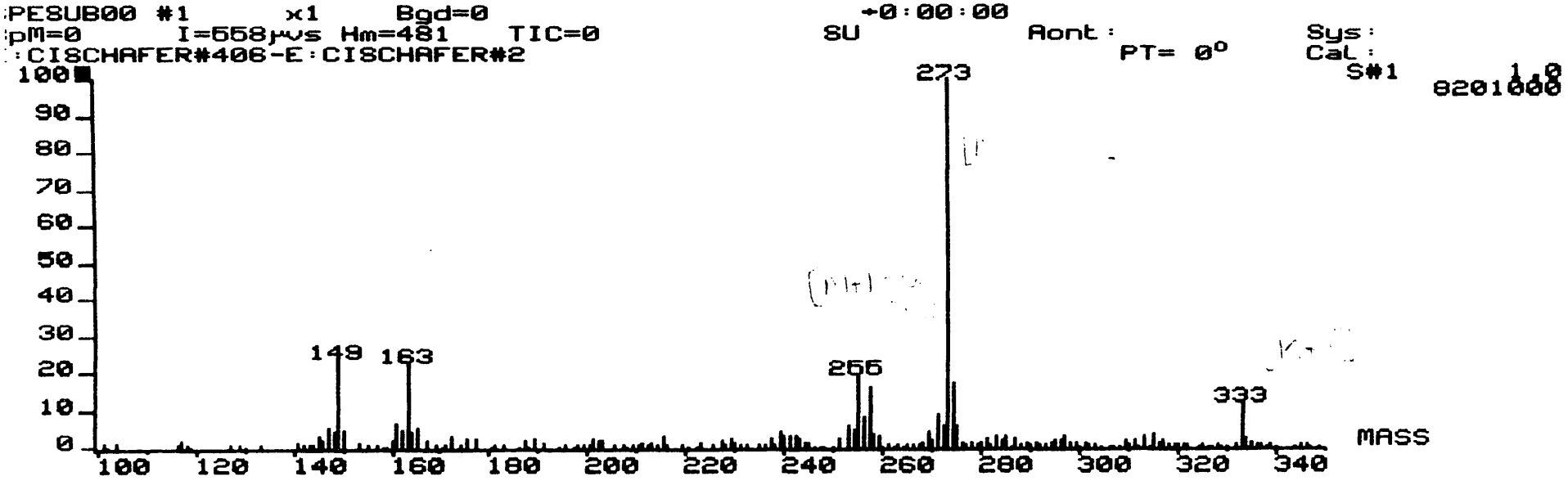


Figure 24. GC-MS (CI) of component I = 2510 (3β-acetoxy-5α-androstanone).

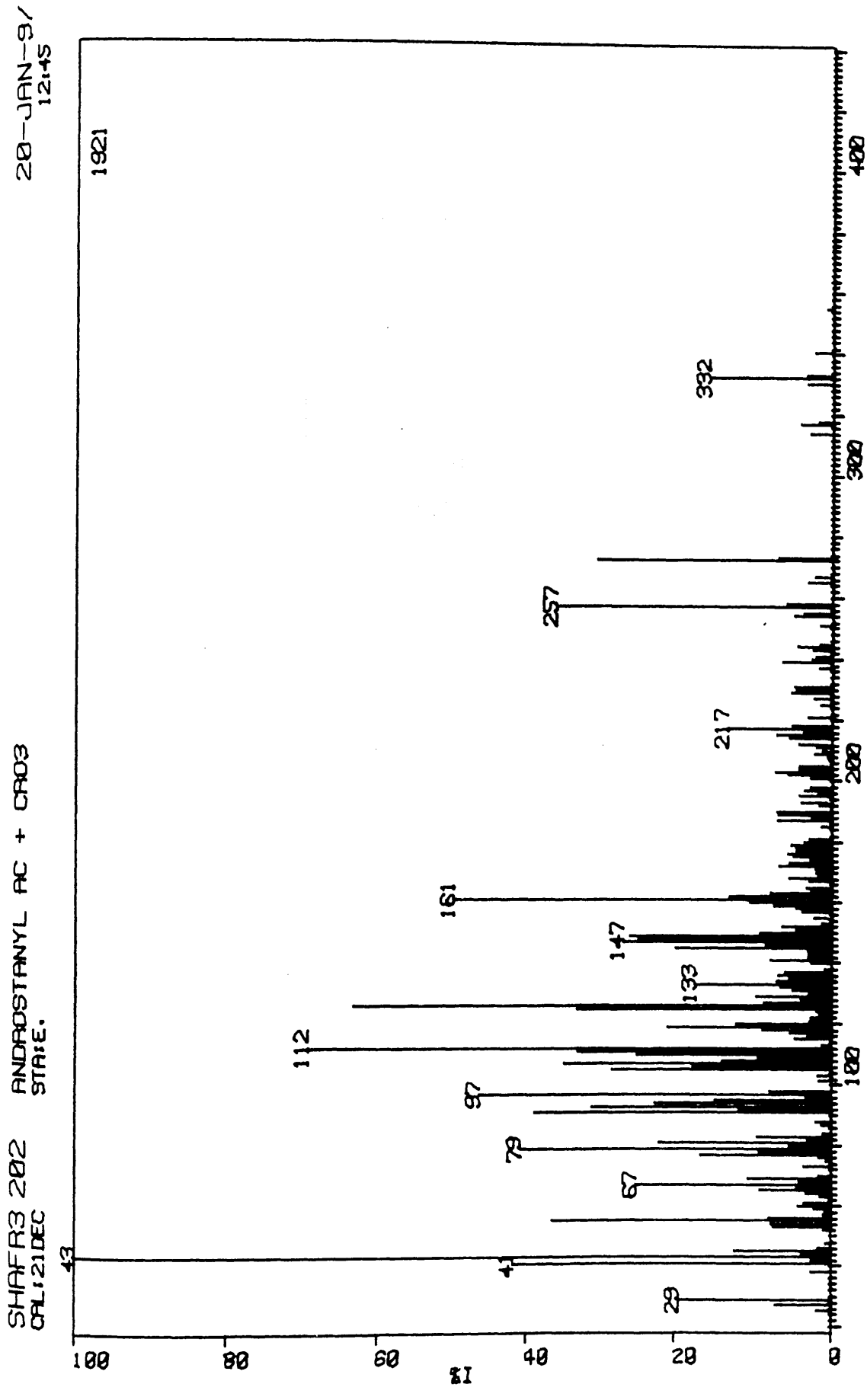


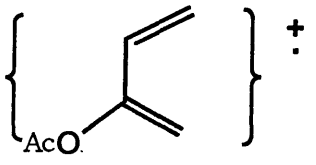
Figure 25. GC-MS (EI) of component I = 2510 (3β-acetoxy-5α-androstanone).

Table 74. Mass spectral data for component I = 2510, a 3β-acetoxy-5α-androstanone (scan 202)

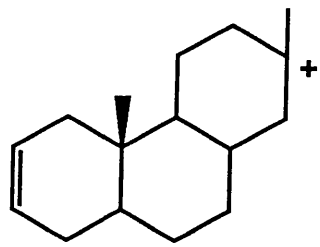
m/z	Ion type	%
332	$[M]^+$	17
317	$[M^{+\cdot}-CH_3^{\cdot}]$	4
314	$[M^{+\cdot}-H_2O]$	3
272	$[M^{+\cdot}-AcOH]$	31
257	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	37
217	$[C_{16}H_{25}]^+$ or $[M^{+\cdot}$-ring A-$H^{\cdot}]$	15
161	$[C_{12}H_{17}]^+$	51
147	$[C_{11}H_{15}]^+$	18
112	$[C_7H_{12}O]^{\cdot+}$	70
43	$[CH_3CO]^+$	100

m/z

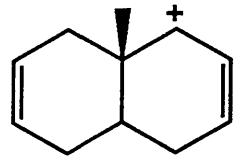
217 $[C_{26}H_{25}]^+$



m/z 112



m/z 217



m/z 147

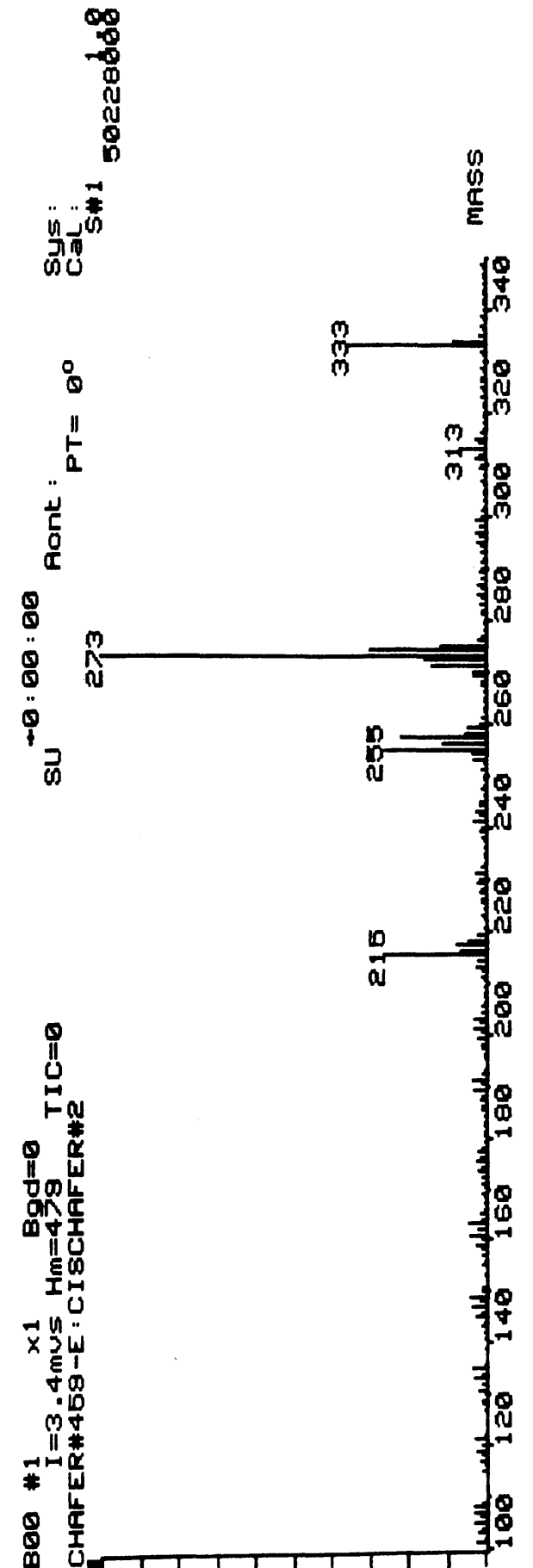


Figure 34. GC-MS (CI) of component I = 2588 identified as 3α-acetoxy-5α-androstan-16-one.

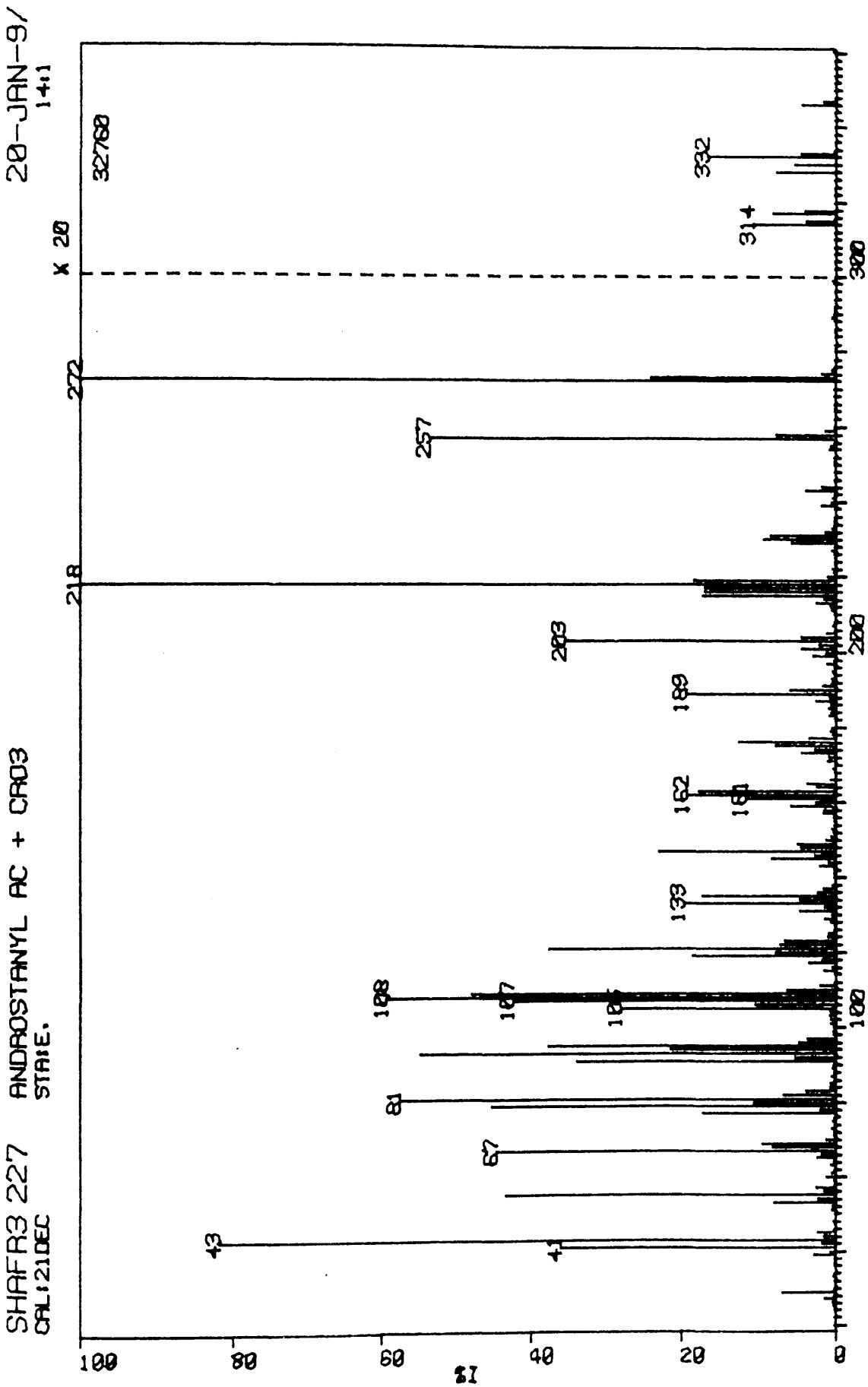
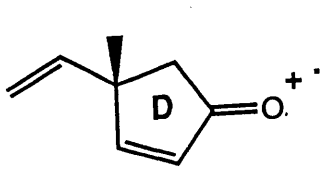


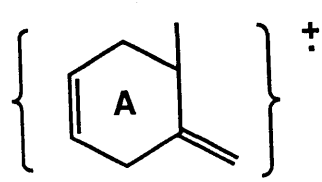
Figure 35. GC-MS (EI) of component I = 2588 identified as 3β-acetoxy-5α-androstan-16-one.

Table 83. Mass spectral data for component with I = 2588, identified as 3β-acetoxy-5α-androstan-16-one (scan 227)

m/z	Ion type	%
332	$[M]^{+\cdot}$	0.8
317	$[M^{+\cdot}-CH_3]^{\cdot}$	0.4
314	$[M^{+\cdot}-H_2O]$	0.5
272	$[M^{+\cdot}-AcOH]$	100
257	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	55
218	$[M^{+\cdot}$-ring A]	100
203	$[M^{+\cdot}$-ring A-$CH_3^{\cdot}]$	36
189		20
162		20
147	$[C_{11}-H_{15}]^{+}$	24
133		21
122	$[C_8H_{11}O]^{+\cdot}$	38
108	$[C_8H_{12}]^{+\cdot}$	60
81		58
43		83



m/z 122



m/z 108

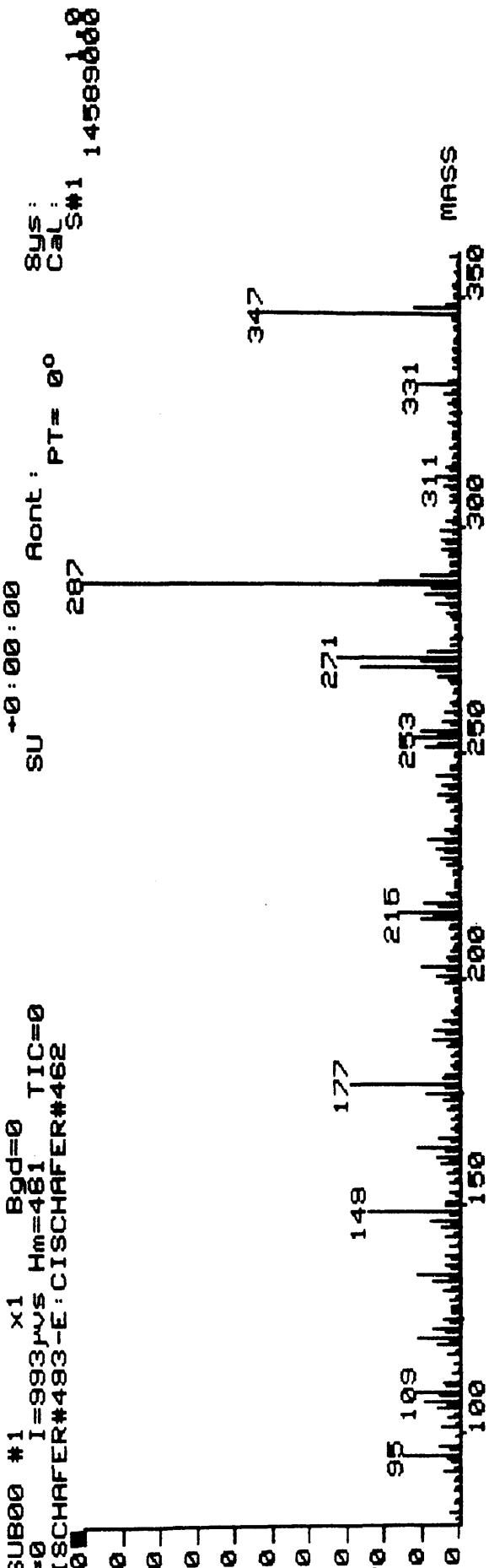


Figure 36. GC-MS (CI) of component I = 2640 (3β-acetoxy-5α-androstan dione).

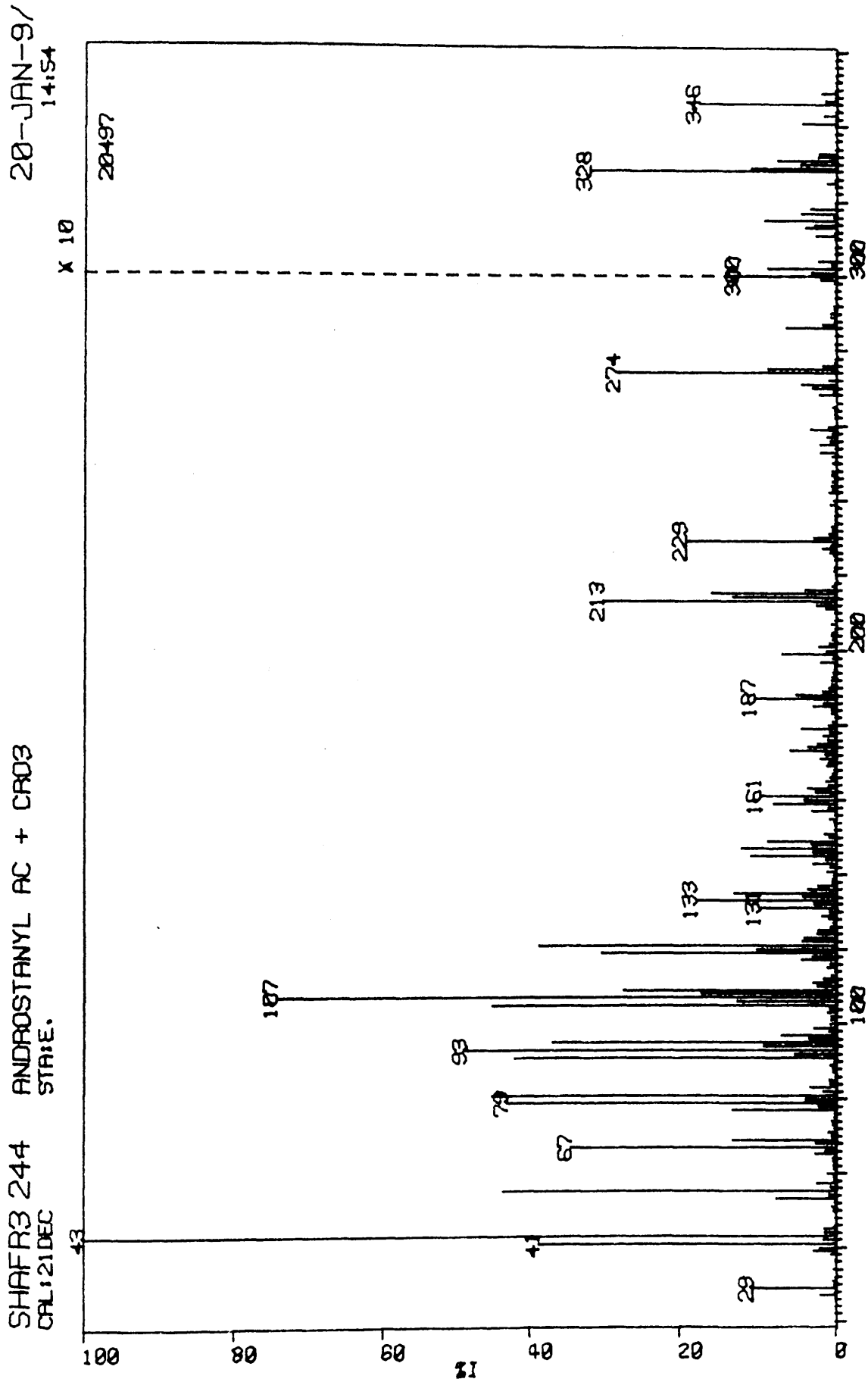
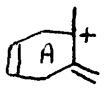


Figure 37. GC-MS (EI) of component I = 2640 (3β-acetoxy-5α-androstan dione.

Table 84. Mass spectral data for component I = 2640, a 3β-acetoxy-5α-androstanedione

m/z	Ion type	%
346	$[M]^+$	1
328	$[M^{+\cdot}-H_2O]$	3
300	$[M^{+\cdot}-CO-H_2O]$	1
286	$[M^{+\cdot}-AcOH]$	7
274	$[M^{+\cdot}-72]$	29
229		20
213	$[M^{+\cdot}-72-AcOH]$	31
187		11
161		10
133		19
121		40
107		76
43	$[CH_3CO_2]^+$	100

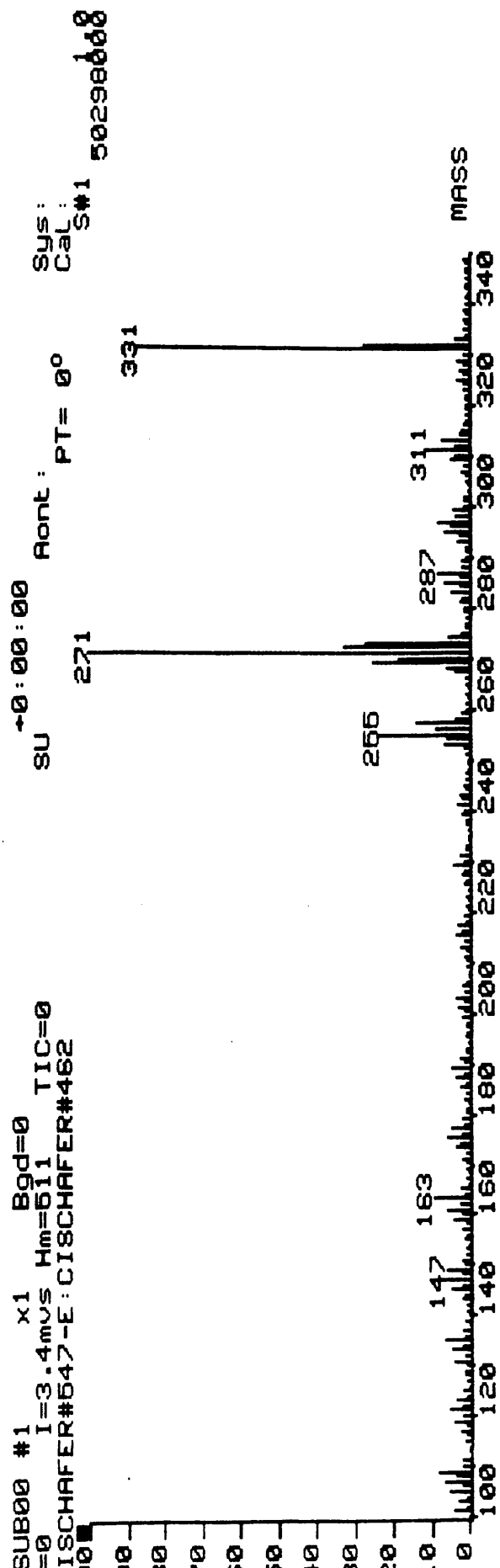


Figure 38. GC-MS (CI) of component I = 2706 identified as 3β-acetoxy-5α-androst-14-en-16-one.

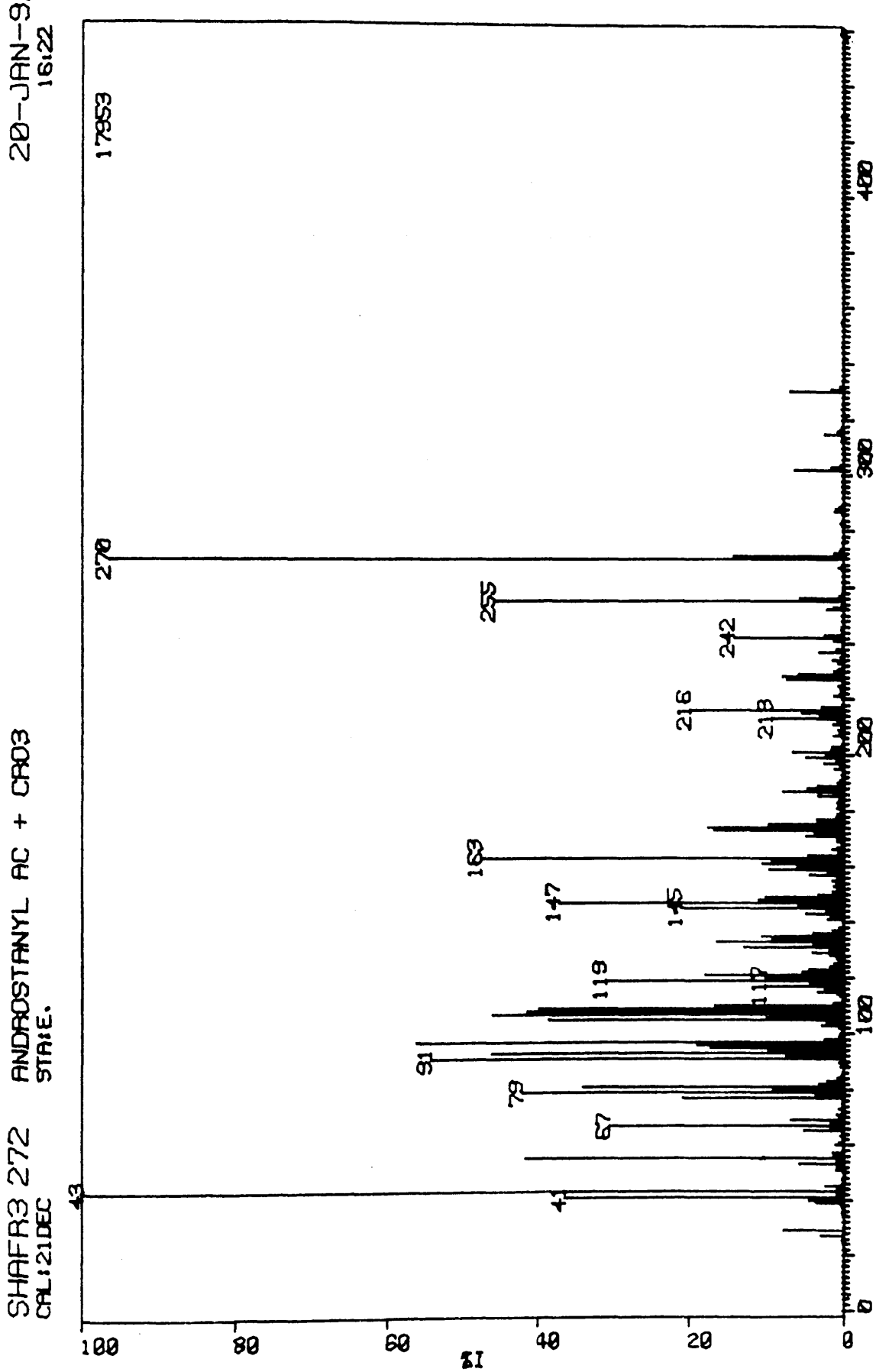
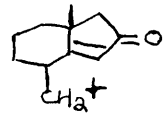


Figure 39. GC-MS (EI) of component I = 2706 identified as 3β-acetoxy-5α-androst-14-en-16-one.

Table 85. Mass spectral data for component I = 2706, identified as 3β-acetoxy-5α-androst-14-en-16-one (scan 272)

m/z	Ion type	%
330	$[M]^{+\cdot}$	7
315	$[M^{+\cdot}-CH_3^{\cdot}]$	2
302	$[M^{+\cdot}-CO]$	6
270	$[M^{+\cdot}-AcOH]$	97
255	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	46
242	$[M^{+\cdot}-AcOH-CO]$	15
216	$[M^{+\cdot}$-ring A$]$	21
163	 $[C_{11}H_{15}O]^+$	48
147		38
43	$[CH_3CO]^+$	100

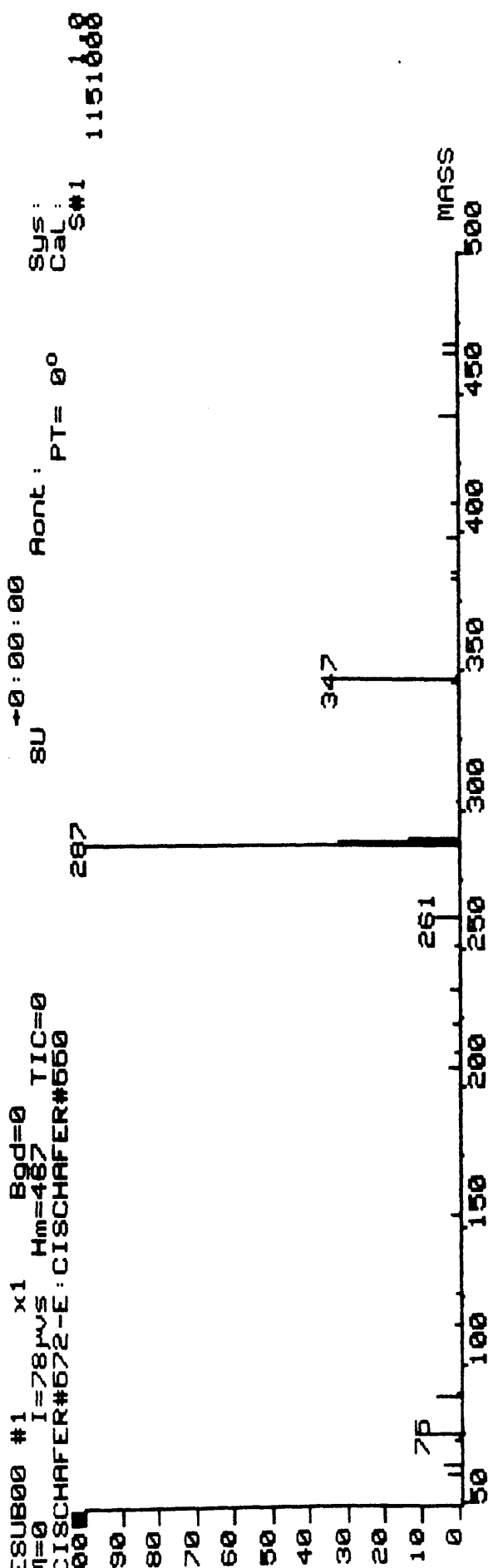


Figure 40. GC-MS (CI) of unknown minor component.

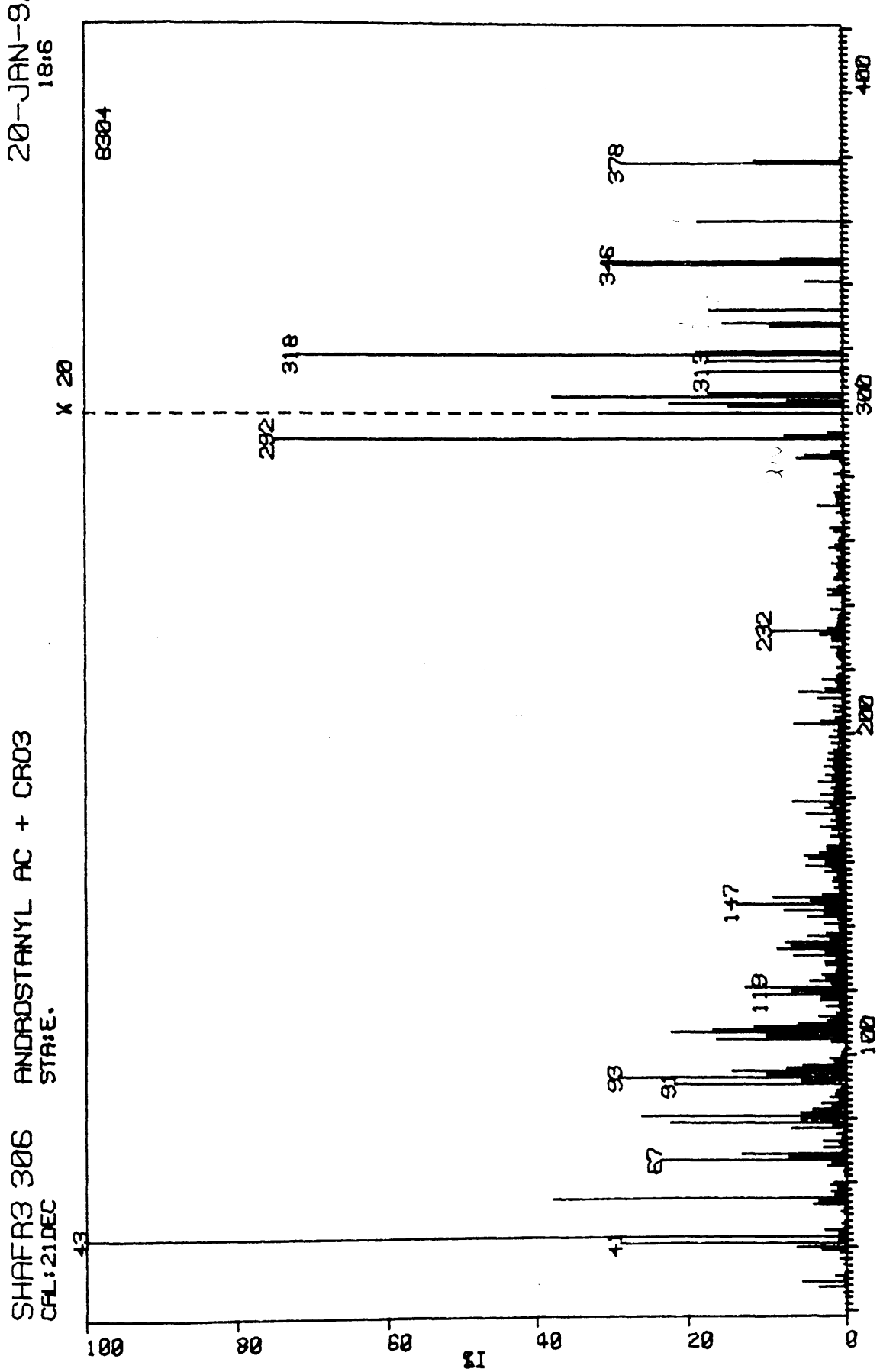


Figure 41. GC-MS (EI) of unknown minor component.

Table 86. Mass spectral data for unknown minor component of scan 306 ($C_{23}H_{36}O_4$)

m/z	Ion type	%
378	$[M]^{+}\cdot$	1.5
360	$[M^{+}\cdot - H_2O]$	1.0
346	$[M^{+}\cdot$-MeOH]	1.6
318	$[M^{+}\cdot$-AcOH]	3.6
292		76
232		10
147		14
93		30
43	$[CH_3CO]^{+}$	100

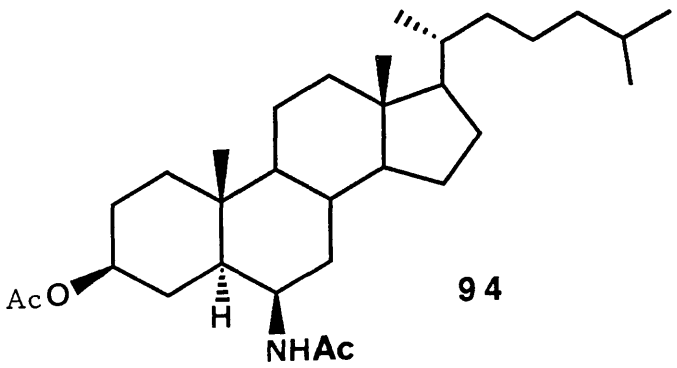
Experimental Section - 5.1.2

CrO_3 oxidation of 5α-androstan-3β-yl acetate under Schäfer's conditions

To a solution of 5α-androstan-3β-yl acetate (41.5 mg, 0.13 mmol) in $CH_2C\ell_2$ (1 ml) and AcOH (0.3 ml)/Ac_2O (0.3 ml) was added powdered CrO_3 (82.3 mg, 0.82 mmol). The reaction mixture was stirred at RT for 18h, then quenched with sodium metabisulphite and worked up as in section 5.1.1.

5.2 CrO_3 Oxidation of 5α-cholestan-3β-yl Acetate

The utilisation of cholesterol in the synthesis of important pharmaceutical steroids is a difficult process. The classic method has been to oxidise the 5,6-dibromide derivative of cholesteryl acetate with chromic acid to give, after regenerating the Δ^5-double bond, 3β-hydroxy-androst-5-en-17-one in 7% overall yield.[57] Cholesterol has also been converted to chol-5-ene-3β,24-diol in 14% overall yield by oxidation of 6β-acetamido-5α-cholestan-3β-yl acetate (94) with trifluoroperoxyacetic acid, then elimination of acetamide and hydrolysis of the 3-acetate.

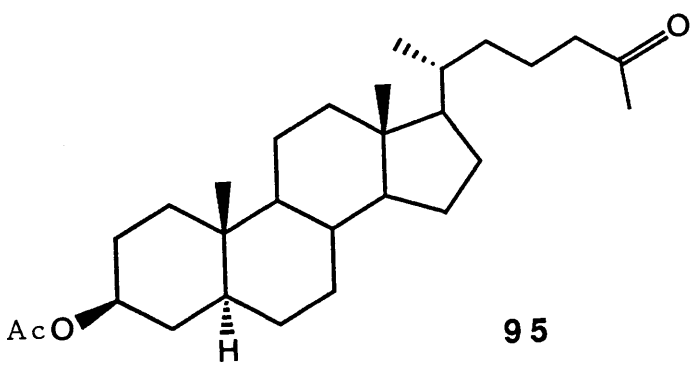


94

The CrO_3 oxidation, under Schäfer's conditions, was attempted on 5α-cholestan-3β-yl acetate to ascertain if oxidation would be directed in ring D with a steroid containing a side-chain.

5α-Cholestan-3β-yl acetate was treated with CrO_3, under Schäfer's conditions (see Experimental section 5.2.1) and the products were identified by comparison with known material using GLC and GC-MS. Table 87 shows the products formed after 25h reaction with CrO_3. CrO_3 selectively attacks tertiary C-H positions in steroids[116] and the expected products from 5α-cholestan-3β-yl acetate oxidation would involve cleavage at these positions. 3β-Acetoxy-5α-androstan-17-one (Table 89 and Figure 42) and 3β-acetoxy-5α-pregnan-20-one (Table 90 and Figure 43) were identified by GC-MS and capillary GLC (Table 87). The major product (49% yield by GLC) was unreacted 5α-cholestan-3β-yl acetate (Table 91 and Figure 44).

Component I = 3354 was identified as 3β-nor-5α-cholestan-25-one (95)

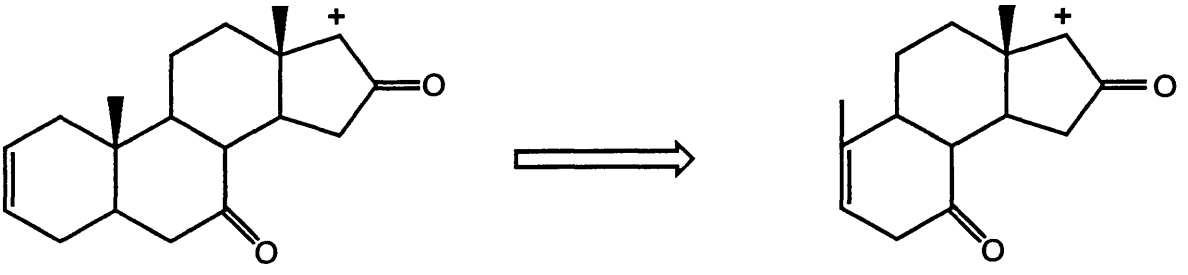


95

The mass spectrum (Table 92, Scan 10 and Figure 45) revealed a molecular ion at m/z 430. The even-electron ions at m/z 317 and m/z 257 correspond to losses of $[C_7H_{13}O]^{\cdot}$ and CH_3CO_2H-$[C_7H_{13}O]^{\cdot}$, respectively.

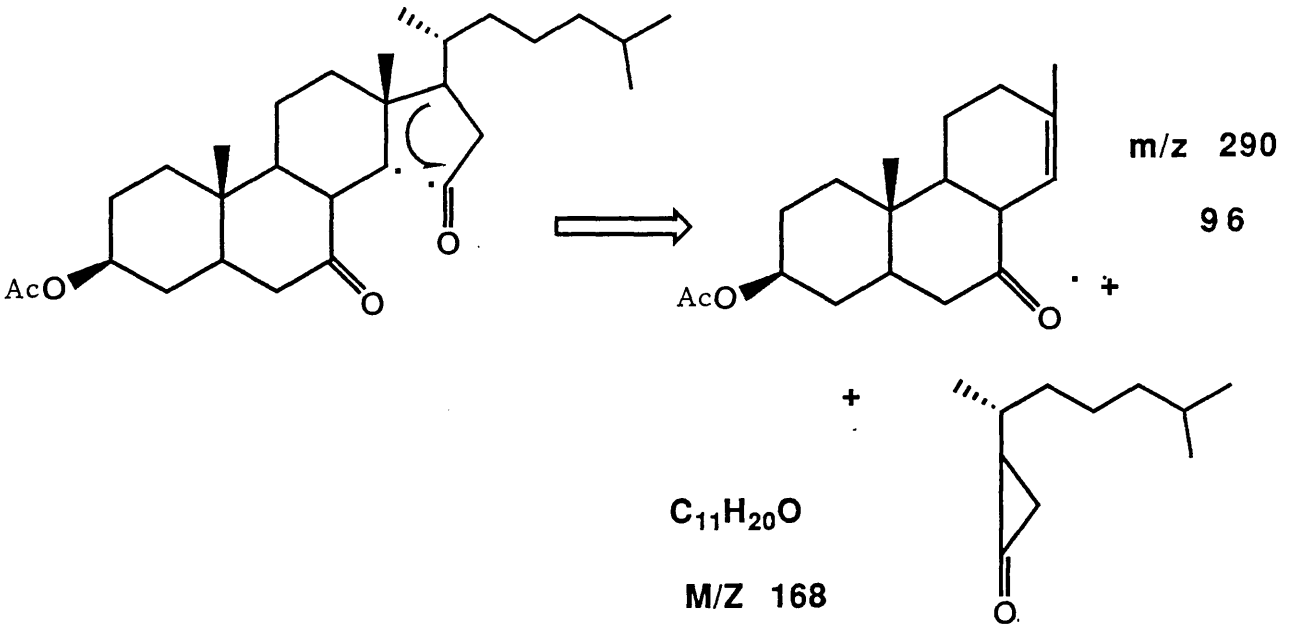
GC-MS failed to separate minor components I = 3393 (2%) and I = 3400 (3%). Two weak molecular ions were observed at m/z 444 and m/z 442 which may be due to a ketone ($C_{29}H_{48}O_3$) and unsaturated ketone ($C_{29}H_{46}O_3$), respectively (Figure 46). One of the compounds, I = 3393, had a similar retention index to 3β-acetoxy-5α-cholestan-7-one (I = 3390) (Table 93).

The molecular ion at m/z 458 of component I = 3430 implies a diketone ($C_{29}H_{46}O_4$). The ΔI for this compound compared to 3β-acetoxy-5α-cholestan-7-one is only 40 units higher, indicating one of the ketone groups is hindered or forms part of a hydrogen bonded β-diketone. No oxidation has taken place in the steroid side-chain as shown by loss of the side-chain [$M^{+\cdot}$-113] in the mass spectrum (Table 94 and Figure 47). Therefore the two ketone groups are located in the steroid nucleus. This is further confirmed by a retro-Diels-Alder process from m/z 285 to m/z 231 (Scheme 16)



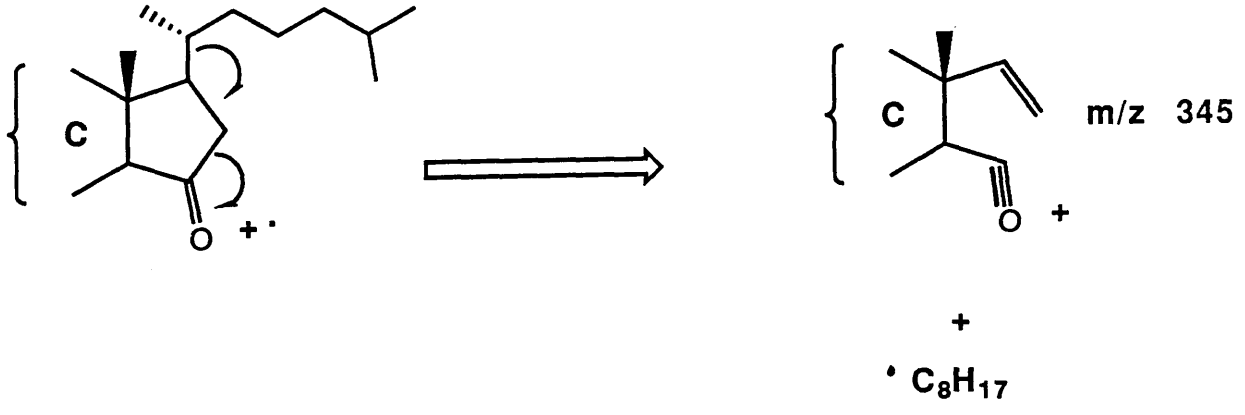
m/z 285 **m/z 231**

The mass spectrum (Table 94 and Figure 47) has a strong peak at m/z 290 [M-168] which may correspond to an ion of type 96 $[C_{16}H_{26}O_3]^{+\cdot}$, for example



Therefore a 7,15-diketone would account for the fragmentation pattern observed and this would also explain the strong ion observed at m/z 345 for loss of the side-chain (Scheme 17)

SCHEME 17



In summary, the major products from oxidation of 5α-cholestan-3β-yl acetate under Schäfer's conditions are degraded steroids in which oxidation has occurred at tertiary side-chain C-H positions giving rise to 3β-acetoxy-5α-androstan-17-one, 3β-acetoxy-5α-pregnan-20-one, and 3β-acetoxy-27 nor-5α-cholestan-25-one. No major oxidation products

were observed in which ring D was selectively attacked (cf. 5α-androstan-3β-yl acetate) although a ketone (3% yield by GLC) and a diketone (5% yield by GLC) were produced that are, as yet, unidentified.

Experimental Section - 5.2.1

CrO_3 oxidation of 5α-cholestan-3β-yl acetate

To a solution of 5α-cholestan-3β-yl acetate (100 mg, 0.23 mmol) in $CH_2C\ell_2$ (2 ml), Ac_2O (0.6 ml) and AcOH (0.6 ml) was added powdered CrO_3 (118 mg, 1.18 mmol). The reaction mixture was stirred at RT for 25h, quenched with sodium metabisulphite and extracted as in Experimental section 5.1.1.

Table 87. Capillary GLC data of products from 5α-cholestan-3β-yl acetate reaction with CrO_3

Retention time min (%)	I	Compound identified
11.67 (6%)	-	3β-acetoxy-5α-androstan-17-one
14.41 (12%)	-	3β-acetoxy-5α-pregnan-20-one
22.75 (49%)	3191	5α-cholestan-3β-yl acetate ($C_{29}H_{50}O_2$)
24.52 (2%)	3264	No MS
26.78 (13%)	3554	3β-acetoxy 27-nor-5α-cholestan-25-one
27.75 (2%)	3393	mixed MS $[M]^{+\cdot}$ = 444
27.95 (3%)	3400	mixed MS $[M]^{+\cdot}$ = 442
28.66 (5%)	3430	diketone $[M]^{+\cdot}$ = 458 ($C_{29}H_{46}O_4$)
29.14 (3%)	-	
30.25 (3%)	-	

Column : CP-Sil-5cb 25m x 0.13 nm 0.13 μm

Carrier : 3 ml/min He 50:1 injection

Level 1: Prog. rate - 30°C/min
Final temp. - 222°C
Final time - 1 min

Level 2: Prog. rate - 2°C/min
Final temp. - 270°C
Final time - 10 min

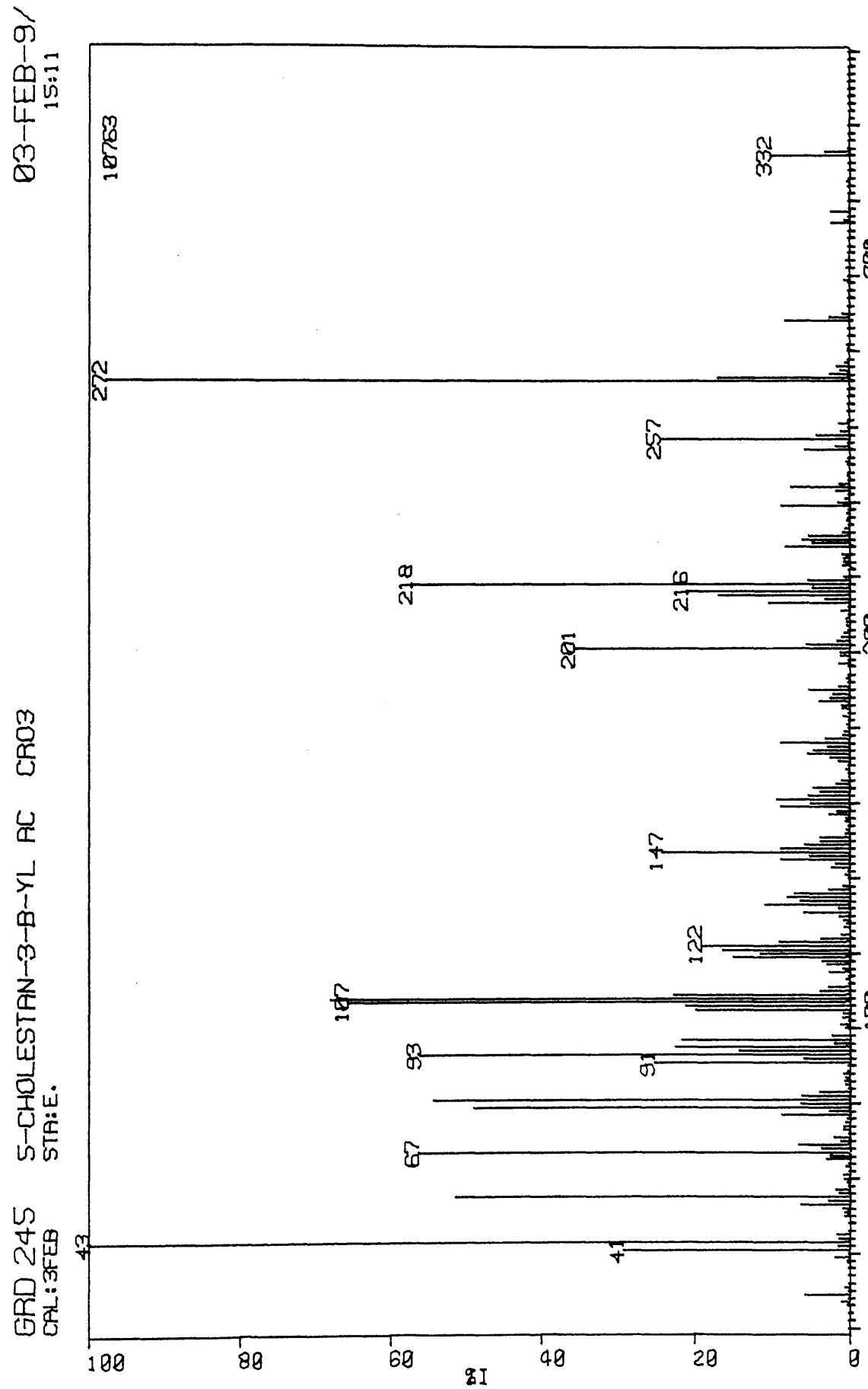
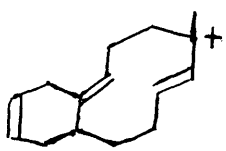


Figure 42. GC-MS (EI) of component 11.67 min. identified as 5α-androstan-3β-yl acetate.

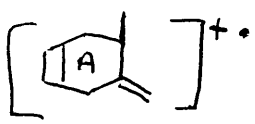
Table 89. Mass spectral data for component 11.67 min. identified as 5α-androstan-3β-yl acetate[113,117]

m/z	Ion type	%
332	$[M]^{+\cdot}$	10
317	$[M^{+\cdot}-CH_3^{\cdot}]$	2
314	$[M^{+\cdot}-H_2O]$	2
288	$[M^{+\cdot}-CH_3CHO]$	8
272	$[M^{+\cdot}-AcOH]$	98
257	$[M^{+\cdot}-AcOH-CH_3]$	25
218	$[M^{+\cdot}$-AcOH-ring A (rDA)	58
201	$[C_{15}H_{21}]^{+}$	36
147	$[C_{11}H_{15}]^{+}$	25
108	$[C_8H_{12}]^{+\cdot}$	66
43	$[CH_3CO]^{+}$	100

m/z 201



m/z 108



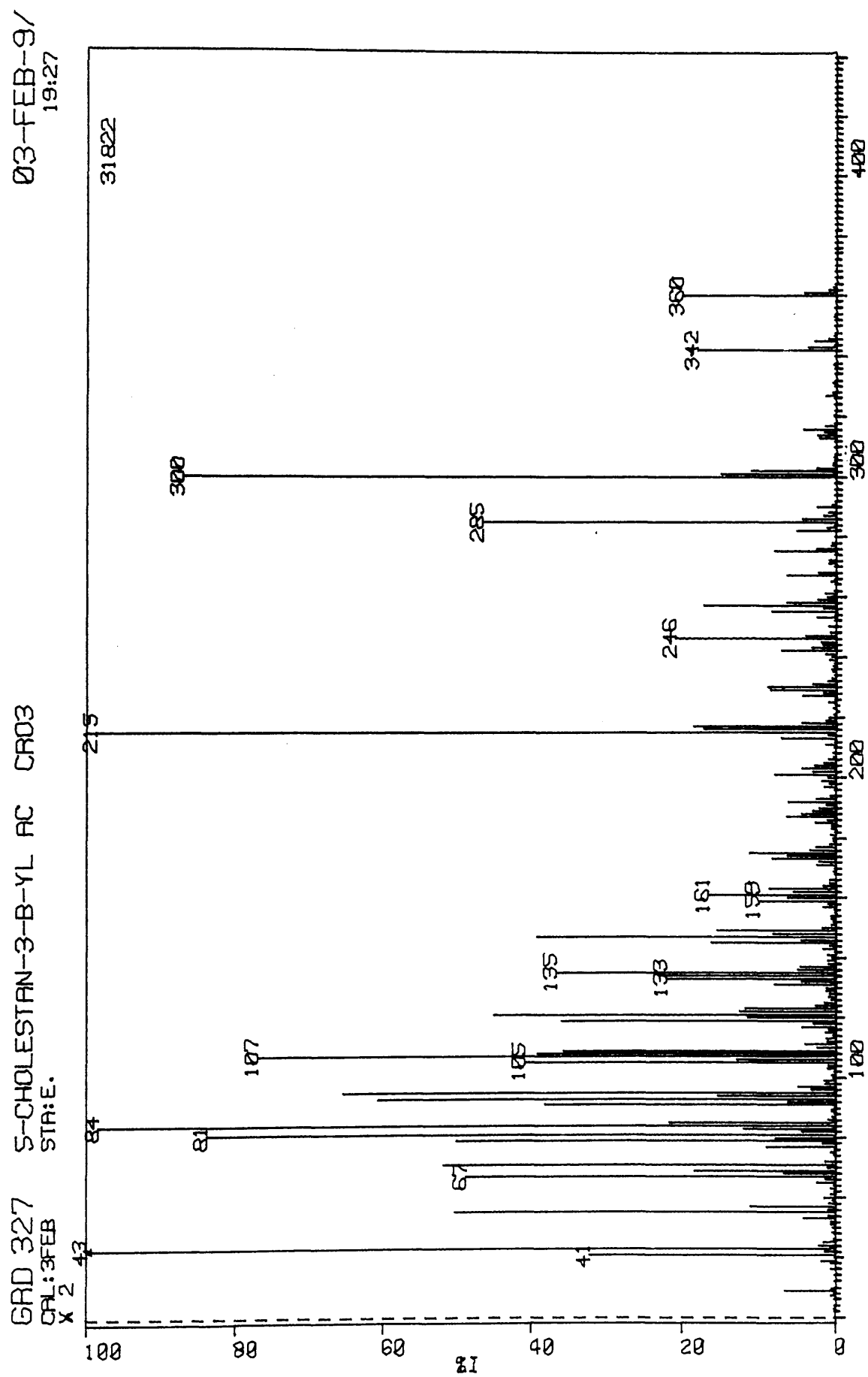
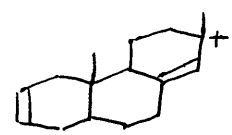


Figure 43. GC-MS (EI) of component 14.41 min. identified as 3β-acetoxy-5α-pregnan-20-one.

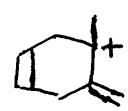
Table 90. Mass spectral data for component 14.41 min. identified as 3β-acetoxy-5α-pregnan-20-one[113]

m/z	Ion type	%
360	$[M]^+$	10
345	$[M^{+\cdot}-CH_3^{\cdot}]$	1
342	$[M^{+\cdot}-H_2O]$	9
327	$[M^{+\cdot}-H_2O-CH_3]$	0.5
316	$[M^{+\cdot}-CH_3CHO]$	2
300	$[M^{+\cdot}-AcOH]$	44
285	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	23
257	$[M^{+\cdot}-AcOH-CH_3\dot{C}O]$	8
246	$[M^{+\cdot}$-AcOH-rDA ring A]	11
215	$[C_{16}H_{23}]^+$	49
107	$[C_8H_{11}]^+$	39
84		49
43	$[CH_3CO]^+$	100

m/z 215



m/z 107



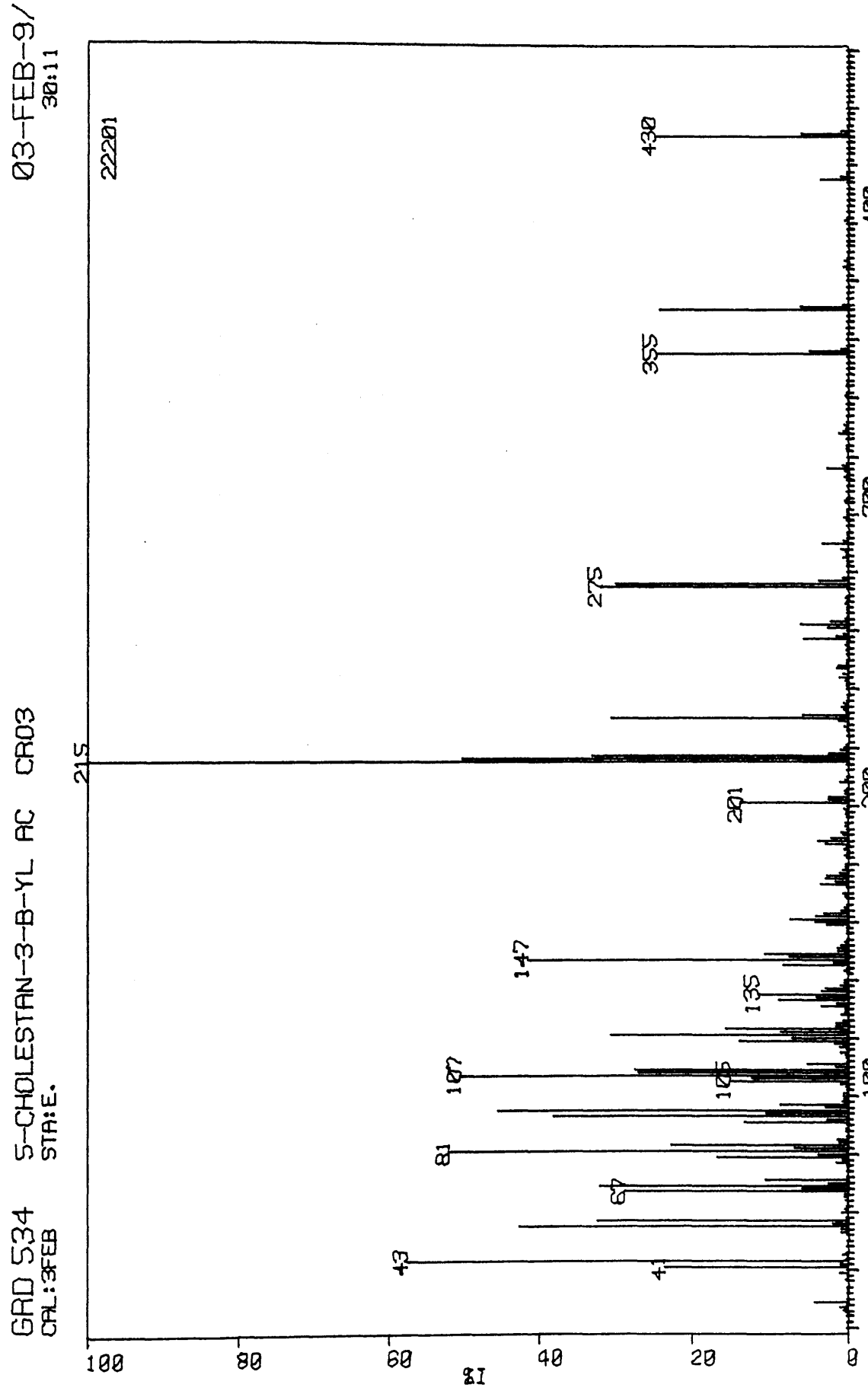
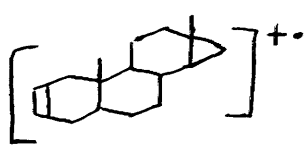
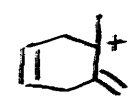


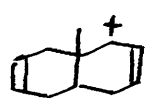
Figure 44. GC-MS (EI) of component I = 3191 identified as 5α-cholestan-3β-yl acetate.

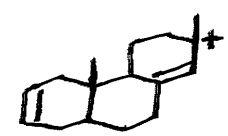
Table 91. Mass spectral data for component I = 3191 identified as 5α-cholestan-3β-yl acetate[113]

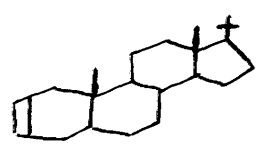
m/z	Ion type	%
430	$[M]^{+\cdot}$	25
415	$[M^{+\cdot}-CH_3^{\cdot}]$	4
370	$[M^{+\cdot}$-AcOH]	25
355	$[M^{+\cdot}$-AcOH-$CH_3^{\cdot}]$	25
316	$[M^{+\cdot}$-AcOH-rDA-ring A]	3
275		32
257	$[M^{+\cdot}$-AcOH-side chain]	6
230	$[C_{17}H_{26}]^{+\cdot}$	31
215	$[C_{16}H_{23}]^{+}$	100
147	$[C_{11}H_{15}]^{+}$	42
107	$[C_8H_{11}]^{+}$	51
81		53
43	$[C_3H_7]^{+}$ or $[CH_3CO]^{+}$	58

m/z 230 

m/z 107 

m/z 147 

m/z 215 

m/z 257 

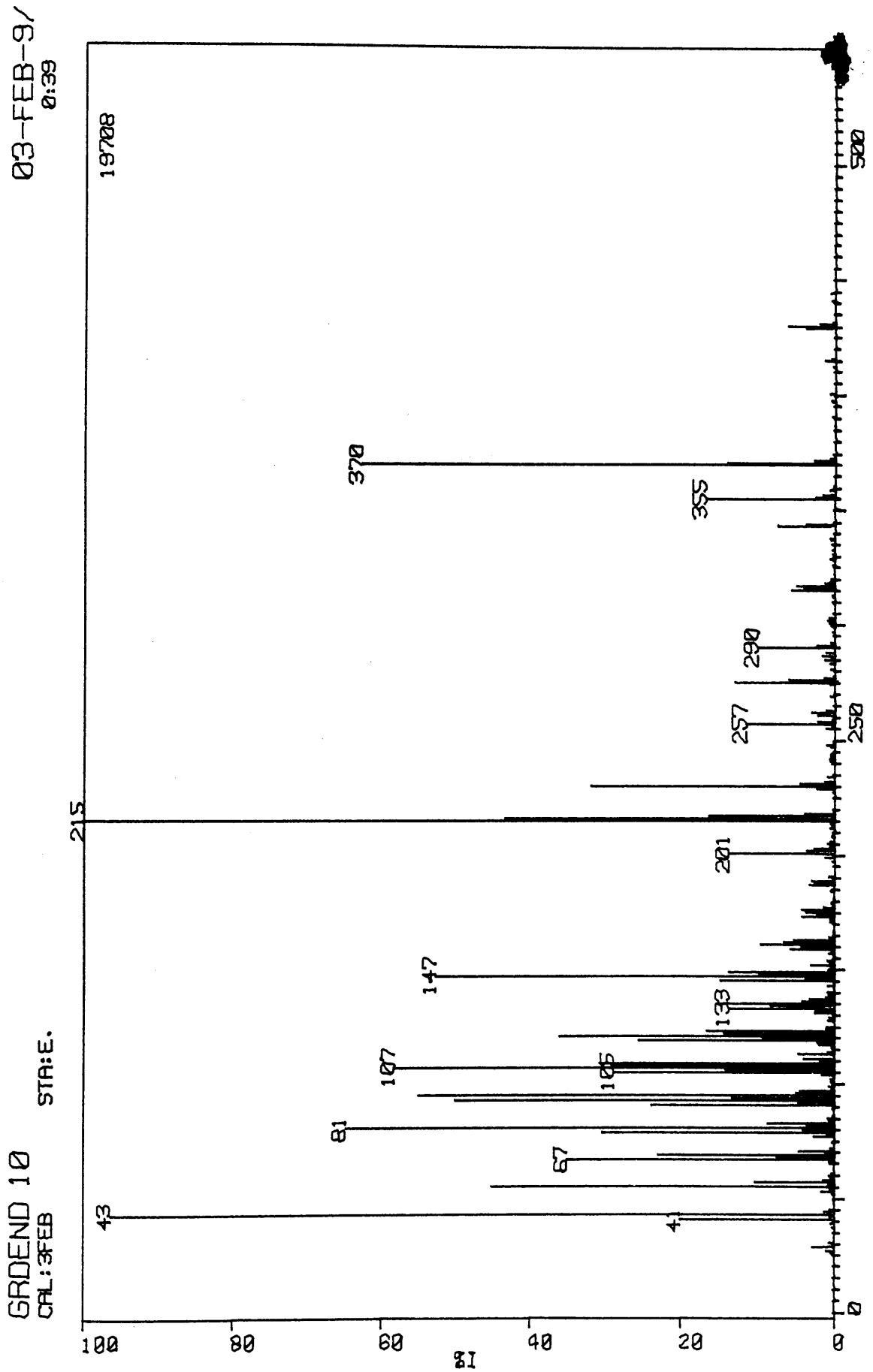


Figure 45. GC-MS (EI) of component I = 3354 identified as 3β-acetoxy-24 nor-5α-cholestan-3β-acetate.

Table 92. Mass spectral data for component I = 3354 identified as 3β-acetoxy-27-nor-5α-cholestan-3β-yl acetate

m/z	Ion type	%
430	$[M]^{+\cdot}$	6
415	$[M^{+\cdot}-CH_3^{\cdot}]$	1
370	$[M^{+\cdot}$-AcOH]	63
355	$[M^{+\cdot}$-AcOH-$CH_3^{\cdot}]$	17
343		7
317	$[M^{+\cdot}$-side chain]	4
315		5
290	$[M^{+\cdot}-140]$ from cleavage through C-13/17 and C-15/16	10
275	290-15	13
257	$[M^{+\cdot}$-AcOH-side chain]	12
230	290-AcOH	32
215	$[C_{16}H_{23}]^+$	100
147	$[C_{11}H_{15}]^+$	54
107	$[C_8H_{11}]^+$	58
43	$[CH_3CO]^+$	94

Table 93. Capillary GLC data

Compound	I
5α-cholestan-3β-yl acetate	3190
3β-acetoxy-5α-cholestan-6-one	3425
3β-acetoxy-5α-cholestan-7-one	3390
3β-acetoxy-27-nor-cholest-5-en-25-one	3341
3β-acetoxy-27-nor-5α-cholestan-25-one	3352

Conditions as in Table 87

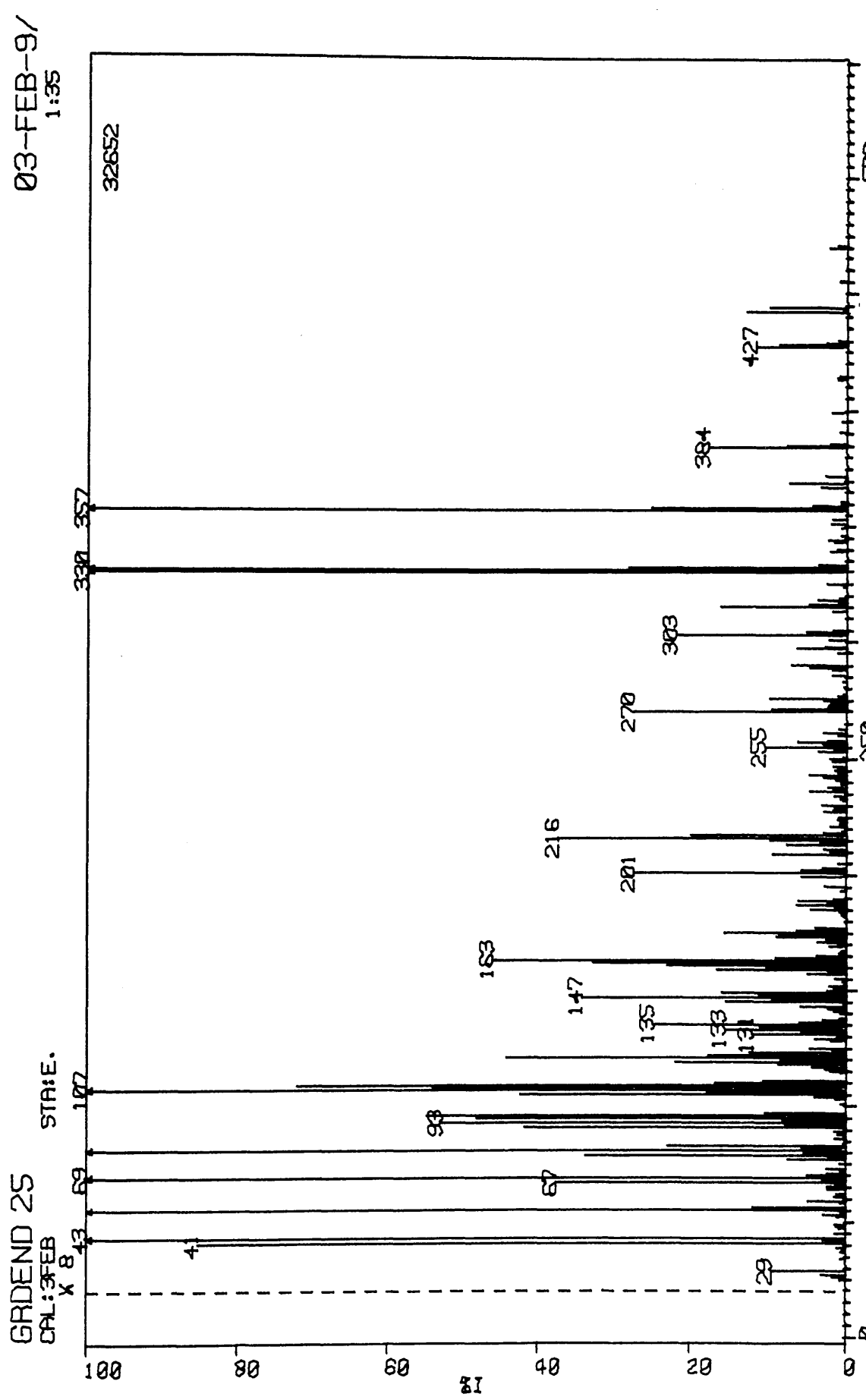


Figure 46. GC-MS (EI) of component I = 3390 and I = 3400.

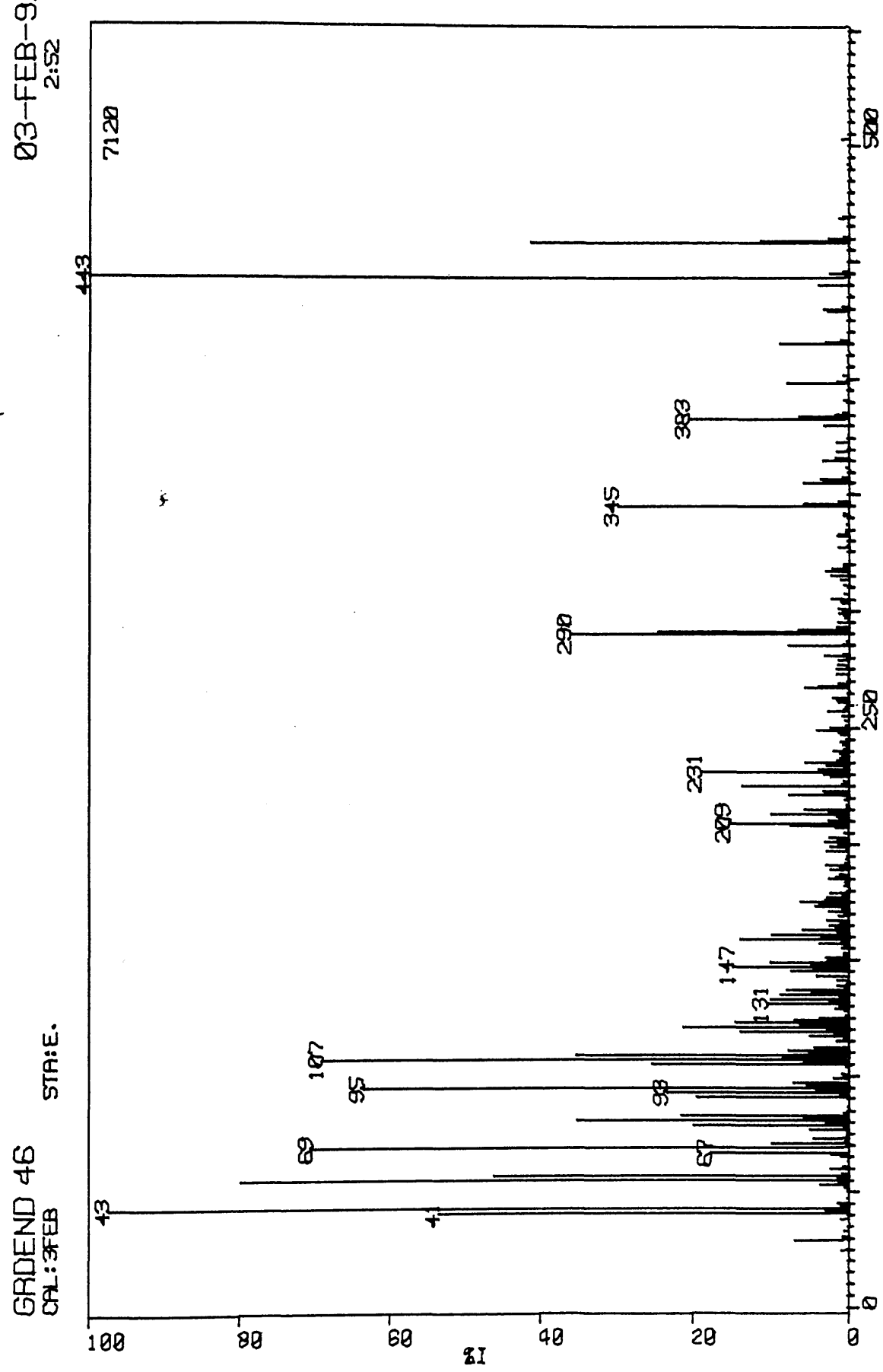


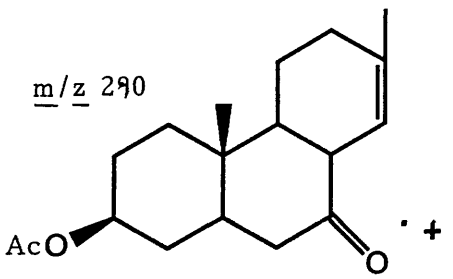
Figure 47. GC-MS (EI) of component I = 3430 identified as 3β-acetoxy-5α-cholestan dione.

Table 94. Mass spectral data for component (I = 3430) provisionally identified as a 3β-acetoxy-5α-cholestanedione (scan 46)

$(C_{29}H_{46}O_4)$

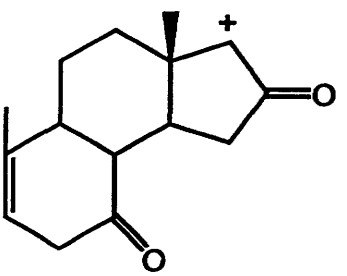
m/z	Ion type	%
458	$[M]^{+\cdot}$	42
443	$[M^{+\cdot}-CH_3^{\cdot}]$	100
415	$[M^{+\cdot}-43]$	9
398	$[M^{+\cdot}-AcOH]$	8
383	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	21
345	$[M^{+\cdot}$-side chain]	30
290	$[C_{18}H_{26}O_3]^+$	37
285	$[C_{19}H_{25}O_2]^+$	8
231	$[C_{15}H_{19}O_2]^+$	19
107	$[C_8H_{11}]^+$	70
43	$[C_3H_7]^+$ or $[CH_3CO]^+$	97

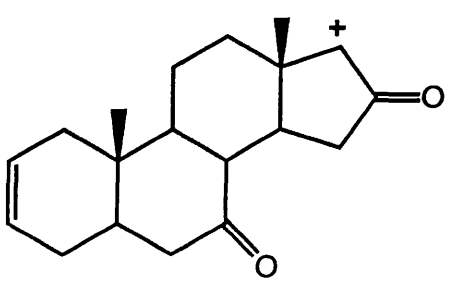
m/z 290



m/z 285

m/z 231





Concluding Section

In an attempted oxidative cyclisation of 25-hydroxy-5α-lanost-8-en-3β-yl acetate using ceric ammonium nitrate, the major products resulted from oxidation at the allylic positions, and included an interesting nitrogenous product. A better substrate would be the saturated steroid, 25-hydroxy-5α-lanostan-3β-yl acetate. However, the synthesis of this derivative would require CrO_3 oxidation to give the Δ^8-7,11-diketone, followed by Birch reduction of the Δ^8-double bond and finally Wolff-Kishner (or Huang-Minlon) reduction of the 7- and the sterically hindered 11-ketone groups. It was also shown that the same type of oxidation products were formed when 5α-lanost-8-en-3β-yl acetate was subjected to the CAN conditions.

As mentioned in Section 3.4, the functionalisation of the C-30 methyl group in lanosterol is important for the biosynthesis of cholesterol. Functionalisation of 7α-hydroxy-5α-lanostan-3β-yl acetate was achieved using $Pb(OAc)_4$ and ceric ammonium nitrate whereas, under more reactive conditions, with $Pb(OAc)_4/I_2/h\nu$ gave a variety of oxidation products. If time permitted, other oxidising agents, such as trivalent iodine species, e.g. $PhI(OAc)_2$, would have been used to attempt these oxidative cyclisations.

Under the template directed radical relay chlorination, introduced by Breslow, 3-pyridylmethyl-3α-acetoxy-5β-cholan-24-oate gave a mixture of chlorinated products which were found difficult to separate. A more rigid template is required where there are small numbers of "degrees of freedom". One possibility may be the 3-pyridylacetate ester attached to the 7α-OH position in the bile acid series. This

derivative, methyl-3α-acetoxy,7α-(3-pyridylacetoxy)-5β-cholan-24-oate, would then be able to deliver a chlorine atom to the C-17 position which could then be dehydrochlorinated to give a mixture of the $\Delta^{17(20)}$ and Δ^{16}-olefins. Removal of the steroid side-chain could then be easily envisaged.

It was shown that the CrO_3 oxidation of 5α-androstan-3β-yl acetate produces 3β-acetoxy-5α-androst-14-en-16-one and 3β-acetoxy-5α-androstan-16-one as the major products, whereas similar oxidation of 5α-cholestan-3β-yl acetate gives degraded steroids as the major products. Future work in this area would involve using other oxidising agents, such as the Gif system or MMPP (catalysed by manganese porphyrins) both known to oxidise unactivated positions.

APPENDIX

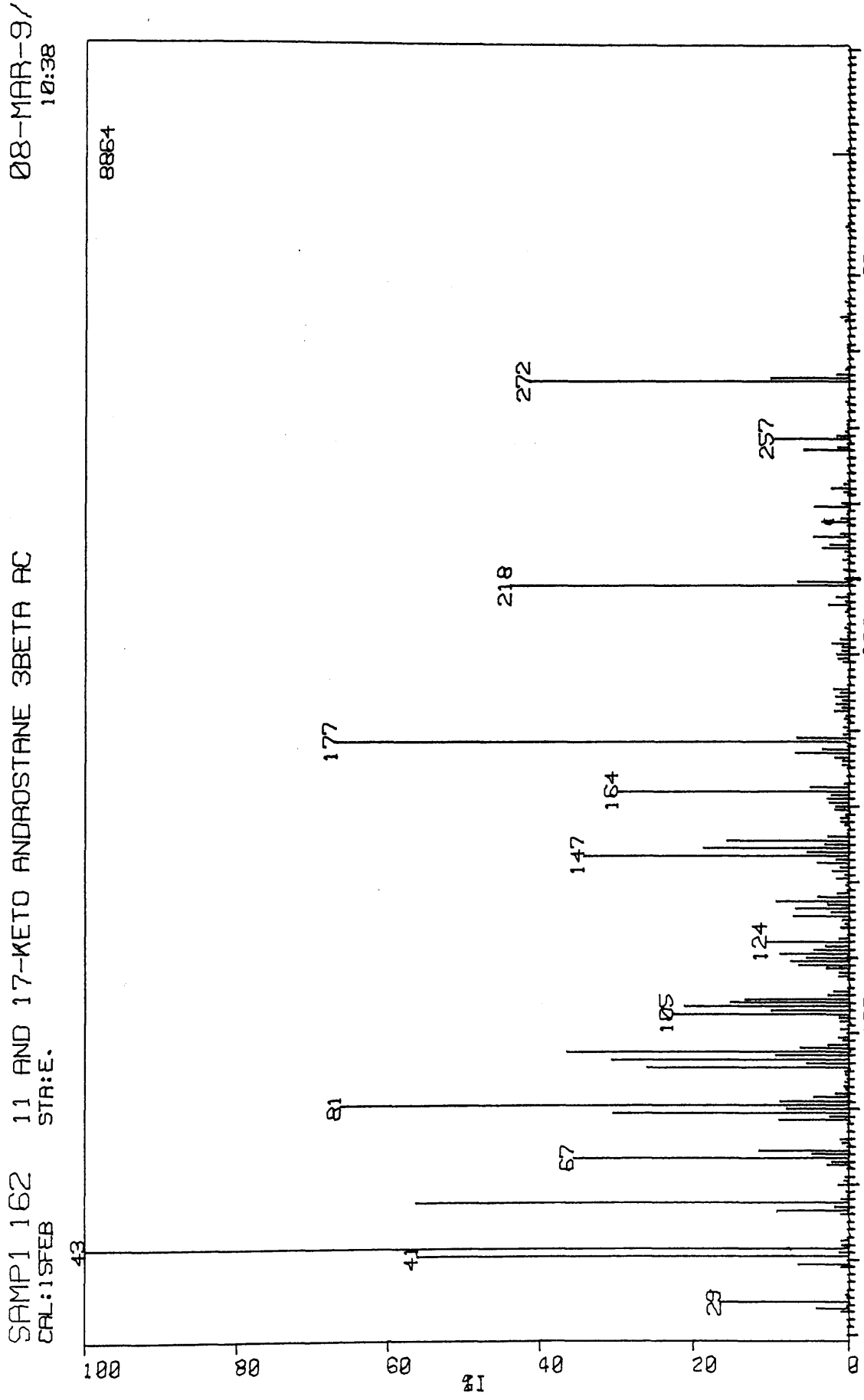
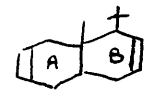
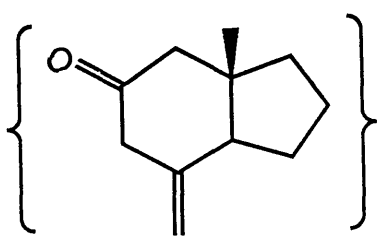


Figure 26. GC-MS (EI) of 3β-acetoxy-5α-androstan-11-one, I = 2484.

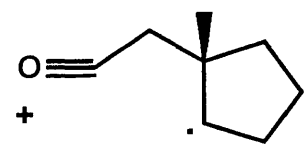
Table 75. Mass spectral data for 3β-acetoxy-5α-androstan-11-one (I = 2484)[112,113]

SAMP 1 162

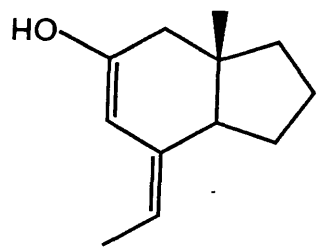
m/z	Ion type	%
332	$[M]^{+\cdot}$	2
272	$[M^{+\cdot}$-AcOH]	42
257	$[M^{+\cdot}$-AcOH-$\dot{C}H_3$]	10
218	$[M^{+\cdot}$-AcOH-rDA ring A]	44
177	cleavage through C-9,10 and C-5,6	68
164	cleavage through C-6,7 and C-9,10	30
147	cleavage through C-8,14 and C-9,11 (A+B) 	35
124	cleavage through C-8,14 and C-9,11 (C+D)	11
107	$[C_8H_4]^+$	12
105		13
95		37
81		67
43	$[CH_3CO]^+$	100



m/z 164



m/z 124



m/z 177

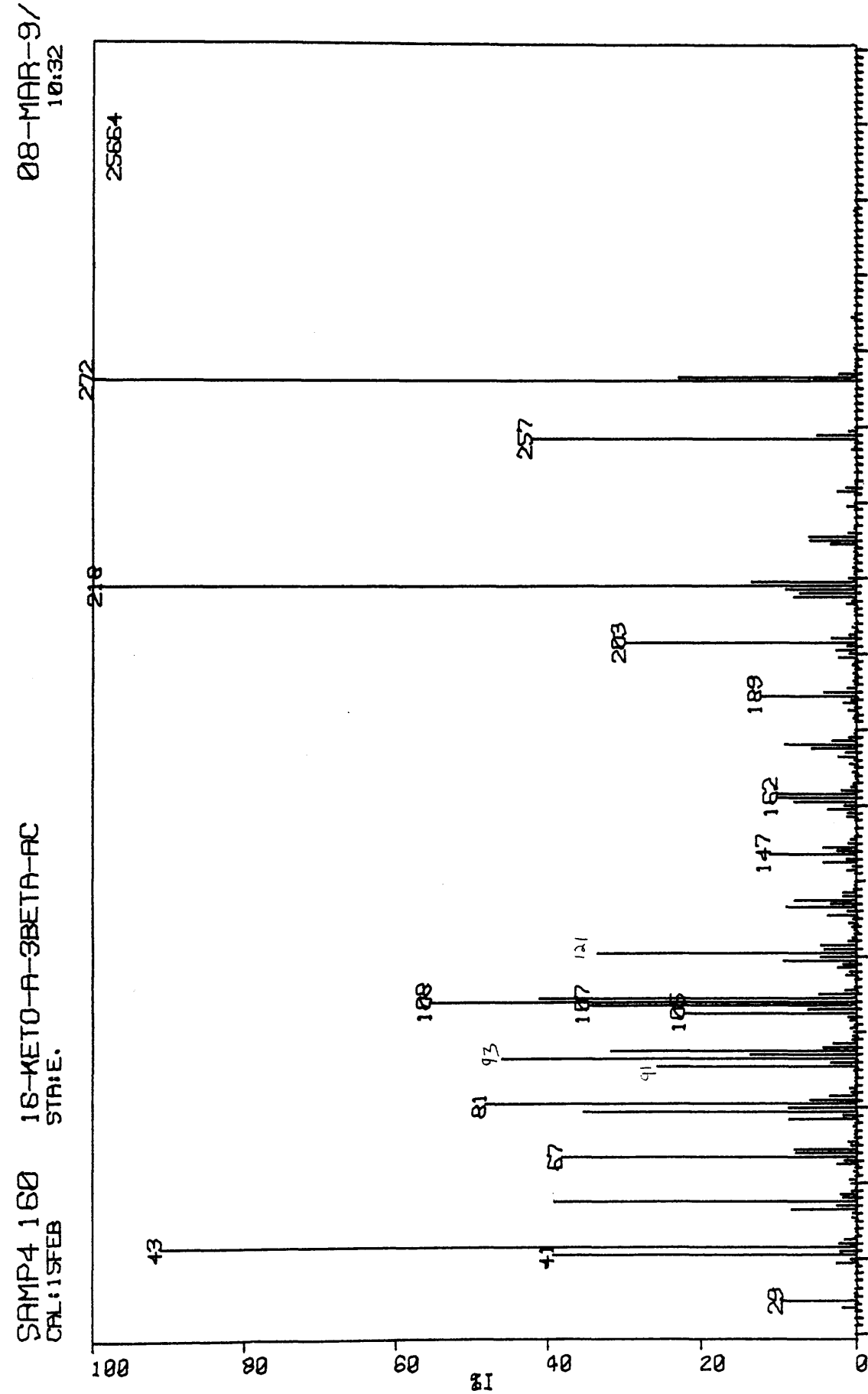
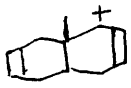
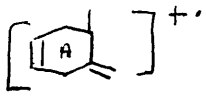


Figure 27. GC-MS (EI) of 3β-acetoxy-5α-androstan-16-one, I = 2586.

Table 76. Mass spectral data for 3β-acetoxy-5α-androstan-16-one (I = 2586)[113,114]

SAMP 4 160

m/z	Ion type	%
272	$[M^{+\cdot}-AcOH]$	100
257	$[M^{+\cdot}-AcOH-\dot{C}H_3]$	43
243		3
201		6
200		6
218	$[M^{+\cdot}$-AcOH-rDA ring A]	100
203	$[M^{+\cdot}$-AcOH-rDA ring A-$CH_3^{\cdot}$]	30
189		13
176		9
162		11
147		12
121		34
108	$[C_8H_{12}]^{+\cdot}$ 	56
93		46
91		26
81		49
43	$[CH_3CO]^+$	92

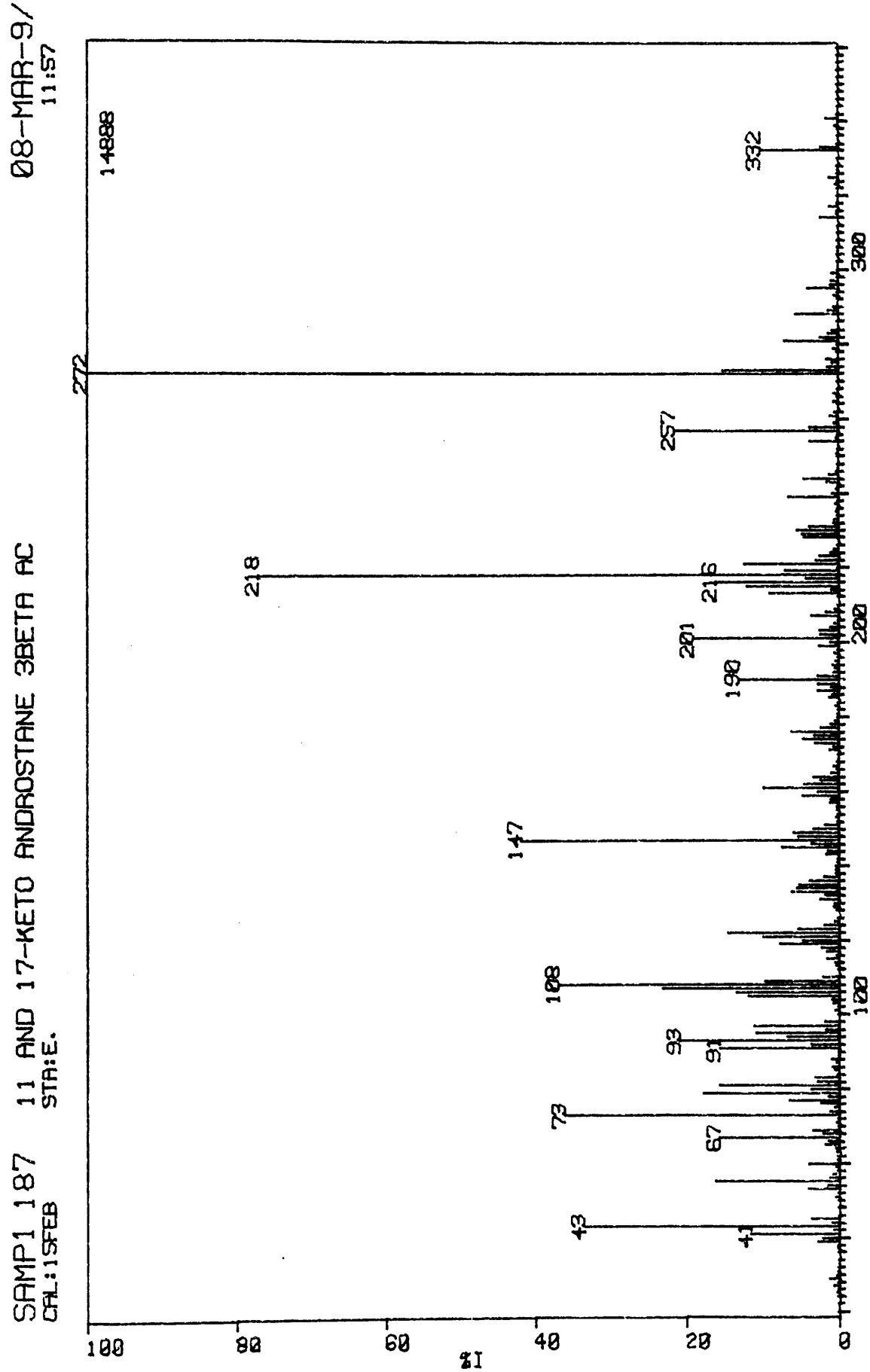
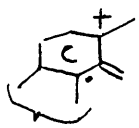


Figure 28. GC-MS (EI) of 3β-acetoxy-5α-androstan-17-one, I = 2584.

Table 77. Mass spectral data for 3β-acetoxy-5α-androstan-17-one ($\underline{I}$ = 2584)[113]

m/z	Ion type	%
332	$[M]^{+\cdot}$	11
317	$[M^{+\cdot}-CH_3^{\cdot}]$	1
314	$[M^{+\cdot}-H_2O]$	3
293		4
288	 $[M^{+\cdot}-CH_3CHO]$	6
281		7
272	$[M^{+\cdot}-AcOH]$	100
257	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	22
218	$[M^{+\cdot}-AcOH$-ring A(rDA)]	80
216	[M-ring D-AcOH $[C_{16}H_{24}]^{+}$	17
201	[M-ring D-AcOH-$CH_3^{\cdot}$]	19
190		14
147		43
108		38
73		36
43	$[CH_3CO]^{+}$	34

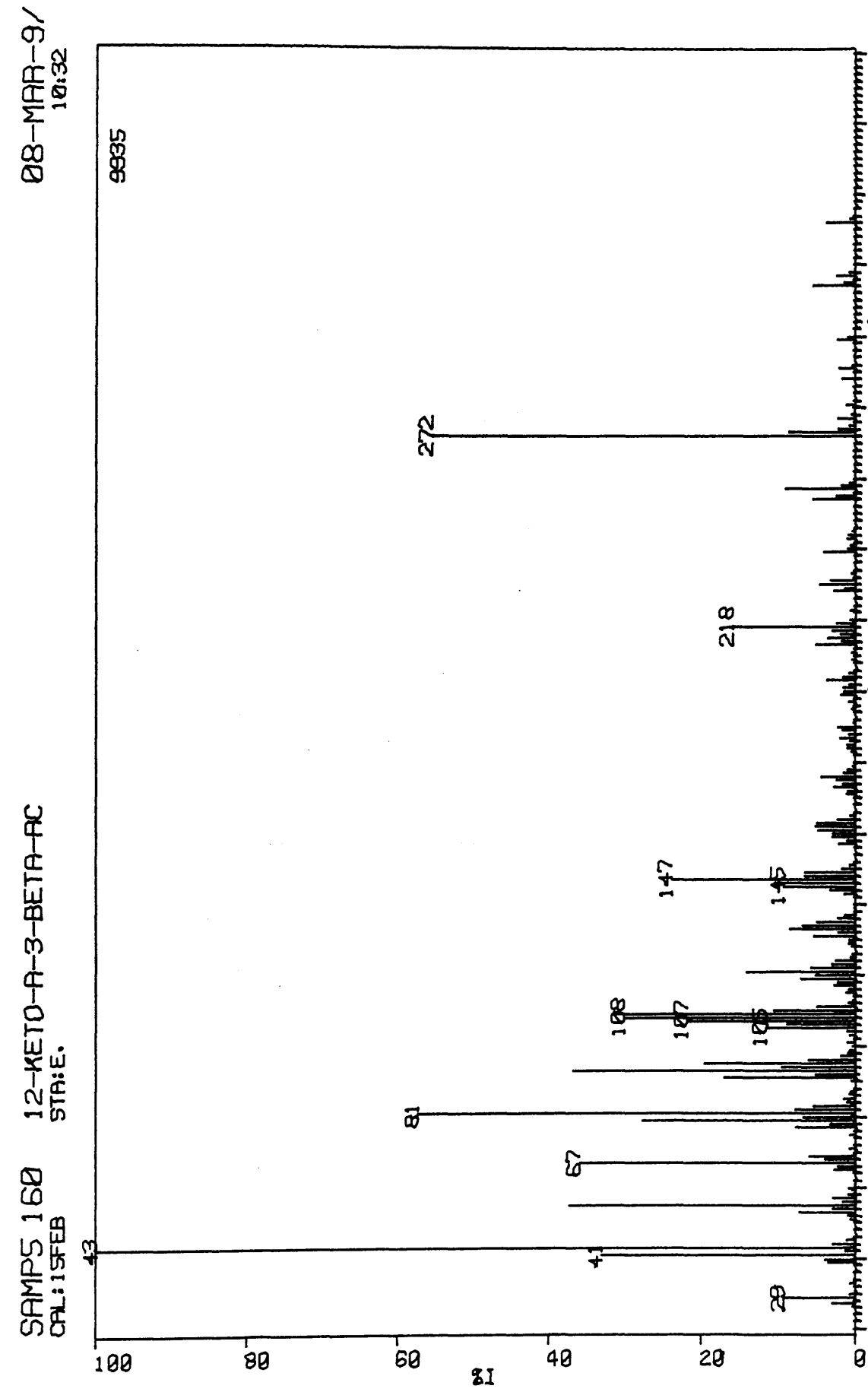


Figure 29. GC-MS (EI) of 3β-acetoxy-5α-androstan-12-one, I = 2589.

Table 78. Mass spectral data for 3β-acetoxy-5α-androstan-12-one (I = 2589)[113]

SAMP 5 160

m/z	Ion type	%
332	$[M^{+\cdot}]$	4
317	$[M^{+\cdot}-CH_3^{\cdot}]$	2
314	$[M^{+\cdot}-H_2O]$	6
272	$[M^{+\cdot}-AcOH]$	56
257	$[M^{+\cdot}-AcOH-CH_3^{\cdot}]$	9
218	$[M^{+\cdot}$-AcOH-rDA ring A]	16
147		24
108		30
81		58
43	$[CH_3CO]^+$	100

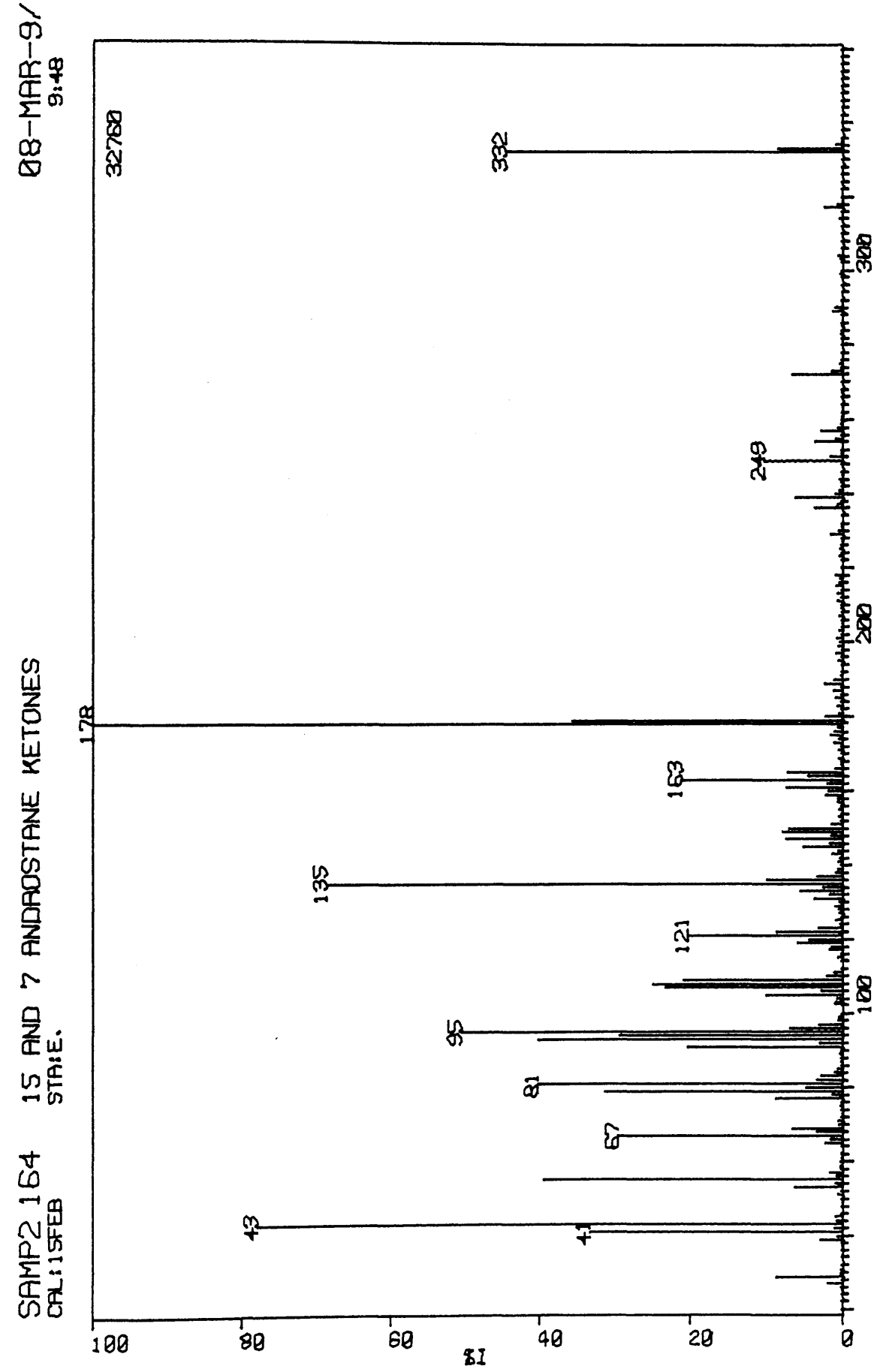
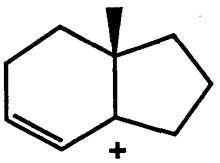


Figure 30. GC-MS (EI) of 3β-acetoxy-5α-androstan-7-one, I = 2540.

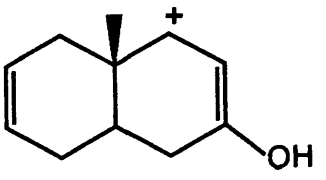
Table 79. Mass spectral data for 3β-acetoxy-5α-androstan-7-one (I = 2540)[113]

SAMP 2 164

m/z	Ion type	%
332	$[M]^{+\cdot}$	45
317	$[M^{+\cdot}-CH_3^{\cdot}]$	2
272	$[M^{+\cdot}-AcOH]$	7
249		10
178	cleavage through C-5,6 and C-9,10 (C+D)	100
163	$[C_{11}H_{15}O]^{+}$	21
135	$[C_{10}H_{15}]^{+}$	69
121		20
95		51
81		41
43	$[CH_3CO]^{+}$	80



m/z 135



m/z 163

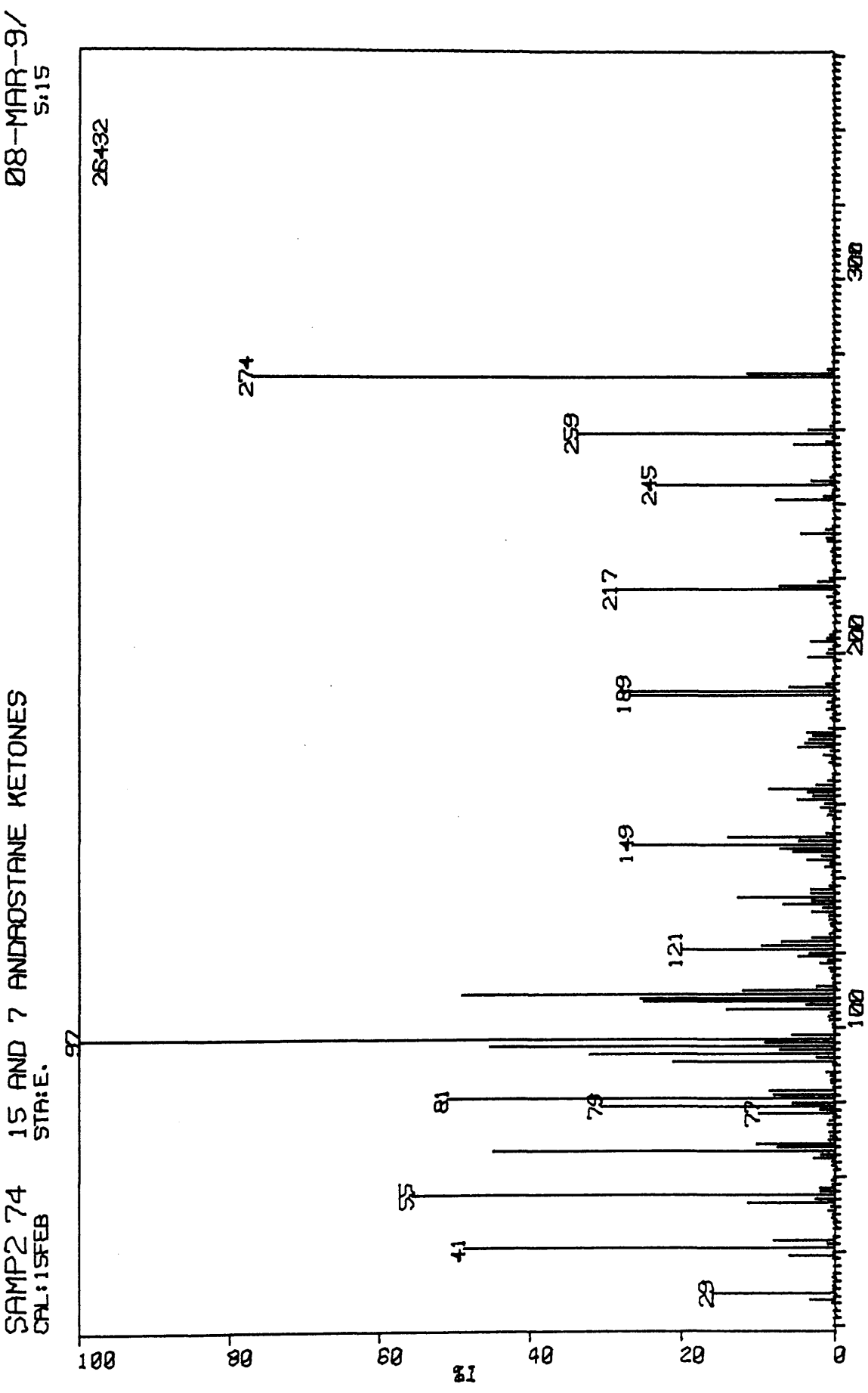


Figure 31. GC-MS (EI) of 5α,14α-androstan-15-one, I = 2206.

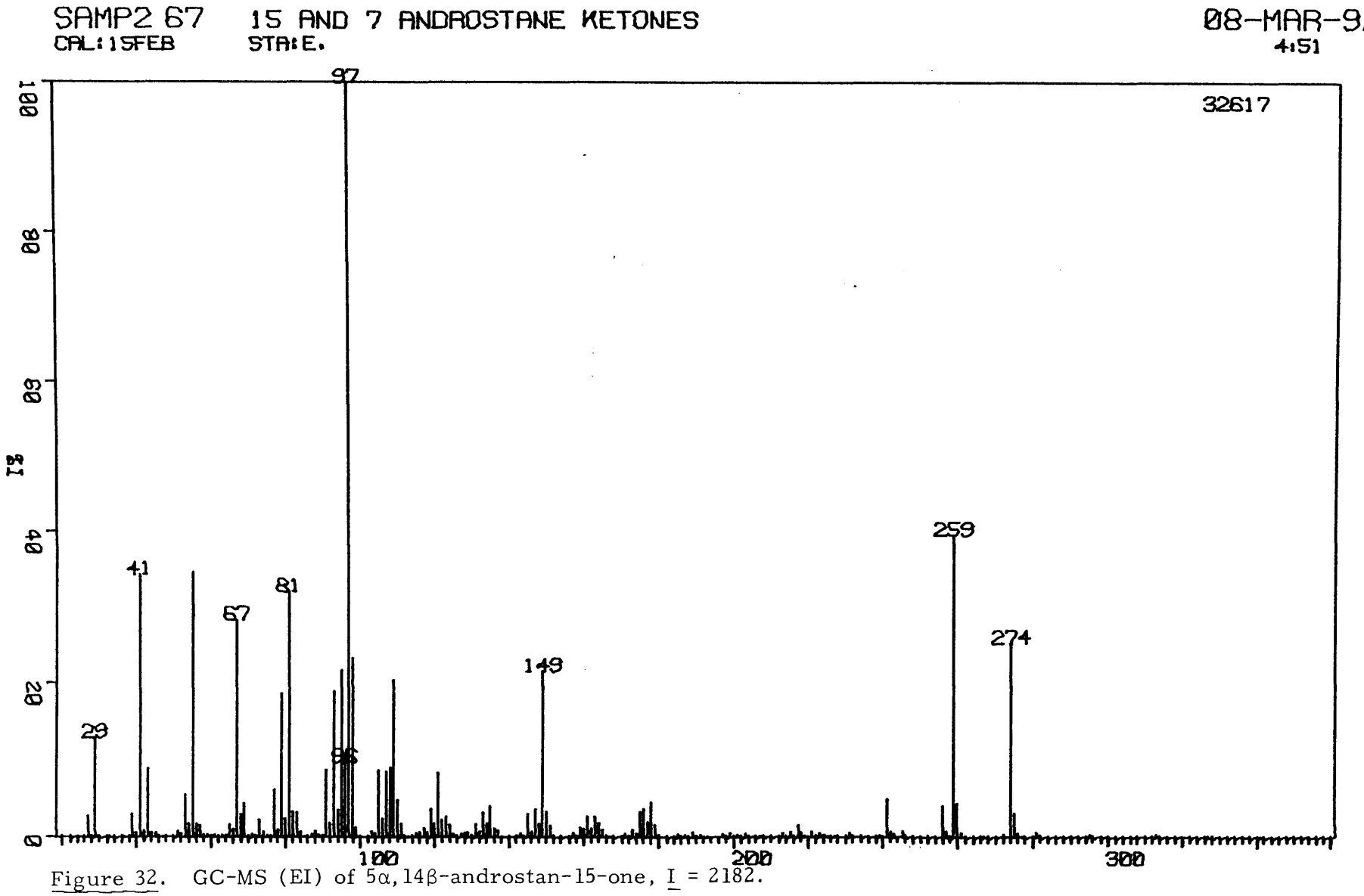
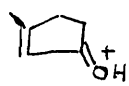


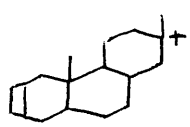
Figure 32. GC-MS (EI) of 5α,14β-androstan-15-one, I = 2182.

Table 80. Mass spectral data for 5α,14α-androstan-15-one (I = 2206)[111,113]

SAMP 2 74

m/z	Ion type	%
274	$[M]^{+\cdot}$	78
259	$[M^{+\cdot}-CH_3^{\cdot}]$	34
245	$[M^{+\cdot}-C_2H_5^{\cdot}]$	24
217	$[M^{+\cdot}-57]$ $[C_{16}H_{25}]^{+}$	30
190	$[C_{14}H_{27}]^{+\cdot}$	28
189	$[C_{14}H_{21}]^{+}$	27
151		14
109		49
97		100

m/z 217

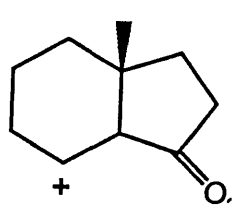


$[C_{16}H_{25}]^{+}$

190 $[C_{14}H_{22}]^{+\cdot}$

189 $[C_{14}H_{21}]^{+}$

} multiple band ruptures



m/z 151

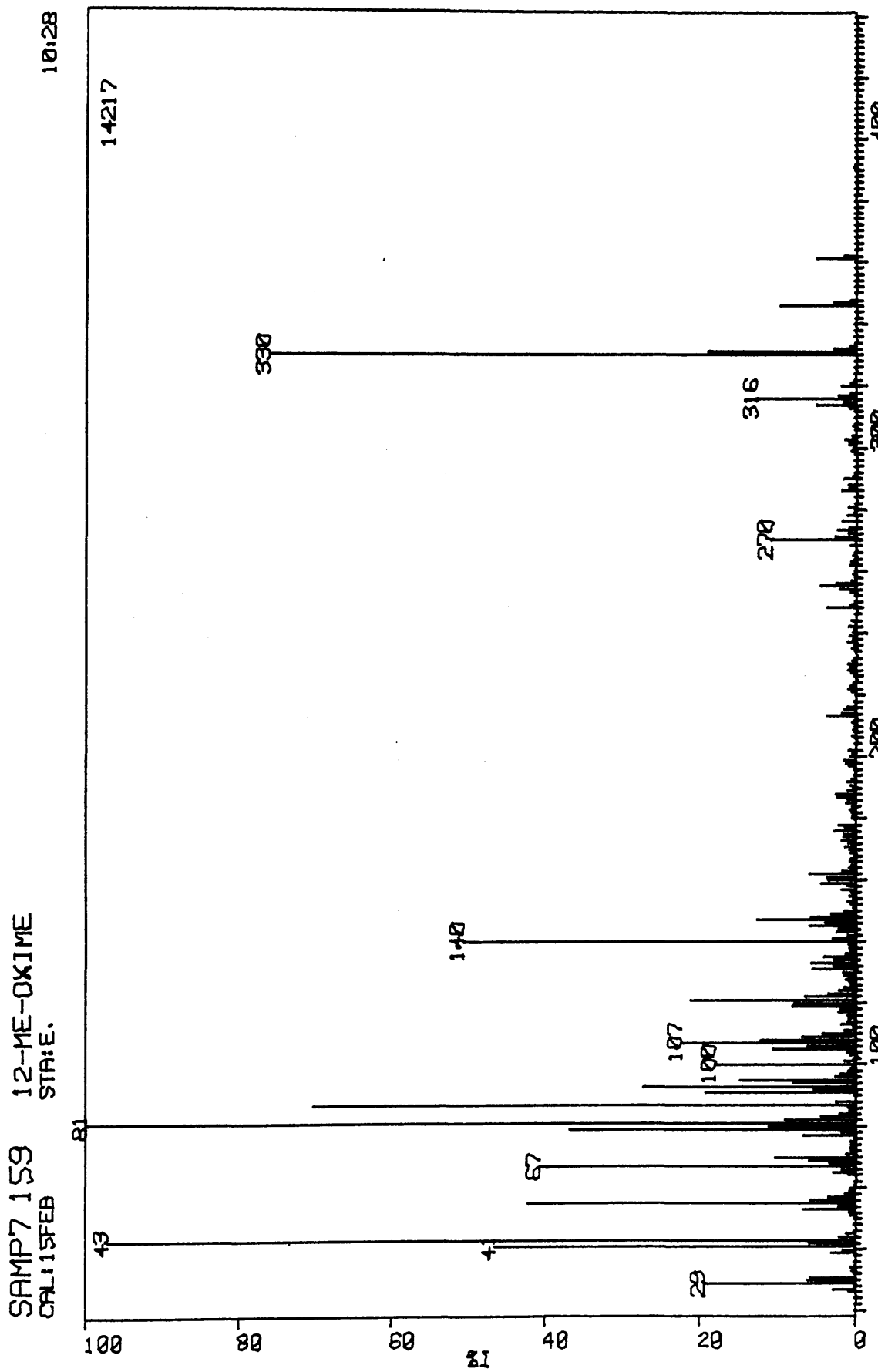


Figure 33. GC-MS (EI) of 3β-acetoxy-5α-androstan-12-one methyl oxime, I = 2597.

Table 81. Mass spectral data for 5α,14β-androstan-15-one (I = 2182)[111,113]

SAMP 2 67

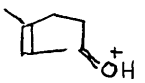
m/z	Ion type	%
274	$[M]^{+\cdot}$	26
259	$[M^{+\cdot}-CH_3^{\cdot}]$	40
241	$[M^{+\cdot}-CH_3^{\cdot}-H_2O]$	5
149	$[C_{11}H_{17}]^{+}$	22
97	$[C_6H_9O]^{+}$ 	100

Table 82. Mass spectral data for 3β-acetoxy-5α-androstan-12-one methyl oxime (I = 2597)

SAMP 7 159 one isomer

m/z	Ion type	%
361	$[M]^{+\cdot}$	5
346	$[M^{+\cdot}-CH_3^{\cdot}]$	10
330	$[M^{+\cdot}-\dot{O}CH_3]$	77
316	$[M^{+\cdot}-(NOCH_3)]$	14
270	$[M^{+\cdot}-AcOH-\dot{O}CH_3]$	12
140		51
81		100
43	$[CH_3CO]^{+}$	98

GLC data for reference compounds (5α-androstanes and androst-5-enes)

Compound	$I^{OV-1}_{215°C}$	ΔI 3β-OH → 3β-OAc
5α-androstane	2050	
5α-androstan-3β-ol	2270	+ 130
5α-androstan-3β-yl acetate	2400	
3β-hydroxy-5α-androstan-17-one	2480	+ 140
3β-acetoxy-5α-androstan-17-one	2620	
3β-hydroxy-androst-5-en-16-one	2470	+ 130
3β-acetoxy-androst-5-en-16-one	2600	
androst-5-en-3β-ol	2260	+ 130
androst-5-en-3β-yl acetate	2390	
3β-acetoxy-5α-androst-14-en-16-one	2730	
3β-hydroxy-5α-androstan-7,17-dione	2645	+ 105
3β-acetoxy-5α-androstan-7,17-dione	2750	
5α-androst-2-en-17-one	2250	

Column: 6 ft 1% OV-1 at 215°C

Carrier gas: N_2 40 ml/min

Detector: FID

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