A Thesis entitled

PARTIAL SYNTHESIS AND REGIOSELECTIVE REDUCTION OF STEROIDAL 11,12-SECO-DIOIC ANHYDRIDES

Submitted in part fulfilment of the requirements for admittance to the

Degree of
Doctor of Philosophy

by

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TO MY MOTHER

AND THE MEMORY OF MY LATE FATHER

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Purification Techniques

SUMMARY

12-seco-5β-cholane-11,12,24-trioic acid in 42% yield, along with two other compounds identified as 3α-hydroxy-11,12-seco-12-nor-5β-cholane-11,24- dioic acid (25%) and 3α,13-dihydroxy-11, 12-seco-12-nor-5β-cholane- 11,24- dioic acid (32%).

Ozonolysis of methyl 3α-ethoxycarbonyloxy-5β-chol-11-enoate afforded the3α-ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12, 24-trioic acid 24-methyl ester, in 25% yield, which was converted to the desired 11,12-seco-dioic anhydride by treatment with acetic anhydride. Reduction of the obtained anhydride with $NaBH_4$ in THF gave in total regio- selectivity the 3α-ethoxycarbonyloxy-11,12-seco-12-hydroxy-5β-cholane- 11,24-dioic acid 24-methyl ester. Treatment of the hydroxy-acid with acetic anhydride led to the corresponding acetoxy-acid, 3α-ethoxycarbonyloxy-11,12-seco-12-acetoxy-5β-cholane -11, 24-dioic acid 24-methyl ester.

Oxidation of methyl 12β-hydroxy-11-oxo-5β-cholanoate with $NaIO_4$ /CrO_3 afforded the corresponding 11,12-seco-dioic acid in good

yield. Treatment with acetic anhydride led to the desired 11,12-seco-5β-cholane-11,12,24-trioic acid-11,12-anhydride 24-methyl ester. Reduction of the anhydride with $NaBH_4$ in THF gave exclusively the 11,12-seco-12-hydroxy-5β-cholane-11,24-dioic acid 24-methyl ester. Treatment of the latter with acetic anhydride afforded the corresponding lactone, methyl 12-oxa-C-homo-11-oxo-5β-cholanoate, in only 6% yield and the acetoxy-acid, 11,12-seco-12-acetoxy-5β-cholane-11,24-dioic acid 24-methyl ester, as the major product.

In the 5α-series, hecogenin was chosen as the starting material. The corresponding 3β-acetoxy- and 3β-benzoyloxy-12-hydroxy- 11-ones derivatives were prepared through the corresponding bromo-ketones. Oxidation of these ketols with $NaIO_4$ /CrO_3 gave the corresponding 11,12-seco-dioic acids which led, upon treatment with acetic anhydride, to the desired 11,12-seco-dioic anhydrides.

Reduction of the obtained 3β-acetoxy-11,12-secotigogenin -11,12-dioic anhydride with $NaBH_4$ in THF gave in total selectivity the

corresponding hydroxy-acid, 3α-acetoxy-11,12-secotigogenin-12-hydroxy-11-oic acid. Treatment of the latter with acetic anhydride afforded in a quantitative yield the corresponding 11,12-seco-12-acetoxy-11-oic acid derivative. When the hydroxy acid was treated with p-toluenesulphonic acid in xylene, 5% of the corresponding lactone was obtained. Treatment of the hydroxy-acid with 2,2'-dipyridyl disulphide in dry benzene afforded 14% of the corresponding lactone.

Gas chromatography technique has been used for qualitative and quantitative analysis. Detailed GLC data of bile acid and hecogenin derivatives, which have been studied in this work, are given in tables 1,4,5,6,7,10,11,12a, and 12b. Mass spectrometry has been also very useful for the identification of different compounds. Interpretations of mass spectral data of all new compounds are given in figures at the appropriate places in Chapter 2. Detailed mass spectral data of most of the investigated derivatives are listed in tables in the Appendix.

In conclusion, 11,12-seco-dioic anhydrides, in both 5α- and 5β-series, having different substituents at C-3 have been prepared.

Treatment of those anhydrides with $NaBH_4$ has led to the reduction in total regioselectivity at C-12. Neither the substituents at C-3 nor the type of A/B ring fusion seem to have an effect on the course of regioselectivity. A rationalisation for this selectivity, in the hydride reduction of these anhydrides, is offered in 2.16. It has also been observed that the products from the reduction process are the corresponding hydroxy-acids.. These hydroxy-acids have proved to resist attempts for cyclisation to the corresponding lactones.

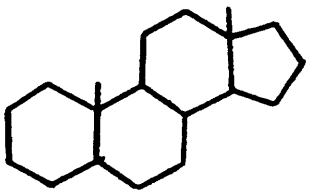
CHAPTER 1

INTRODUCTION

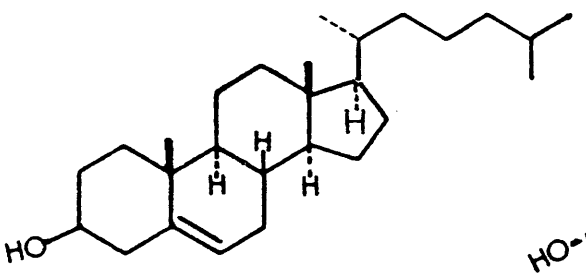
1.1 Steroids

Steroids are a group of important naturally occurring compounds containing a hydrogenated 1,2-cyclopentenophenanthrene nuclear structure (1). They are derived biogenetically from the triterpenoids by the loss of three methyl groups. The general name 'steroid' was introduced in 1936 to cover all compounds possessing sterol-like skeletons.

The steroids have been known for almost 180 years. Cholesterol (2) was first described in 1812, and owes its discovery to Michel Eugene Chevreul. The bile acid, cholic acid (3), was isolated by Leopold Gmelin from ox bile in 1828. However, the correct steroid skeleton was not known until 1932, when Rosenheim and King (National Institute of Medical Research, London) suggested that all the classical work, the formula of Diels'



(1)



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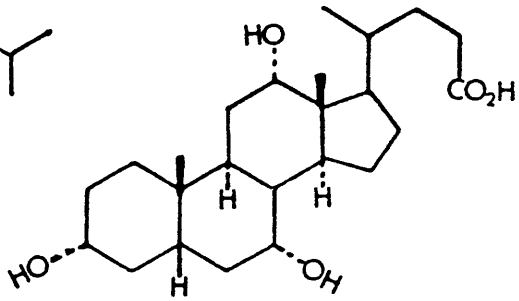

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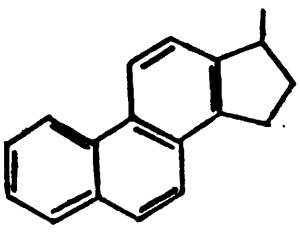

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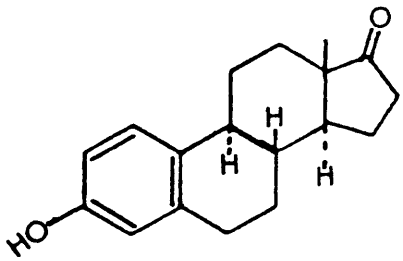


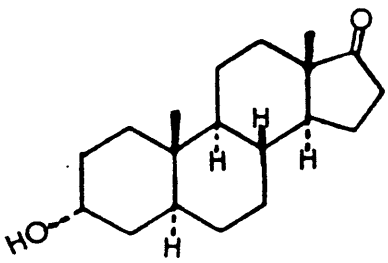
(5a)

hydrocarbon (4) and Bernal's X-ray data, could be rationalized in terms of the skeletal structure (1). A similar proposal was made almost simultaneously by Wieland. This proved to be correct and ultimately led to the establishment of the complete structure of the bile acids.

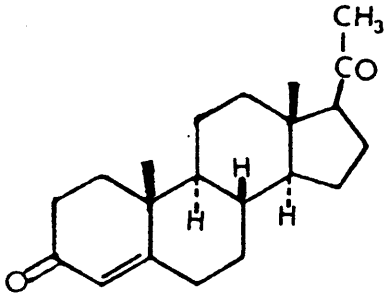
Amongst the steroids are substances of considerable medicinal importance. These include the sex hormones such as oestrone (5a), androsterone (5b) and progesterone (6), the adrenocortical hormones such as cortisone (7), the antirachitic vitamin [vitamin D_2 (8)], the cardiac glycosides which are sugar derivatives of steroids like digitoxigenin (9) and the steroidal antibiotic, fusidic acid (10). Related to these compounds are a wide variety of analogues that have been introduced into therapy to correct hormone deficiencies, to act as anabolic agents, to alleviate rheumatoid arthritis and various skin conditions and, more recently, as oral contraceptives.

1.2 Occurrence

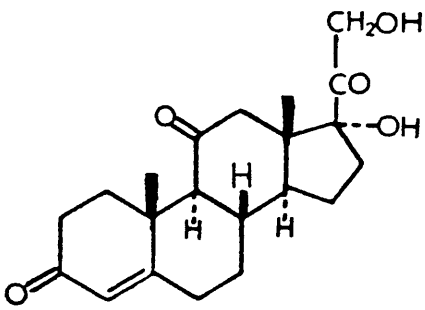
The steroids occur widely in nature, both in their free forms and as derivatives: - typically, sterols occur free and as esters of higher aliphatic acids, while more highly functionalised steroids such as alkaloids, sapogenins, etc. occur largely as glycosides. They may be classified on the


HO
H
H
H
H
O

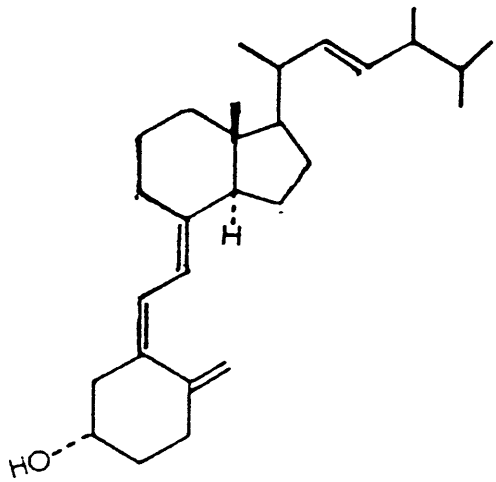
(5b)


CH3
CO
O
H
H
H

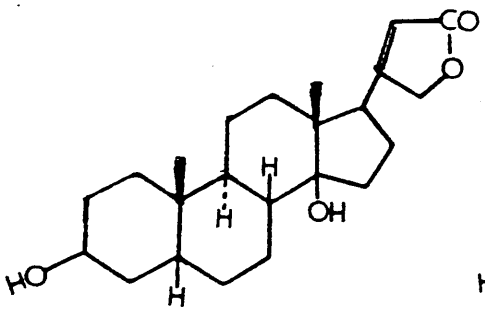
(6)


CH2OH
CO
OH
O
O
H
H
H

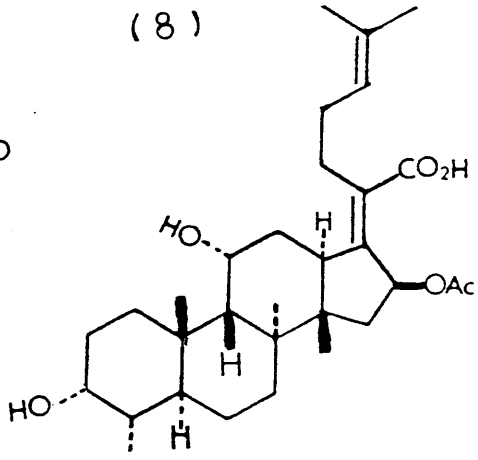
(7)


HO
H

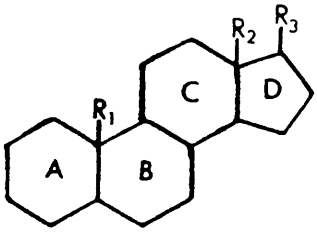
(8)


CO
O
HO
OH
H
H
H

(9)


CO2H
HO
OAc
HO
H
H
H

(10)

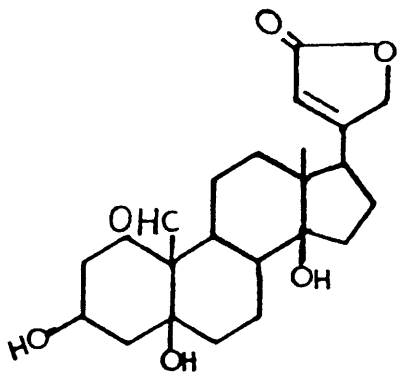

R2
R3
C
D
R1
A
B

(11)

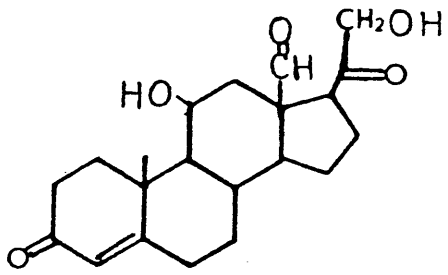
basis of occurrence as zoosterols (from animal sources), phytosterols (plant sources), mycosterols (fungal/yeast sources), and marine sterols (sponges, etc.). Cholesterol is the characteristic sterol of higher animals. Stigmasterol is widely distributed in plants, but only calabar bean and soya bean contain sufficient to serve as useful sources. The commonest of the mycosterols is ergosterol, which occurs together with 5α, 6-dihydroergosterol in yeast. Examples of marine sterols include ostreasterol from sponges, oysters and clams, stellasterol and its 22:23-dehydroderivative, obtainable from starfish. Steroids are also found in insects, though the insects are known to be incapable of synthesising the steroid skeleton. The insects consume steroids in their diet and convert them to their own purpose.

1.3 Steroid Types

The steroids are classed together because of their structural similarity. They all possess or have been derived from the tetracyclic carbon skeleton (11). Apart from minor differences, due to the presence of nuclear substituents and sometimes the degree of unsaturation, the diverse types of compounds comprised by the group arise mainly from variation in the entities R_1, R_2, R_3. R_1 is absent when ring A and/or ring B is aromatic.



(12)



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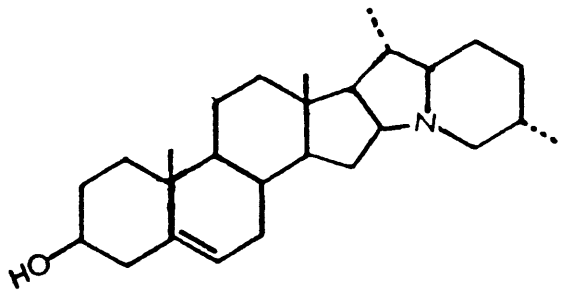
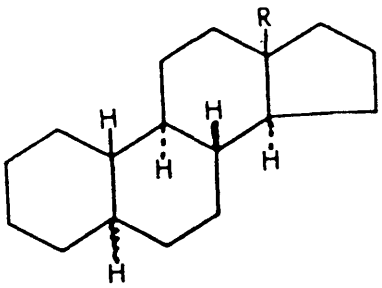


(14)

R_1 and R_2 are generally methyl groups, although incomplete residues such as primary alcohol or aldehyde groups are sometimes found, as in strophanthidin (12) and aldosterone (13), respectively. The side chain R_3 may be absent altogether, in which case the position is usually oxygenated, or it may consist of two, four, five, seven, eight, nine, ten, eleven, or twelve carbon atoms in either a branched chain or a ring system. The side chain in some azasteroids consists of eight carbon atoms associated with an atom of nitrogen, as in solanidine (14).

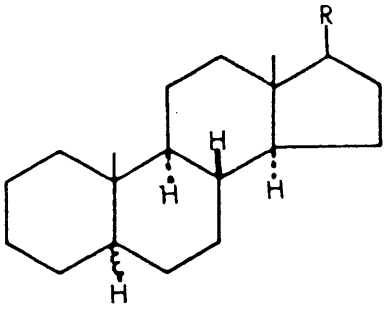
1.4 Nomenclature

Steroid nomenclature is now based on CIBA conference rules, adopted with slight modifications in 1957 by IUPAC. A systematic naming procedure requires simple names for parent hydrocarbon skeletons to which prefixes and suffixes are added to indicate the nature of substituents. These parent compounds include gonane (15), oestrane (16), androstane (17), pregnane (18), cholane (19), cholestane (20). Others include ergostane (24-methyl cholestane), stigmastane (24-ethyl cholestane), lanostane (4,4,14α-trimethyl-5α-cholestane), cardanolide for cardiac aglycones and



(15) ; R = H

(16) ; R = CH_3

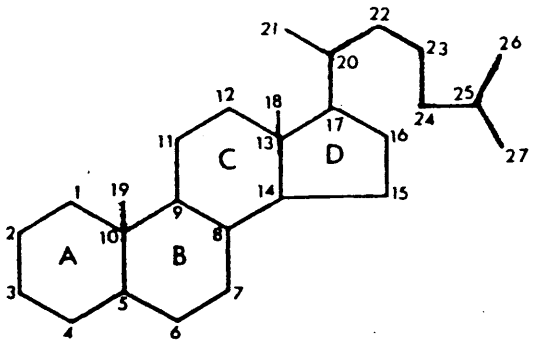


(17) ; R = H

(18) ; R = CH_2CH_3

(19) ; R = $CHMe(CH_2)_2CH_3$

(20) ; R = $CHMe(CH_2)_3CHMe_2$



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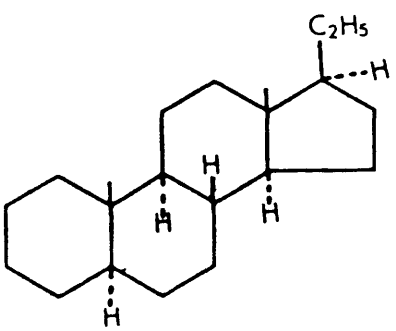

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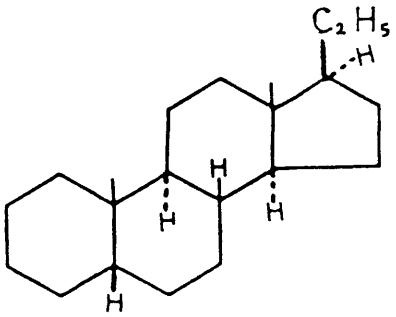
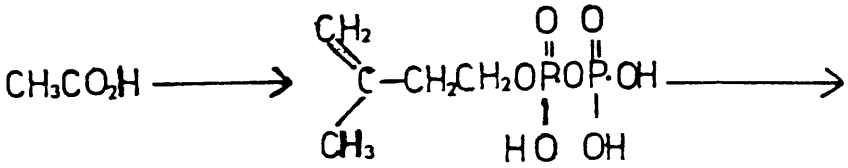


(23)

spirostan for sapogenins. The name 'etianic' acid may be used for androstane-17β-carboxylic acid.

The steroids are numbered as shown in (21). When any of the carbon atoms are missing, the numbering of the others remains unchanged. The names imply the stereochemistry shown, except at C-5, where it has to be defined expressly as 5α or 5β because there are two A/B ring fusions which occur naturally. Thus the compound (22) represents 5α-pregnane, while compound (23) is 5β-pregnane. All natural steroids belong to the absolute stereochemical series shown in which the 18- and 19-methyl groups (if present) are on the near face of the ring system as it is drawn. All substituents cis to these methyl groups are defined as β and are indicated by a thickened line in the drawings of their structural formulæ; all substituents trans to these methyl groups are defined as α and are represented by a broken line in the drawings of their formulæ. Where the configuration is unknown, this is indicated by the Greek letter ξ and in the formulæ by a wavy line. Stereochemistry at chiral centres in the side chain cannot be described in this way. For these the (R) and (S) system is used.



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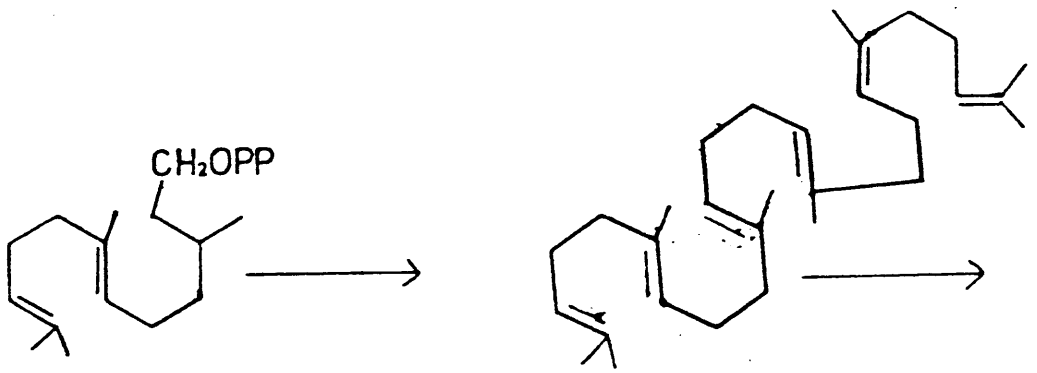
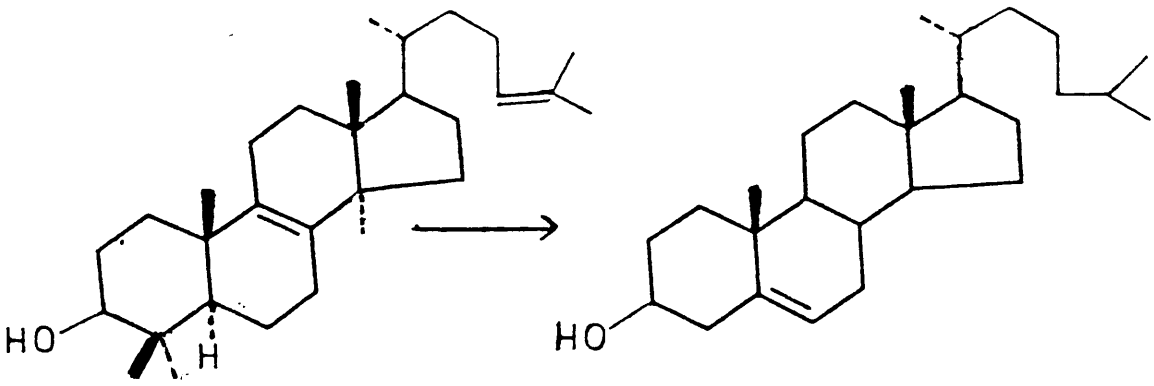


(25)

(26)



(27)

(2)

Fig. 1 Some Key Stages in Cholesterol Biosynthensis

1.5 The Biosynthesis of the Steroids

Detailed studies of cholesterol biosynthesis have been made in the recent past, particularly by Bloch, Cornforth and Popják, and Lynen, and have resulted in a clarification of the essential features of the pathway.

It has been found that the biosynthesis of cholesterol, which occupies a central position in the biosynthesis of many other steroids, is achieved, as shown (Fig.1), through the following stages[1]:

A The conversation of acetate into isopentenyl pyrophosphate (24), IPP, which is an irreversible intermediate in the biosynthesis of cholesterol (2).

B Isomerisation of IPP to dimethyl pyrophosphate (DMPP).

C The coupling of DMPP with IPP to yield geranyl pyrophosphate (GPP), and of GPP with IPP to produce farnesyl pyrophosphate (FPP), (25).

D The reductive coupling of two units of FPP to form squalene (26).

E The cyclization of squalene 2,3-epoxide to lanosterol (27).

F The conversion of lanosterol into cholesterol.

In 1956 Tavormina, Gibbs and Huff[1] showed that cell-free extracts from liver were able to incorporate radioactively-labelled

mevalonic acid (28) into cholesterol. Cell-free extracts from yeast and liver were utilized in the study of the biosynthesis of squalene from mevalonic acid.

The conversion of the mevalonic acid (28) into isopentenyl pyrophosphate involves stepwise ATP-dependent phosphorylation to form successively the 5-monophosphate (5-phosphomevalonic acid) and the 5-pyrophosphate (5-diphosphomevalonic acid). The latter undergoes ready decarboxylation and elimination to form isopentenyl pyrophosphate (24), as shown in Fig.2. The intermediate was isolated by addition of an enzyme inhibitor, iodoacetamide. The intervention of farnesyl pyrophosphate in the biosynthesis of squalene was demonstrated by omitting NADPH from the cell-free enzyme system used in the conversion of mevalonic acid into squalene. Under these conditions farnesyl pyrophosphate accumulated. The first step in the conversion of isopentenyl pyrophosphate into farnesyl pyrophosphate was shown to be its enzymic isomnerization to dimethylallyl pyrophosphate (29), (a reaction inhibited by iodoacetamide). This unit formed the terminal isopropylidene group of farnesyl pyrophosphate. Geranyl pyrophosphate (30) and farnesyl pyrophosphate have been identified as precursors of squalene in yeast systems.[1]

The equilibrium of isopentenyl pyrophosphate (24) with

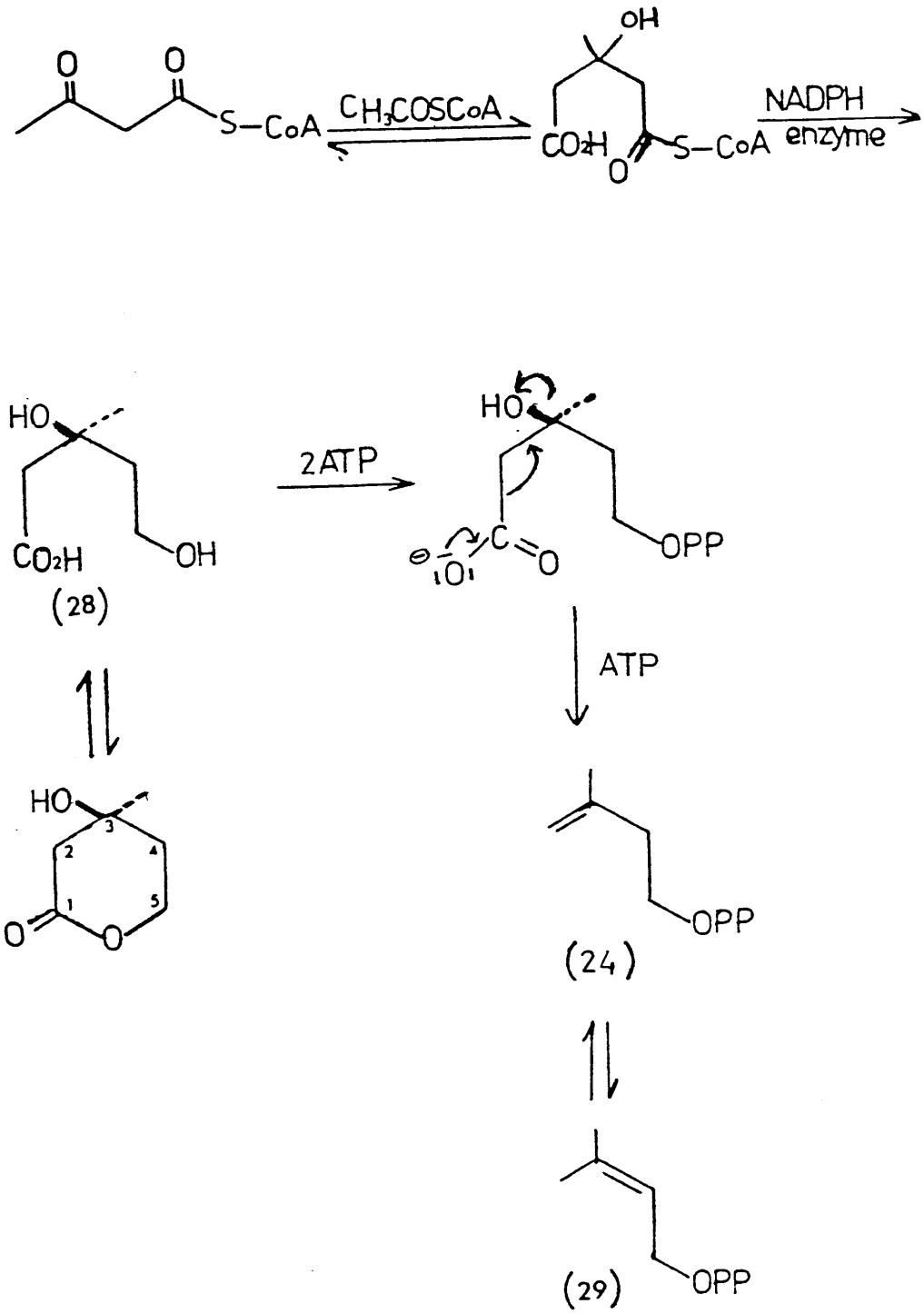

O
O
S–CoA
CH3COSCoA
OH
CO2H
O
S–CoA
NADPH
enzyme
HO
CO2H
OH
(28)
2ATP
HO
C
O
OPP
ATP
HO
3
2
4
1
5
O
O
OPP
(24)
OPP
(29)

Fig. 2

dimethylallyl pyrophosphate (29) is catalysed by the enzyme isopentenyl-diphosphate Δ-isomevase (E.C.5.3.3.2.) while the condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate to produce geranyl pyrophosphate (30) and the condensation of the latter with isopentenyl pyrophosphate to yield farnesyl pyrophosphate (25) are both catalysed by dimethylallyl-transtransferase (E.C.2.5.1.1.), which is often described as prenyltransferase. It has been suggested recently that the enzyme-catalysed condensation of isopentenyl pyrophosphate with geranyl pyrophosphate proceeds by the mechanism illustrated in Fig.3.

When radioactive farnesyl pyrophosphate was incubated with yeast enzymes and NADPH, radioactive squalene was formed.[1] Two units of the pyrophosphate condense in a tail-to-tail manner with the formation of squalene. An understanding of how this coupling takes place came with the isolation and characterisation of an intermediate between farnesyl pyrophosphate (27) and squalene (3) known as presqualene pyrophosphate (31).[2] Its mode of formation and conversion into squalene is summarised in (Fig.4). The first step involves electrophilic addition of the tail of one farnesyl unit to the corresponding double bond of the other. The resulting presqualene pyrophosphate contains a potential cyclopropyl carbinyl cation which rearranges as shown and is eventually reduced by

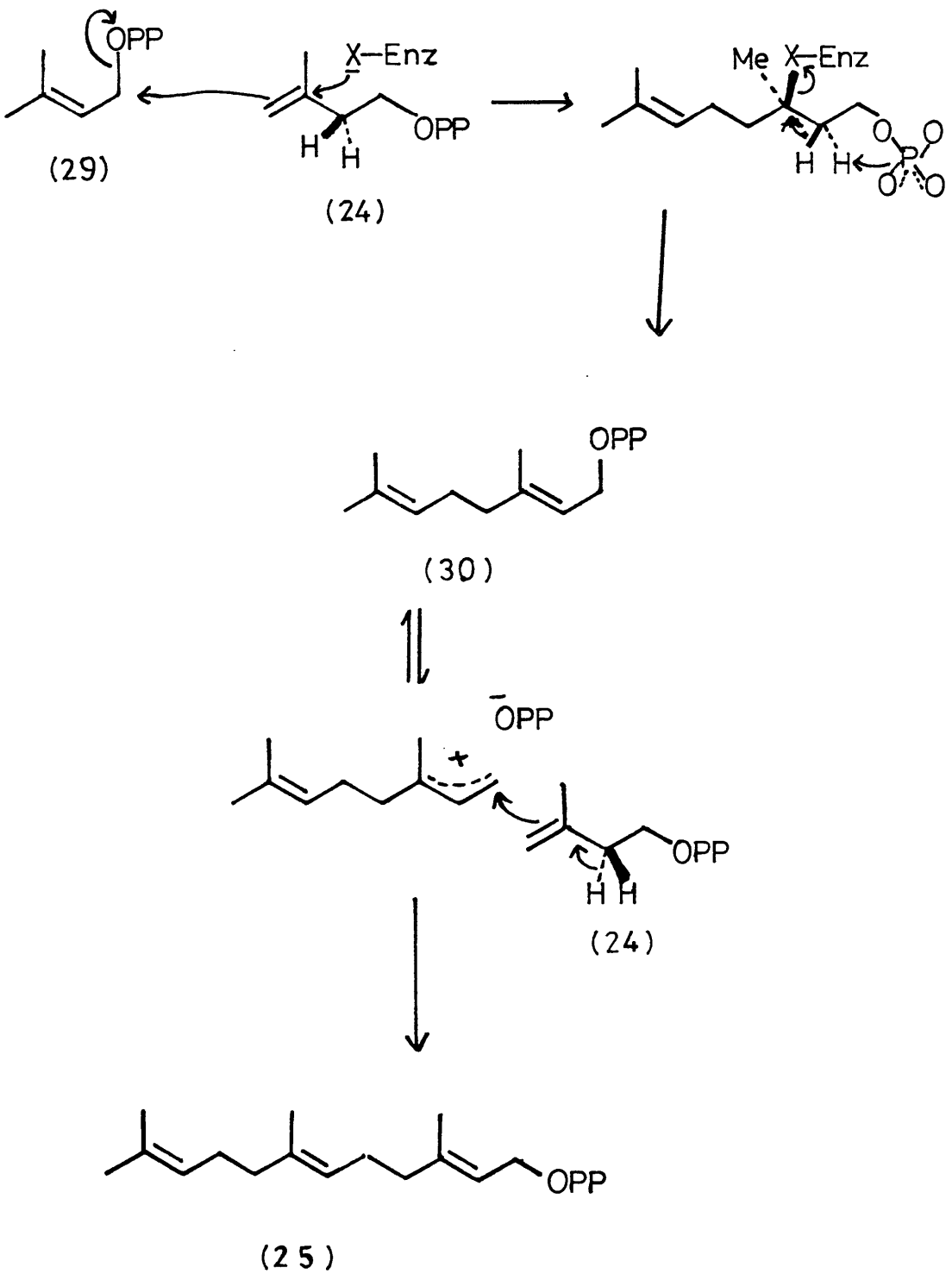


Fig. 3

NADPH to squalene.

The scheme of cyclisation of squalene put forward by Woodward and Bloch[1] implied that lanosterol or some closely related compound should be an intermediate in the biosynthetic pathway between squalene and cholesterol. After this intermediate role of lanosterol had been clearly established, a major aspect of subsequent investigations became the elucidation of the mechanism of conversion of squalene into lanosterol.

The nature of the cyclizing agent was at first the subject of speculation. Oxygen from H_2O^{18} was incorporated into lanosterol and hence species such as OH^+ were involved. However, squalene, 2,3- epoxide (32) has been shown to be very efficiently converted into lanosterol and the steroids by rat-liver systems. It is established that squalene is selectively oxidised by enzymes at a terminal double bond and the resulting 2,3-epoxide then undergoes a concerted enzyme-controlled cyclisation to give a polycyclic product. The triterpene which is produced depends upon the enzyme system and on the conformation which the squalene chain is forced to adopt. Thus, concerted cyclisation of the all-chair conformation (A) (Fig. 5a) leads to a pentacyclic product. To obtain lanosterol (4), the epoxide must adopt the chair-boat-chair conformation (B): protonation and

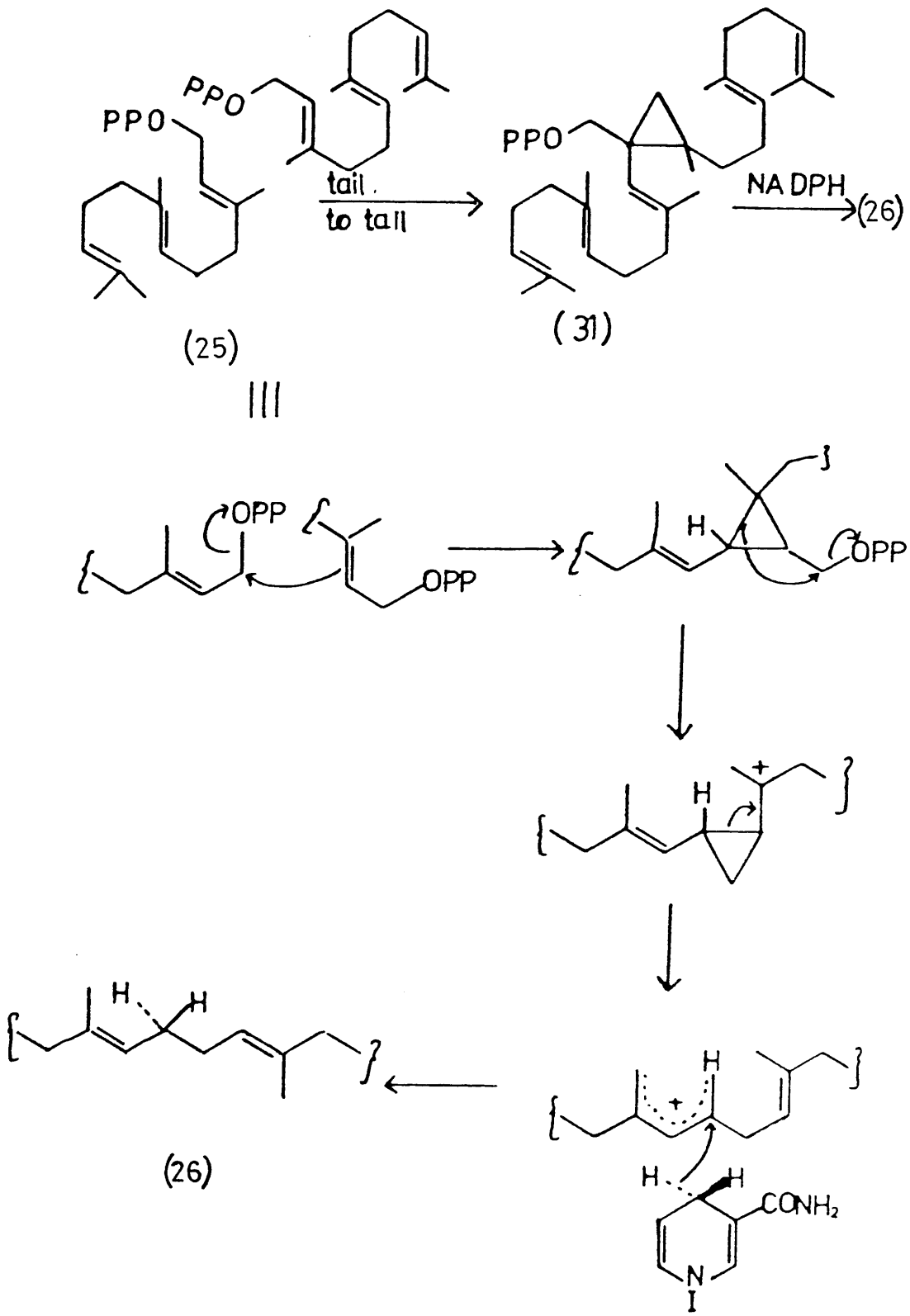
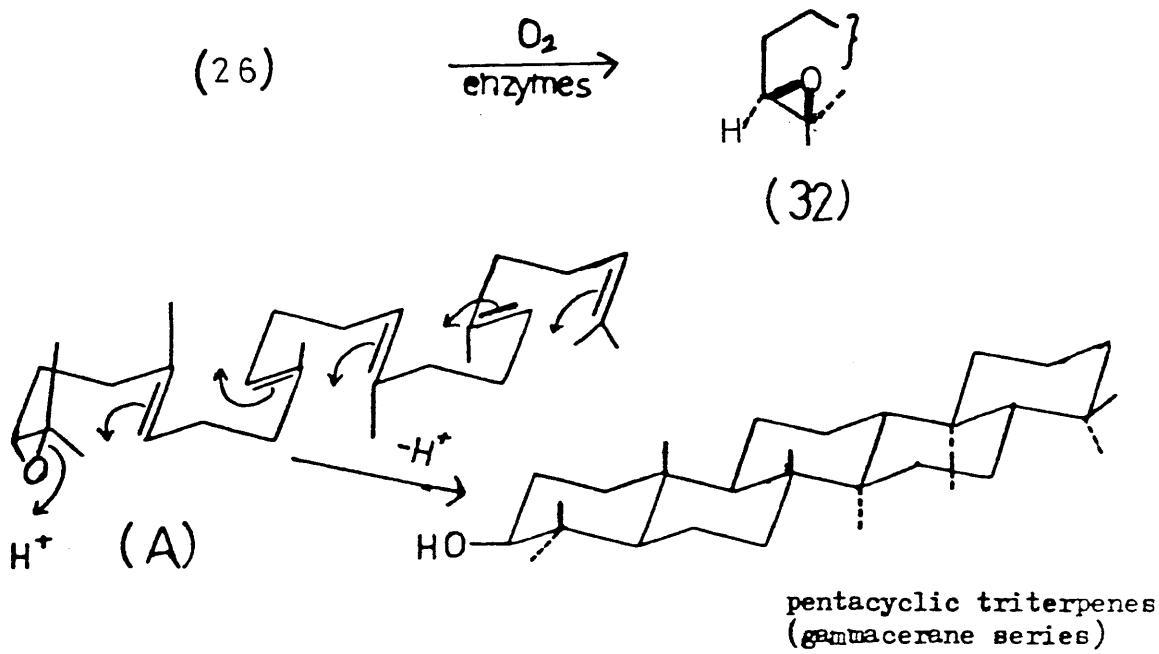


FIG. 4 The formation of squalene(3) from farnesyl pyrophosphate(27) via presqualene pyrophosphate(31).


(26)
O_2
enzymes
H
O
(32)
$-H^+$
H^+
(A)
HO
pentacyclic triterpenes
(gammacerane series)

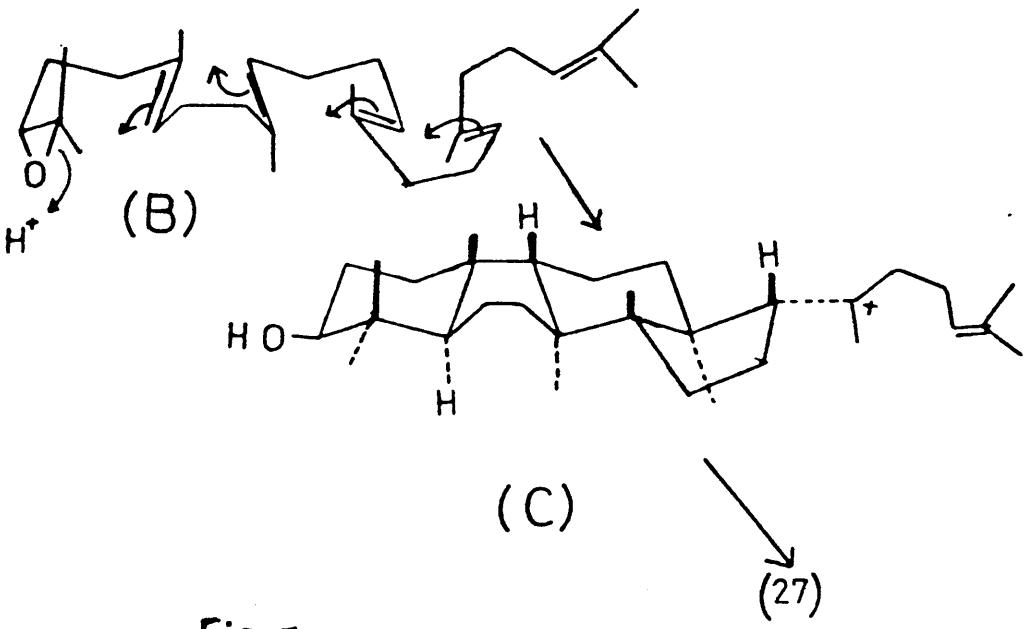

O
H^+
(B)
H
H
HO
H
(C)
(27)
Lanosterol

Fig.5a

synchronous cyclisation of the latter leads to the C-20 cation (C). This is followed by a series of 1,2-hydride and methyl shifts ending with loss of the C-9 proton and the formation of lanosterol (27).

The conversion of lanosterol (27) into cholesterol (2) involves the sequential replacement of the methyl groups at C-4 and C-14 with hydrogen atoms, the migration of the Δ^8 double bond to Δ^5 and the saturation of the side chain. A number of stages in this conversion involving the oxidation of the methyl groups and their elimination as carbon dioxide have been established.[2] Since there is evidence for the intervention of 4,4'-dimethylcholesta-8,24-dien-3β-ol (33) in this process, it appears that the loss of the methyl group at C-14 must occur initially followed by the removal of the two methyl groups at C-4. This probably takes place in a stepwise fashion, as shown in (Fig. 5b). The C-27-steroid resulting from the demethylation of lanosterol (27) is cholesta-8, 24-dienol (34) known as zymosterol. The conversion into cholesterol involves reduction of the side-chain double bond and rearrangement of the nuclear double bond. This rearrangement is dependent on oxygen and the process seems to involve isomerization to Δ^7 cholesterol, oxidation to 7-dehydrocholesterol and finally cholesterol (2).

In the biosynthesis of many other steroids, cholesterol

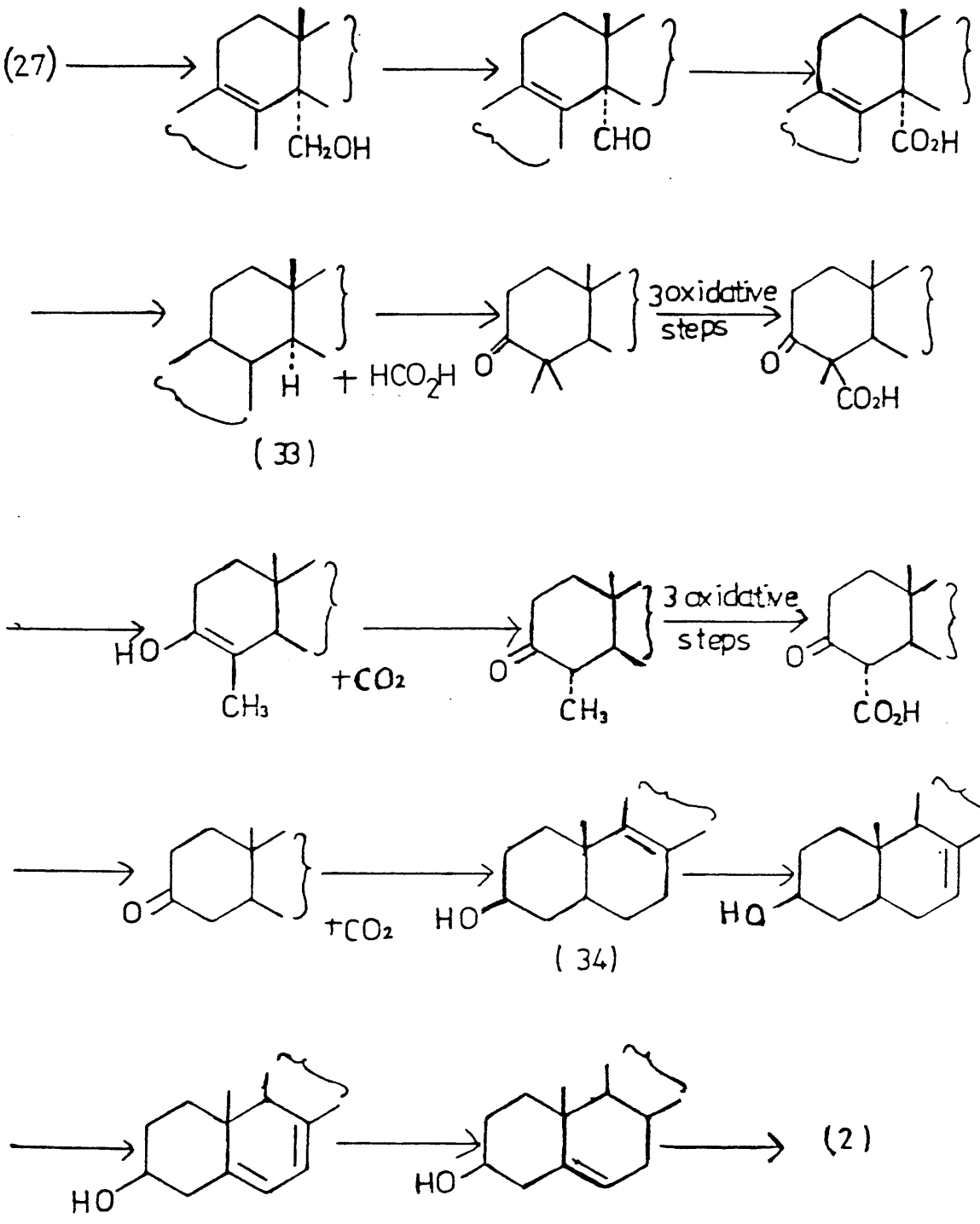
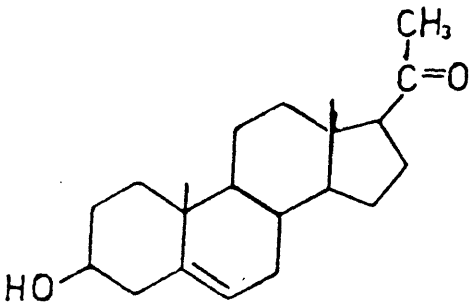
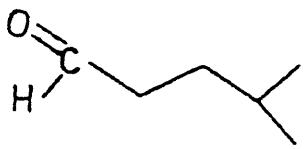


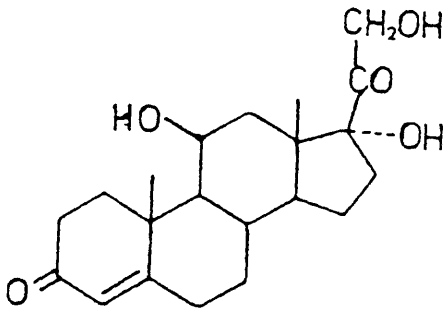
Fig. 5b Conversion of Lanosterol into cholesterol via Δ^{24} intermediates.



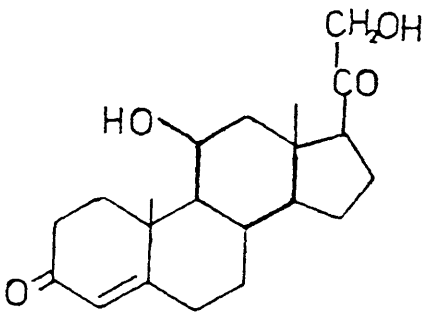
(35)



(36)



(37a)



(37b)

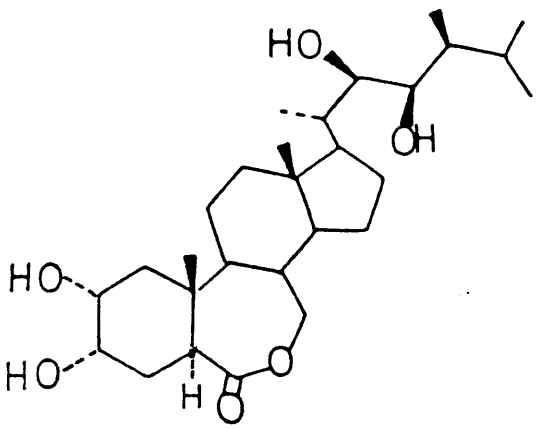
occupies a central position. Thus, it has been shown to be converted into the bile acids such as cholic acid (3).[3] In this biosynthesis it would appear that the appropriate nuclear transformations, such as hydroxylations and reduction of the double bond, take place prior to cleavage of the side-chain.[3] On the other hand, the formation of the steroid hormones involves cleavage of the side-chain to form pregnenolone (35), the remaining carbon atoms appearing as isocaproic aldehyde (36). Further conversion of pregnenone (35) can lead to androsterone (5b) or by successive hydroxylation and oxidation to the cortisol (37a) or corticosterone (37b).

1.6 Brassinolide and Related Steroids

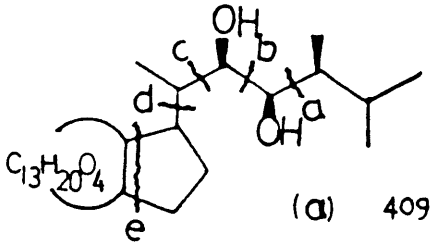
a. The Discovery of Brassinolide

Recently, a novel plant growth-promoting substance named brassinolide (38) has been isolated from the pollen of rape (Brassica napus L.) and its structure determined as (22R, 23R, 24S)-2α, 3α,-22, 23-tetra-hydroxy-24-methyl-7-oxa-B-homo-5α-cholestan-6-one.[4]

More recently, Ikeda and co-workers[5] reported the isolation and identification of brassinolide and its analogues in insect galls of the chestnut tree (Castanea crenata Sieb. et Zucc.), while Ikekawa and his group[6] have identified the brassinolide and its analogues in three different



(38)



(39)

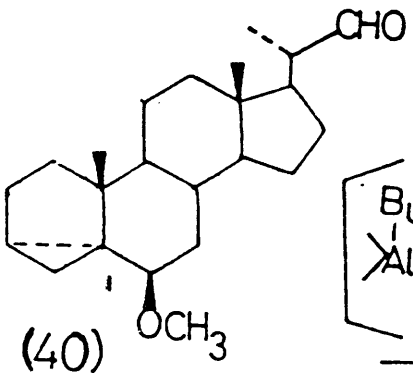
(a) 409 , 71

(b) 379, 101, 361, 343,

(c) 349, 131,

(d) 321, 303,

(e) 279.



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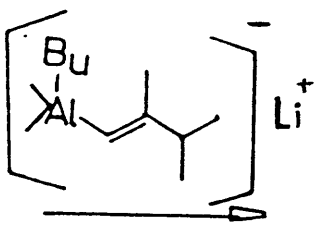

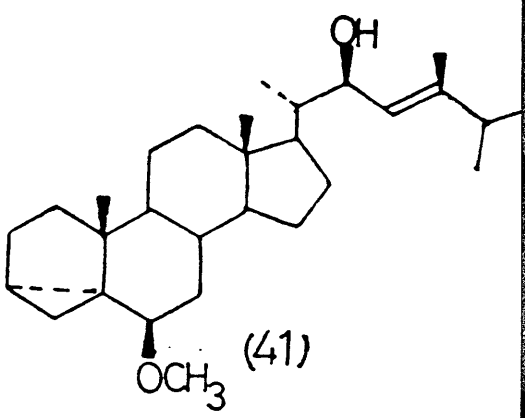

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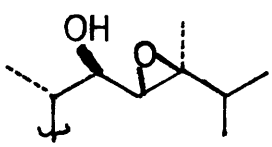

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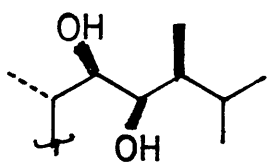


(43)

plants; the immature seeds and sheaths of Chinese cabbage, Brassica campestris var. pekinensis, the leaves of green tea, Thea sinensis and the chestnut tree, Castanea spp, by means of selected ion monitoring.

Brassinolide (38) is a new type of plant growth hormone and the first known natural steroid containing a seven-membered B-ring lactone. When tested in the bean second-internode bioassay, it promotes both cell elongation and cell division, resulting in curvature, swelling and, more dramatically, splitting of the internode when applied at levels as low as 10ng per plant.[4] Brassinolide shows a strong activity in the lamina inclination of rice seedlings at 0.005 ppm,[7] possessed a broad spectrum of biological activities compared with the known plant hormones, [8,9] and increases agricultural crop yield.[10]

b Brassinolide Isolation and Characterisation

Grove [4] and co-workers reported that a 2-propanol extract of bee-collected Brassica napus L. (rape) pollen (40 kg) was subjected to successive chromatography on silica gel, C_{18} Hi-flosil and high performance chromatography on μ-Bondapak C_{18} (methanol:water 63:35) and brassinolide (4 mg) was obtained. By purely physical techniques, which included mass spectrometry, infra-red, and proton ^{13}C magnetic resonance

spectrometry, the gross structure of a tetrahydroxy steroid lactone was deduced.

Elemental analysis of crystalline brassinolide (m.p. 274-275°C) (methanol) showed it as $C_{28}H_{48}O_6$. High resolution field desorption mass spectrometry gave m/z 481.3489 $(M+1)^+$, calcd. 481.3528. Functional group analysis by infra-red spectrometry indicated the presence of hydroxy groups (υ_{max} 3,450 cm^{-1}). The carbonyl stretching frequency of 1,690 cm^{-1} (KBr) moved in dichloromethane solution to 1,720 cm^{-1} suggesting that this might represent a carboxyl carbonyl group.

Proton magnetic resonance analysis gave signals (0.72, s, 3H; 1.03, d, 6H, J=6.3Hz; 1.04, s, 3H; 1.10, d, 3H, J=6.4Hz; 1.13, d, 3H, J=6.5Hz) indicative of six methyl groups, which were confirmed by ^{13}C magnetic resonance analysis (10.3, 11.9, 12.1, 15.6, 20.8 and 21.0). Additionally, five C-O carbons and C=O carbon were shown to be present. These data in combination with evidence from infra-red spectrometry permitted the assumption of the presence of four hydroxy groups and a lactone structure. Acetylation gave a tetraacetate [m/z 649 $(M+1)]^+$, by CI-MS (isobutane reagent gas)] hence confirming the four hydroxy groups.

Evidence from mass-spectrometric analysis confirmed a

dihydroxylated side chain and steroid skeleton. Field desorption mass spectrometry produced ions consistent with four losses of H_2O; 463, 445, 409 and with C-22, C-23 cleavage (m/z 379, 361 (379-H_2O)). Chemical ionization mass spectrometry (isobutane reagent gas) showed ions at m/z 481, 463, 445, 427 (M+1-3H_2O)$^+$, 409, 379, 361, 349, 343, 321, 303, and 279. The diagram (39) gives a graphic interpretation of this fragmentation pattern which is consistent with a dihydroxylated side chain and ring D of a steroid. The structure of brassinolide was established completely by an X-ray diffraction technique on a single crystal (from methanol) which belonged to the monoclinic space group $P2_1$.

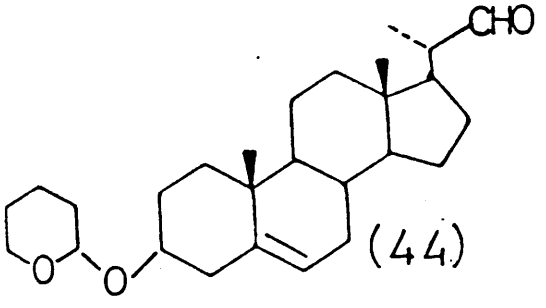
A perspective drawing of brassinolide (less hydrogens) was obtained using experimentally determined atomic positions at an intermediate stage least-squares refinement. The results confirmed brassinolide as a tetrahydroxylated, 7-membered ring B lactonic steroid; 22α, 3α, 22R,23R-tetrahydroxy-24S-methyl-7-oxa-B-homo-5α-cholestan-6-one (38).

c Synthetic Studies on Brassinolide

Chemical synthesis of brassinolide has been achieved by several groups of scientists.[11,12,13,14] Siddall[11] used as a starting material

the 3,5-cyclo-steroidal aldehyde (40), obtained from i-stigmasterol methyl ether, by selective ozonolysis of the side chain.[15,16,17] The chiral C-20 group was used to generate asymmetry at C-22 and this in turn was to control the stereochemistry of C-23 and C-24 during hydroxy-directed epoxidation of (41). Thus, in the alkylation of the aldehyde (40) with lithium butyldimethyl-(E)-2,3-dimethylbutenyl-alanate,[18,19] a 46% yield of the major 22(S)-allylic alcohol isomer (41) and 8% yield of the less polar 22(R)-isomer were obtained along with a trace of aldehyde after chromatography on silica gel. The 23(R), 24(R) epoxide, 24-methyl side chain synthesis was achieved by Siddall in a hydroxy-directed epoxidation of (41) with m-chloroperbenzoic acid, with a remarkable stereoselectivity of (95:5). Recrystallisation of the product afforded the pure epoxide (42). Regioselective (3:1) anti-Markovnikov reduction of the epoxide (42) with inversion of configuration at C-24 completed the 22(R)/23(R)-dihydroxylated side chain to afford compound (43). Thus reduction with lithium borohydride, BH_3-THF complex at 50°C for 20 hours gave a major vicinal diol and a minor 1,3-diol, separated via acetonide formation.

Ikekawa[12] reported that the aldehyde (44) derived from the readily available 3β-hydroxy-dinor-5-cholenoic acid[20] was similarly alkylated with 3-methyl-but-1-ynyl-lithium and afforded a 1:1 epimeric



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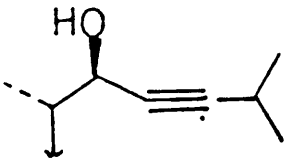

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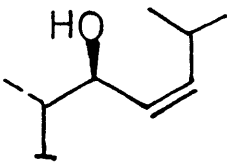

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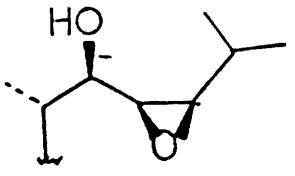
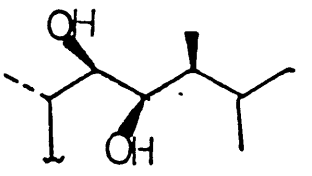
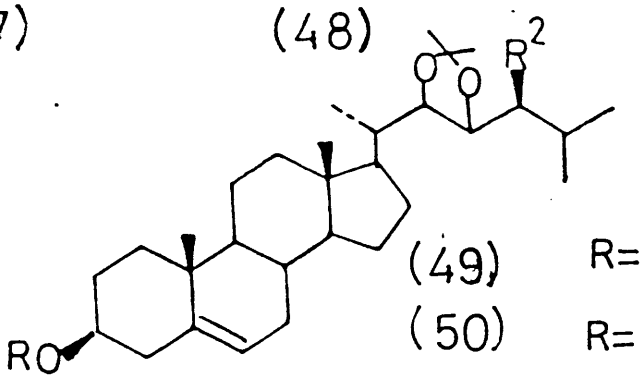


(47)

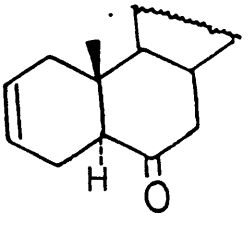
(48)



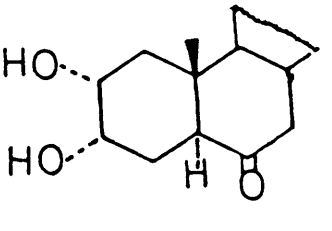


(49) R=Ac R^2= CH_3

(50) R=Ms R^2= CH_3



(51)



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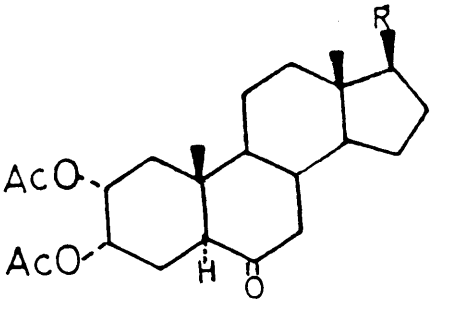
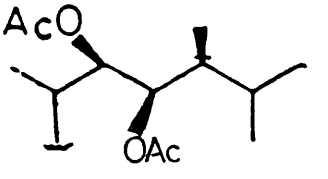
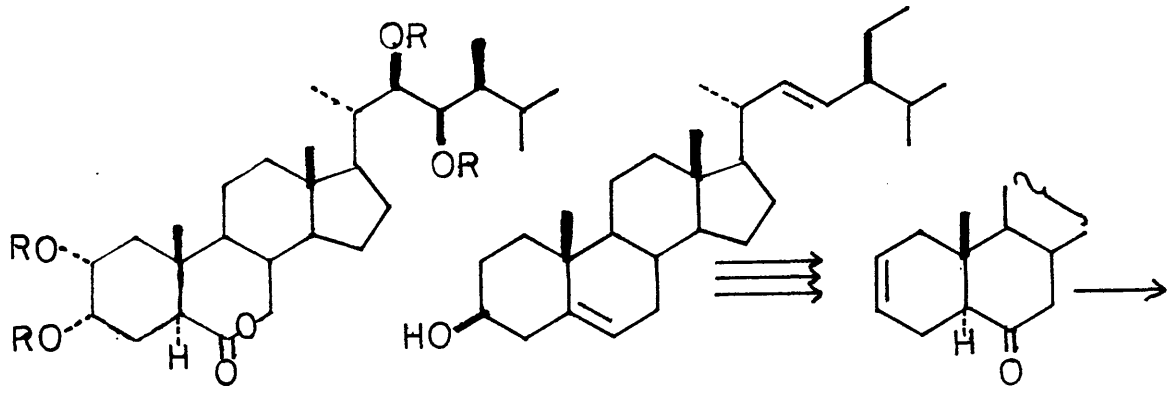


R =



(53)

mixture of 22-alcohols from which the more polar 22(R) isomer (45) was isolated by a single recrystallisation in 38% yield. The 23(R), 24(R)-epoxide was stereospecifically prepared, in 85% yield, from cis-allylic alcohol by means of the t-butyl hydroperoxide-oxovanadium acetylacetone epoxidation reaction. The next synthetic goal of Ikekawa's group was to open the epoxy function to yield the vicinal diol and C-24 methyl group of the desired stereochemistry. Ring opening and simultaneous C-24 methylation of the epoxide (47) using various reagents such as Me_2CuLi and MeMgBrCuI were reported to be unsuccessful by Ikekawa and co-workers. The epoxide was then converted to a C-24 nitrile via hydrocyanation[21] with excess HCN-Et_3Al, the C-22 acetoxy group was saponified and the vicinal diol protected as acetonide. ^{13}C-n.m.r. proved the result to be a single compound and from the established mode of trans-opening of epoxide rings, the stereochemistry at C-24 was established. The nitrile was then reduced and hydrolysed to the aldehyde. The formyl group was transformed via acetylation, borohydride reduction, methanesulphonation, iodide substitution, and Bu_3SnH reduction to a methyl group in 77% overall yield. The 6-carbonyl function was generated from (50) by hydroboration in excess of BH_3-THF complex and alkaline H_2O_2 followed by oxidation with

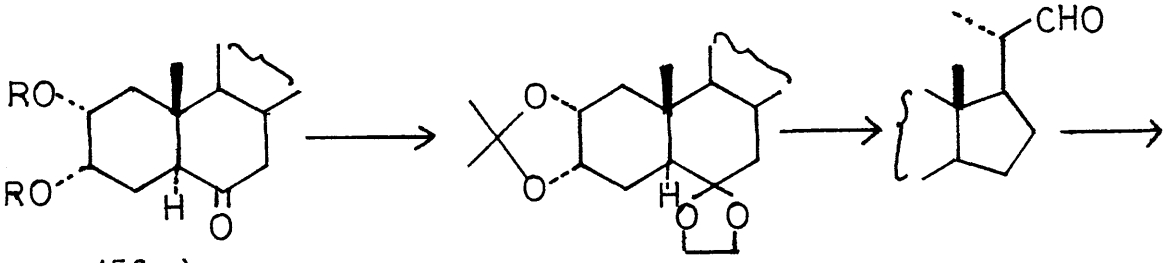


(38) R=H

(65) R=Ac

(54)

(55)

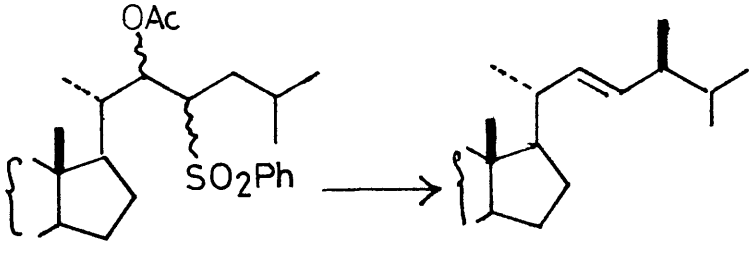


(56a) R=H

(56b) R= $\rangle C Me_2$

(56c)

(57)

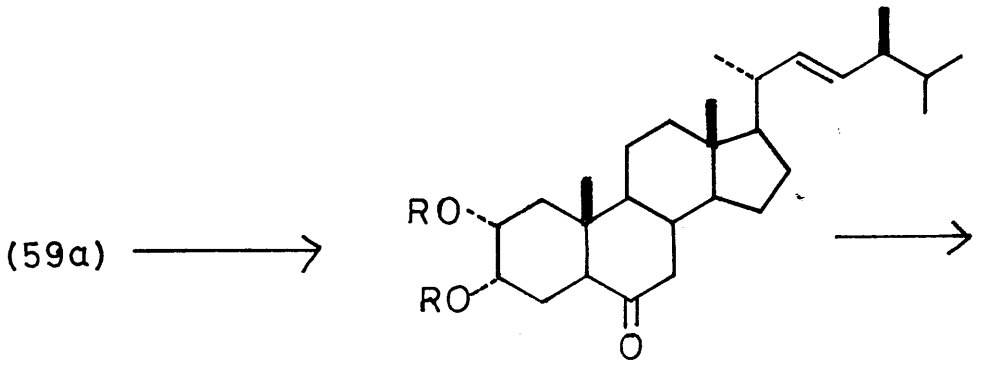


(58)

(59a)

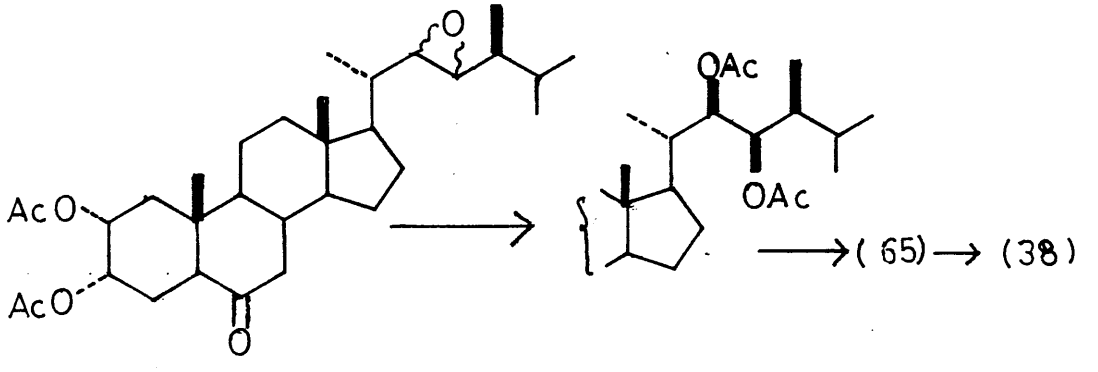
pyridinium chlorochromate. The resulting 3-methanesulphonyl ester underwent elimination smoothly with LiBr in refluxing dimethylformamide to the ketone (51). Cis-α-hydroxylation of (51) with a catalytic amount of osmic acid and N-methyl-morpholine-N-oxide gave the 2α,3α-diol (52) which was acetylated to tetra-acetate (53). Baeyer-Villiger oxidation of the tetra-acetate followed by hydrolysis gave brassinolide in 68% yield.

Sakakibara and co-workers[13] reported the synthesis of brassinolide using stigmasterol (54) as a starting material. The stigmasterol was converted to the dienone (55).[22] This was oxidised with osmium tetroxide and N-methylmorpholine N-oxide in aqueous acetone [23] to give a diol (56a). This was converted to the corresponding acetonide (56b) in quantitative yield by treatment with 2,2-dimethoxypropane and TsOH. After protection (butanone ethylene acetal and TsOH) of the carbonyl groups as an ethylene acetal, (56C) was treated with ozone. Reductive work-up (dimethyl sulphide in the presence of sodium bicarbonate) of the resulting ozonide yielded an aldehyde (57), in 60% yield from (56b). Formation of the oleginic side-chain with (24S)-methyl group was accomplished by the Kocienski olefin synthesis[24] employing (57) and a phenyl sulphone (64c). This sulphone (64c) was prepared from optically pure (R)-(+)-citronellic acid (62). This was converted to the acid (63)[25,26]

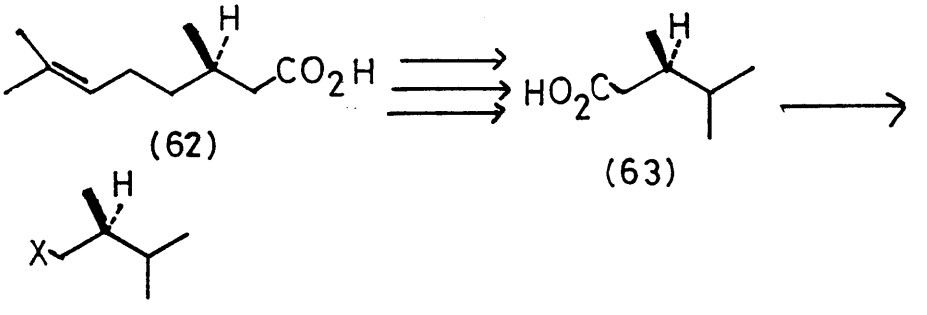


(59b) R=H

(59c) R=Ac



(60) (53)



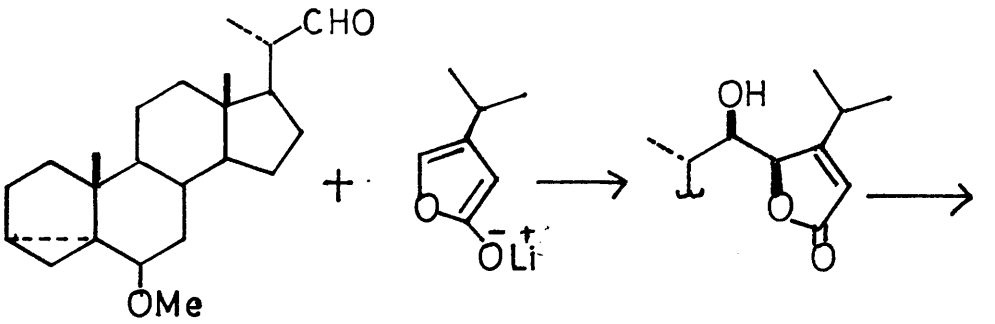
(62) (63)

(64)

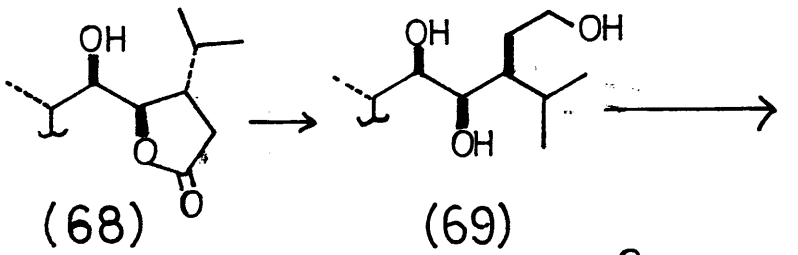
(a) X=I ; (b) = SPh ; (c) = SO_2Ph

which was in turn transformed to the desired sulphone, in 49.2% overall yield, via (64a) and (64b).[27] Addition of (57) to the carbanion derived from the sulphone (64c) was followed by acetylation to give a β-acetoxy sulphone (58). Reduction of (58) with sodium-amalgam in methanol-ethyl acetate (2:1) gave the olefinic product (59a) which, upon deprotection, furnished the dihydroxy enone (59b), in 31% overall yield from (57). The corresponding acetate (59c) was epoxidized with m-chloroperbenzoic acid to give the epoxide (60) as a steroisomeric mixture in 62% yield. The epoxy ring in (60) was cleaved with 30% hydrobromic acid in acetic acid to give a bromoacetate by trans-ring-opening. Another inversion at the carbon bearing the bromine atom was effected by heating with acetic acid-water (4:1) at 100-120°C for 19 hr. The product was acetylated with acetic anhydride and 4-(N,N-dimethyl-amino)pyridine to give the desired tetraacetoxy ketone (53) in 25.3% yield from (60) after chromatographic purification. The Baeyer-Villiger oxidation of (53) with trifluoroperacetic acid in the presence of disodium hydrogen phosphate in methylene chloride yielded brassinolide tetraacetate (65), in 82.9% yield after chromatographic purification. Hydrolysis of (65) by sodium hydroxide followed by acidification gave the desired brassinolide (38).

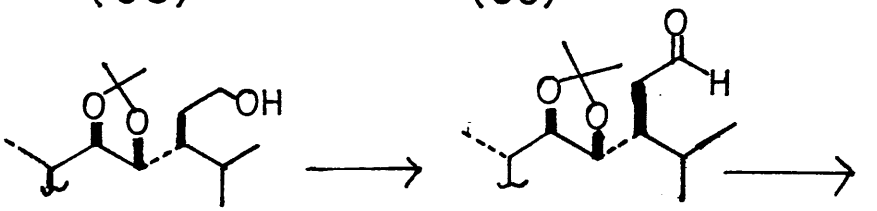
Most recently, Donaubauer et al.[14] reported a new method



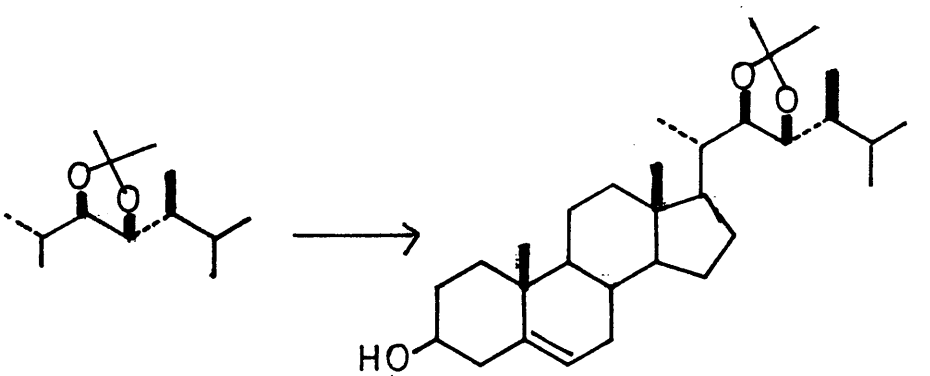
(40) (66) (67)



(68) (69)



(70) (71)

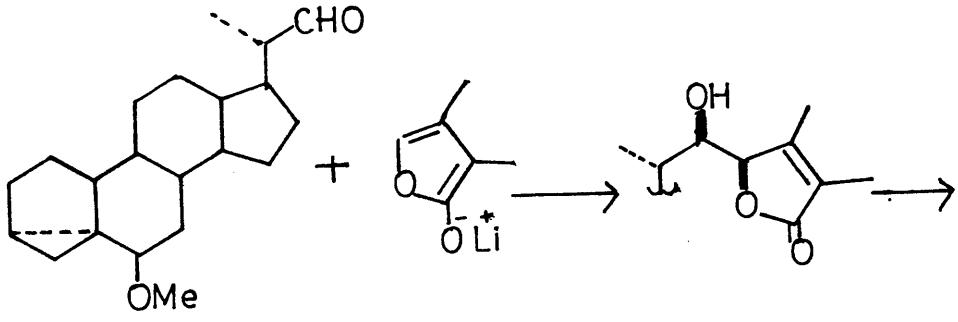


(72) (73)

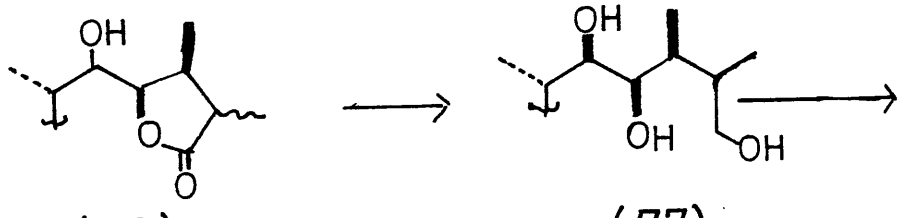
the synthesis of brassinolide from stigmasterol. Stigmasterol was converted to the (20S)-6β-methoxy-3α-5-cyclo-5α-pregnane-20-carbox- aldehyde (40), according to well-known procedures.[28,29] This aldehyde was then used in an aldol reaction with the anion from 3-isopropylbut-2-enolide (66).[30] The anion was generated in tetrahydrofuran from the butenolide and lithium diisopropylamide and was cooled to -78°C before addition of the aldehyde. The temperature was maintained below -70°C for 5 hr and the reaction was quenched with dilute hydrochloric acid at this temperature. Under these conditions, the 22R, 23R intermediate (67) was obtained in 65% yield. When the aldol reaction mixture was allowed to warm up to 0°C before quenching, the major product was the 22R, 23S isomer. Apparently, the reversible nature of the aldol reaction led to the more stable product at higher temperature. Catalytic hydrogenation of the intermediate butenolide (67), (Pt/activated carbon, freshly distilled dioxane, H_2 at 1 atm), gave a 78:22 mixture of isomers in virtually quantitative yield. The coupling constant for H-23 to H-24 (4.9 Hz) in the major isomer (68), was smaller than that for H-23 to H-24 (6.8 Hz) in the minor isomer and the stereochemistry at C-24 in (68) was therefore tentatively assigned as 24S. Reduction of lactone (68) with $LiAlH_4$ afforded the trihydroxy derivative

(69) in 90% yield, and this compound was heated with 2,2-dimethoxypropane (55-60°C) and pyridinium p-toluenesulphonate to form the 22,23-acetonide and a mixed ketal at C-29. The crude product was refluxed in methanol for 1h to give the C-29 hydroxy compound (70) in an overall yield of 92%. Oxidation of (70) with pyridinium dichromate gave the aldehyde (71) in 94% yield. This compound was decarbonylated with tris(triphenylphosphine)rhodium chloride to give the 24S methyl derivative (72) in 78% yield. The overall yield for the side-chain synthesis starting from the aldehyde (40) was 32%. The stereochemistry of the side-chain was confirmed by converting the i-sterol methyl ether (72) to the known 3β-hydroxy derivative (73), in 98% yield.

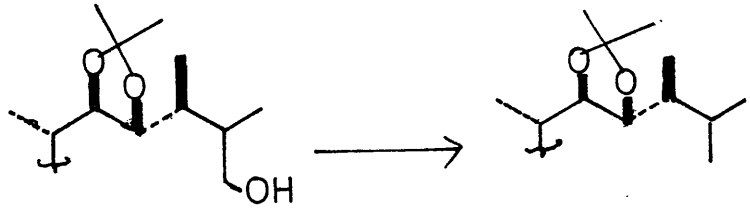
Donaubauer[14] also described a shorter way of constructing the side-chain. This route employed a similar sequence of reactions, as above, but starting with 2,3-dimethylbutenolide which was readily obtained by reduction of 2,3-dimethylmaleic anhydride with sodim borohydride in tetrahydrofuran.[31] Aldol reaction of the anion (74) of this compound with the aldehyde (40) at -78°C afforded the hydroxy butenolide (75) in 74% yield. A small amount of the 22S, 23S isomer (6%) and of the 22S, 23S isomer (10%) were also obtained. Catalytic hydrogenation of (75) as for (67) gave the saturated lactone (76) in 77% yield. Reduction of the lactone with



(40) (74) (75)



(76) (77)

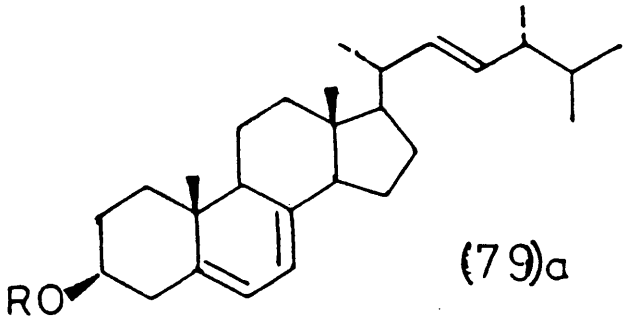


(78) (72)

$LiAlH_4$ gave the triol (77) which was converted to the acetonide (78). This compound was treated with methanesulphonyl chloride and pyridine, and the product was reduced with $LiAlH_4$ to give the same intermediate (72), (22R, 23R, 24S), as had been obtained in the earlier sequence. The overall yield for the four steps from the saturated lactone (76) was 70%. Finally, conversion of the intermediate (73) to brassinolide was achieved in 15% yield by employing procedures similar to those described by other workers.

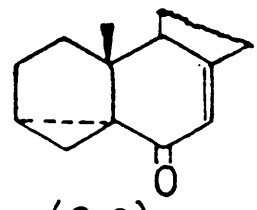
d Synthetic Analogues of Brassinolide

M.J. Thompson et al.[32] reported the first synthetic analogues of brassinolide. These analogues are prepared from ergosterol (79). They differ from brassinolide in the stereochemistry of the 22,23-dihydroxy and 24-methyl groups. The synthetic approach by Thompson[32] and his group to these analogues has since been extended to the synthesis of brassinolide, and its C-22, C-23 isomer from 22-dehydrocampesterol (80).[33] 24-Ethyl brassinosteroids have been later prepared from stigmasterol (81).[34] The approach involved the selective transformation of Δ^5-olefin to the desired carbonyl functionalised ring B, generation of the Δ^2-olefin in ring A, and per-hydroxylation of the diene intermediate to the tetrahydroxy-ketone. In the final step, the ketone was oxidised to the lactone, brassinolide.

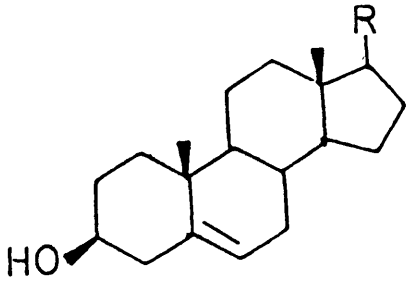


(79)a R = H

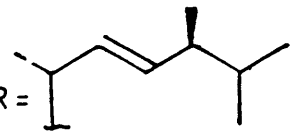
b R = Ts



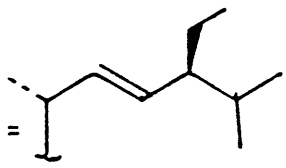
(82)

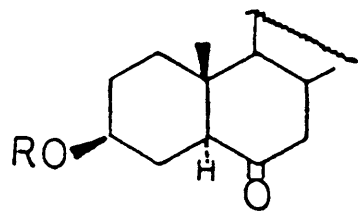


(80) R =



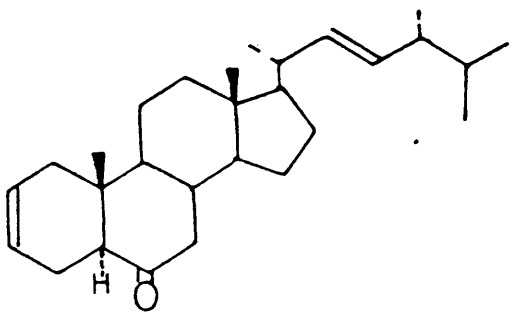
(81) R =



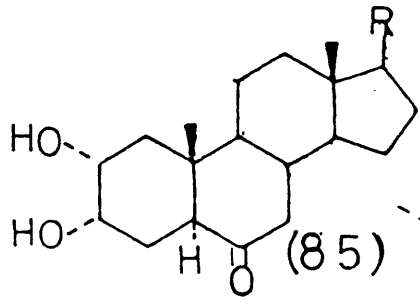


(83a) R=H

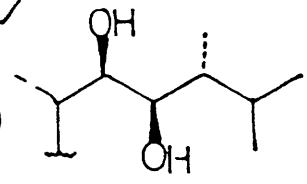
(b) R=Ts

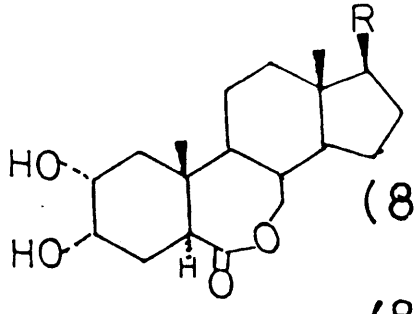


(84)

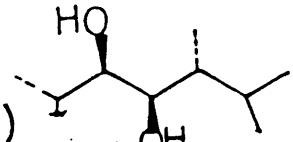


(85)

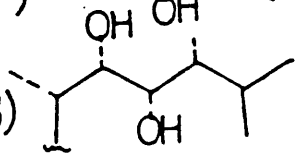




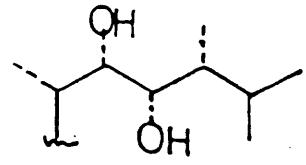
(87)



(88)

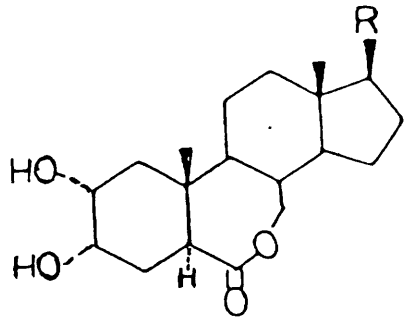


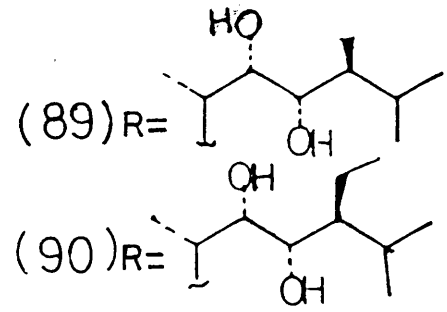
(86)



Ergosterol tosylate (79b) was solvolysed[35] to i-ergosterol and converted by oxidation with chromic acid in pyridine to the α,β-unsaturated ketone (82). Reduction of (82) (with Li/liquid NH_3) gave the 7,8-dihydro-derivative from which the 3β-hydroxy group was regenerated via acid-catalysed rearrangement. Treatment of the tosylate with LiBr in boiling dimethylformamide led to smooth elimination to give the diene intermediate (84). Osmium tetroxide per-hydroxylation of (84) in pyridine followed by reductive cleavage of the osmate ester gave a 1:1 mixture of the tetrahydroxy ketones (85) and (86). Separation was achieved on a column of alumina (grade III) eluted with chloroform-methanol to give pure compounds. Baeyer-Villiger oxidation[36] of the respective tetraacetate derivatives in chloroform with m-chloroperbenzoic acid for two weeks at room temperature gave predominantly the crude tetra-acetoxy-7-oxa-6-oxo steroids. These lactones were purified by chromatography and deprotected to (87) and (88). Brassinolide and its C-22, C-23 isomer (89) have been prepared from 22-dehydrocampesterol via this approach by Mandava et al. [37] Stigmasterol (81) has also been transformed to two isomeric brassinosteroids, 2α, 3α, 22S, 23S-tetrahydroxy-24S-ethyl-7-oxa-B-homo-5α-cholestan-6-one (90), and the minor isomer (C - 22R, C-23R).

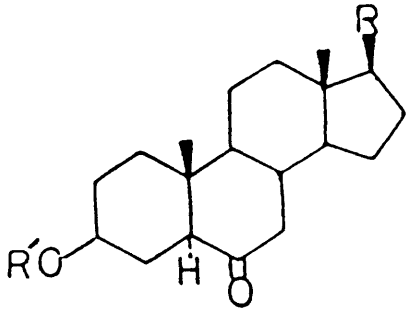
The synthesis of the brassinosteroid (90) from stigmasterol has also been reported by Wada and Marumo[34] in a procedure slightly different from that of Mandava et al. Analogues with modified ring A substituents were also reported by this group. As in Mandava's work, compound (91) was similarly generated from stigmasterol tosylate by solvolysis, oxidation of the i-stigmasterol and acid-catalysed regeneration of the 3β-hydroxy-5-ene function. Osmylation of (91) in dry benzene and a trace of pyridine followed by reductive cleavage of the osmate ester gave an isomeric mixture (94:6) of C-22S, 23S and C-22R, C-23R compounds (92a) and (93). The diacetate of the major isomer (92a) underwent elimination smoothly with LiBr in boiling dimethylformamide to the Δ^2-olefin derivate (94). Stereospecific α-face hydroxylation with osmium tetroxide and reductive cleavage of the osmate ester, followed by protection as acetate yielded the tetra-acetoxy ketone. Oxidation with m-chloroperbenzoic acid afforded two isomeric lactones (83:17), the 7-oxa and 6-oxa compounds. The tosylate (91) was converted to the 7-oxa lactone (95) by sequential Baeyer-Villiger oxidation and detosylation with LiBr in dimethylformamide. Catalytic hydrogenation yielded the 2,3-dihydro derivative (96). 2β-Methoxy, 3α-hydroxy and 2β, 3α- diol analogues were synthesised in a





(89) R=

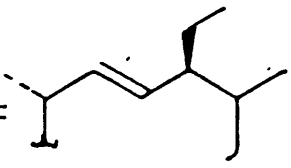
(90) R=



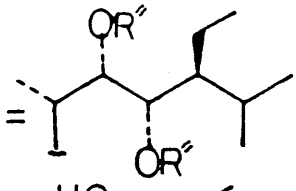
R' = H

R' = Ts

(91) R=



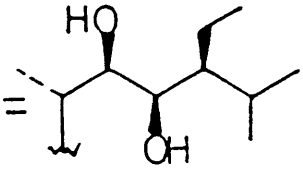
(92) R=

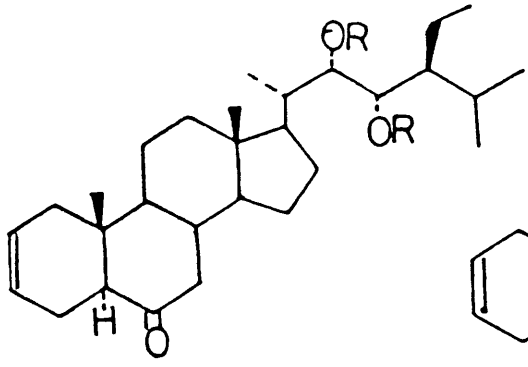


(a) R'' = H

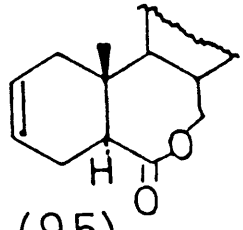
(b) R'' = Ac

(93) R=

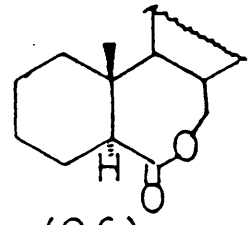




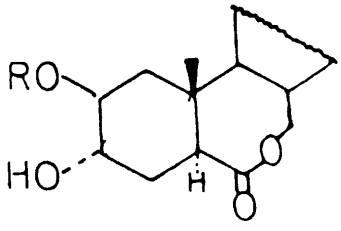
(94)



(95)

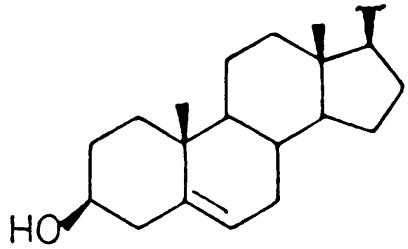


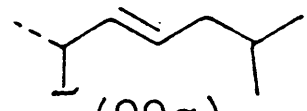
(96)



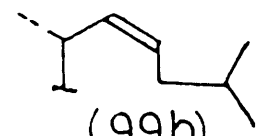
(97) R = H

(98) R = CH_3





(99a)



(99b)

sequence involving α-face expoxidation of the α^2-olefin (94) followed by diaxial fission[37] in 5N-H_2SO_4 or aqueous tetrahydrofuran, respectively.

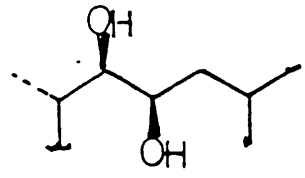
The synthesis of four C-22, C-23 stereoisomers of 28-nor-brassinolide from 22(E) and 22(Z)-dehydrocholesterol precursors have been reported[38] also. One of these isomers was transformed into 28-nor brassinolide. The Z-olefin (99b) was made according to Hutchins et al.[39] The E-isomer was obtained from the ester (100) in a sequence involving reduction (LAH/THF), mesylation (MsCl/pyridine) and a further reduction to the terminal methyl group.

Catalytic OsO_4 and N-methylmorpholine-N-oxide-perhydroxylation of the E-olefin afforded 22R, 23R- and 22S, 23S-dihydroxycholesterols in 76% yield. The Z-olefin with OsO_4 in ether for 10 days gave the isomeric 22R, 23S and 22S, 23R-dihydroxycholesterols. Configurational assignments were proved via an alternative synthesis of 22R, 23R from (LAH/THF) reduction of 22R, 23R-epoxide and Grignard reaction on (103) followed by saponification. The 22R, 23S-isomer, one of the products of osmylation of the Z-olefin was isolated along with the 22R, 23R-isomer in Grignard synthesis. Proof of the configuration of the second Z-olefin-derived dihydroxy compound came from the alternative synthesis

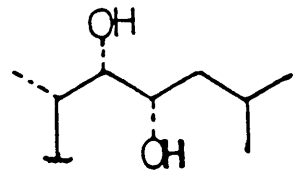
via the 22S, 23S-epoxide of the ester (100).

The 22R, 23R-dihydroxycholesterol was transformed into 28-nor-brassinolide via the enone (104). 22R, 23R-dihydroxycholesterol was converted by a sequence of acetonide formation, mesylation hydroboration-oxidation and pyridinium chlorochromate oxidation into 3β-mesyloxy-22R, 23R-dihydroxy-cholestan-6-one 22, 23-acetonide. Treatment with LiBr in boiling dimethylformamide eliminated MsOH to give the enone (104). The α-face hydroxylation with catalytic OsO_4 and N-methylmorpholine-N-oxide followed by acetylation gave the diacetate (105). Baeyer-Villiger oxidation of (105) (using CF_3CO_3H-Na_2HPO_4/CH_2Cl_2) and subsequent base treatment furnished the 28-nor-brassinolide (106).

Recently, Konda and Mori[40] reported the synthesis of four brassinolide analogues, (107), (108), (109) and (110), starting from cholesterol, stigmasterol or pregnenolone. It was found that an analogue possessing a hydroxyl group at C-17, (110), instead of the steroidal side chain was only 0.001% as active as brassinolide upon lamina-inclination testing with rice seedlings, while the other analogues were 1-2% as active as brassinolide. This indicates the indispensable role of the side chain for the plant growth promoting activity of brassino-steroids. The synthetic routes



22R 23R



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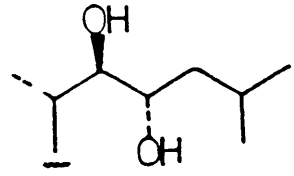

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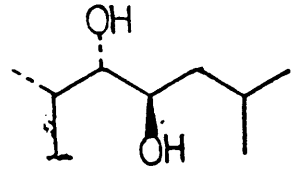

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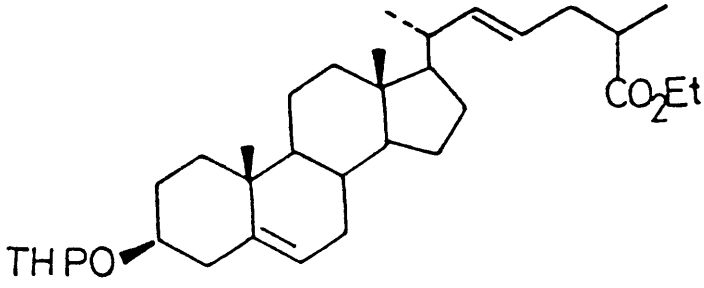

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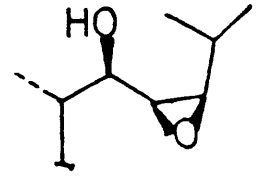

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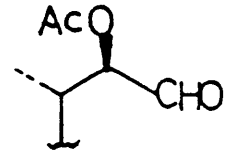

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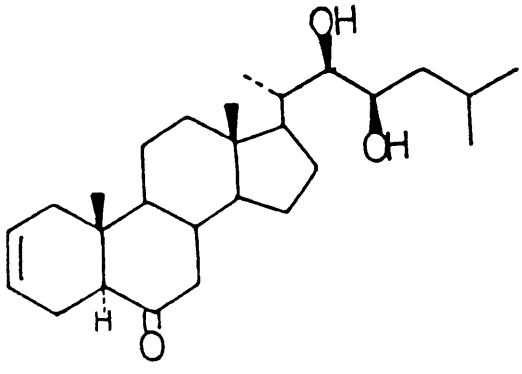

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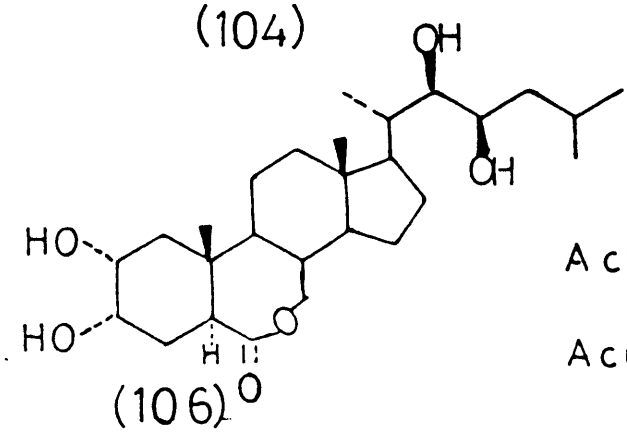

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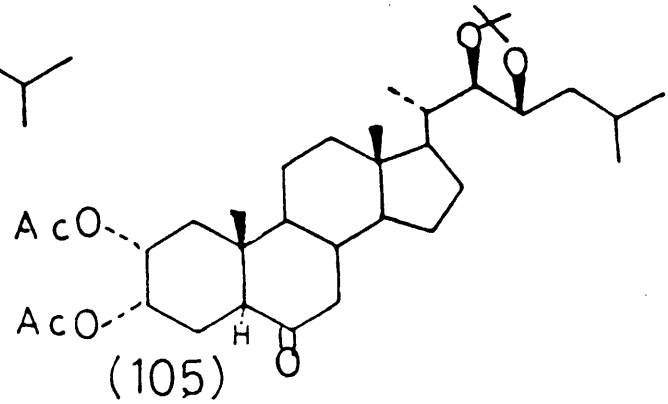
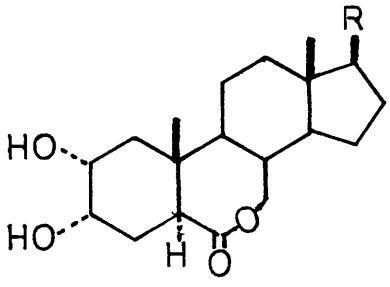
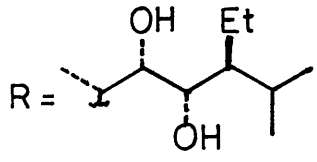


(105)

to these four analogues were similar to those employed for the synthesis of (22S, 23S)-homobrassinolide (90)[41,42] as shown in Fig.6. The starting material for the synthesis of (107) was cholesterol (2). This was converted to the ketone (115) via (111) and (113).[42-44] Treatment of (115) with p-toluenesulphonic acid in sulpolane gave (117). Hydroxylation of (12) with osmium tetroxide and N-methylmorpholine-N-oxide yielded a diol (118a). The corresponding acetate (118b) was submitted to the Baeyer-Villiger oxidation to give the lactone (119). This was converted to the desired 22,23-bisdeoxy-28-nor-brassinolide (107) in the conventional manner. For the synthesis of (108) and (109), the known aldehyde (57) was employed as the starting material, which was in turn prepared from stigmasterol.[41] Addition of isoamylmagnesium bromide to (57) afforded (120). The (S) configuration tentatively assigned to the newly generated hydroxyl group at C-22 was based on the assumption that Cram's rule was applicable in this particular case as it was in others.[45,17] After removing the acetonide protective group, the triol (121a) was obtained. The Baeyer-Villiger oxidation of the corresponding acetate (121b) yielded the lactone (122). This gave 23-deoxy-28-nor-brassinolide (108). To synthesise (109), the aldehyde (57) was reduced with LAH to (123). Removal of the protecting group yielded the triol (124a); the derived triacetate (124b) was oxidised





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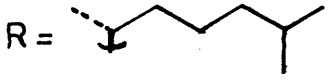

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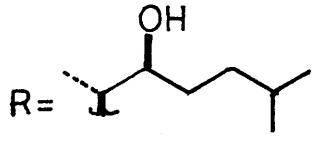


(108)

(109)

R = OH

(110)

with trifluoroperacetic acid to give the lactone (125). This gave the brassinolide analogue (109) with a shortened side chain.[47]

The synthesis of (110) started from pregnenolone (112a). Solvolysis of pregnenolone tosylate (112b) yielded (114).[43] This was oxidised to the ketone (116). Acid treatment of (116) gave the unsaturated ketone (126). This yielded the glycol (127a) upon oxidation with osmium tetroxide. The corresponding diacetoxy diketone was submitted to the Baeyer-Villiger oxidation to give the triacetoxy lactone (128). This gave the nuclear analogue (110) of brassinolide in the usual manner.

More recently, Mori et al.[48] reported the synthesis of seven new plant growth-promoting steroids as well as a new method for the synthesis of brassinolide (38). The seven new analogues are; castasterone (129), dolicholide (130), dolichosterone (131), homodolicholide (132), homodolichosterone (133), 6-deoxocastasterone (134), and 6-deoxodolichosterone (135). The three types of the steroidal side-chain of brassinosteroids as seen in brassinolide (38), dolicholide (130) and homodolicholide (132), were all constructed by the regio- and stereoselective ring-opening reactions of the appropriate expoxides, 23, 24-epoxides, as shown in (Fig. 7.). These epoxides were prepared from stigmasterol.

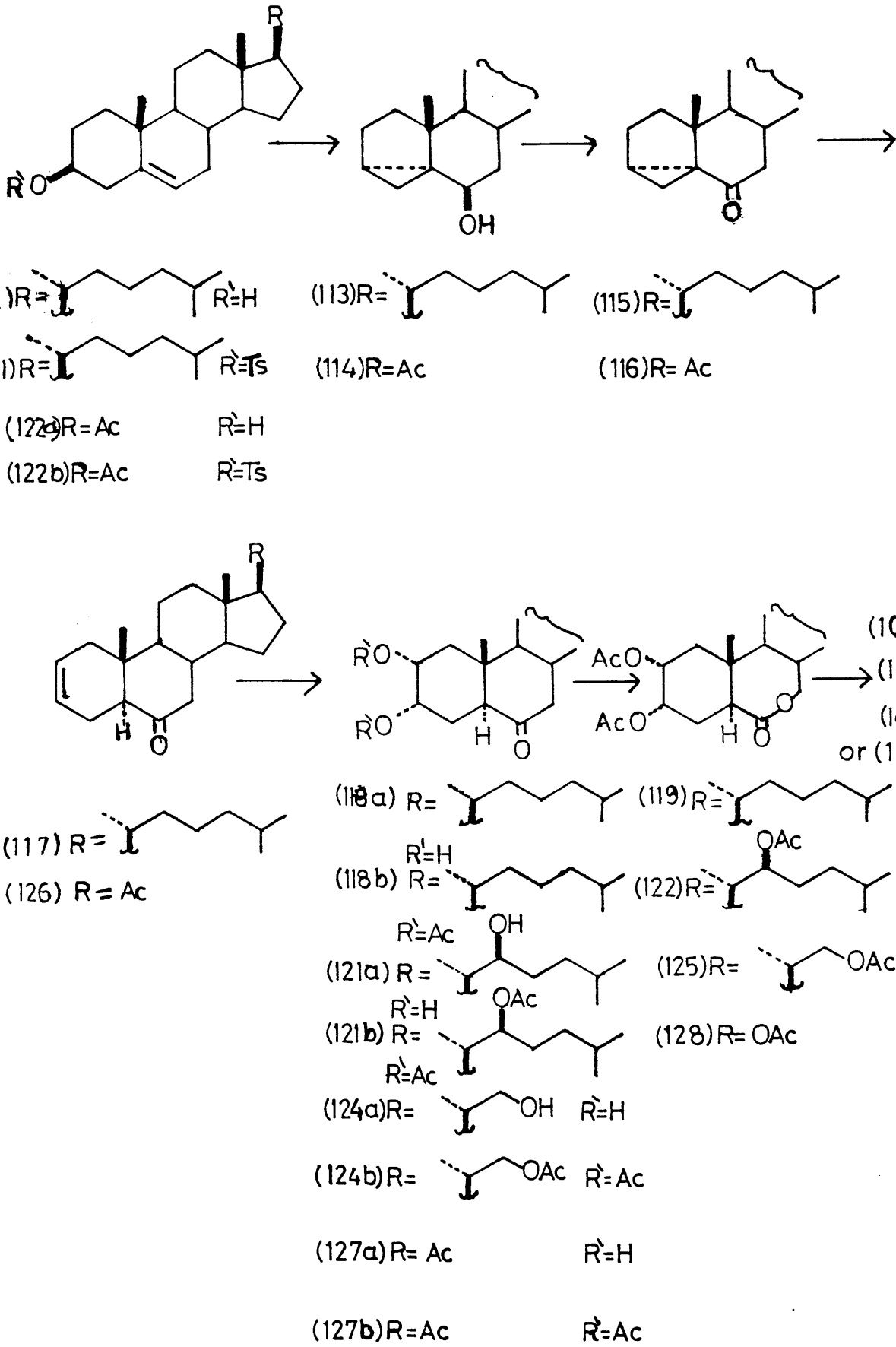


Fig. 6

Synthetic routes to brassinolide analogues.

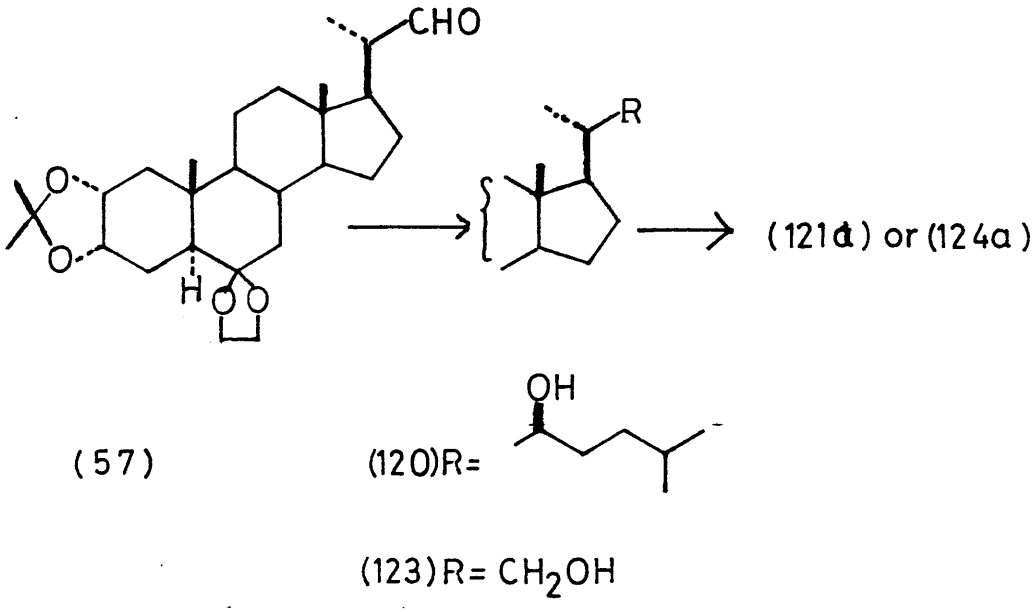
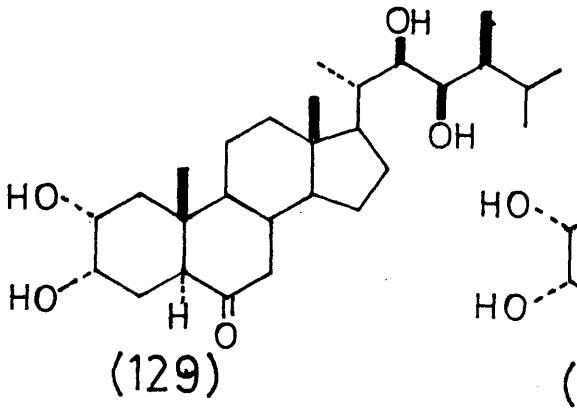


Fig. 6 (contd.)



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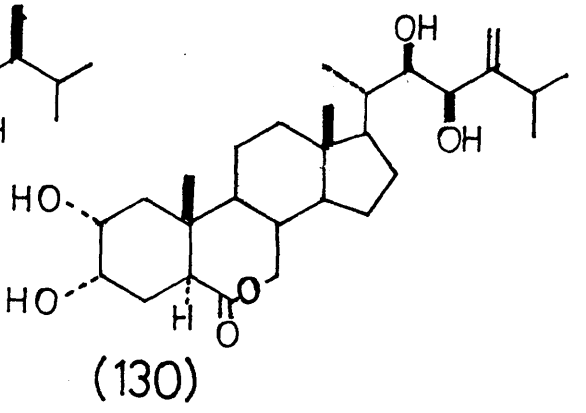

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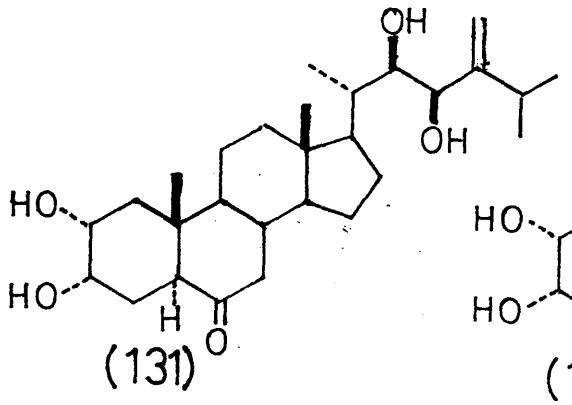

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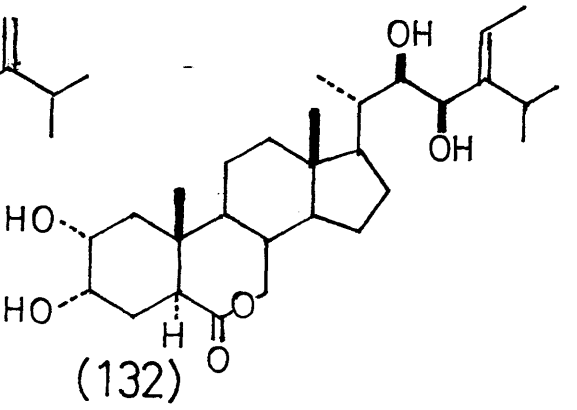

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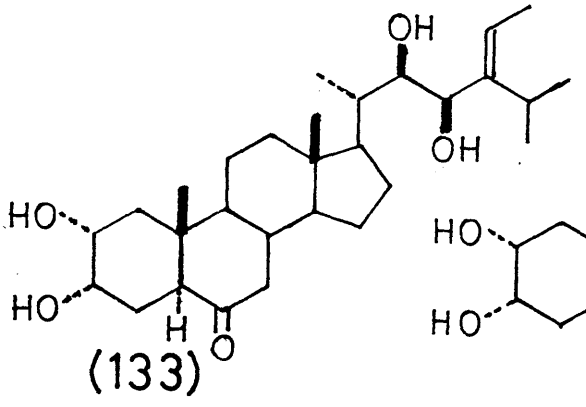

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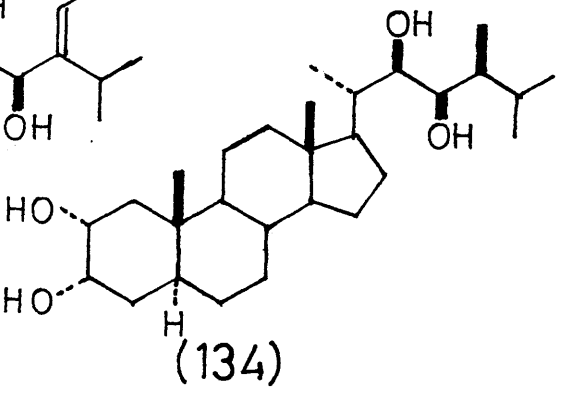

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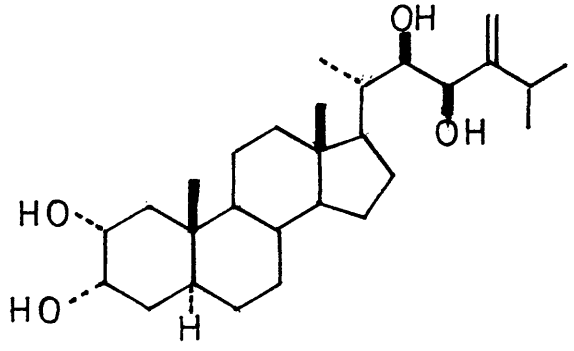

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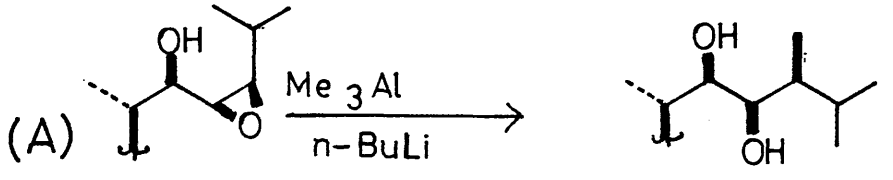
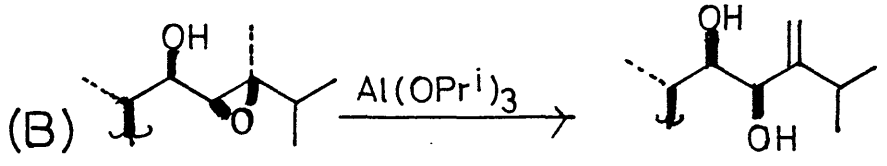




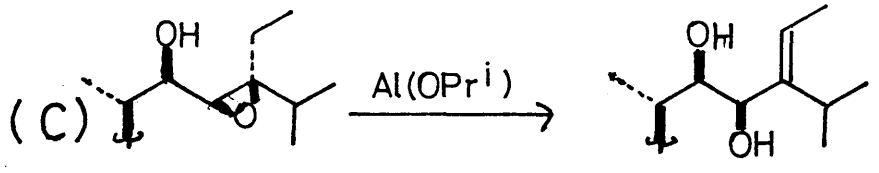
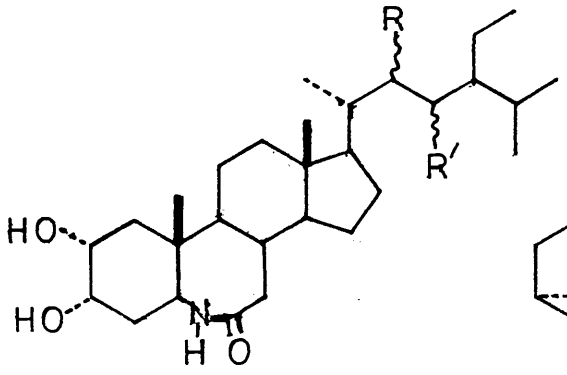


Fig.7

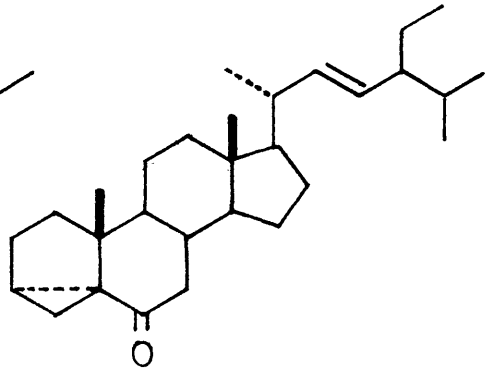
Epoxide cleavage reactions employed for the construction of the steroidal side-chain.

Anastasia et al.[49] have reported the synthesis of an aza-analogue of brassinolide, (2R, 3S, 22S, 23S)-2,3,22,23-tetrahydroxy-6-aza-B-homo-5α-stigmastan-7-one (136a). Its isomer (136b) was also obtained as a minor product. The starting material for the synthesis was stigmasterol which was converted to the known (22E)-3α, 5-cyclo-5α-stigmast-22-en-6-one (137).[50] This ketone was transformed to the isomeric Δ^2-ene by quantitative conversion of (137) to (22E)-3β-chloro-5α-stigmast-22-en-6-one (138) by treatment with hydrochloric acid in acetic acid and successive dehydrohalogenation of (138) to (22E)-5α-stigmasta-2, 22-dien-6-one (55) by heating with lithium bromide in dimethylformamide.[51] Treatment of (55) with 1 mol equivalent of hydroxylamine hydrochloride gave a single oxime (139), the stereochemistry of which was assigned on the basis of its behaviour in the Beckman rearrangement in non-equilbrating conditions. Thus, thionyl chloride treatment[52] of (139) immediately gave a single lactam identified as (22E)-6-aza-B-homo-5α-stigmasta-2, 22-dien-6-one (140). No traces of the isomeric lactam (141) were detected. The same lactam (140) was obtained less conveniently from (55) with the Schmidt reaction using sodium azide and polyphosphoric acid.[53] The oxidation of the di-unsaturated lactam



(136a) R=β-OH, R′=β-OH

(136b) R=α-OH, R′=α-OH



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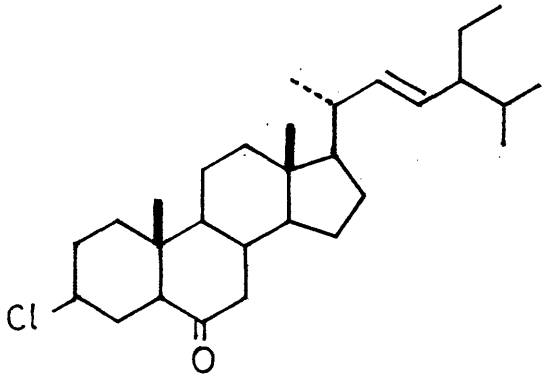

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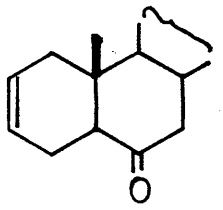

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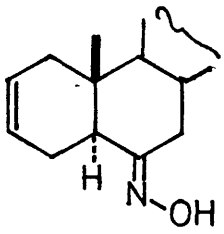

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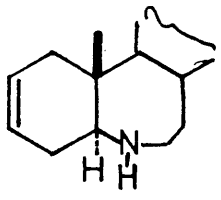

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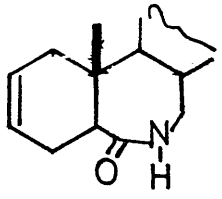


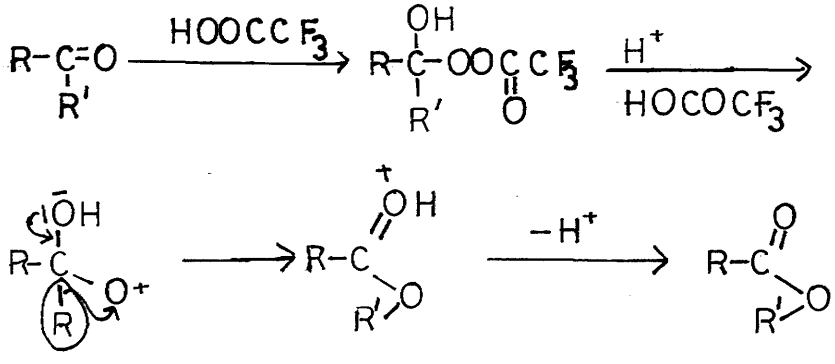
(141)

(140) with osmium tetroxide afforded the 22S, 23S-dihydroxyaza analogue (136a) of the homobrassinolide accompanied by minor amounts of its 22R, 23R isomer (136b).

Although, it is well known that in the peracid oxidation of cyclohexanones a tertiary carbon atom migrates in preference to a secondary one, we have seen, in the above reported methods for the synthesis of brassinolide and its analogues, an exception to this rule. It has also been observed that, in the Baeyer-Villiger oxidation of 6-keto steroids, the presence of C-3 substituents leads to more pronounced migration of C-7 than in their absence.[46a] Moreover, the bulk of the C-3 substituents has been reported to have an effect on the preferred migratory aptitude of C-7 in relation to C-5. So far, there is no satisfactory explanation for the migration of C-7 instead of C-5, in the Baeyer-Villiger oxidation of 6-keto steroids. However, it seems that this behaviour is motivated by the conformation of the substrate and the effect of the steric hindrance on the stability of the formed intermediate (peracid adduct).

The nature of the oxidising agent is also important. Trifluoroperacetic acid (CF_3CO_3H), for instance, is the most efficient reagent in the Baeyer-Villiger oxidation of cyclic ketones. While methyl cyclopropyl ketone is completely inert to perbenzoic acid, it afforded a high yield of the

corresponding ester by treatment with (CF_3CO_3H).[46b] The oxidation of 5α-6-keto steroid with trifluoroperacetic acid is 1000 times faster than oxidation with m-chloroperbenzoic acid and also is more regioselective.[11] The small size and the strong acidity of trifluoroacetic acid may be the reasons behind its efficiency. Trifluoroacetic acid probably functions as a catalyst (Scheme 1) because of its strong acidity with respect to other acids (see Table below).



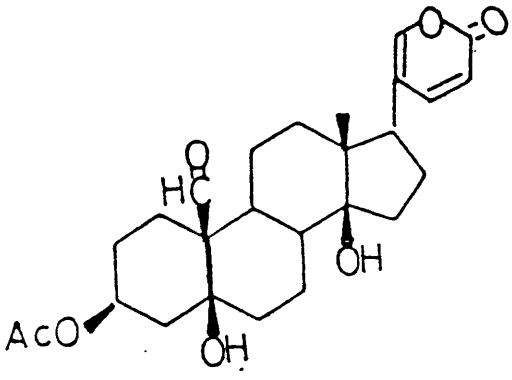
(Scheme 1)

Acid	pKa
CF_3CO_2H	0.30
CH_3CO_2H	4.80
$C_6H_5CO_2H$	4.20
m-Cl- $C_6H_4CO_2H$	3.82

1.7 Steroidal Ring-C Lactones

Many of the naturally occurring sesquiterpenes, the higher terpenes, steroids, alkaloids, and sugars isolated in recent times, contain lactone functions. In the steroids, the lactones are often encountered as 5- or 6-membered extra-nuclear structures. Examples include hellebrigenin (142), strophanthidin (143), withaferin (144), and antheridiol (145). The biological activity of some of these compounds has attracted considerable interest towards their synthesis.[54] Lactones, on the other hand, have themselves served as key intermediates in the synthesis of medicinally important molecules. Thus some -lactones have been of some considerable interest in the synthesis of prostaglandins[55,56] and anti- tumour agents.[57]

In the literature, some 6- and 7-membered ring-C lactones and the aza analogues of some of them have been reported. Kutney et al.[58] have reported the synthesis of 3β-hydroxy-11-oxa-5α-22β-spirostan-12-one (148) by reduction of the keto acid (147) with sodium borohydride. The same keto acid (147) [prepared by ozonolysis of the 9,11-dehydrohecogenin acetate (146)], when treated with anhydrous ammonia in a sealed tube at 150°C for 15 hours, gave an excellent yield of the enol lactam (149).



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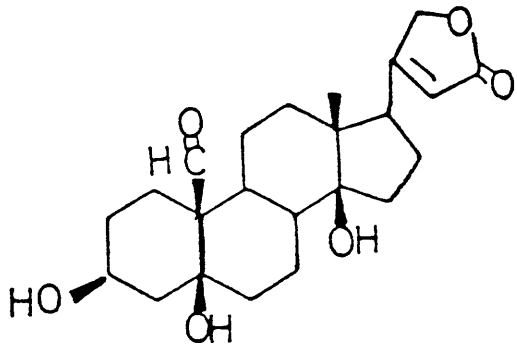

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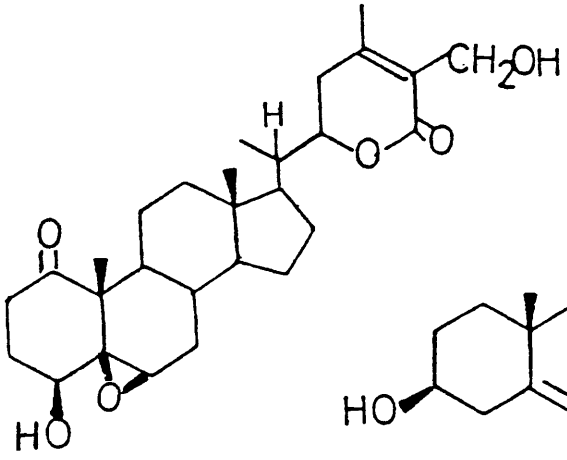

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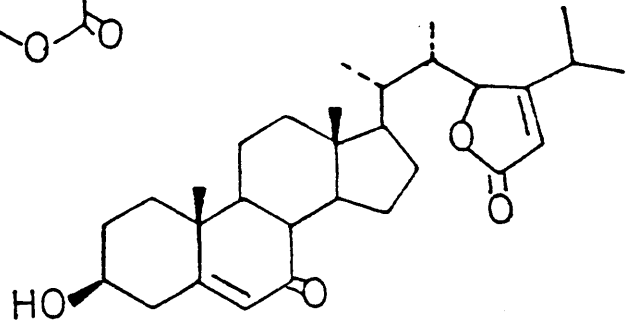

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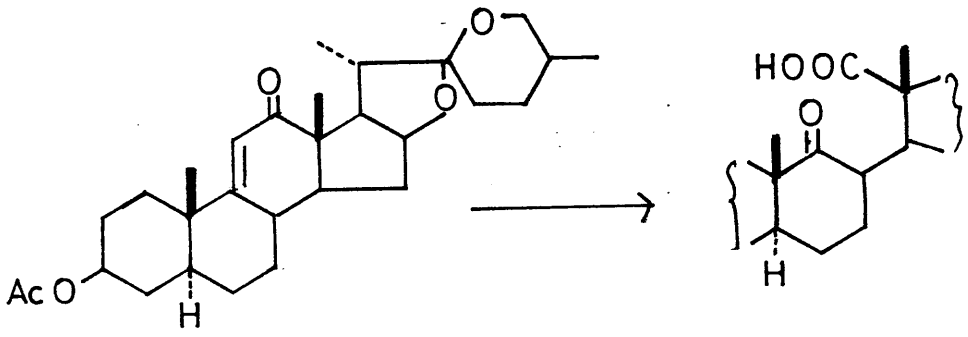

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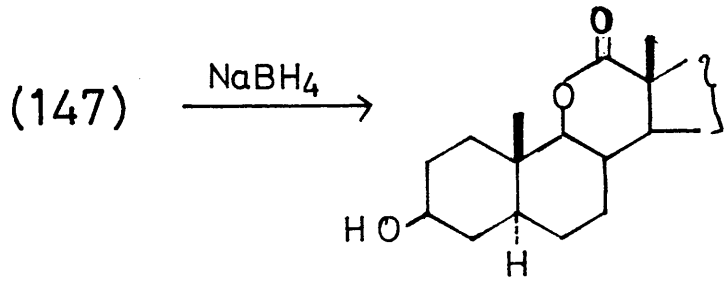

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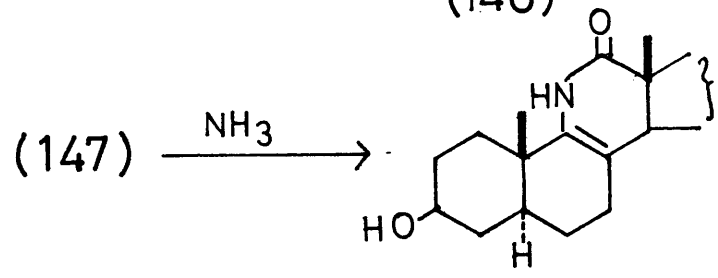
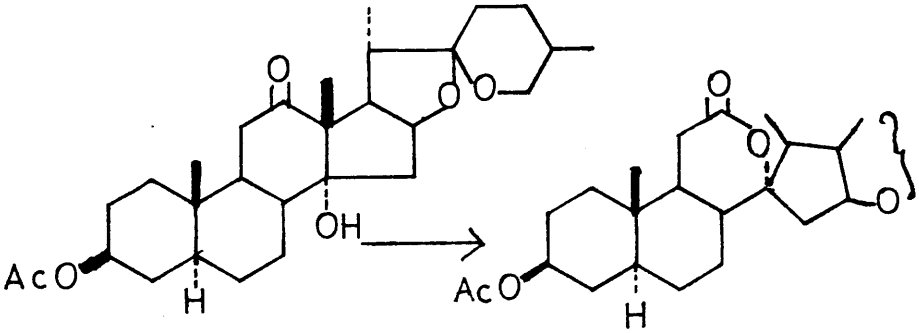


(149)

The spirolactone (151) was prepared [59] by irradiation of a solution of 14α-hydroxyhecogenin (150) in dioxan, in a quartz flask, by a 500-W mercury lamp at the boiling point.

Rothman et al.[60] first reported the preparation of steroidal seven-membered ring-C lactones. They described the Baeyer-Villiger oxidation of both hecogenin acetate (152) and the bile acid derivative (154) to the corresponding lactones (153) and (155) respectively. The hecololactone (153) was prepared by treatment of hecogenin acetate (152) with perbenzoic acid in chloroform solution containing 9.3 volume percentage of sulphuric acid. The reaction proceeded smoothly at room temperature for 12 days, giving 98% yield. Similarly, the methyl 3α-cathyloxy-12-ketocholanate (154) oxidised in the same manner afforded the corresponding lactone (155). Degradation of the hecololactone (153) to the 12, 13-seco-16-allopregnene-20-ketone (156) was done by Rothman and Wall.[61] The three-step Marker[62] procedure of sapogenin degradation involving the isomerization of the sapogenin acetate with acetic anhydride at 200°C to a pseudosapogenin diacetate, subsequent oxidation to a 16-acyloxypregnane-20-one, and hydrolysis to a Δ^{16}-20-ketone is applicable to hecololactone acetate. However, the hydrolytic conditions in the third step were modified in order to obtain the desired product (156).



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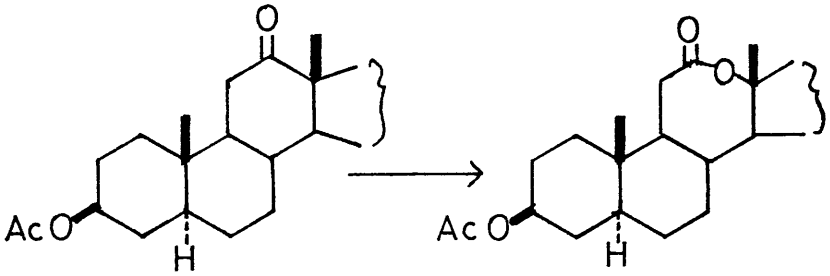
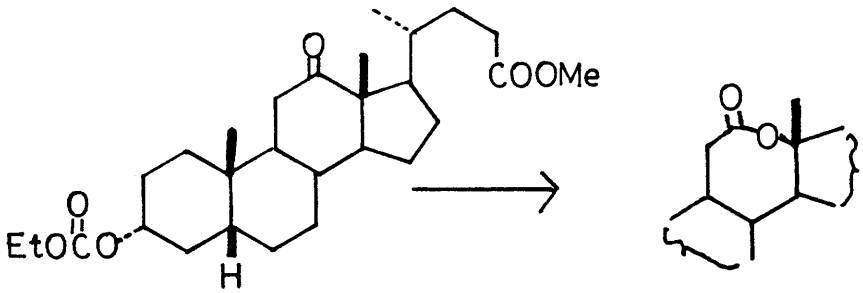


(152) (153)



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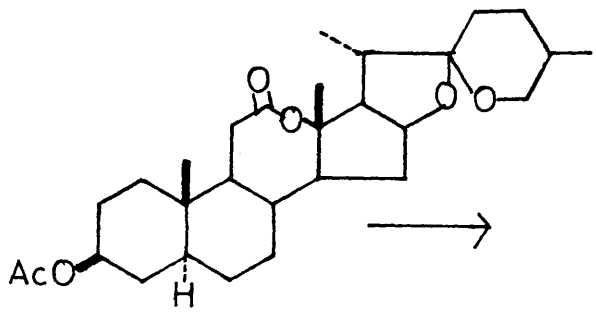

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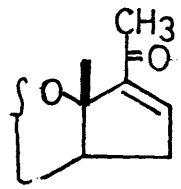

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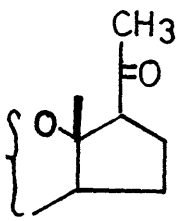

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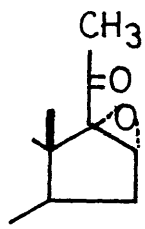

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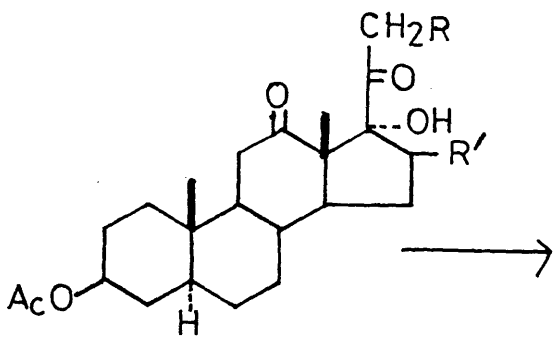
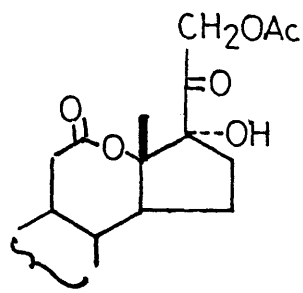


(159) R = H ; R′ = Br

(160) R = H ; R′ = H

(161) R = Br ; R′ = H

(162) R = OAc ; R′ = H

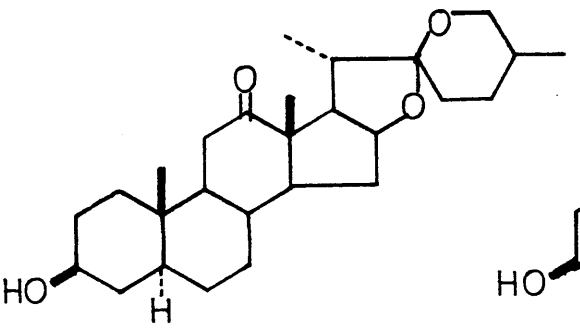


(163)

The acyloxy oxidation product of the pseudosapogenin was dissolved in t-butyl alcohol and treated with 5% aqueous sodium bicarbonate to give the 12,13-seco-16-allopregnen-12,13-lactone derivative (156). This gave the saturated lactone (157) upon hydrogenation. Treatment of (156) with alkaline hydrogen peroxide afforded the lactone epoxide (158).

Rothman and Wall have also reported the preparation of 12-carboxy-12, 13-seco-allopregnane-3β, 13α-17α,21-tetrol-20-one12,13-lactone 3, 21-diacetate (163).[63] They started with 16-bromoallopregnane-3β,17α-diol-12,20-dione 3-acetate (158), a compound first synthesised by Mueller et al.[64] This was converted to (160) in 96% yield by the use of palladium on calcium carbonate.[65-67] Monobromination of (160) followed by acetolysis gave the 3β, 21-diacetoxy-17 α-5α-allopregn-12,20-dione (162). Baeyer-Villiger oxidation of (162), using one equivalent of perbenzoic acid in the presence of sulphuric acid as catalyst for 70 hr, afforded the lactone (163). The oxidation occurred at C-12 only.

Bladon and McMeekin[68] investigated the Baeyer-Villiger oxidation of hecogenin (164). Oxidation of this with peracetic acid in a two-phase system in the presence of sulphuric acid gave a lactone mixture. The crude product was hydrolysed directly to give a mixture of acids that, on crystallisation, gave 82% of hecolic acid (165) and 5% of a second,



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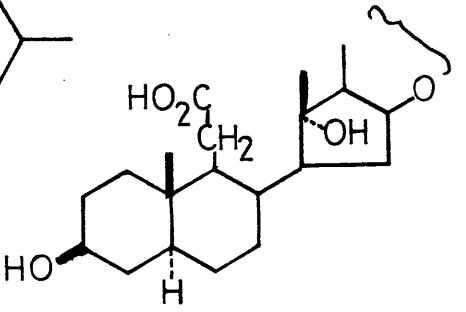

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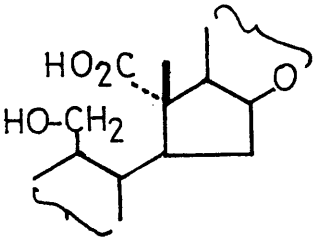

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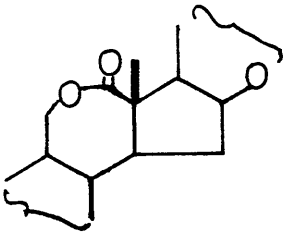

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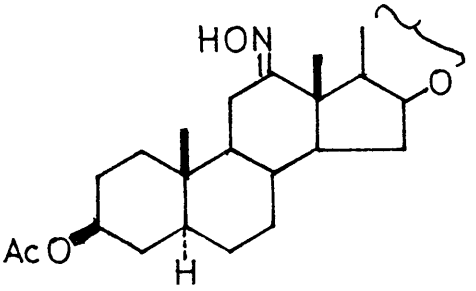

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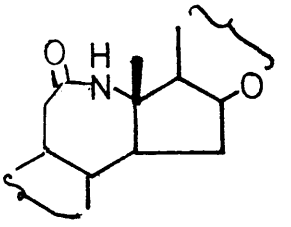

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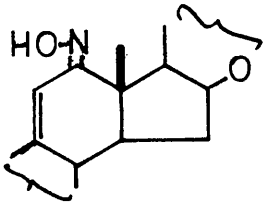

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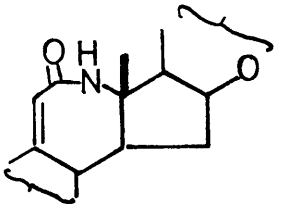

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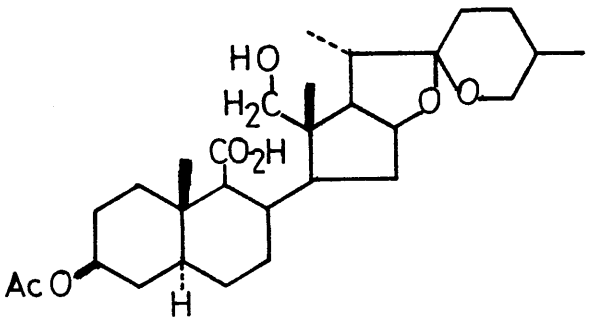

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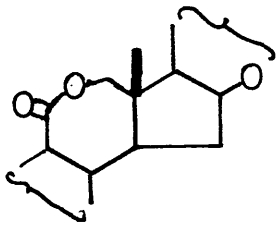

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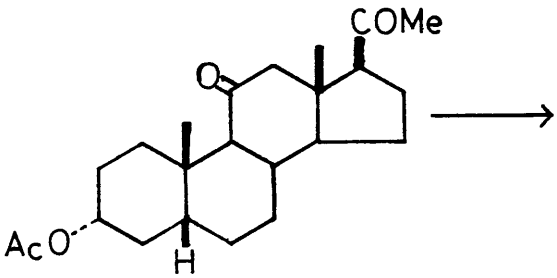

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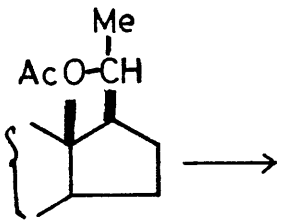

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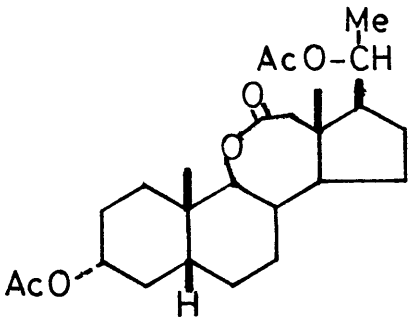


(176)

isomeric acid which was called isohecolic acid (166).[68] Sublimation of (166) afforded the lactone (167). Bladon and McMeekin[68] have also reported the rearrangement[69] of the oxime [70] of hecogenin acetate (168) catalysed by toluene-p-sulphonyl chloride in pyridine to give the lactam, hecololactam (169). Similarly, the oxime (170) was converted to the unsaturated lactam (171).

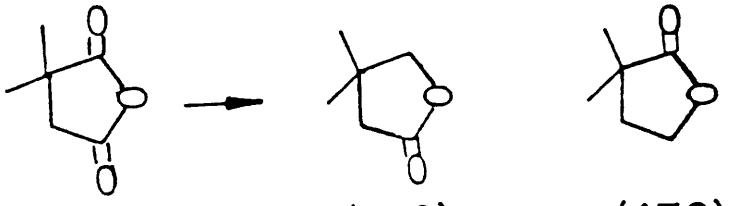
Meanwhile, Zderic et al.[71] have reported the synthesis of the lactone (173) by treatment of the corresponding hydroxy-acid (172) with pyridine and acetic anhydride at room temperature.

The synthesis of the 3α,20β-diacetoxy-9β-hydroxy-9,11-seco-5α-pregnan-11-oic acid 9, 11-lactone (176) was also reported.[72] The lactone (176) was obtained from Baeyer-Villiger oxidation (using trifluoro-peracetic acid) of 3α, 20β-dihydroxy-5β-pregnan-11-one (175). The latter was, in turn, prepared from the sodium borohydride reduction of 3α-acetoxy-5β-pregnan-11, 20-dione (174) followed by acetylation with acetic anhydride and pyridine.

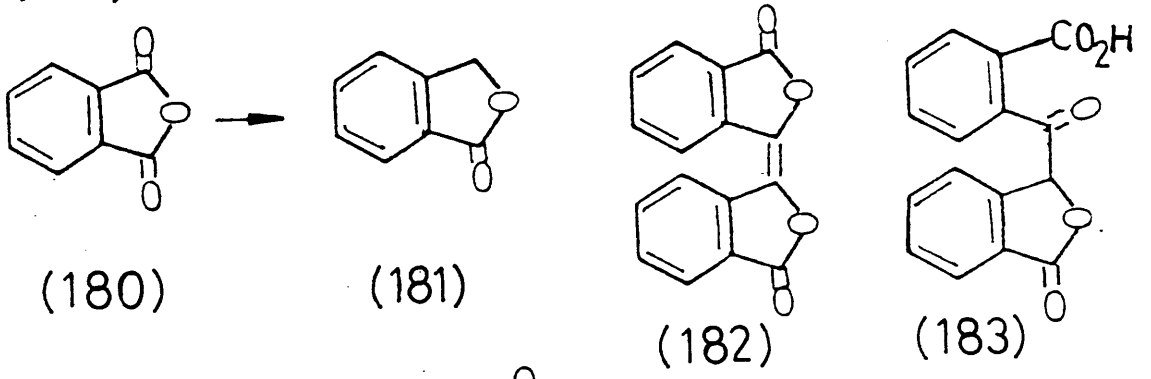
1.8 Metal Hydride Reduction of Cyclic Anhydrides

The reduction of organic carbonyl compounds with metal hydrides was first observed in 1939 by Brown et al.[73] Before this

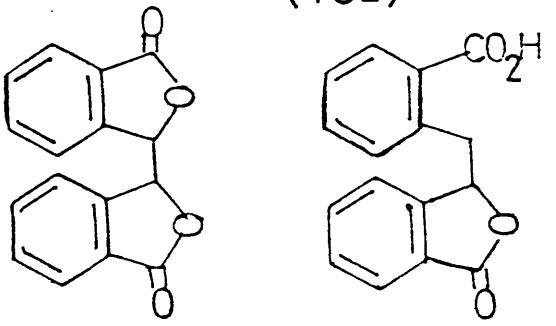
publication, methods requiring the use of such reagents as zinc/acetic acid or sodium metal dissolving in methanol were widely employed in these reductions.[74-76] Other methods[77-82] were reintroduced as improved procedures. These methods have many drawbacks since these reactions often required elevated temperatures, long reaction times, and gave low yields on the desired products. The early observations of metal hydride reduction due to Herbert C. Brown led to the discovery of sodium borohydride[83] and lithium aluminium hydride. [84] After the exploration of the reducing properties of these reagents, many more remarkable reducing hydrides have been introduced to organic chemistry. These reagents have substantially improved procedures for the regio-, stereo- and chemoselective reduction of various functional groups. [85,86] Sodium cyanoborohydride, [87] for example, has been reported to effect efficient and selective reduction of alkyl halides to alkanes,[88] imines to amines,[89] and tosylhydrazones derived from aldehydes and ketones to their corresponding alkanes[90] all in excellent yields. Hindered trialkylborohydrides, e.g. lithium tri-isoamylborohydride, show the unusual ability to introduce major steric control in the reduction of cyclic ketones.[91,92] The investigations in the past years have made available to organic chemists a choice of hydride reagents for achieving specific synthetic transformations. Accordingly,



(177) (178) (179)



(180) (181) (182) (183)



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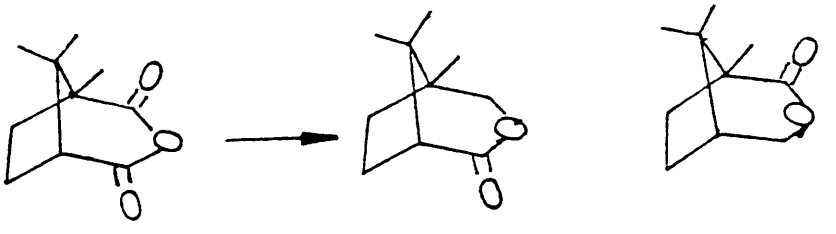


(186) (187) (188)

numerous applications for these reagents have been reported and are still appearing in the literature.

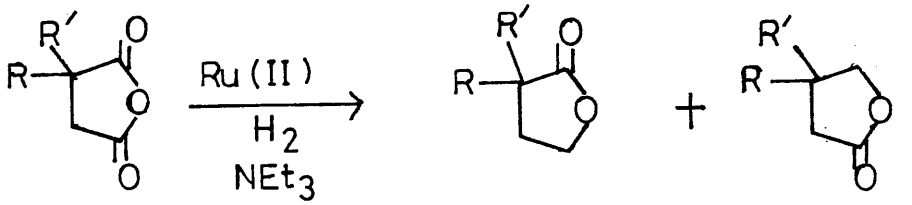
However, before metal hydrides were introduced, there had been reports in the literature which indicated that lactones are possible products from the reduction of cyclic anhydrides. A variety of conditions have been used in the transformation of one carbonyl group to methylene. Phthalic anhydride, for example, has been reduced with zinc in acetic acid[93] or Raney nickel to afford phthalide,[94] while upon amyl reduction with sodium in pentyl alcohol,[95] phthalic anhydride afforded hexahydrophthalide, the corresponding hydroxy-acid and hexahydro-α-toluic acid. For non-aromatic anhydrides, examples include the reduction of succinic anhydride with sodium amalgam[96] in 12% yield, reduction of cis-1-methyl-cyclohexane-1,2-dicarboxylic anhydride[97] with sodium in ethanol to the corresponding isomeric lactones and of 2, 2-dimethylsuccinic anhydride (177) to the lactone (179).[98] Blanc has reported that the lactone (179) was the only product of the latter reaction, other than significant amount of the parent dicarboxylic acid. When Morand and Kayser[99] employed an improved analytical procedure, they observed that the 2,2-dimethylsuccinic anhydride was reduced to a mixture of lactones (178) and (179) in a ratio (11:9). Similarly, Brewster and Fusco[100] re-examined

the reduction of phthalic anhydride (180) with zinc in acetic acid and showed that in addition to phthalide (181), a series of bimolecular products was obtained, (182) to (185). The proportions of these products depended on the reaction conditions.

The reduction of anhydrides to lactones via hydrogenation has also been reported. Phthalic anhydride with hydrogen in the presence of platinum and acetic acid afforded hexhydrophthalide, the corresponding hydroxy-acid and hexahydro-o-toluic acid.[101] The reduction of cyclooctene-1, 2-dicarboxylic anhydride under these conditions gave the corresponding saturated lactone.[102] McCrindle et al[103] obtained hexahydrophthalide via hydrogenation of hexhydrophthalic anhydride. Camphoric anhydride (186) yielded β- and α-campholide, (187) and (188), succinic anhydride gave butyrolactone accompanied by butyric acid as a minor product, while cyclobutane-1,2-dicarboxylic anhydride uniquely gave the methyl acid, (2-methylcyclobutanecarboxylic acid) under these conditions. However, in acetic acid no uptake of hydrogen was observed in the reduction of camphoric anhydride. Recently, ruthenium (II) complexes have been shown to be efficient catalysts in the selective homogeneous hydrogenolysis of carboxylic acid anhydrides.[104] Lyons et al. applied this method to the synthesis of butyrolactone and phthalide from their

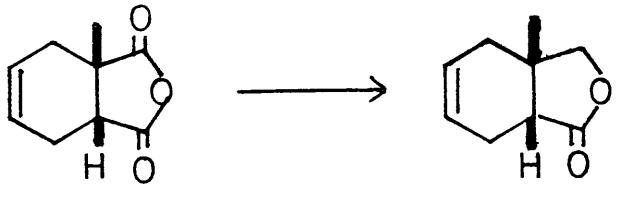
corresponding anhydrides.[105] For some unsymmetrical anhydrides, the procedure has been demonstrated to be regioselective and to provide lactones that are inaccessible via BH_4 reduction. Accordingly, Morand et al.[99] obtained from the reduction of 2,2-dimethylsuccinic anhydride via this method, the isomeric lactones (178) and (179) in a ratio of (1:9). More recently, Ikariya et al.[106] have reported that the hydrogenation of cyclic anhydrides (189) catalysed by ruthenium complexes produced the corresponding isomeric lactones (190) and (191), where the reaction preferentially occurred at the less hindered carbonyl group to give (190) selectively. This is a contrast in that the reduction of cyclic anhydrides $LiAlH_4$ or $NaBH_4$ tends to take place selectively at the more hindered carbonyl group.[107-110] A major drawback of this method is the high pressure required for the hydrogenolysis.

Of all available procedures for effecting the conversion of cyclic anhydrides to the corresponding lactones, reduction with metal hydrides is the simplest and most effective one. The most commonly used metal hydrides for this purpose have been $LiAlH_4$, $NaBH_4$; to a lesser extent $LiBH_4$, $Li[t\text{-}BuO)_3AlH]$ and very recently, lithium and potassium tri-sec-butylborohydrides (K- or L-Selectrides). The reaction of the

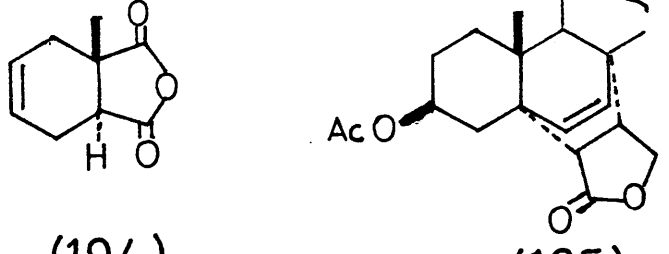


(189) (190) (191)

R = Me , R′ = H
or R = R = Me



(192) (193)



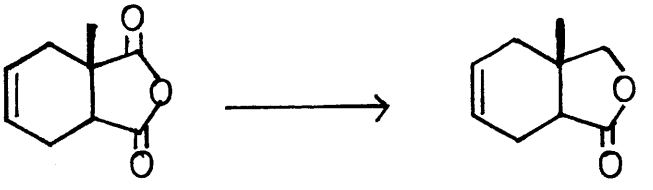
(194) (195)

anhydrides with these reagents has generally been carried out in aprotic solvents. These solvents include tetrahydrofuran, ethyl ether, diglyme, ethanol, and isopropyl alcohol. Sodium borohydride forms a hetero-geneous mixture with tetrahydrofuran, but $LiBH_4$ is very soluble in this solvent, though it has not been used as extensively as $NaBH_4$ in the reduction of anhydrides.[111] Much of the early literature reports on the controlled reduction of cyclic anhydrides to their lactones were based on reduction with $LiAlH_4$. Examples of these include the work of Darrini et al. [112] and of Farnum and Snyder.[113] The reduction of cis-1-methyl-cyclo-hex-4-ene-1,2-dicarboxylic anhydride (192) to the lactone (193) with $LiAlH_4$, provided the first indication of the regio-selectivity obtainable via metal hydride reduction.[114] This result, along with the observation of Brown and co-workers, [115,116] that lactones could generally be made from cyclic anhydrides by reduction with $Li[(t\text{-}BuO)_3AlH]$, prompted the investigation of the steric course of $LiAlH_4$ reduction of some 5-membered cyclic anhydrides. Thus Bloomfield et al.[117] reduced cis and trans-1-methyl-cyclohex-4-ene-1,2-dicarboxylic anhydrides (192) and (194) and their saturated analogues with $LiAlH_4$. The more hindered carbonyl group was

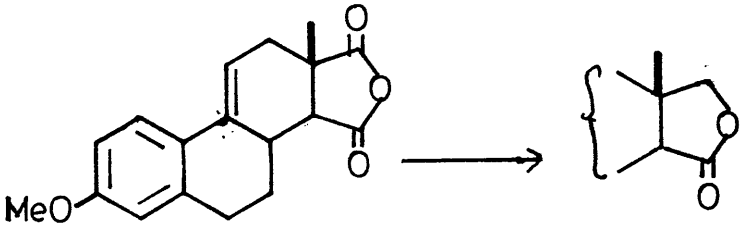
reduced yielding the lactones, which confirmed the findings of Granger et al.[114] In the steroid series, the Inhoffen adducts of ergosteryl acetate (or the 3β-methyl ether) and maleic anhydride were shown to be selectively reduced at the more hindered carbonyl group with $LiAlH_4$ to afford the lactone (195).[118]

Recently Kayser et al.[119] have observed that the conformation of the parent molecule should be considered in predicting regioselectivity of nucleophilic additions to cyclic anhydrides to form the corresponding lactones, since $LiAlH_4$, $NaBH_4$ or Selectride reduction of (196) and (198) gave exclusively the lactones (197) and (199), but the reduction of (200), under the same conditions, afforded the lactone (201) only, i.e. the reduction occurred at the more hindered carbonyl group in the first case but happened at the less hindered carbonyl function in the second. Among the few examples of 6-membered ring anhydrides that have been reduced with $LiAlH_4$ is the anhydride (202) derived from Gibberellin A_{13} which selectively afforded the lactone (203).[120] More examples of reduction under these conditions came from the work of Morand et al.[121] in which camphoric anhydride afforded α- and β-campholides in a ratio of (2:3).

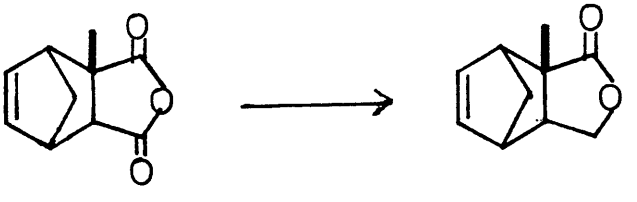
However, sodium borohydride has been the more extensively



(196) (197)



(198) (199)

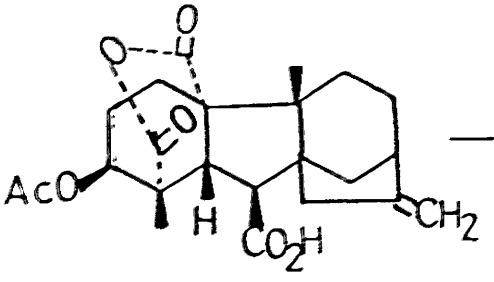


(200) (201)

used metal hydride for the conversion of cyclic anhydrides to lactones. Chaikin and Brown[122] first showed that anhydrides are reduced, though only slowly, on prolonged heating with $NaBH_4$. Very few reports[123,124] of $NaBH_4$ reduction of acid anhydrides appeared in the literature. These early reports included the reduction of carboxylic carbonic anhydride[125] and thiophthalic anhydride.[126] Bailey and Johnson, in 1970, investigated the course of regioselective reduction of some unsymmetrical 5- and 6-membered cyclic anhydrides with $NaBH_4$. Sodium borohydride, in contrast to early reports, was noted as being a convenient reagent, affording the corresponding δ-lactones in 60-85% yields. The unsymmetrical anhydrides, under these conditions were selectively reduced at the more hindered carbonyl group, affording results analgous to those obtained via $LiAlH_4$.[127] The reductions of several other simple anhydrides with $NaBH_4$ to lactonic products have been reported.[128-133]

Recently, a number of lithium and potassium trialkylborohydrides have emerged as powerful regio- and stereoselective reducing agents for organic functional groups.[134-136] The remarkable applicability of these reagents for the reduction of symmetrical anhydrides to lactones was demonstrated by the conversion of cis-1,2-cyclohexane-

dicarboxylic anhydride, cis-4-cyclohexene-1,2-dicarboxylic anhydride, and phthalic anhydride to their respective lactones in yields of 85-93%.[137] The regioselective reduction of some unsymmetrical anhydrides with these reagents has also been reported.[138-140] Krishnamurthy et al.[137] obtained the pairs of lactones (179 and 178) and (204 and 205) in ratios of 92:8 and 88:12 respectively, via lithium trisoamyl-borohydride reduction of 2,2-dimethylsuccinic anhydride and 3-methylphthalic anhydride. Sodium borohydride reduction of these anhydrides yielded the pairs of lactones in the corresponding ratios of 9:100 and 43:57. Similar results were obtained in the reduction of 1-methoxy-2,3-naphthalenedicarboxylic anhydride (206) with L-Selectride[139] and in the K-Selectride reduction of 2,2-dimethylsuccinic anhydride.[140] The immediate result of the reversal of regioselectivity in the reduction of some anhydrides with these reagents is the much simpler route to those lactones (from reduction at the less hindered carbonyl) that may otherwise only be accessible via hydrogenation under high pressure.[139,140] However, it has been shown[141] in a recent study of reductions of unsymmetrically substituted maleic anhydrides by a variety of metal hydride reagents that the high regioselectivity observed in these reactions is controlled chiefly by electronic factors.



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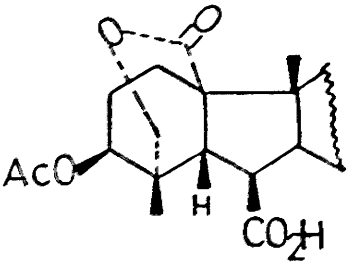

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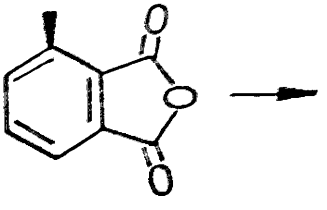

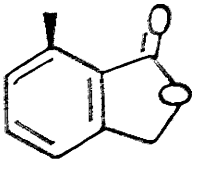

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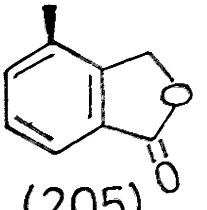

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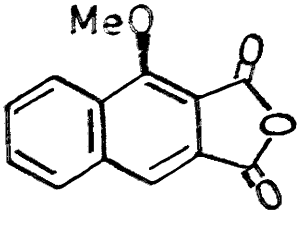

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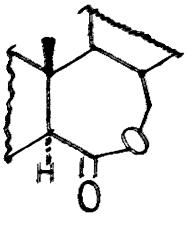

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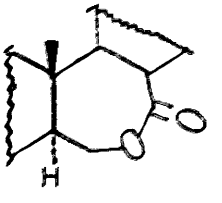


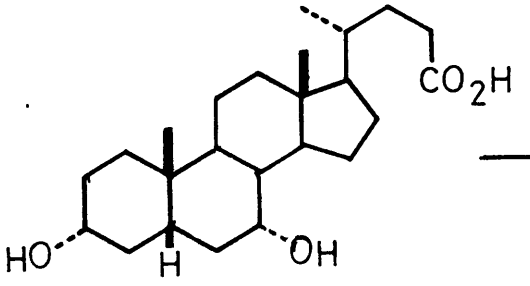
(208)

1.9 Oxidative Cleavage of Steroid Rings

There are many reports in the literature describing a variety of methods leading to the oxidative cleavage of the different rings in the steroid skeleton.

Oxidation of chenodeoxycholic acid (209) with hypobromite was reported to give the 7α-hydroxy-3,4-seco-dioic acid (210).[142,143] D.M. Piatak et al.[144] have reported the cleavage of a number of steroidal conjugated ketones with ruthenium tetroxide. Oxidation of testosterone acetate (211) and 17β-acetoxy-3-oxo-5α-androst-1-ene (212) afforded the 17β-hydroxy-5-oxo-3,5-seco-4-norandrostan-3-oic acid (213) and 17β-1, 3-seco-2-nor-5α-androstane-1,3-dioic acid (214), respectively.

The keto-acid (213) has also been prepared by ozonolysis of testosterone,[145-7] or testosterone acetate followed by alkaline hydrolysis.[148] L. Milewich and L.R. Axelrod[149] have also reported the synthesis of (213) by oxidation of testosterone acetate (212) with sodium periodate and potassium permanganate in the presence of potassium carbonate. In a similar way, 5-oxo-3,5-seco-4-norcholestan-3-oic acid (216) has been prepared from the corresponding cholest-4-en-3-one (215).[150] The 2-bromocholestan-3-ones (217) and (218) were converted to the dicarboxylic acids (219) and (220) respectively, through three stages.[151]



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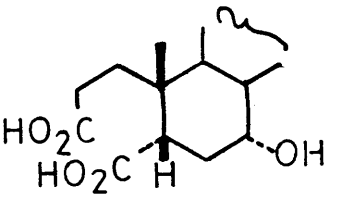
HOBr



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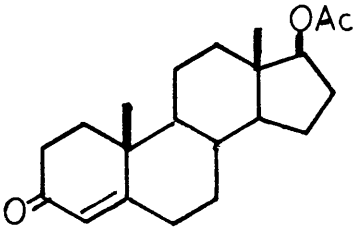

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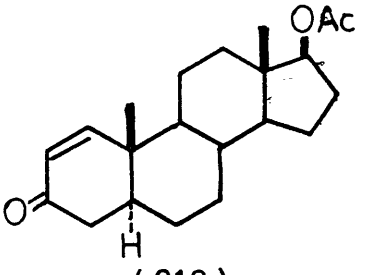

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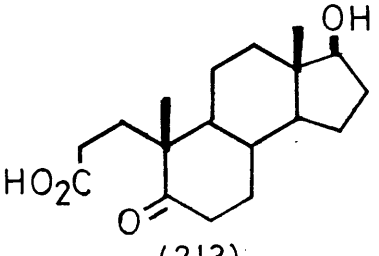

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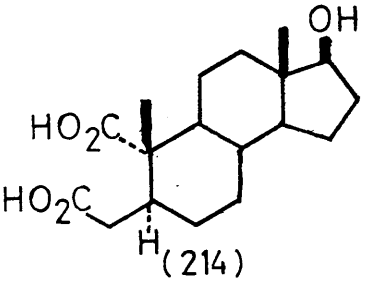

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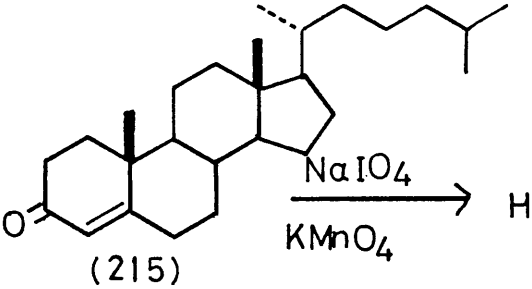
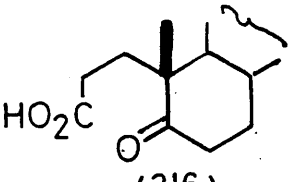


(215)

$NaIO_4$

$KMnO_4$



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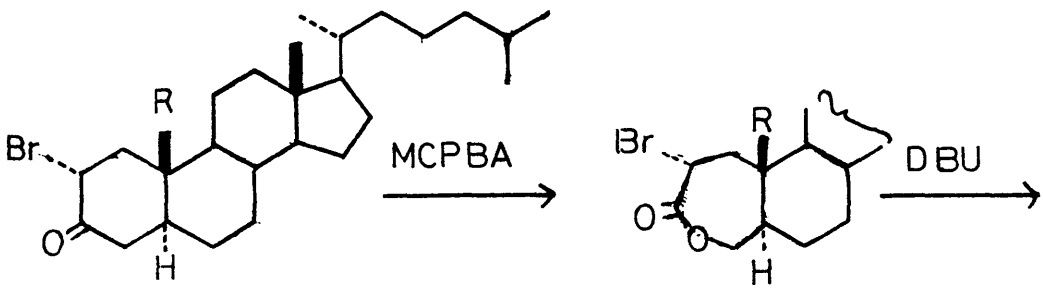
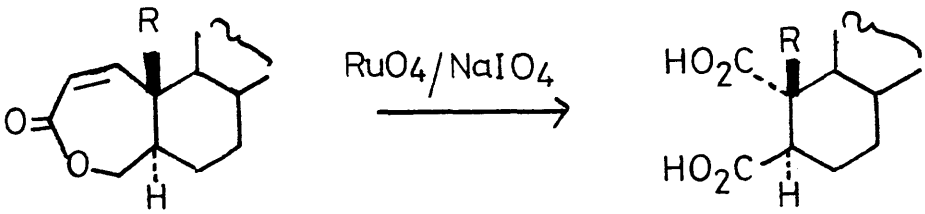


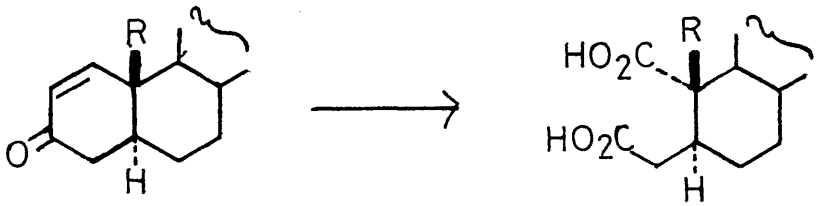
(217) R = Me

(218) R = CO_2Me



(219) R = Me

(220) R = CO_2Me

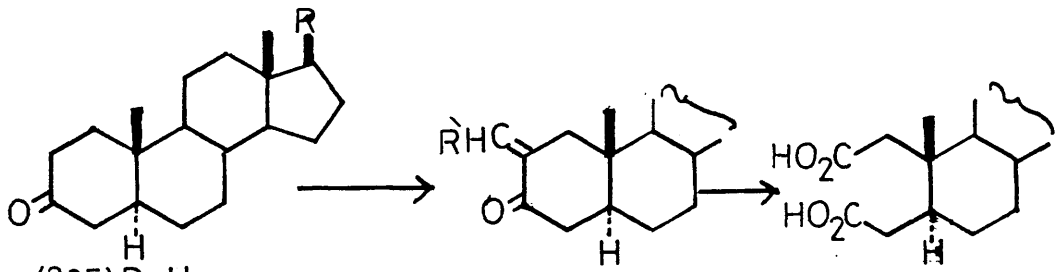


(221) R = Me

(222) R = CO_2Me

(223) R = Me

(224) R = CO_2Me



(225) R = H

(226) R = C_8H_{17}

(227) R = H; R' = C_4H_3O

(228) R = C_8H_{17}; R = Ph

(229) R = H

(230) R = C_8H_{17}

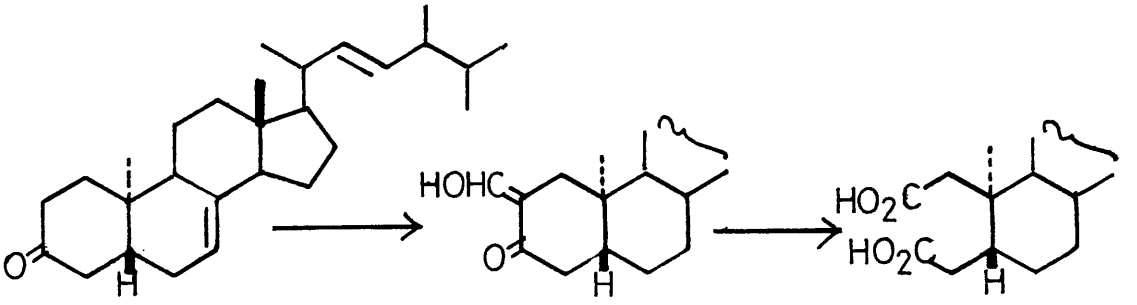
The reaction proceeded by treatment of the bromoketones with MCPBA (m-chloroperoxybenzoic acid), followed by reaction of the products with DBU (1,8-diazabicyclo[5.4.0] undec-7-ene), and finally oxidation of the obtained unsaturated lactones with ruthenium tetroxide and sodium periodate. Similarly, the dicarboxylic acids (223) and (224) were prepared from the corresponding α,β-unsaturated ketones (221) and (222).[151]

E.R.H. Jones et al.[152] have reported the synthesis of 2,3-seco-dioic acids in high yield. 5α-Androstan-3-one (225) was treated with furfuraldehyde to give 2-furfurylidene-5α-androstan-3-one (227) in 93% yield, while the reaction of 5α-cholestan-3-one (226) with benzaldehyde afforded the corresponding benzylidene-ketone (228) in 95% yield. By oxidation of the ketones (227) and (228) with alkaline hydrogen peroxide, the desired seco-dicarboxylic acids (229) and (230) were obtained with 75% and 82% yields, respectively. In a similar manner, 5β-lumista-7,22-dien-3-one (231) was treated with ethyl formate and sodium hydride to give the 2-hydroxymethylene derivative (232) which was oxidised with alkaline hydrogen peroxide to afford 2,3-seco-5β-lumista-7-, 22-diene-2, 3-dioic acid (233).[153] Peroxy acid has also been used to cleave the 3,5-dienolic ether (234) to the carbo-

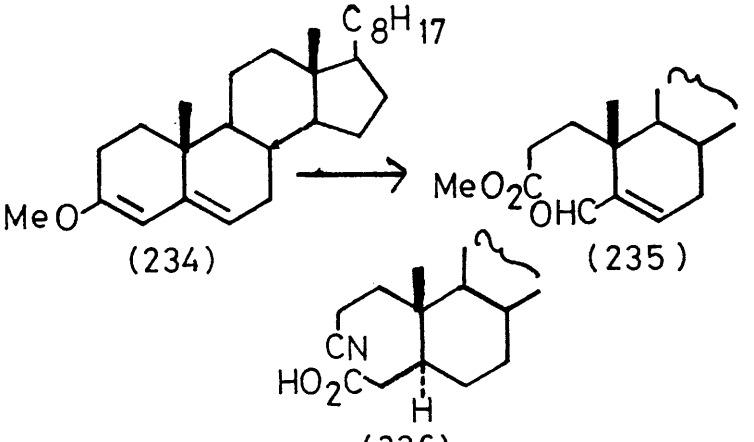
methoxy-aldehyde (235).[154]

Treatment of α-azido-ketones with bromine in acetic acid was reported to lead to ring cleavage giving the corresponding cyano-carboxylic acids. By this method, 2α-azido-5α-cholestan-3-one afforded the cyano-carboxylic acid (236).[155]

In the oxidative cleavage of ring B, a variety of ring A substituted 6,7-seco-dioic acids have been prepared. 3β-Chlorohydroxy-, and acetoxy-6, 7-dioic acids have been reported in the 5α-cholestane series while in the 5β-series 3β-chloro-, hydroxy-, acetoxy-, benzoyloxy- and 3-oxo-6, 7-seco-dioic, Δ^4-cholestane-6, 7-seco-dioic, and 3α, 5-cyclo-5α-cholestane-6,7-seco-dioic, acids have also been made. From bile acids, 5β-cholanoic acid has been converted to the 5β-6, 7-seco-dioic acid. Most of these C_6-C_7-seco acids have been made via direct oxidative cleavage of the bond in 6- or 7-oxo-steroids. Windaus et al.[156] were the first to utilize the direct oxidative cleavage of 6-oxosteroids in the synthesis of seco-6, 7-dioic acids. By this method, 3β-chloro-5α-cholestan-6-one was oxidised with concentrated nitric acid to afford 3β-chloro-6,7-seco-5α-cholestane-6, 7-dioic acid. From this dioic acid and its 3α-chloro epimer, obtained in a



(231) (232) (233)



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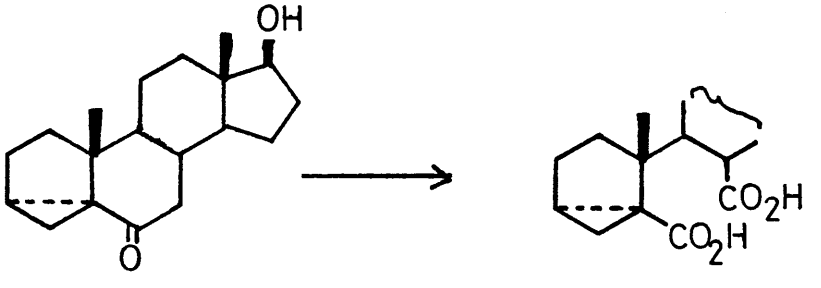
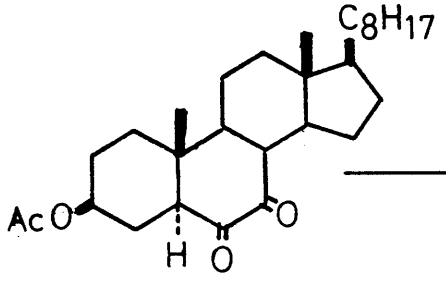


(237) (238)

similar manner, C.W. Shoppee[157] made the corresponding 3α- and 3β-hydroxy-6, 7-dioic acids via hydrolysis with KOH and proved the stereochemical orientation of the hydroxy group in cholesterol. Yamasaki et al.[158] in the oxidation of 6-keto-cholestanyl acetate and 7-ketocholestanyl acetate have shown that 3β-acetoxy-6,7-seco-5α-cholestane-6,7-dioic acid is accessible via nitric acid oxidation. 3β-Acetoxy-6-keto- and 3β–acetoxy-7-keto-cholanoic acids were also oxidised to the 6,7-dioic acids.[158] It should be mentioned that the reaction yields of dioic acids from the direct nitric oxidation of 6- or 7-oxosteroids are usually low.

It has been reported that ring B fission to the dicarboxylic acid in 3, 5-cyclo-6-oxo-steroids has been achieved in very good yields, using aqueous potassium hypobromite in pyridine.[159] Oxidation of 3, 5-cyclo-6-ketone (237) with potassium hypobromite gave the 6,7-seco-dicarboxylic acid (238).[160] However, the application of this procedure is limited, as the oxidation state of a 6-ketosteroid makes the opening of the 3,5-cyclopropane ring difficult. With 6,7-diketosteroids, such fission has been accomplished using alkaline hydrogen peroxide.[157] 3β-Acetoxy-6, 7-seco-5α-cholestane-6,7-dioic acid (240) was obtained



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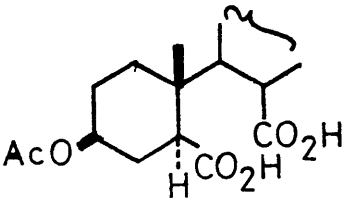

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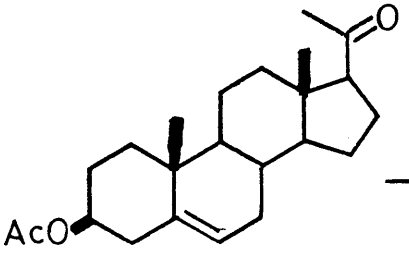
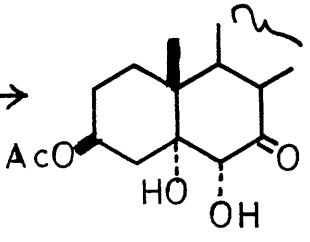


(241)

KMnO4



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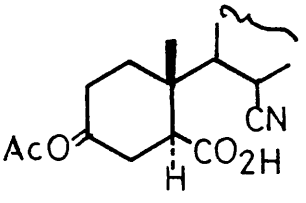

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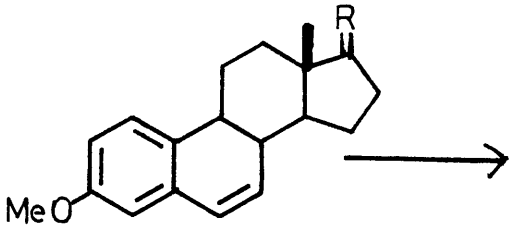
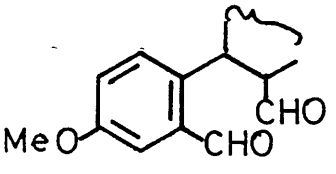


(244) R=O

(245) R=β-Ac ; H



(246) R=O

(247) R=β-Ac ; H

accordingly from 3β-acetoxy-5α-cholestane-6,7-dione (239). Δ^5-7-Oxo-steroids have also been oxidised to the seco-6,7-dioic acids[138] by treatment with trifluoroperoxyacetic acid. In the cholestane series, this method was reported to afford 3β-acetoxy-, benzoyloxy-, and chloro-6, 7-seco-5β-cholestane-6, 7-dioic acids from the corresponding Δ^5-7-oxo-compounds.

Potassium permanganate oxidation of pregnenolone acetate (241) in pyridine has been shown to afford 3β-acetoxy-5α,6α-dihydroxy-7,20dioxopregnane (242).[161] Similarly, keto-triols were obtained from cholesteryl acetate and from androst-5-ene-3β, 17β-diol diacetate. From these keto-triols, seco-6, 7-dioic acids were made by treatment with Jones reagent. 3β-Acetoxy-7α-azido-5α-cholestan-6-one has been shown to undergo ring-cleavage to form the cyano-carboxylic acid (243) on treatment with bromine in acetic acid.[155] Oxidations of the 4-methyloestratetraene (244) and the 4-methyl-19-norpregnatetra-ene (245) with OsO_4-$NaIO_4$ have been reported to give the correspnding 6,7-seco-dialdehydes (246) and (247) respectively.[162]

In studies by V. Ekhato in the Chemistry Department, University of Glasgow, oxidation of ketols with sodium periodate followed by treatment with chromic acid led to the preparation of

6,7-seco-dicarboxylic acids. When 7β-hydroxy-5αcholestan-6-one, its 3β-methoxy-, benzoyloxy-, acetoxy-, chloro-, and 2α, 3α-diacetoxy-substituted derivatives were sequentially treated with $NaIO_4$ in aqueous dioxan, and CrO_3 in acetic acid, they afforded the corresponding 6,7-seco-dioic acids [(248) to (253)].[163] Some natural bile acids, such as hyocholic acid , have vicinal diol systems which could be easily cleaved to give seco-dioic acids by applying the latter method.

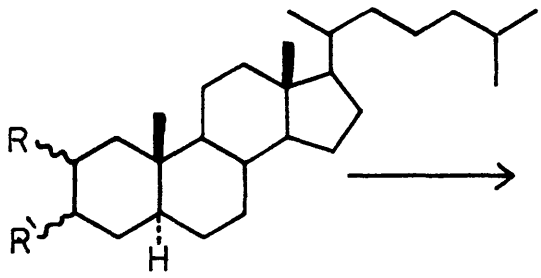
Oxidative cleavage of ring C in both 5α- and 5β- series has been reported. Oxidation of 11, 12-dihydroxy-5β-cholan-24-oic acid (254) with chromium trioxide gave the 11,12-seco-5β-cholane-11,12,24-trioic acid (255).[164] The same trioic acid was obtained from CrO_3 oxidation of the corresponding ketol (256). [164] The α, β-unsaturated ketone (257) was cleaved by oxidation with ruthenium trioxide to give the keto acid, methyl 3α-acetoxy-12- carboxy-9,11-seco-11-nor-9-oxo-5β-cholan-24-oate (258).[142] Oxidation of 3α, 12β-dihydroxy-11-keto-5β–cholaoic acid (259) with sodium periodate has been reported to afford the aldehydo-acid (260), in 70% yield.[165] Acetylation of the latter followed by treatment with chromium trioxide gave 3α-acetoxy-11,12-seco-5β-cholane-11,12,24-trioic

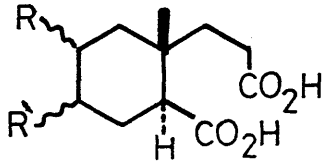
acid (262), in 42% yield.[165]

Ring C oxidative cleavage in the 5α-series has also been reported. Cleavage of 11β,12β-dihydroxytigogenin (263) with lead tetraacetate leads, in good yield, to the corresponding 11,12-seco-dialdehyde (264). Treatment of (263) with benzoyl chloride gave the corresponding 3β-benzoyloxy-11,12-seco-dialdehyde (265). Treatment of dialdehydes (264) and (265) with 8N chromium trioxide led respectively to the 11,12-seco-diacids (266) and (267).[71] J P Kutney et al.[58] have reported that 9,11-dehydrohecogenin acetate (268) affords, by ozonolysis or treatment with Lemieux reagent (IO_4 + $KMnO_4$),[166,167] the 11,12-seco-keto acid (269). The ozonolysis method has been shown to give a lower yield of the product.[58] The analogous 5β-11-nor-12-seco-keto acid (271) has also been prepared by ozonolysis of the corresponding 2α, 20β-diacetoxy-5β-pregn-9(11)-en-20-one (270).[72]

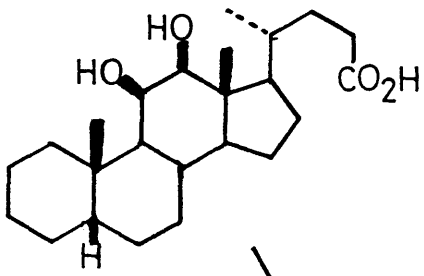
Cleavage of ring D has also been reported. Oxidation of 5α-androstan-17-one (272a) with iodine and base, with careful control of conditions, afforded the corresponding 16,17-seco-dioic acid (273a) in 75% yield.[152,168] Hypoidite oxidation of (272b) yielded, on acidification, a mixture of α-monoester (273b) and diester (273c).[169] Treatment of the

Δ^{14}-17-oxo-androstane (274) with alkaline hydrogen peroxide led to three ring C cleaved products, (275), (276) and (277).[170]

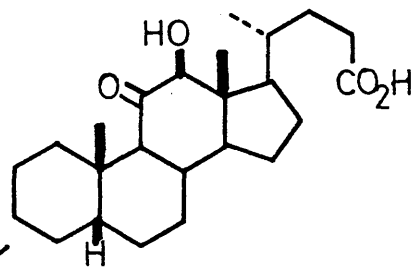




	R	R'
(248)	H	H
(249)	H	3β-MeO
(250)	H	3β-$PhCO_2$
(251)	H	3β-AcO
(252)	H	3β-Cl
(253)	2α-AcO	3α-AcO



(254)



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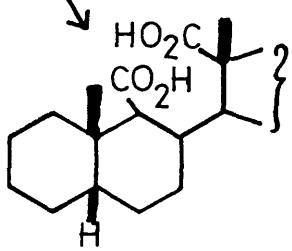

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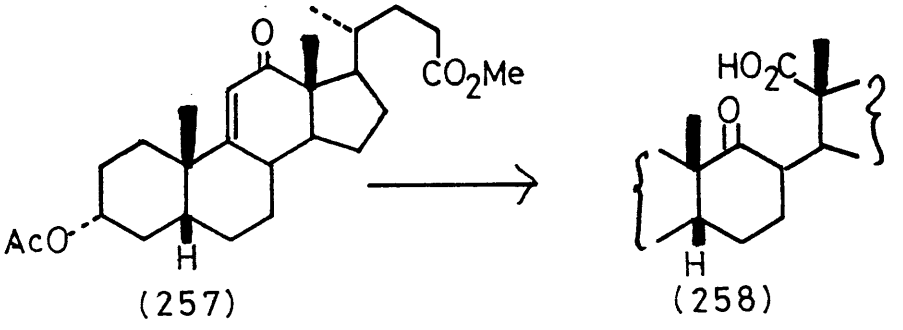
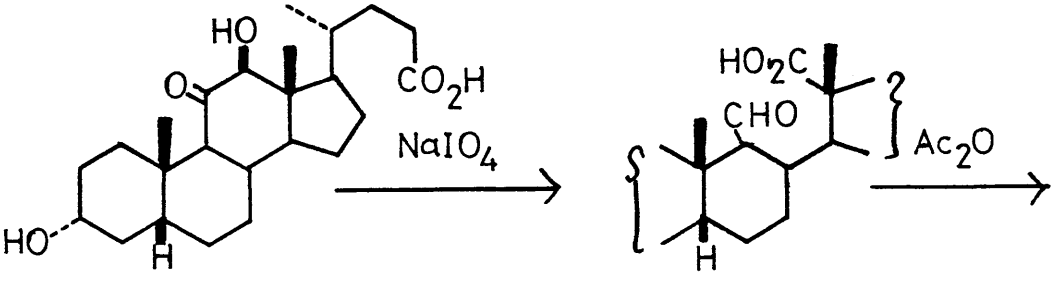


(257) (258)



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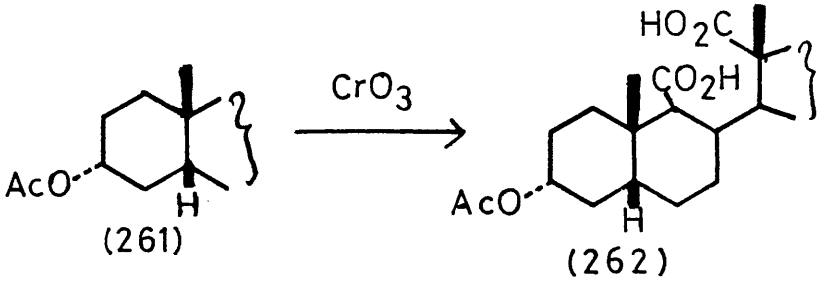

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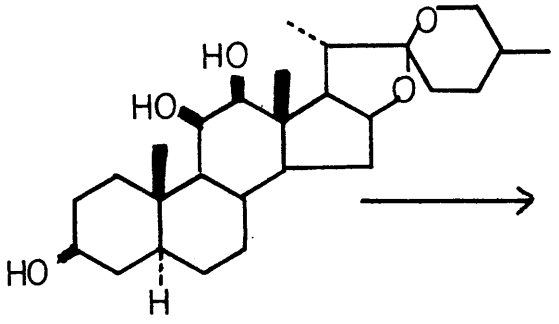

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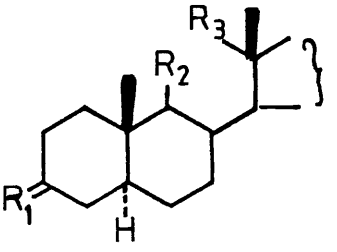
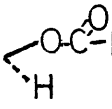
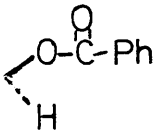
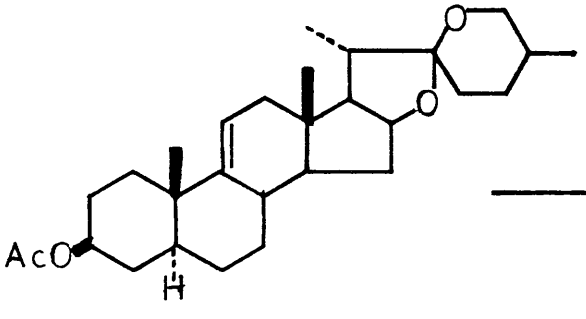


(264) R_1 =  ; $R_2=R_3=CHO$

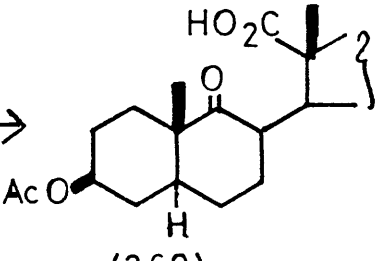
(265) R_1=  ; $R_2=R_3=CHO$

(266) $R_1=O$; $R_2=R_3=CO_2H$

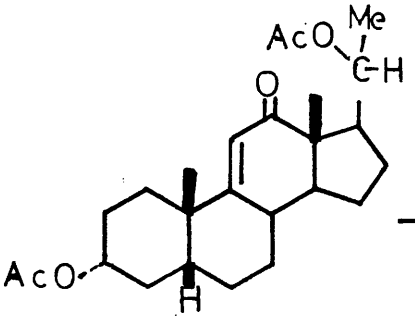
(267) R_1=  ; $R_2=R_3=CO_2H$



(268)



(269)



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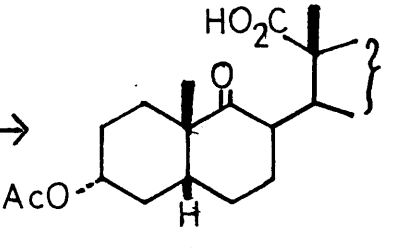
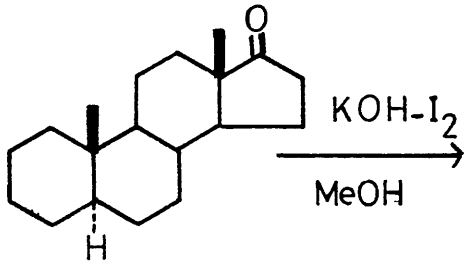
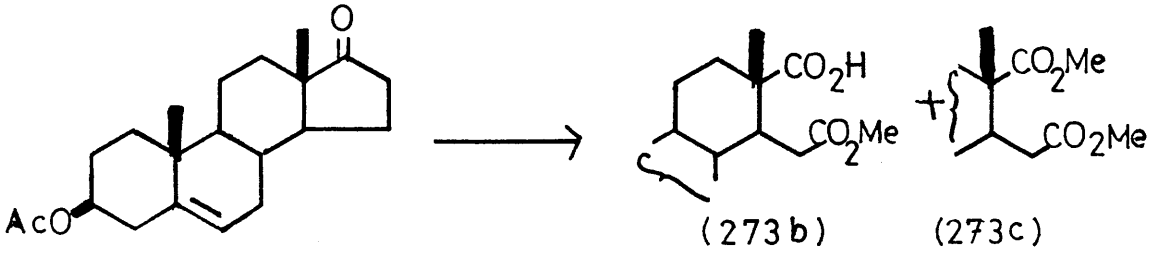


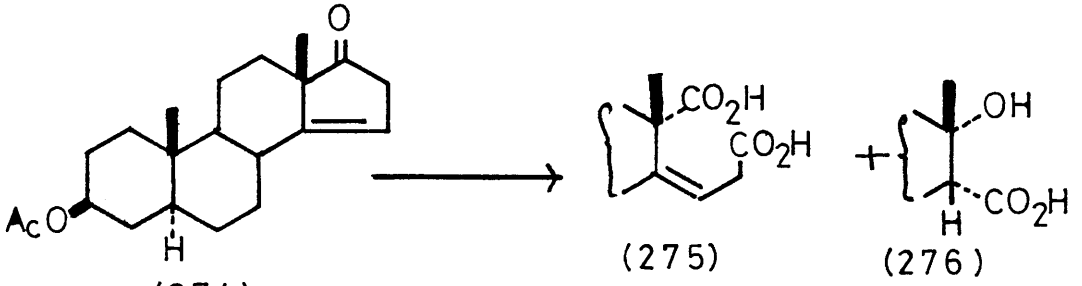
(271)



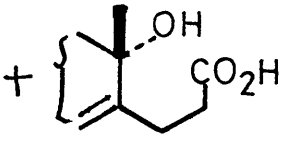
(272a) (273a)



(272b) (273b) (273c)



(274) (275) (276)



(277)

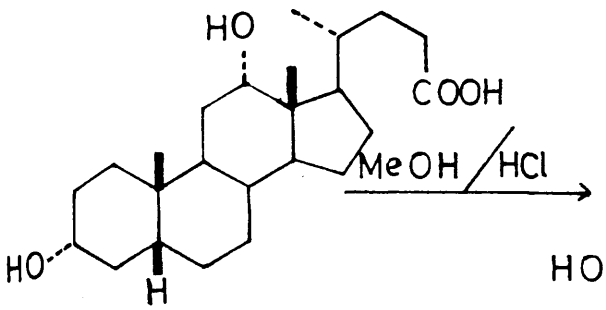
CHAPTER TWO

RESULTS AND DISCUSSION

CHAPTER 2

RESULTS AND DISCUSSION

Lactones have been useful intermediates in the synthesis of medicinally important compounds. Some γ- lactones in particular have been used in the synthesis of prostaglandin[55,56] and anti-tumour agents.[57] Among naturally occurring steroids, the lactones are usually found as 5- or 6-membered extra-nuclear structures. Since these steroidal lactones are biologically active, extensive investigations have been directed towards their synthesis.[54] However, the first naturally occurring ring B steroidal lactone, brassinolide, isolated from extracts of Brassica napus L. (rape) pollen, showed plant growth accelerating potency. The ring B 7-membered lactone in the brassinosteroid was suggested to be essential for its hormonal action. The reduction of cyclic anhydrides with complex metal hydrides and, in particular, with $LiAlH_4$ or $NaBH_4$, [120,171] has been shown to offer a feasible route to the synthesis of the corresponding lactones. In this department, the synthesis and reduction of ring B seco-5α-steroid anhydrides have been investigated.[163] A high regioselectivity has been shown upon the sodium borohydride reduction of seco-6, 7-dioic anhydrides to afford the corresponding lactone grouping characteristic of brassinolide.[172]



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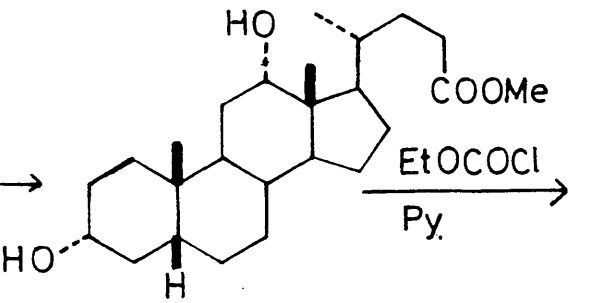

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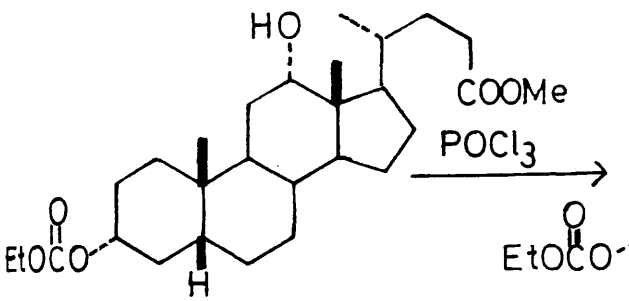

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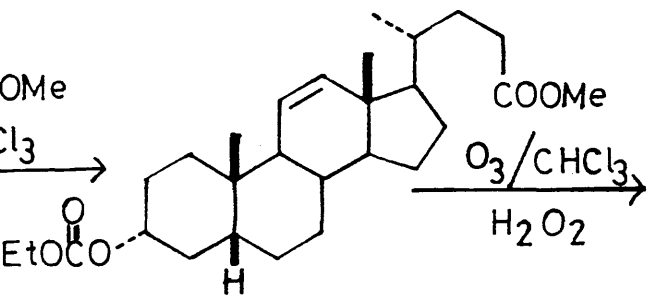

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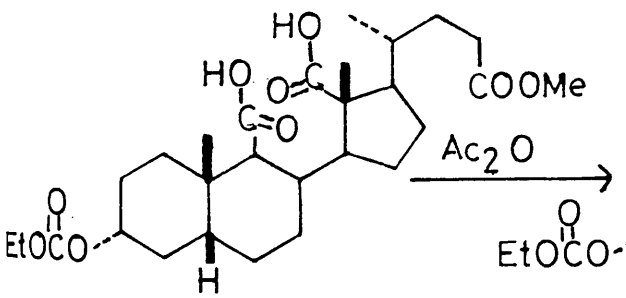
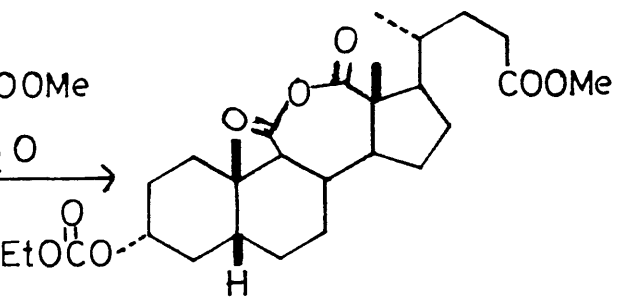


(282)



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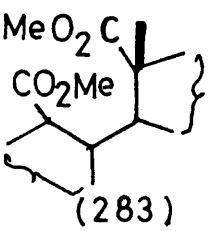

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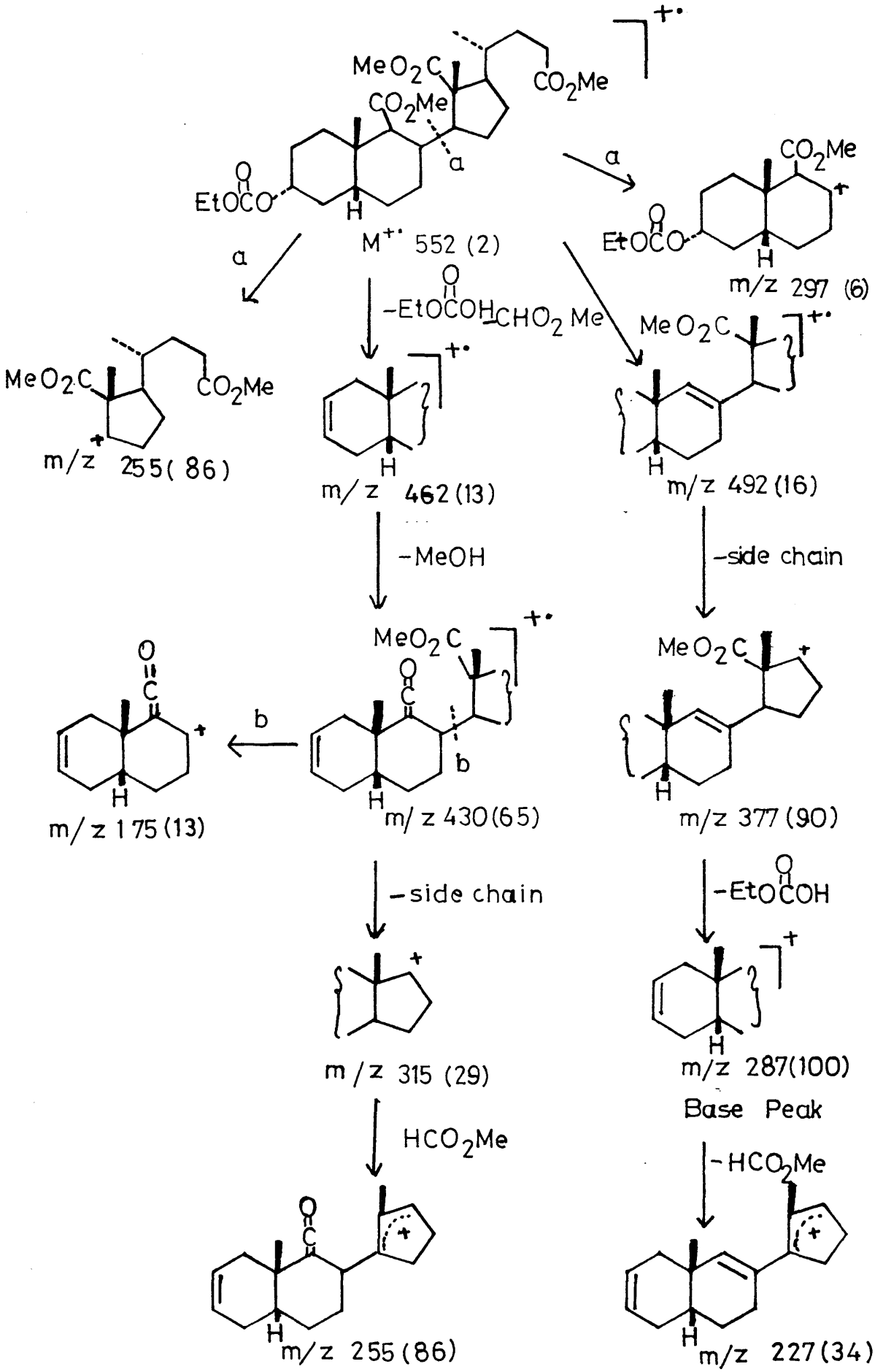

MeO2C
CO2Me
CO2Me
EtOCO
a
M+· 552 (2)
m/z 297 (6)
m/z 255 (86)
-EtOCOH
-CHO2Me
m/z 462 (13)
m/z 492 (16)
-MeOH
-side chain
b
m/z 175 (13)
m/z 430 (65)
m/z 377 (90)
-side chain
-EtOCOH
m/z 315 (29)
m/z 287 (100)
Base Peak
HCO2Me
-HCO2Me
m/z 255 (86)
m/z 227 (34)

Fig. 9

In the present work, we have been interested in the basis of regioselectivity of hydride reductions of cyclic anhydrides. Our specific interest has been the investigation of the synthesis of ring C seco-anhydrides, in both 5α- and 5β-series, and the possibility of regio selective reduction of these anhydrides to the corresponding lactones.

We have chosen the commercially available bile acid, deoxycholic acid (278) as a starting material in the 5β-series. Deoxycholic acid was very convenient for focusing on the desired ring C reactions. It possesses an axial hydroxyl group at C-12 and an equatorial hydroxyl function at C-3. Therefore the latter could be converted to the corresponding acetoxy, benzoyloxy, cathyloxy, ... etc, keeping the 12-hydroxyl intact, with no need for protecting group for 3-OH. However, deoxycholic acid was conveniently converted to the corresponding methyl ester (279), in 91% yield, by treatment with methanolic HCl at room temperature for 24h.

2.1 3α-Ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12, 24-trioic acid 24-methyl ester (282)

Methyl deoxycholate (279) was treated with ethyl chloro-carbonate in pyridine to give the corresponding 3α-ethoxycarbonyloxy (cathylate) derivative (280), in 87% yield. Dehydration of (280), applying the method of Nakada and Yamazaki,[173b] by treatment with phosphorus

oxychloride afforded methyl 3α-ethoxycarbonyloxy-5β-chol-11-enoate (281), in 50% yield. Oxidative cleavage of the Δ^{11}-cholenoate (281) by ozonoysis and subsequent treatment with hydrogen peroxide led to the desired cathylate (282), a new derivative of the known 3β-hydroxy-11,12-seco-dioic acid.[174] The mass spectrum (recorded by GC-MS) of the dimethyl ester (283), obtained by treatment with diazomethane, showed the molecular ion at m/z 552 in 2% abundance. The principal ions fit the fragmentation pattern outlined in Fig. 9, which showed the loss of the functional group at C-3 together with an additional proton (EtOCOH), the bile acid side chain, $MeCO_2H$, and MeOH. The mass spectrum also exhibited two fragments, one of them with high abundance (86%) resulting from cleavage of the bond between C-8 and C-14.

Several attempts to improve the yield of the dicarboxylic acid from the corresponding Δ^{11}-cholenoate (281) have not been successful. Clark [175] has reported the oxidative cleavage of norbornene (284) by sodium periodate and ruthenium dioxide to give cis-1,3-cyclopentane-dicarboxylic acid (285), in 80-90% yield. When we applied this procedure on the Δ^{11}-cholenoate (281), no reaction took place and starting material was recovered. Oxidation of olefinic compounds into the corresponding mono or dicarboxylic acids has also been achieved by simultaneous reaction with

potassium permanganate and sodium periodate.[176,177] Unfortunately, we have also found this method not applicable to the Δ^{11}-cholenoate (281).

2.2 3α-Ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12,24-trioic acid 11,12-anhydride 24-methyl ester (286)

Treatment of 11,12-seco-dioic acid (282) with acetic anhydride at 130°C for 1h afforded the desired 11,12-seco-dioic anhydride (286). in 36.2% yield. The infra red spectrum showed in addition to the band at 1270 cm^{-1} attributable to the presence of the cathyloxy group (EtO-C-O), two bands due to the cyclic anhydrides at 1750 and 1795 cm^{-1}. The latter band was more intense than the former band. However, in 5- and 6-membered ring anhydrides, such as phthalic anhydride and 2,3-diphenylglutaric anhydride, the lower frequency band is only fractionally more intense than that of the higher frequency band. In the ^{1}H-nmr spectrum, three 3H singlets at δ0.92, 1.18, and 3.65 are due to the methyl groups at C-19 and C-18, and the methyl ester at C-24 respectively; a 3H doublet at δ1.06 is attributed to the methyl group at C-21; the ethoxy group shows a 3H triplet at δ1.40 and a 2H quartet at δ4.28; the 3β-H gives a 1H multiplet signal at δ4.60. In the mass spectrum, recorded by GC-MS, the parent ion appeared at $M^{+\cdot}$ = 506 (1%) and the principal ions are in agreement with the fragmentation pattern summarised in Fig.10. This

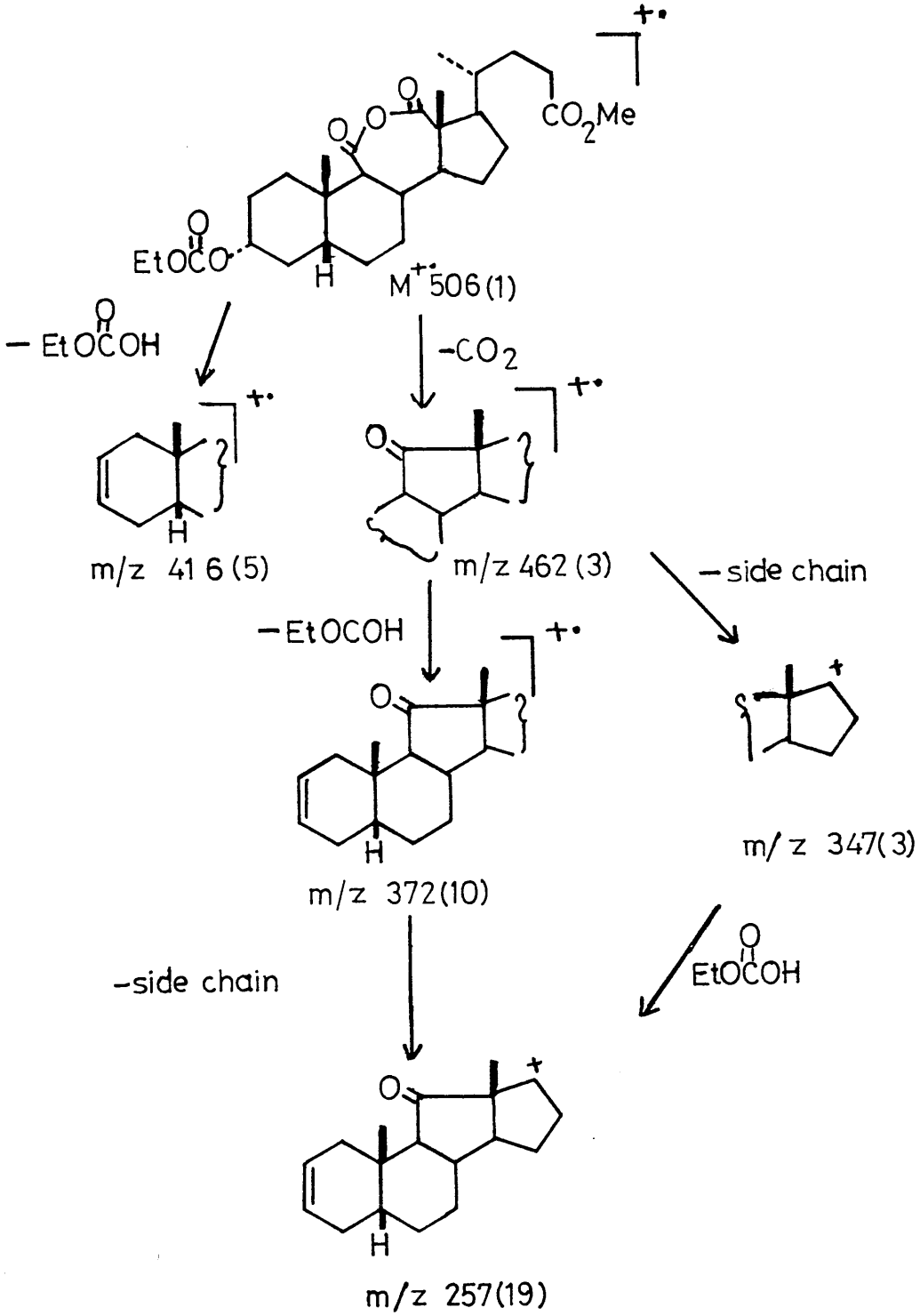

CO2Me
EtOCO
M+· 506 (1)
– EtOCOH
–CO2
m/z 416 (5)
m/z 462 (3)
– side chain
–EtOCOH
m/z 372(10)
m/z 347(3)
–side chain
EtOCOH
m/z 257(19)

Fig. 10

spectrum showed the molecular ion at 1% abundance, $(M-CO_2)^{+\cdot}$, $(M-CO_2-EtOCOH)^{+\cdot}$, and $(M-CO_2-EtOCOH-\text{the bile acid side chain})^{+}$; in addition to other fragments.

However, there was no evidence of change in the configuration at C-9, since the GLC analysis (see Table 5) showed one peak and the ^{1}H-nmr showed no sign to suggest so.

2.3 Synthesis of methyl 3α-ethyoxycarbonyloxy-11α-bromo-12-oxo-5β-cholanoate (288)

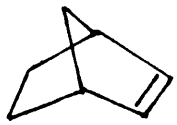
We decided to prepare 11-bromo-12-ketone, convert it to the corresponding ketol, and to explore the oxidative cleavage of the latter to the desired 11,12,-seco-dicarboxylic acid.

Treatment of methyl 3αethoxycarbonyl-deoxycholate (279) with Jones' reagent afforded methyl 3α-ethoxycarbonyloxy-12-oxo-5β-cholanoate (287). Reaction of the ketone (287) with bromine in acetic acid,[178] at 50-60°C overnight, led to the 11α-bromo-12-ketone (288) having the 24-methyl ester partially hydrolysed. Pure methyl 3α-ethoxy-carbonyloxy-11α-bromo-12-oxo-5β-cholanoate (288) was collected after the crude product had been treated with diazomethane and passed through a column of silica gel. The infra-red spectrum showed

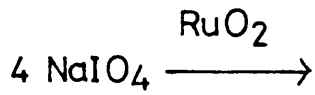
strong bands at 1740 cm^{-1}(broad) (C-24 methyl ester and C-12 ketone) and 1260cm^{-1} (3α-EtOC-O-). The ^{1}H-nmr spectrum exhibited three 3H singlets at δ1.00, 1.18 and 3.66 due to the methyl groups at C-19 and C-18, and the methyl ester at C-24; a 3H doublet at δ1.30 is attributed to the methyl group at C-21; the 11β-proton is evident as a doublet at δ4.95 (J=10.0 Hz); the ethoxy group at C-3 showed a 3H triplet at δ1.25 and 2H quartet at δ4.16 while the 3β-H appeared as a multiplet at δ4.62 ppm. The two highest ions in the mass spectrum appeared at m/z 476 $(M-79)^{+\cdot}$ and 475 $(M-80)^{+\cdot}$, both in 6% abundance. Detailed data of the mass spectrum are given in the appendix. Similarly, the 3α-benzoyloxy-12-ketone (290). The infra-red spectrum showed strong bands at 1710 and 1280, 1700 and 1730 cm^{-1}, consistent with the presence of the 3α-benzoyloxy group, 12-ketone, and the C-24 methyl ester. In the ^{1}H-nmr spectrum, three 3H singlets appeared at δ1.01, 1.20, and 3.67 due to the methyl groups at C-19 and C-18 and the methyl ester at C-24, respectively; a 3H doublet at δ1.30 is attributed to the methyl group at C-21; the appearance of a 1H doublet at δ4.98 (J=10.0 Hz), due to the 12β-H, confirms the presence of the 11α-bromide, a 1H multiplet at δ4.95 and a 5H multiplet at δ7.18-8.02 can be attributed to the 3β-H and the 3α-benzoate, respectively. The mass

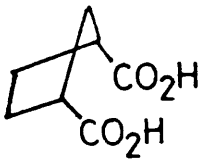
spectral data are given in the appendix.

The conversion of α-bromoketones to the corresponding ketols by alkaline hydrolysis is one of the funadmental reactions in organic synthesis. In the literature, there has been considerable application of this reaction to the synthesis of steroidal ketols. Gallagher[178] has reported that reaction of 3α-acetoxy-11α-bromoketone with ethanolic sodium hydroxide under reflux afforded the rearranged 3α, 12β-dihyroxy-11-ketone (292). Recently Numazawa et al.[179-180] have reported a controlled alkaline hydrolysis of 16-bromo-17-ketosteroid without rearrangement of the corresponding ketol product. They also demonstrated the synthesis of a 16α-hydroxy-17-oxosteroid in which the 3β-sulphate substituent was unaffected by the reaction.[179] It was thought that this procedure could be applied to our system and perhaps would yield the desired 11,12-ketol with the ring A substituent remaining in the product. The 3α-ethoxycarbonyloxy-11α-bromo-12-ketones, (288) and (291), were recovered unchanged after subjection to the mild conditions of the reported[179,180] methods of controlled alkaline hydrolysis. However, when (288) and (291) were treated with alcoholic sodium hydroxide under reflux, both led to the rearranged 3α, 12β-dihydroxy-11-ketone (292). The mass spectral data of the methylated ketol (293) are given in the appendix.

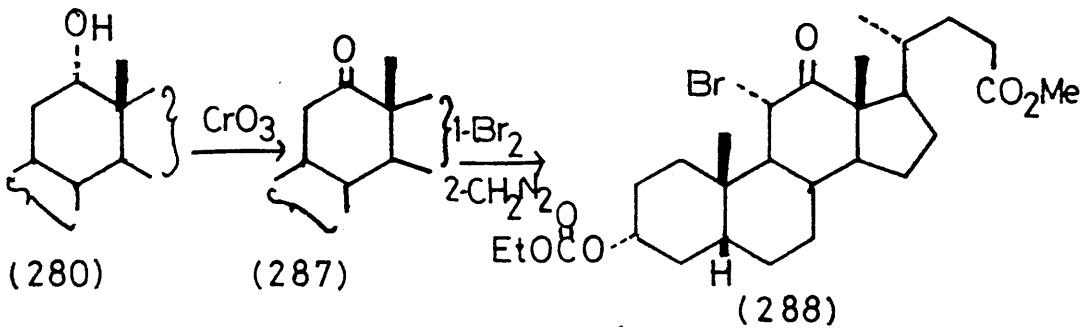


(284)

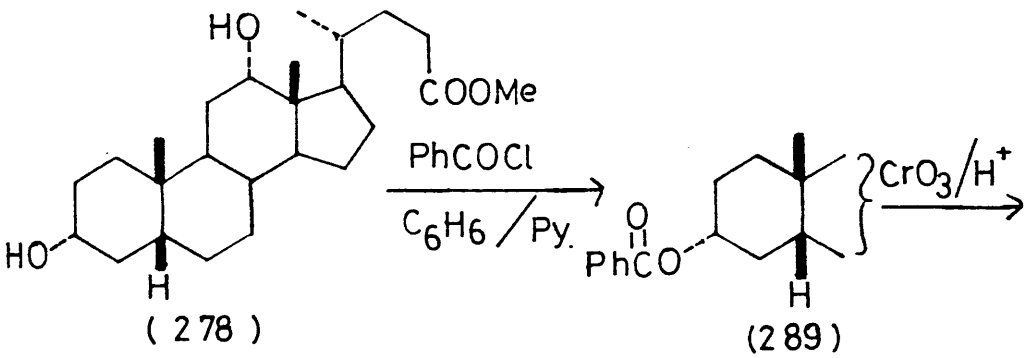




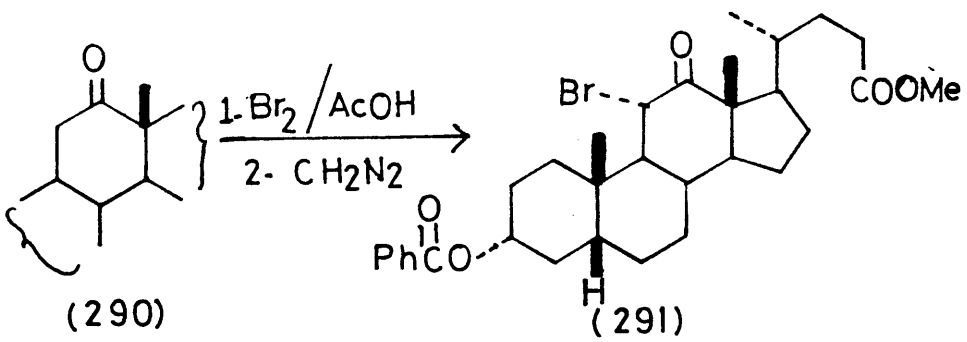
(285)



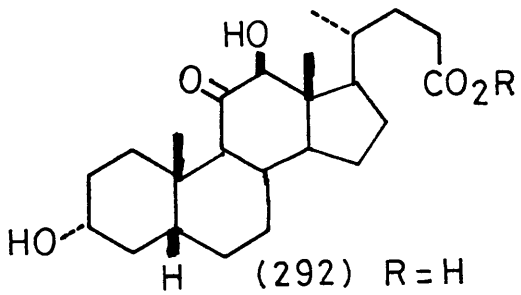
(280) (287) (288)



(278) (289)

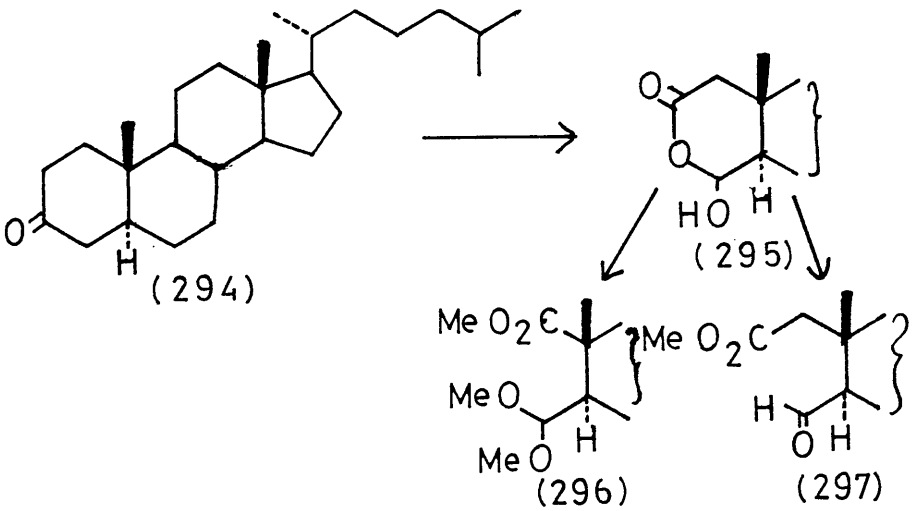


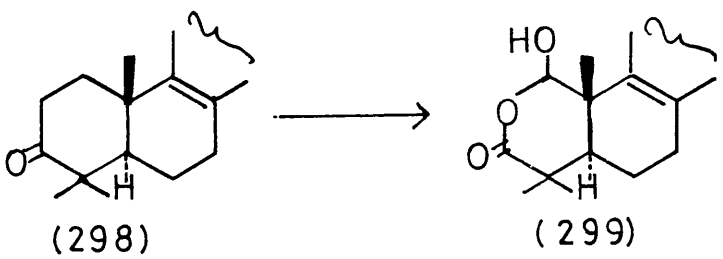
(290) (291)

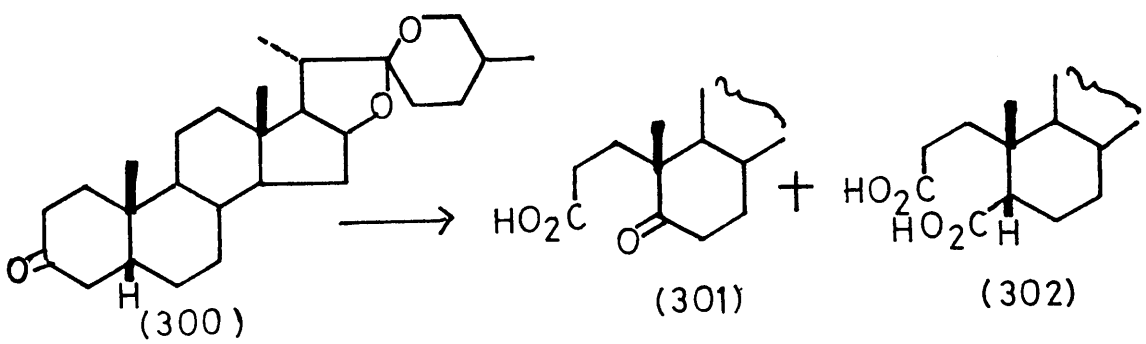


(292) R = H

(293) R = Me







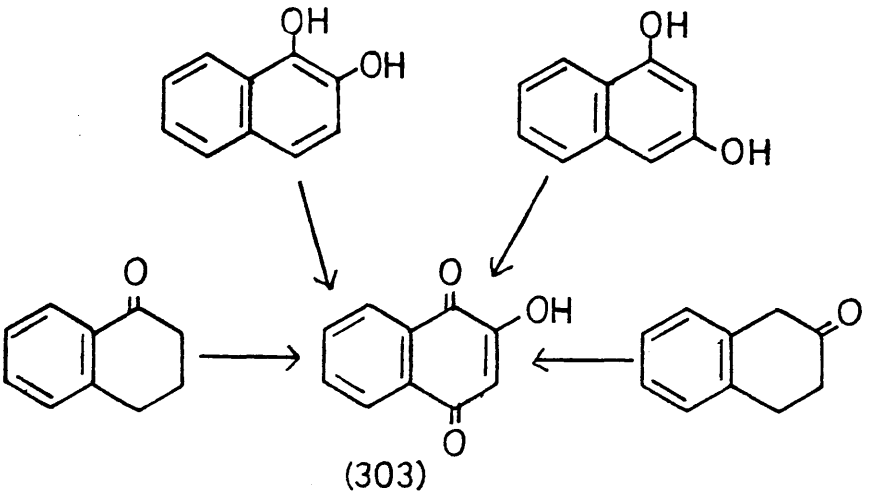
2.4 Oxidative Ring-Cleavage by Superoxide

There is now a considerable interest in the role of superoxide ion in chemical (and biochemical) oxidation reactions.[181] The biochemistry of superoxide ion is an area that has also attracted attention.[182, 183] The use of potassium superoxide (KO_2) as a versatile synthetic reagent has increased rapidly since the discovery[184] that it is solubilized in non-polar solvents by crown ethers. It is well known that the superoxide ion can be easily generated in aprotic solvent under phase transfer conditions using either crown ethers or quaternary ammonium salts. Although Lee-Ruff[185] has reported that isolated ketones are inert to superoxide oxidation, it has been shown that some monocyclic ketones can be cleaved by superoxide under catalytic phase transfer conditions to give the corresponding dicarboxylic acids without loss of carbon atoms. Lissel and Dehmlow have reported that mono or dicarboxylic acids are produced by oxidative cleavage of the corresponding ketones, using potassium superoxide[186] in the presence of phase transfer catalyst.

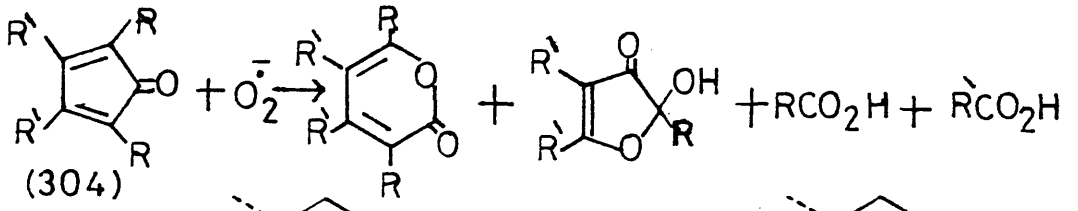
Treatment of cyclopentanone with potassium superoxide in benzene in the presence of "Aliquat 336" (tricaprylmethylammonium

chloride) afforded glutaric acid in 89% yield. A similar oxidation of camphor gave camphoric acid in 93% yield of the converted material, which was, however, only 19%.[186] When we repeated the latter reaction, a similar result was obtained.

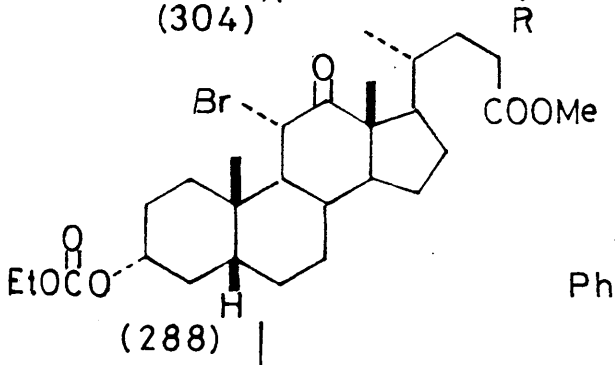
Reactions of carbonyl compounds with superoxide have been described recently. Filippo et al.[187] have reported that treatment of α-keto, α-hydroxy, and α-halo-ketones, esters and carboxylic acids with the superoxide ion in benzene in the presence of phase transfer catalyst (18-crown-6-ether) resulted in oxidative cleavage of these compounds to carboxylic acids (42-98% yield). Under these conditions, 3-bromocamphor afforded 54% of camphoric acid while camphoroquinone and benzoin gave camphoric acid (87%) and benzoic acid (9%), respectively. More recently, Alvarez et al.[188] have shown that the reaction of potassium superoxide with 5α- and 5β-3-keto steroids in benzene in the presence of 18-crown-6-ether gave the corresponding oxidised compounds in which one carbon atom is lacking. Under these conditions, 5α-cholestan-3-one (294) afforded the lactol (295) in 50% yield. Methylation with methanolic hydrogen chloride led to the methyl ester dimethyl acetal (296), while esterification with diazomethane gave the methyl ester (297). Reaction of 3-keto-dihydrolanosterol (298) with potassium superoxide, under similar



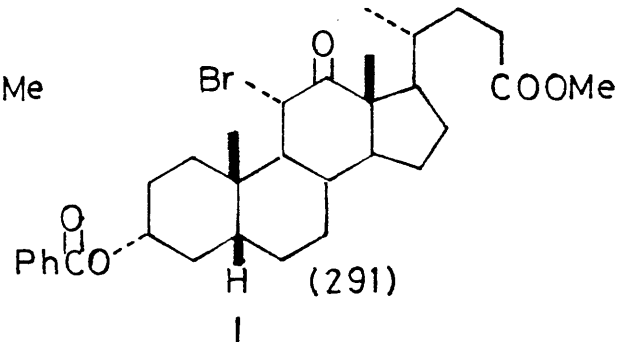
(303)



(304)



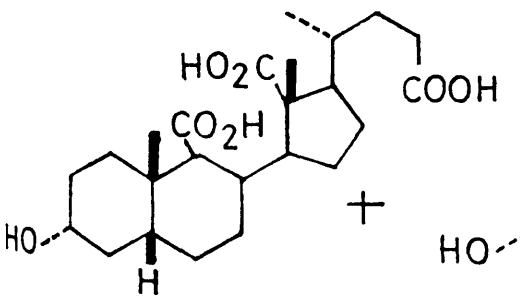
(288)



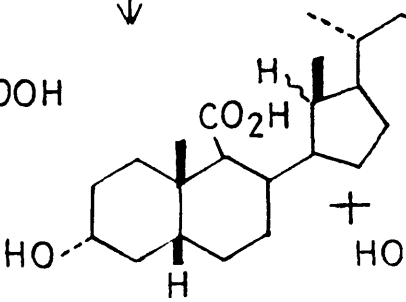
(291)

KO_2 / Aliquat 336

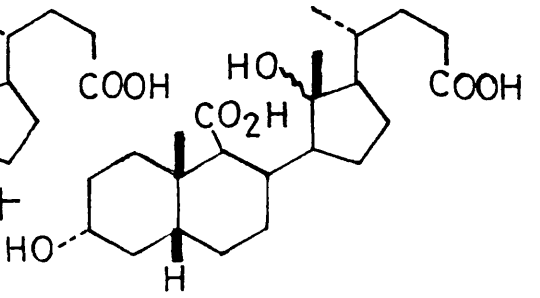
C_6H_6 ; r.t.; 48 hr.



(305)



(306)



(307)

conditions, afforded the lactol (299) in 60% yield. Treatment of 5β-spirostan-3-one (3-keto-smilagenin) (300) with KO_2, gave a mixture of dicarboxylic acids, (301) and (302) in 45 and 7% yields, respectively. Oxidation of 1, 2- or 1,3-dihydroxynaphthalene with KO_2 was reported to give 2-hydroxy-1, 4-naphthoquinone (303) in good yield.[189] The latter was also prepared by treatment of α- and β-tetralone with potassium superoxide in THF in the presence of crown ether. Indoles undergo ring cleavage to afford o-formyl and o-acyl amino ketones or N-acylanthranilic acids and ring expansion to give 2-quinolones, on reaction with KO_2. The products have been reported to be substituent dependent.[190] It is thought that the reaction mechanism involves proton abstraction by O_2 to give an indolyl radical which reacts further with O_2 to give indoxyl hydroperoxide followed by intramolecular nucleophilic addition and ring cleavage. Neckers and Nauck[191] have reported that treatment of tetracyclones (304) [R=Ph, p-ClC_6H_4, p-$MeOC_6H_4$; R'=p-tolyl, p-ClC_6H_4, p-$MeOC_6H_4$] with KO_2 in dry benzene in the presence of dicyclohexano-18-crown-6 led to RCO_2H and $R'CO_2H$ as well as pyranones and furanones. The mechanism is thought to involve electron transfer to produce tetracyclone radical anion.

In this work, trial experiments on the treatment of 11-keto steroid with potassium superoxide inthe presence of phase transfer catalyst have not led to the desired oxidative cleavage of ring C. The starting material was mainly recovered, in addition to a few unidentified components having much lower retention indices (GLC) and molecular ions in spectra obtained by GC-MS. It was decided then to explore the possibility of superoxide oxidation of 11-bromo-keto-steroids.

Treatment of methyl 3α-benzoyloxy-11α-bromo-12-keto-5β-cholanoate (291) with potassium superoxide in dry benzene in the presence of "Aliquat 336", for 48h at room temperature, afforded a mixture of three main components, monitored by TLC and GLC (see data in Table 1). These components were separated on a column of silica gel. The major product (42.1% of the crude mixture, GLC analysis) was identical with the 3α-hydroxy-11, 12-seco--5β-cholane-11,12,24-trioic acid (305), described by Gallagher.[174] The mass spectrum of its trimethyl ester 3-TMS ether derivative (308) showed the molecular ion $M^{+\cdot}$ 552 (7%). The other principal ions are in agreement with the postulated fragmentation pattern (Fig.11) of this compound. The least polar component (25.5%) was isolated as a gummy oil. The ^{1}H-nmr spectrum of the methylated derivative exhibited a 3H doublet at δ0.74 which can be attributed to the C-18 methyl group

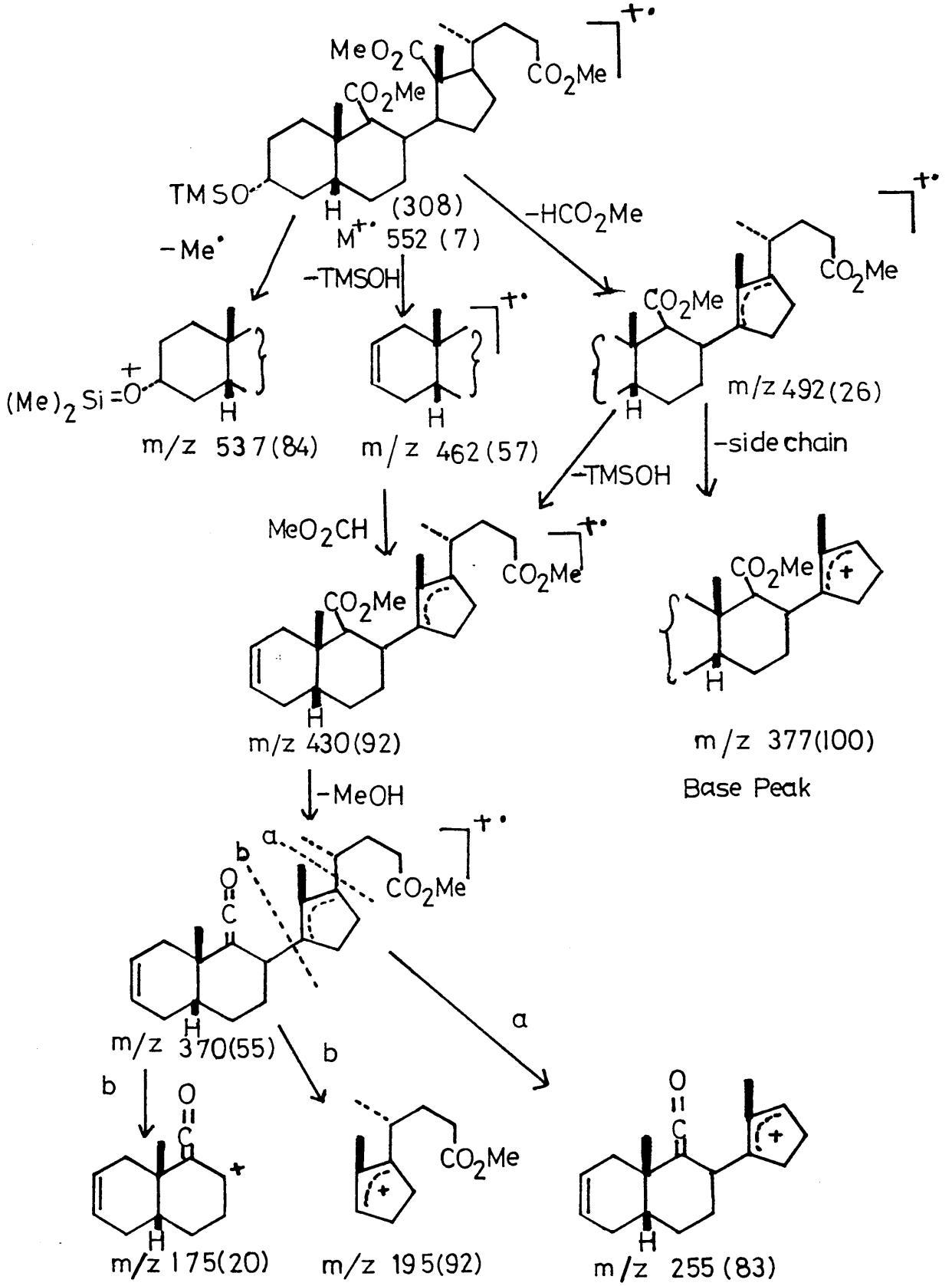

MeO2C
CO2Me
CO2Me
TMSO
H
(308)
M+· 552 (7)
-Me·
-TMSOH
-HCO2Me
(Me)2 Si=O
m/z 537(84)
m/z 462(57)
m/z 492(26)
-side chain
-TMSOH
MeO2CH
m/z 430(92)
m/z 377(100)
Base Peak
-MeOH
a
b
O=C
m/z 370(55)
b
a
m/z 175(20)
m/z 195(92)
m/z 255(83)

Fig. 11

group being attached to carbon bearing a proton; two 3H singlets at δ3.65 and 3.67 are due to two methyl ester groups at C-24 and possibly C-11; in addition to a 3H singlet at δ0.7, α 3H doublet at δ1.06, and a 1H multiplet at δ3.7 are evidence for the presence of the 19-methyl group at C-19, the methyl group at C-21, and the 3β-H, respectively. According to these data, the compound was provisionally assigned the structure (306), 3α-hydroxy-12-nor- 11,12-seco-5β-cholane-11,24-dioic acid. The mass spectrum of the methylated TMS ether derivative (309) showed the parent ion, $M^{+\cdot}$ = 494, corresponding to the formula $C_{28}H_{50}O_5$ Si, and the other principal ions are consistent with the fragmentation pattern outlined in Fig.12.

The third isolated compound (32.4% of the mixture) was crystalline material. This compound was methylated (with diazomethane) and then treated with acetic anhydride for 4h at 100°C. The ^{1}H-nmr spectrum of the resulting derivative showed two 3H singlets at δ3.65 which could be attributed to methyl ester groups at C-24 and C-11, respectively; two 3H singlets at δ2.00 and 2.03 are due to two acyl groups, at C-3 and possibly C-13, in addition to two 3H singlets at δ0.68 and 1.37, a 3H doublet at δ1.20, and a 1H multiplet at δ4.65 expected for the present of the methyl

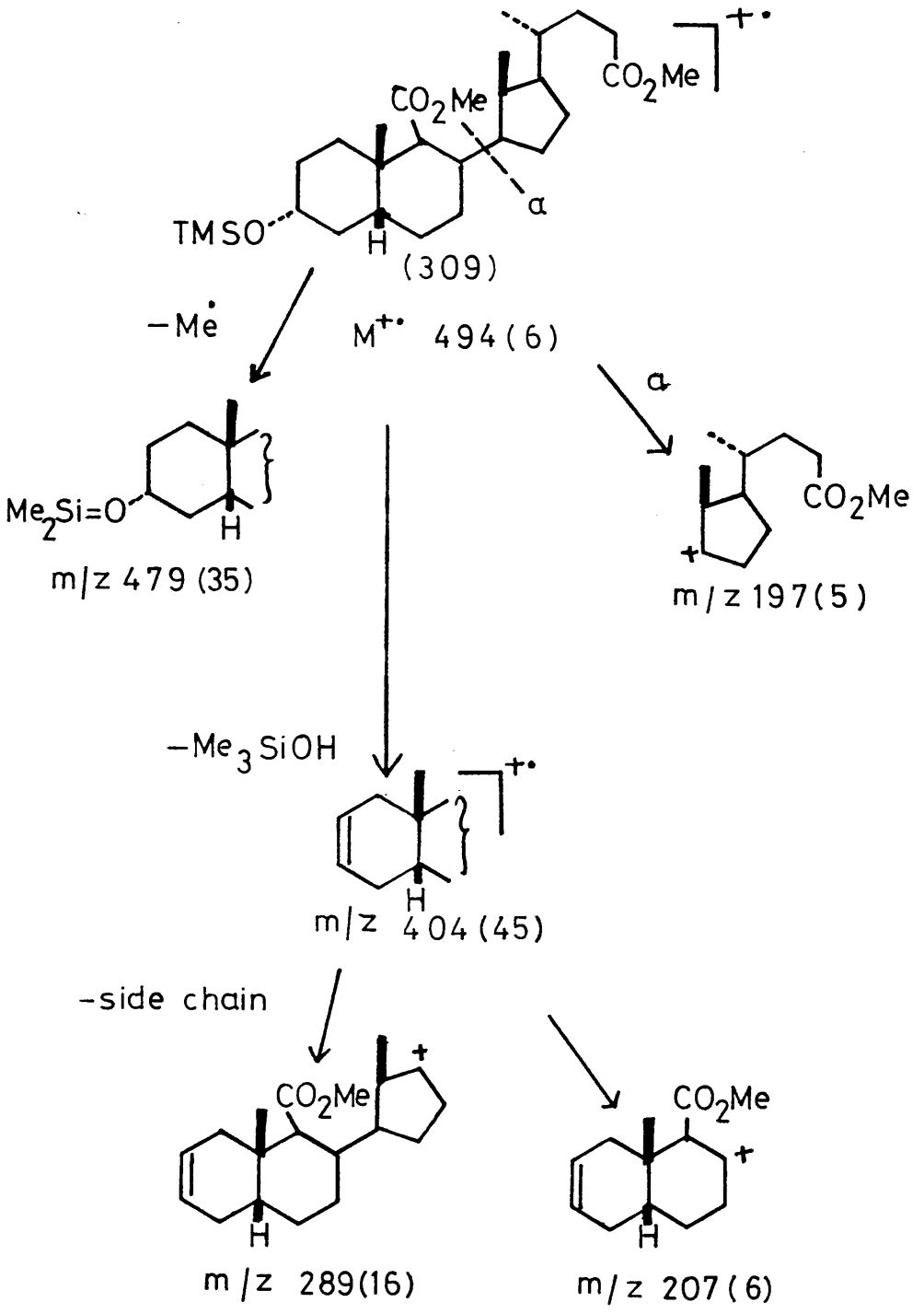
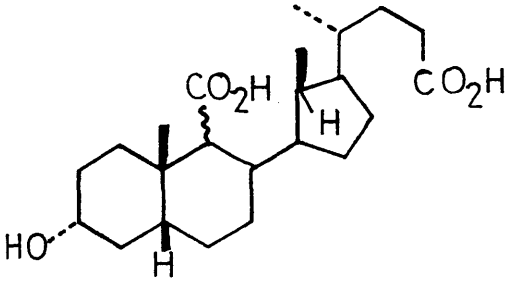
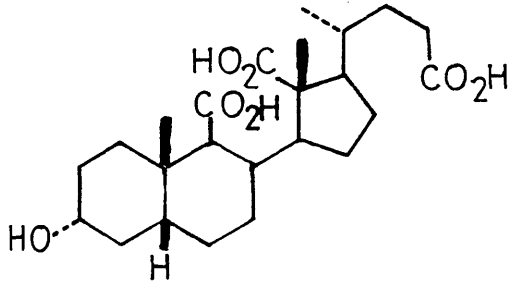
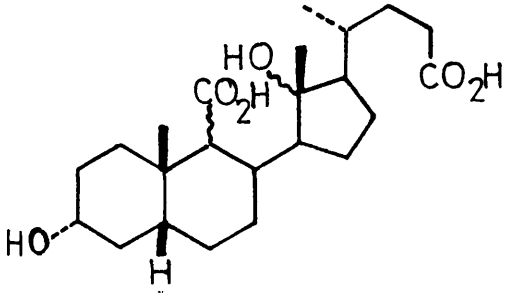

CO2Me
CO2Me
α
TMSO
H
(309)
−Me·
M+· 494 (6)
α
Me2Si=O
H
m/z 479 (35)
CO2Me
m/z 197 (5)
−Me3SiOH
H
m/z 404 (45)
-side chain
CO2Me
H
m/z 289 (16)
CO2Me
H
m/z 207 (6)

Fig. 12

groups at C-19 and C-18, the methyl group at C-21, and the 3β-H, respectively. The compound was given the structure (307), 3α, 13-dihydroxy-11,. 12-seco-12-nor-5β–cholane-11, 24-dioic acid. The mass spectrum supported this assignment when samples of di-TMS-ether and diacetoxy derivative of the methylated diacid were injected into the GC-MS system. The spectrum of the diacetoxy-dimethyl ester (310) showed the parent ion $M^{+\cdot}$ = 552, corresponding to the formula $C_{29}H_{46}O_8$, while the di-TMS-ether-dimethyl ester (311) exhibited the parent ion $M^{+\cdot}$ = 582, in agreement with the expected formula $C_{31}H_{586}O_6Si$. The other principal ions in the spectra of both (310) and (311) are consistent with the fragmentation patterns outlined respectively in Figs. 13a and 13b.

Table 1: Data of GLC and TLC analysis of the products obtained from the reaction of 11α-bromo-ketone (291) with KO_2.

Compound	m.p.	R_f[8:2; EtOAc: pet.ether]	$I^{1\%\ OV-1}_{-270°C}$ (after methylation)
	- (oil)	0.57	3340
	258-261°C	0.39	3280
	242-245°C	0.26	3295

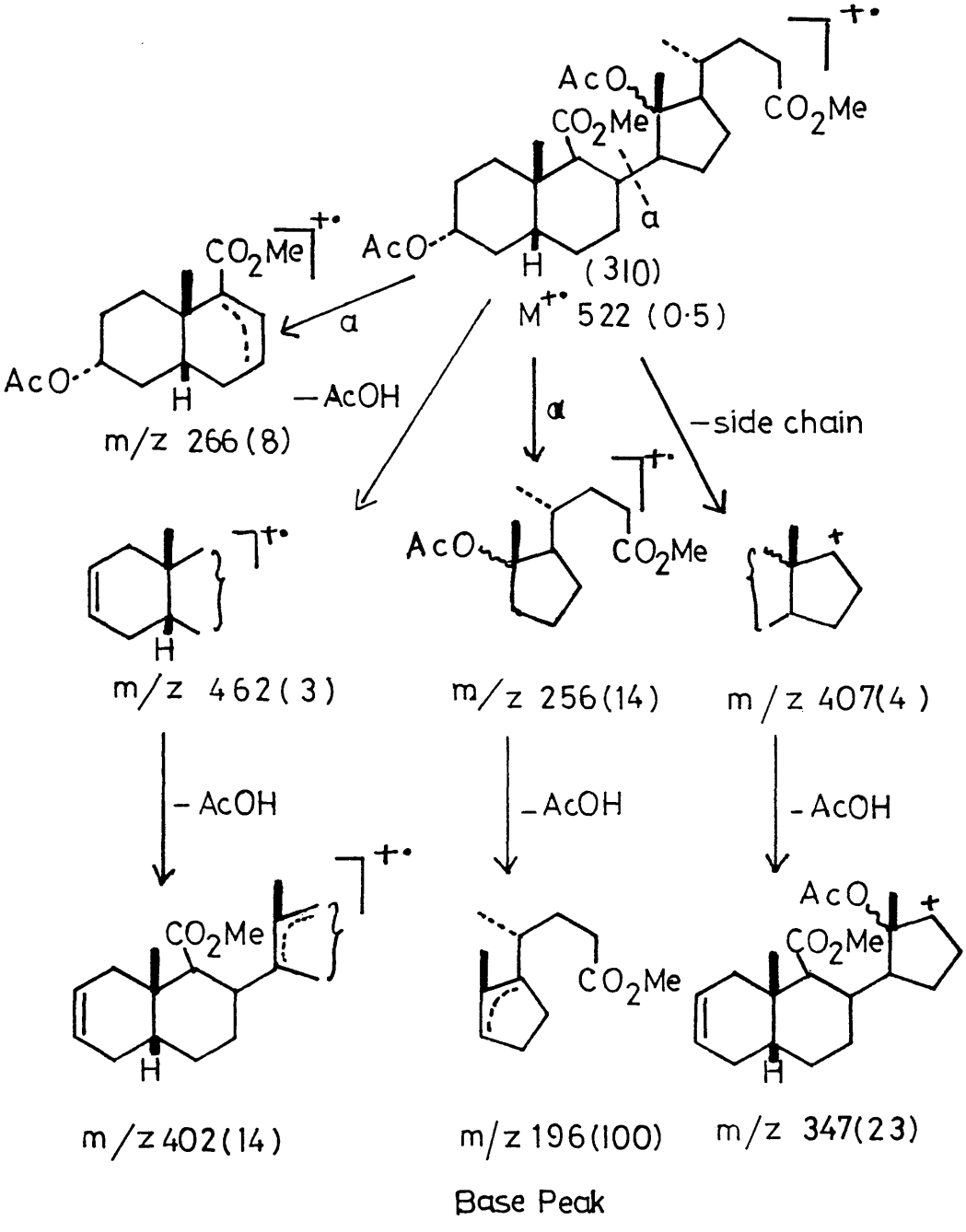

AcO
CO2Me
CO2Me
a
H
(310)
M+• 522 (0·5)
CO2Me
AcO
a
H
m/z 266 (8)
−AcOH
a
−side chain
m/z 462 (3)
AcO
CO2Me
m/z 256 (14)
m/z 407 (4)
−AcOH
−AcOH
−AcOH
CO2Me
H
m/z 402 (14)
CO2Me
m/z 196 (100)
Base Peak
AcO
CO2Me
H
m/z 347 (23)

Fig. 13 a

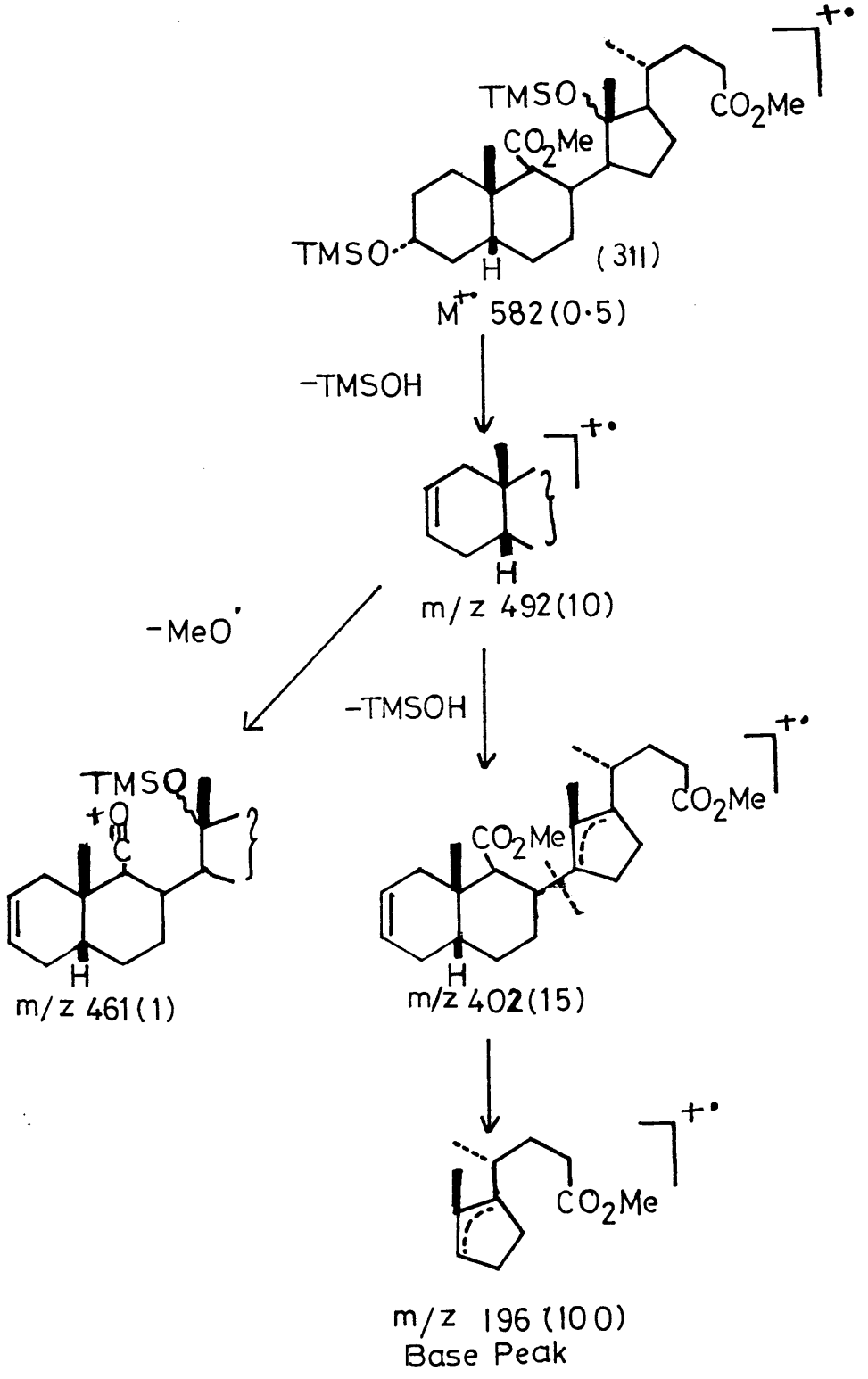
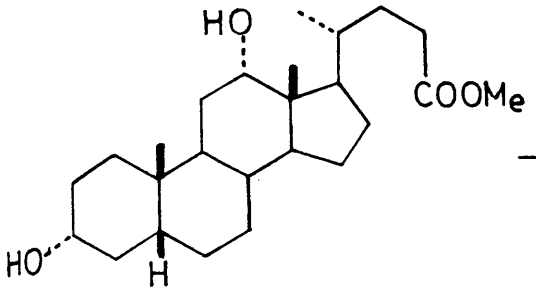

TMSO
CO2Me
CO2Me
TMSO
H
(311)
M+• 582(0·5)
-TMSOH
m/z 492(10)
-MeO•
-TMSOH
TMSO
+O
C
H
m/z 461(1)
CO2Me
CO2Me
H
m/z 402(15)
CO2Me
m/z 196 (100)
Base Peak

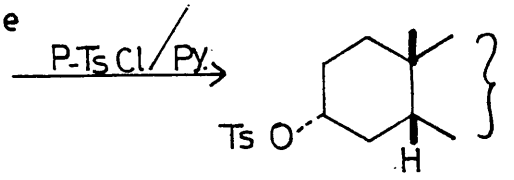
Fig. 13b

2.5 Methyl 11α-bromo-12-oxo-5β-cholanate (316)

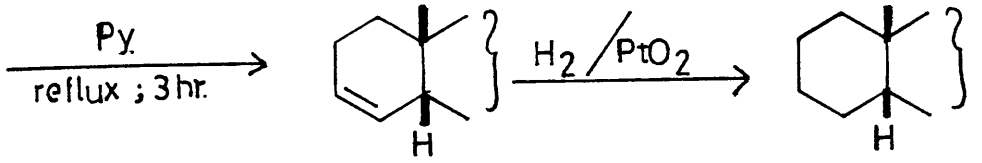
The ketol (317) was prepared from methyl deoxycholate (279) according to Barnett and Reichstein's method.[192] Methyl deoxycholate (279) was treated with p-toluenesulphonyl chloride in dry pyridine to give the correspnding 3-tosylate (312) which led to methyl 12α- hydroxy-5β-chol-3-enoate (313) upon reflux for 3h in dry pyridine. Hydrogenation of (313), in the presence of reduced PtO_2 afforded methyl 12-hydroxy-5β-cholanoate (314), which upon oxidation with chromium trioxide gave the ketone (315): this was brominated to afford the desired bromo-ketone (316). The ^{1}H-nmr spectrum confirmed the structure by showing a doublet at δ4.95 ppm (J=10.0 Hz) due to the 11β-proton- which is in accordance with the data reported by Zürcher [193] - in addition to the other usual signals.



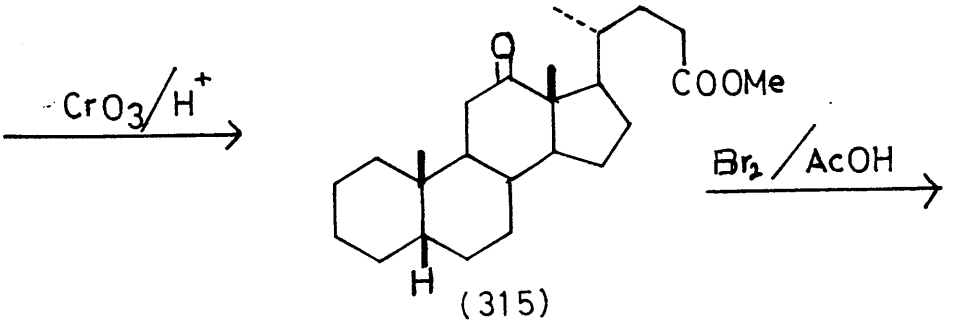
(279)



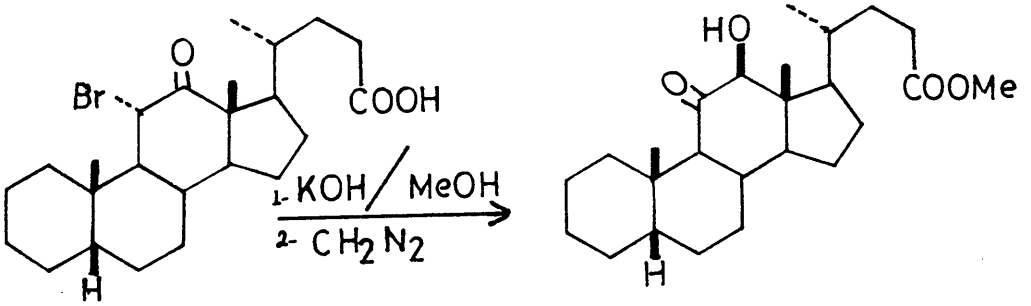
(312)



(313) (314)



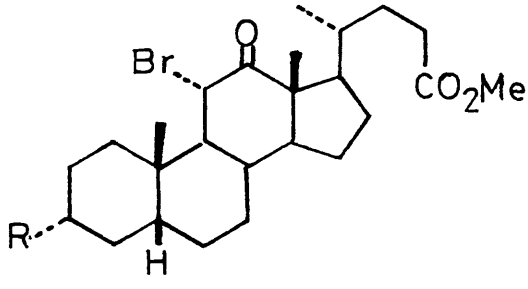
(315)



(316) (317)

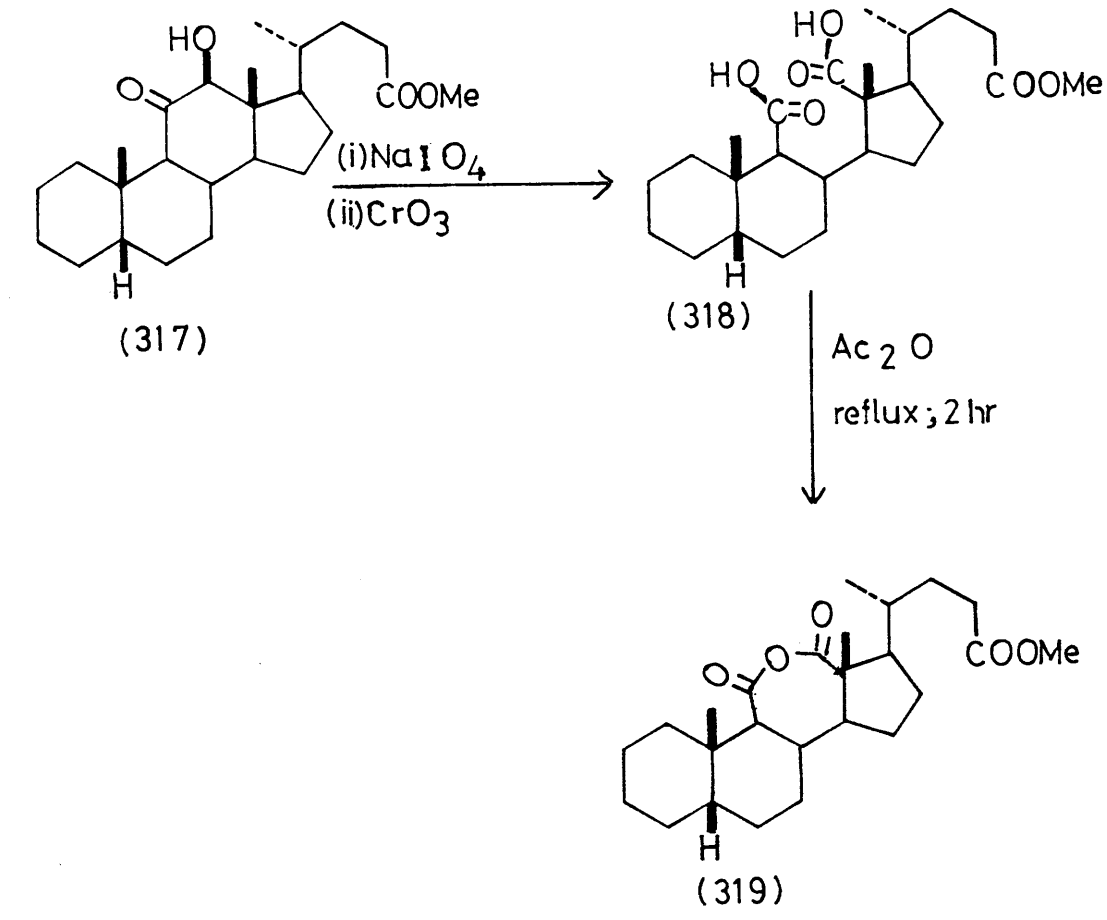
Table 2: 90 MHz ^{1}H-nmr Data for 11α-bromo-12 ketones.

(Solvent : $CDCl_3$)



δ (ppm)

R	3β-H	11β-H	18-Me	19-Me	21-Me
H	-	4.95d (J=10.10Hz)	1.14s	1.09s	1.32d
EtOCO	4.62m	4.95d (J=10.10Hz)	1.18s	1.00s	1.30d
PhCO	4.95m	4.98d (J=10.0Hz)	1.20s	1.01s	1.32d


HO
O
COOMe
(i)NaIO4
(ii)CrO3
H
(317)
HO
HO
O=C
C=O
COOMe
H
(318)
Ac2O
reflux; 2 hr
O
O
O
COOMe
H
(319)

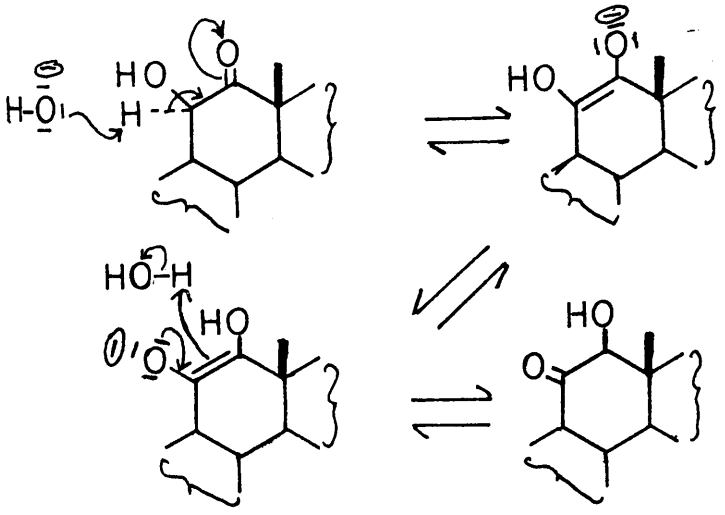

H-O
HO
H
O
HO
O
HO-H
HO
O
HO
O

Fig. 14

2.6 11,12-seco-5β-Cholane-11,12,24-trioic acid 24-methyl ester (318)

Hydrolysis of 11α-bromo-12-oxo-5β-cholanoate (316) (KOH/MeOH), followed by methylation (CH_2N_2) led to the rearranged ketol (317), methyl 12β-hydroxy-11-oxo-5β-cholanoate.[192] It is thought that the rearrangement takes place through the enolate ions as outlined in Fig.14.

Reaction of the ketol (317) with sodium periodate in dioxan, followed by treatment with chromium trioxide in acetic acid at room temperature led to the desired 11,12-seco-trioic acid 24-methyl ester (318), a new derivative of the known 11,12-seco-11,12,24-trioic acid,[164] characterised by m.p., elemental analysis, and by the mass spectrum of the 11,12-seco-11,12,24-trioic acid trimethyl ester; (see Fig.15 for the postulated fragmentation modes). The mass spectrum showed: the ions $(M\text{-}HCO_2Me)^{+\cdot}$, $(M\text{-}MeOH)^{+\cdot}$, in addition to other fragments whereas the base peak was shown as $(M\text{-}HCO_2Me\text{-side chain})^{+\cdot}$.

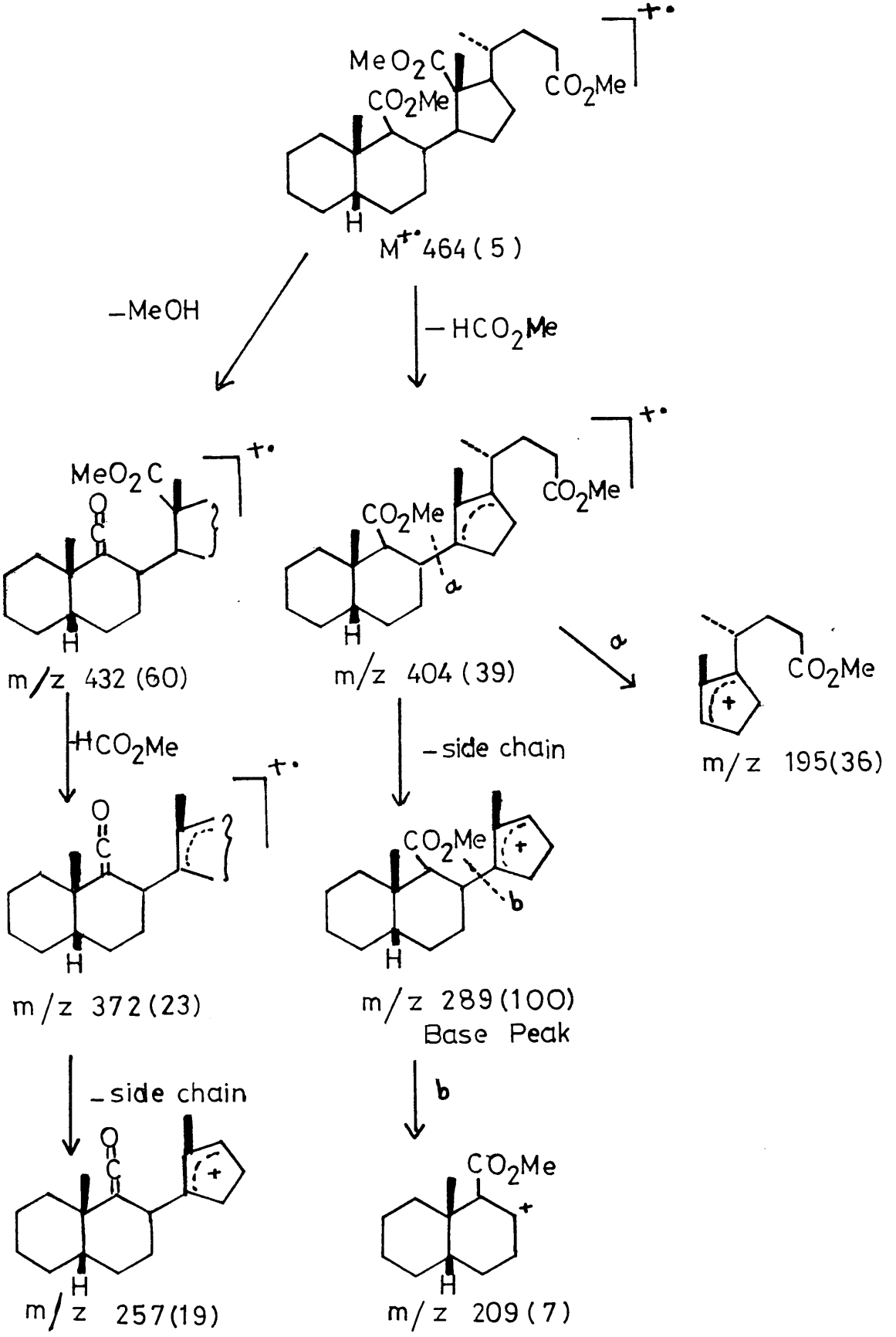

MeO2C
CO2Me
CO2Me
H
M+• 464 (5)
–MeOH
–HCO2Me
MeO2C
O
C
H
m/z 432 (60)
CO2Me
CO2Me
a
H
m/z 404 (39)
a
CO2Me
m/z 195(36)
HCO2Me
O
C
H
m/z 372(23)
–side chain
CO2Me
b
H
m/z 289(100)
Base Peak
–side chain
O
C
H
m/z 257(19)
b
CO2Me
H
m/z 209 (7)

Fig. 15

2.7 11,12-seco-5β-Cholane-11,12-24-trioic acid 11,12-anhydride 24-methyl ester (319)

Treatment of the 11,12-seco-5β-cholane-11,12,24-trioic acid 24-methyl ester with acetic anhydride for 2h under reflux afforded the 11,12-seco-anhydride (319),[194] in 36.5% yield. The infra-red spectrum of the product showed typical bands of a cyclic anhydride at 1750 and 1795 cm^{-1}. Again, there is a distinct difference in the intensity of the higher frequency band in the spectrum of this compound as compared with that of the 5- and 6-membered ring anhydrides. In the latter case, the signal is strong, while in the former it is considerably weaker. The anhydride was also characterised by elemental anlysis, ^{1}H-nmr spectrum, and GC retention index, and by the mass spectrum (see Fig.16 for postulated fragmentation pattern). The mass spectrum showed the molecular ion at 7% abundance, $(M-CO_2)^{+\cdot}$; and $(M-CO_2$-the bile acid side chain$)^{+\cdot}$; in addition to other fragments.

It should be mentioned that there was no sign of conversion in the configuration at C-9. The GLC showed one peak (see Table 4) and there was no evidence in the ^{1}H-nmr spectrum to support this case.

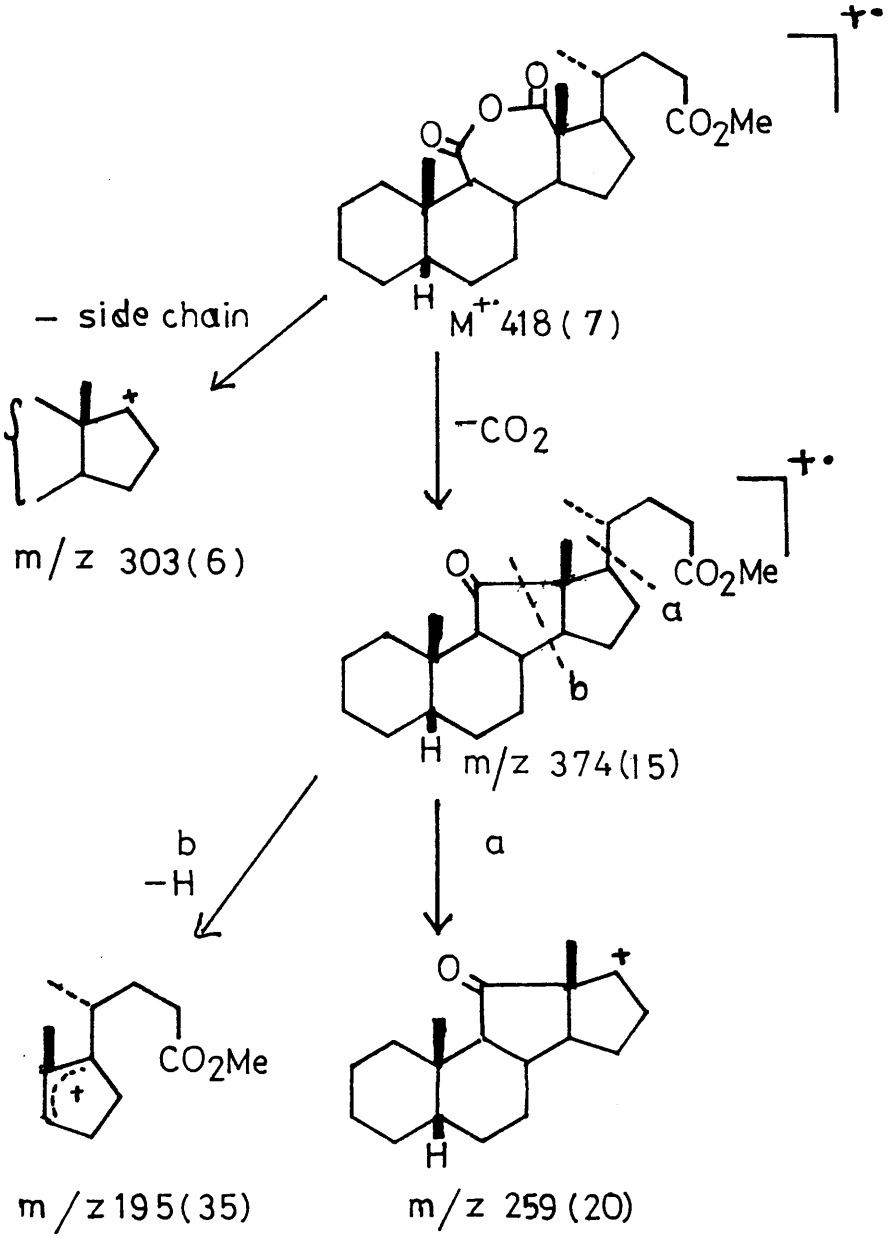
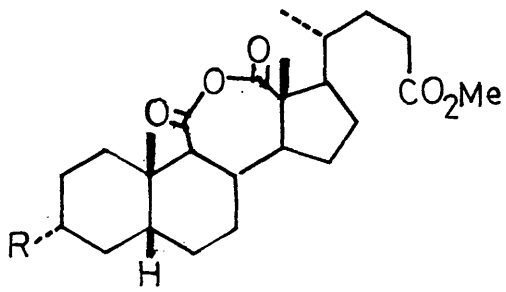

CO2Me
M+· 418 (7)
– side chain
m/z 303(6)
–CO2
CO2Me
a
b
m/z 374(15)
b
–H
a
CO2Me
m/z 195(35)
m/z 259 (20)

Fig. 16

Table 3: 90 MHz ^{1}H-nmr Data for 11,12-seco-dioic acid anhydrides.

(Solvent : $CDCl_3$)

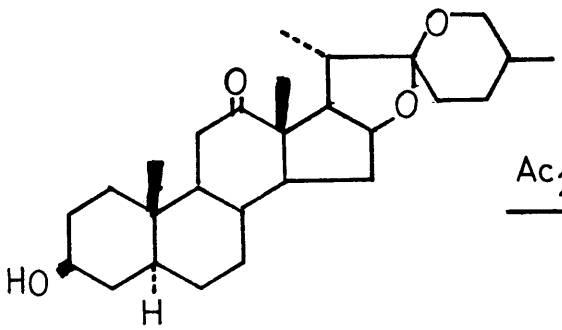


δ (ppm)

R	3β-H	18-Me	19-Me	21-Me	24-Me (ester)
H	-	1.18s	0.92s	1.06d	3.66s
EtOCO (O)	4.60m	1.00s	0.82s	0.98d	3.65s

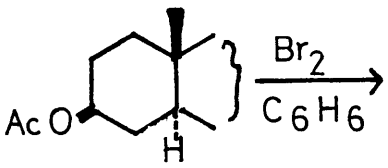
2.8 3β-Acetoxy-12β-hydroxy-5α:25R-spirostan-11-one (327)

Hecogenin (320) is commercially available and could be conveniently converted to the corresponding ketol, then to the desired 11,12-seco-dioic acid anhydride (through the seco-dioic acid). Therefore, it was chosen as a starting material for the investigation in the 5α-series. Hecogenin acetate (321)[62] was converted to 3β-acetoxy-12β-hydroxy-5α: 25R-spirostan-11-one (327) according to the method of Elks et al.,[201] through the following stages. Hecogenin acetate was first treated with bromine in dry benzene to give 11α:23a-dibromo-hecogenin acetate (322). Hydrolysis of (322) led to the 23-bromo-ketol (323) which, upon actylation, gave the 23-bromo-ketone diacetate (324). Debromination of (324) (Zn/AcOH)[210,202] afforded 3β,12β-diacetoxy-5α:spirostan-11-one (326) which led to the desired 3β-acetoxy-12β-hydroxy-5α:25R-spirostan-11-one (327) by treatment with acetic anhydride in acetic acid.[201] ^{1}H-nmr spectrum showed: δ0.60ppm (s,3H$_9$18-Me); 0.80 (d,3H,27-Me); 0.95 (d,3H,21-Me); 1.5 (s,3H,19-Me); 2.00 (s,3H,3β-AcO); 3.44 (d,2H,26-CH$_2$); 3.81 (s,1H,12α-H); and 4.60 (m, 3α-H).



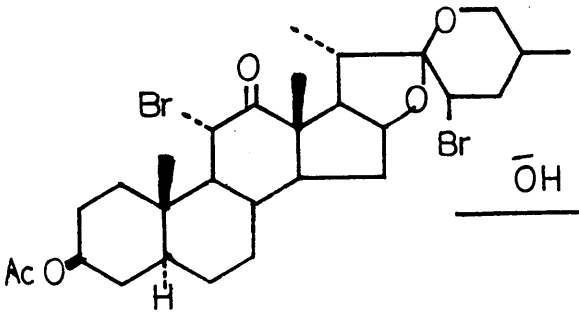
(320)

Ac_2O / Py.



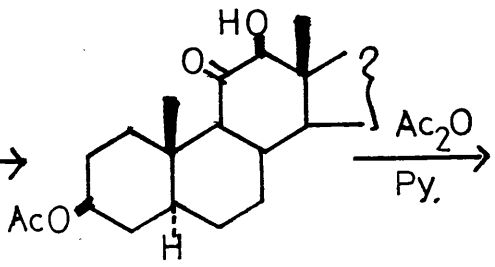
(321)

Br_2 / C_6H_6



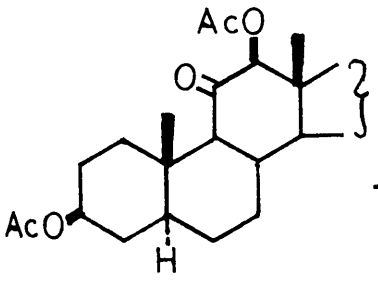
(322)

$\bar{O}H$



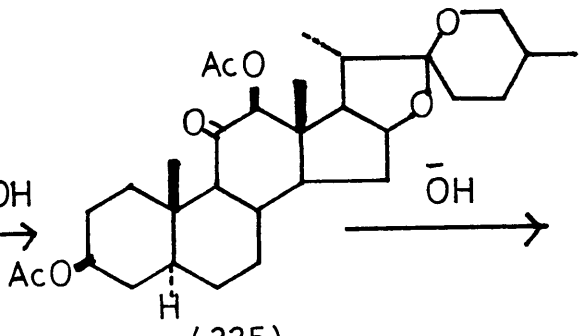
(323)

Ac_2O / Py.



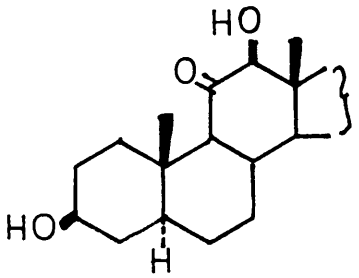
(324)

Zn / AcOH

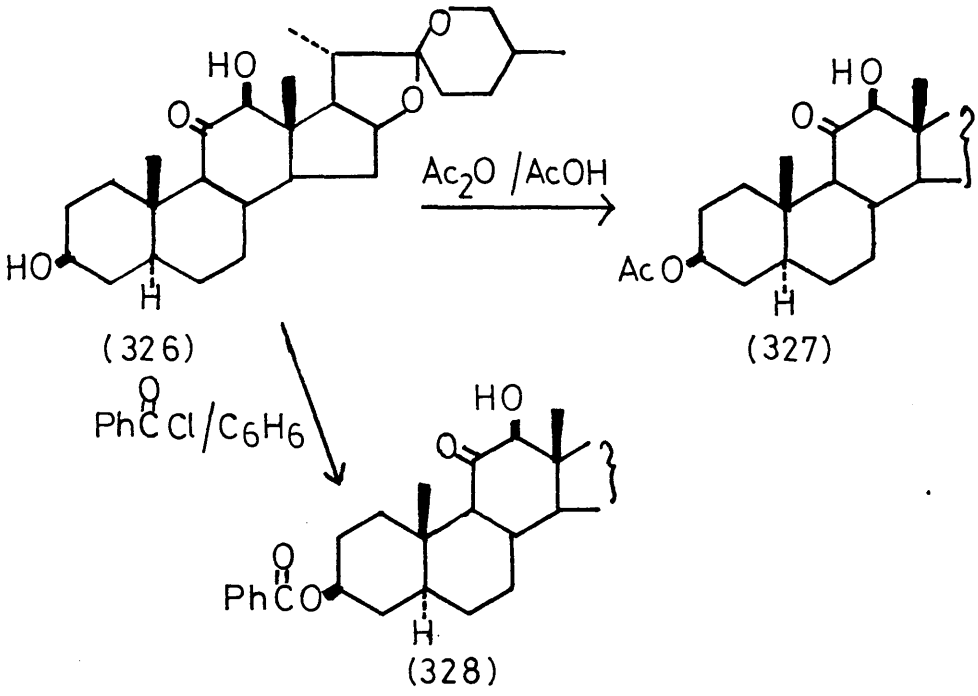


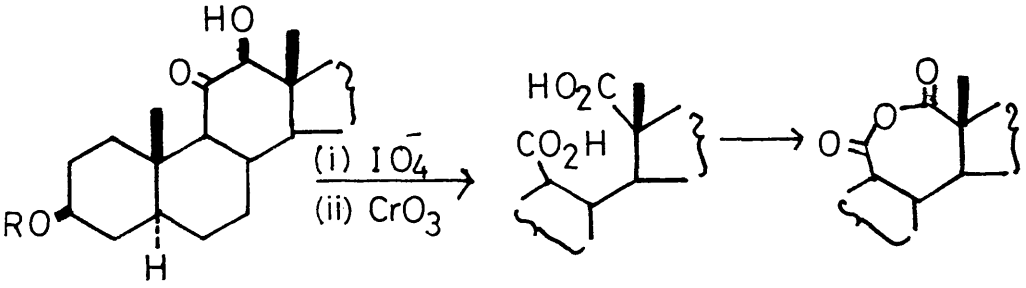
(325)

$\bar{O}H$



(326)





(327) R = Ac (329) R = Ac (330) R = Ac

(328) R = PhCO (332) R = PhCO (333) R = PhCO

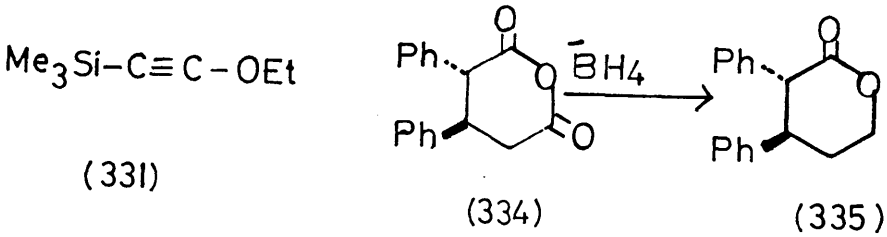
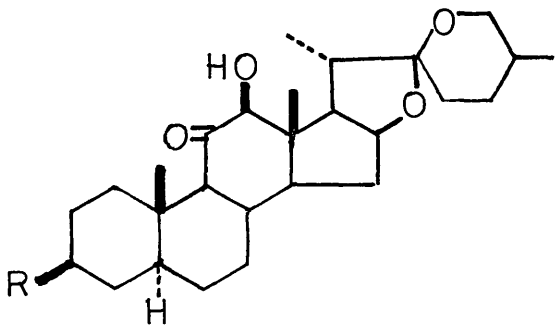


Table 8: 90 MHz ^{1}H-nmr Data of Ring-C Ketols Derived from Hecogenin. (Solvent : $CDCl_3$)



R	3α-H	12α-H	18-Me	19-Me	21-Me
			δ (ppm)		
AcO	4.60m	3.81s	0.60s	1.05s	0.95d
PhC(=O)-O	4.90m	3.81s	0.60s	1.12s	1.03d

2.9 3β-Benzoyloxy-12β-hydroxy-5α:25R-spirostan-11-one(328)

Treatment of 3β,12β-dihydroxy-5α:25R-spirostan-11-one (326) with benzoyl chloride in dry benzene[203] in the presence of a small amount of pyridine (1:40; volume of py./volume of C_6H_6) afforded 3β-benzoyloxy-12β-hydroxy-5α:25R-spirostan-11-one (328). The mass spectrum showed the molecular ion at m/z 550 in 5% abundance. The ^{1}H-nmr spectrum exhibited a 5H multiplet at δ7.40-8.20, a 1H multiplet at δ4.90, and a 1H singlet at δ3.81 ppm attributed to the 3β-benzoate, 3β-H, and the 12α-H respectively.

2.10 11,12-Secotigogenin-11,12-dioic acid 3β-acetate (329)

Treatment of the ketol (327), 3β-acetoxy-12β-hydroxy-5α: 25R-spirostan-11-one, with sodium periodate in dioxan followed by reaction with chromium trioxide in acetic acid at room temperature, led to the desired 11,12-seco-dioic acid (329), characterised by m.p., elemental anlysis, and by the mass spectrum [molecular ion, m/z 548 (6)]. The other principal ions fit the fragmentation pattern illustrated in Fig.18; the base peak occurred at m/z 139, attributed to $(C_9H_{15}O)^+$(see fragment (d) in Fig 17.)

Fig. 17: A summary of some of the main fragmentations of the spiroketal system found in the mass spectra of some sapogenins.[205]

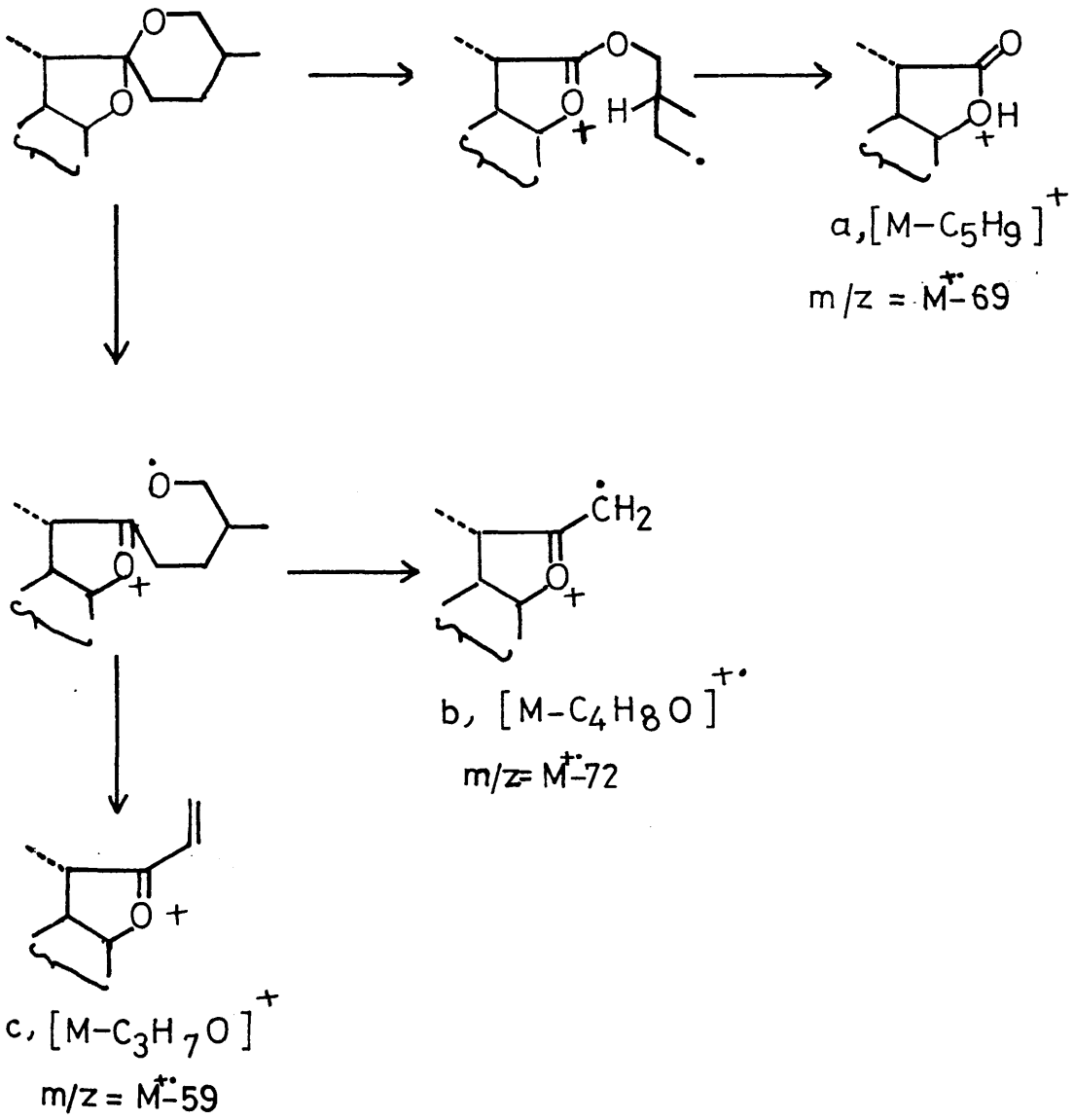
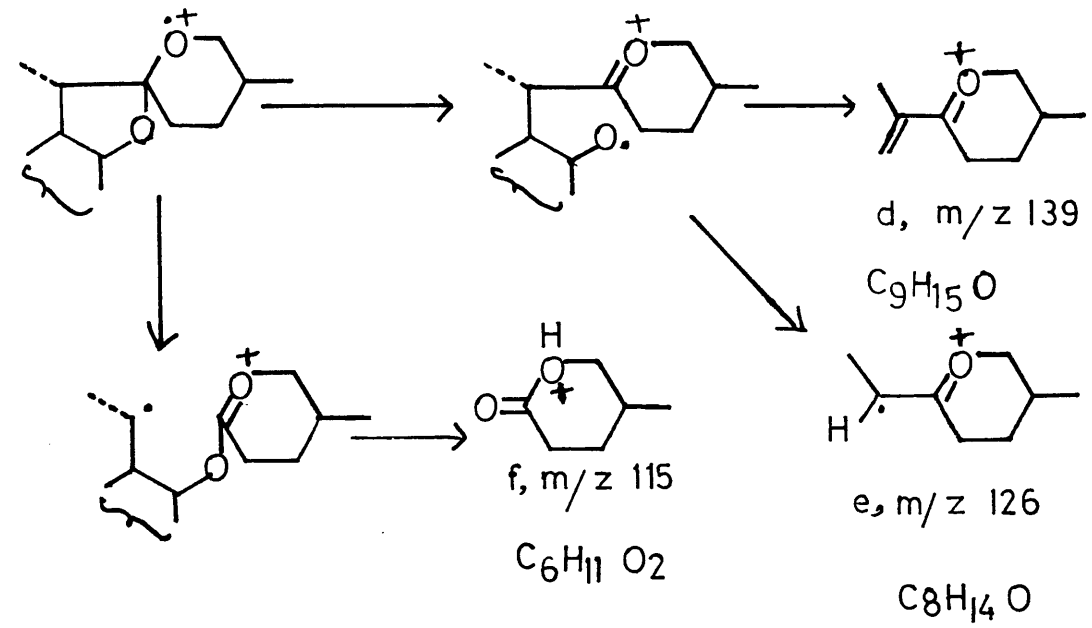
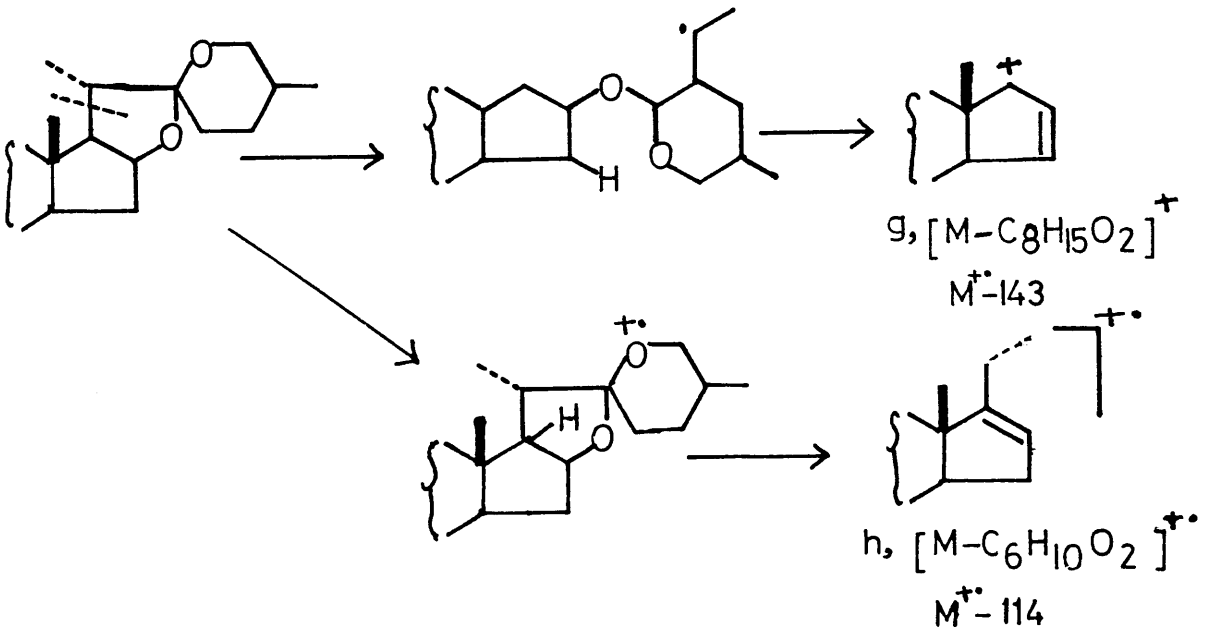


Fig. 17: (contd.)


d, m/z 139
$C_9H_{15}O$
f, m/z 115
$C_6H_{11}O_2$
e, m/z 126
$C_8H_{14}O$


g, $[M-C_8H_{15}O_2]^+$
$M^{+\cdot}-143$
h, $[M-C_6H_{10}O_2]^{+\cdot}$
$M^{+\cdot}-114$

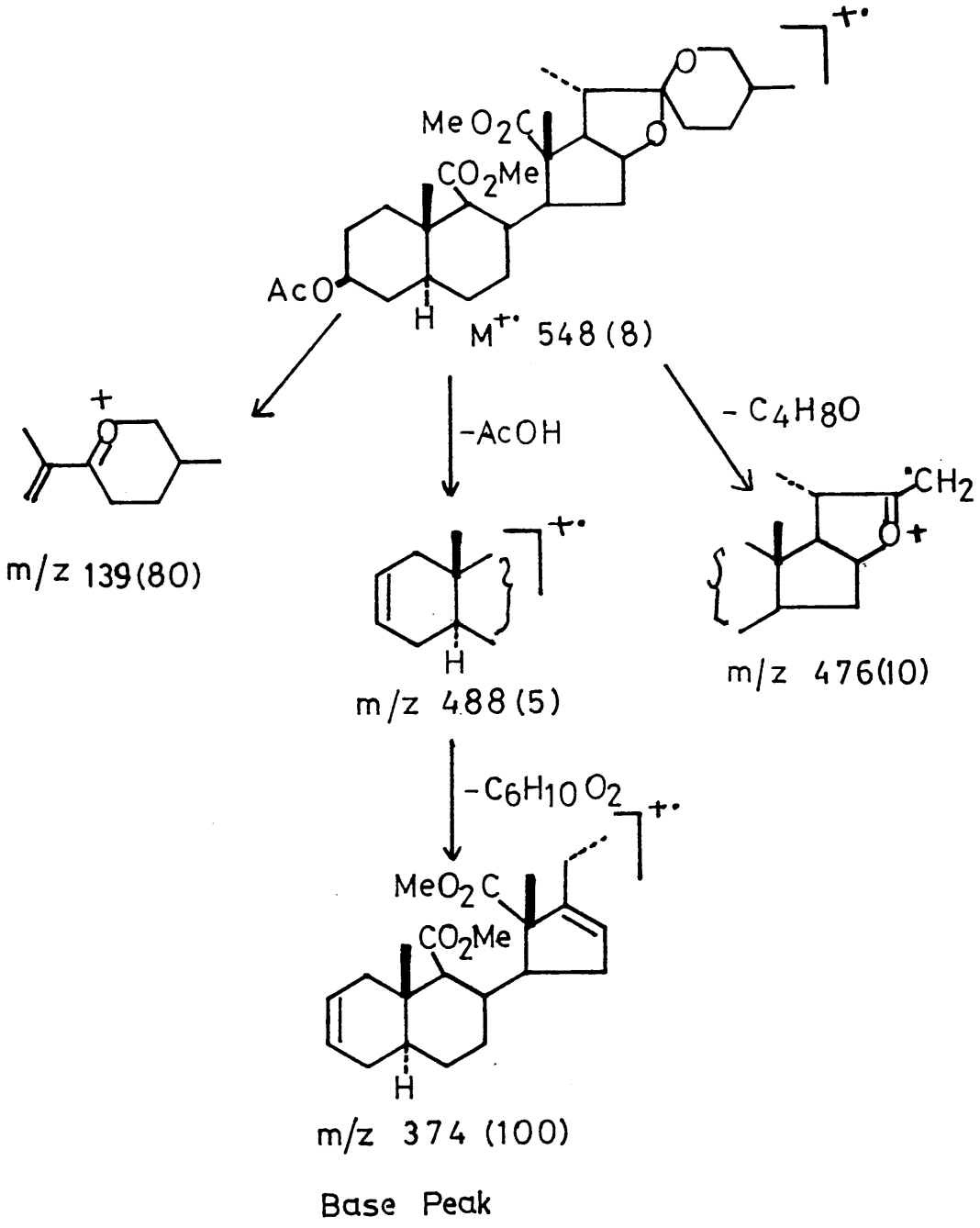

MeO2C
CO2Me
AcO
H
M+· 548 (8)
-AcOH
- C4H8O
m/z 139(80)
m/z 488 (5)
m/z 476(10)
-C6H10O2
m/z 374 (100)
Base Peak

Fig. 18

2.11 11,12-Secotigogenin-11,12-dioic acid anhydride 3β-acetate (330)

At the time of this work, Y. Kita and co-workers [204] reported that trimethylsilylethoxyacetylene (331), obtainable by the trimethylsilylation of the commercially available ethoxyacetylene, provides an effective method for dehydration of various types of carboxylic acids to give the corresponding carboxylic anhydrides under mild conditions, and allows easy isolation of pure products in almost quantitative yields. The reported cyclic anhydrides were of five- and six-membered ring types only. When 11,12-secotigogenin-11,12-dioic acid (309) was treated trimethyl- silylethoxyacetylene, the starting material was recovered. The dicarboxylic acid (329) was then refluxed in acetic anhydride to give the desired 11,12-secotigogenin-11,12-dioic acid anhydride 3β-acetate (330) in 37.3% yield. The infra-red spectrum showed the expected bands at 1750 and 1795 cm^{-1} (cyclic anhydride) and at 1730 and 1250 cm^{-1} (acetate). In the ^{1}H-nmr spectrum, three 3H singlets at δ1.20, 1.24 and 2.04 ppm are due to the methyl groups at C-18 and C-19, and the acetyl group at C-3; two 3H doublets at δ0.82 and 1.10 ppm are attributed to the methyl groups

at C-27 and C-21: two 1H multiplets at δ4.40 and 4.70 ppm indicate the protons at C-16 and C-3 respectively. In the mass spectrum, recorded by GC-MS, the parent ion appeared at $M^{+\cdot}$ = 502 (9) and the other principal ions are in agreement with the fragmentation outlined in Fig.19. The spectrum showed: $(M-AcOH)^{+\cdot}$ and $(M-CO_2)^{+\cdot}$; in addition to other fragments.

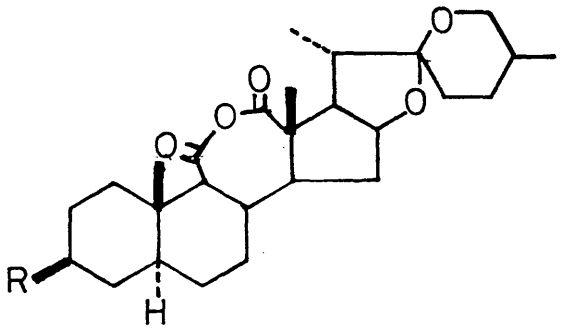
The GLC analysis (Table 10) showed that the compound is homogeneous by showing only one sharp peak. However, there was no evidence in the ^{1}H-nmr to suggest a conversion of the configuration at C-9.

2.12 11,12-Secotigogenin-11,12-dioic acid anhydride 3β-benzoate (333)

As in 2:11, 3β-benzoyloxy-12β-hydroxy-5α:25R-spirostan-11-one (328) was treated with sodium periodate in dioxan followed by reaction with chromium trioxide in acetic acid to give the 11,12-secotigogenin-11,12-dioic acid 3β-benzoate (332).[71] Treatment of the dioic acid (332) with acetic anhydride afforded the desired 11,12-seco-dioic acid anhydride (333) in 39.2% yield. The infra-red spectrum showed absorption bands at 1800 and 1750 cm^{-1} (cyclic

anhydride), the latter is much greater intense than the former, at 1715^{-1} (ester), and at 1600 and 1290 cm^{-1} (benzoyloxy). In the ^{1}H-nmr spectrum, two 3H singlets at δ1.19 and 1.26 ppm are attributed to the methyl groups at C-18 and C-19; two 3H doublets at δ0.82 and 1.15 ppm are due to the methyl groups at C-27 and C-21 respectively; the aromatic function at C-3 gives a 5H multiplet at δ7.45-8.05 ppm; in addition, there are the other usual signals (see Experimental). In the mass spectrum, the parent ion appeared at $M^{+\cdot}$ = 546 (1) and the principal ions fit the postulated fragmentation modes illustrated in Fig.20. The mass spectrum showed: $(M-CO_2)^{+\cdot}$, $(M-CO_2-PhCOOH)^{+\cdot}$, as well as other fragments.

Table 9: 90 MHz ^{1}H-nmr Data for Anhydrides Derived from Hecogenin. (Solvent : $CDCl_3$)



δ(ppm)

R	3α-H	18-Me	19-Me	21-Me
AcO	4.70m	1.20s	1.24s	1.10d
O ‖ PhC-O	4.95m	1.19s	1.26s	1.15d

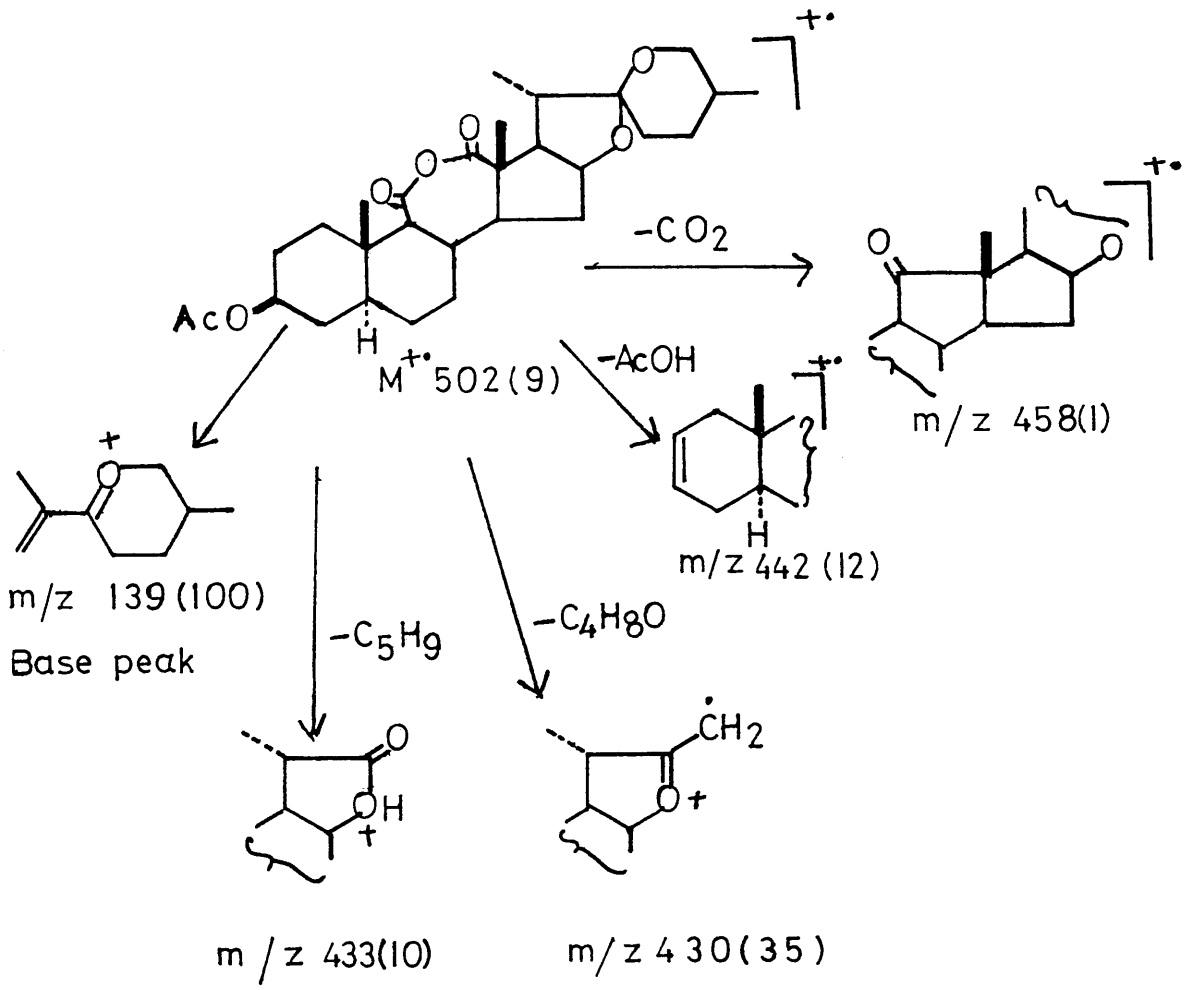

AcO
M+• 502(9)
-CO2
m/z 458(1)
-AcOH
m/z 442 (12)
m/z 139 (100)
Base peak
-C5H9
-C4H8O
m/z 433(10)
m/z 430(35)

Fig. 19

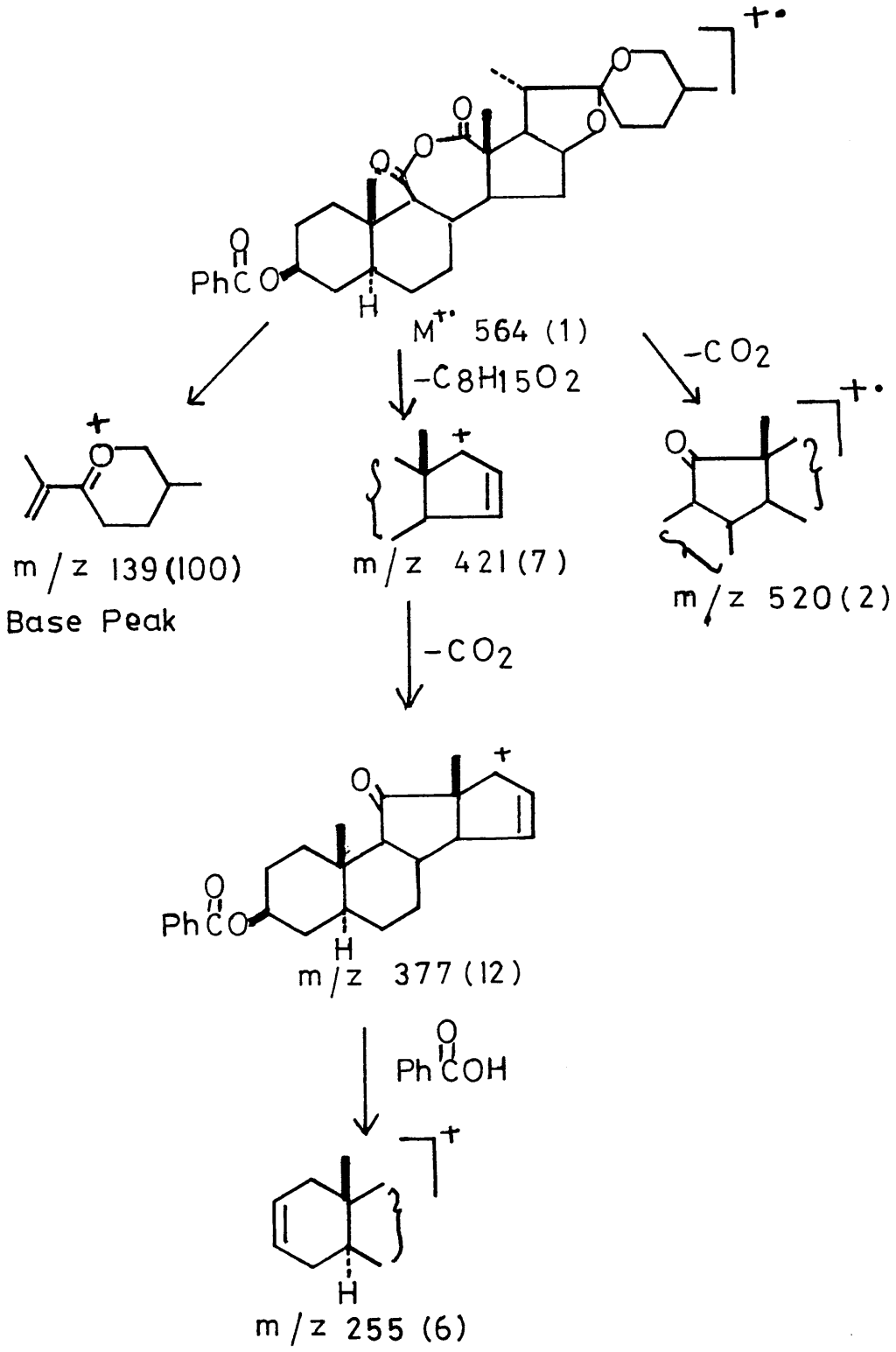

PhCO
M+· 564 (1)
-C8H15O2
-CO2
m/z 139 (100)
Base Peak
m/z 421 (7)
m/z 520 (2)
-CO2
PhCO
m/z 377 (12)
PhCOH
m/z 255 (6)

Fig. 20

2.13 Gas-Liquid Chromatography (GLC) Data for some Bile Acid and Hecogenin Derivatives

Gas-liquid chromatography techniques, since their investigation in 1952, have been the most useful analytical methods for the separation and estimation of organic compounds other than polymers or other molecules of high molecular weight. In GLC, the stationary phace is a liquid or gummy film held on a solid support or on the wall of the column in open-tubular (capillary) GLC, and the mobile phase is a gas which flows steadily over the surface of the film. If the solid support is suitably inert, adsorption is negligible, and the process is almost pure partition chromatography. Mobility of a compound is determined by its partition between the mobile and stationary phase. The concentration of a sample in the gas phase will be primarily determined by its vapour pressure at the operation temperature. Therefore, large separations depend mainly on marked differences in the volatility of the components in any mixture. GLC is a good complement to liquid-phase chromatography, since the mobilities of the latter depend only on functionality and polarity and are insensitive to molecular weight (apart from gel filtration)..

There has been a wide-ranging research aimed at elucidating the relationships between structure and retention data. The object of these

investigations was to allow comparison between the retention data measured in different laboratories and to make it possible to draw a conclusion from the retention data about the structure of the investigated material. This could be achieved by introducing a system for the presentation of retention data which makes it possible to eliminate the effects on the retention data of as many of the gas chromatographic parameters as possible. The simplest, but most primitive method of characterising the gas chromatographic behaviour of a compound, is the direct presentation of its "retention time": apart from the structure of a material and the nature of the stationary phase, this depends on the concentration of the latter and on the size and shape of the column, its temperature and the carrier gas flow-rate.

A more suitable technique is to relate the retention time to that of a standard material measured under the same circumstances,[194] for then the effect of the latter parameters (with the exception of the column temperature) can be eliminated.

Modern methods of presentation of the relationship between stucture and and chromatographic behaviour are based on the determination of the relative retention times. All of these methods depend on the fact that within a homologous series, at isothermal temperatures,

there is a relationship between the numbers of carbon atoms in the molecules of the materials and the logarithms of their relative retention times. Among the methods based on this principle, the "retention index" system of Kováts[195] is the most internationally accepted and is used in this work. The Kováts retention index (I) can be obtained by multiplying by 100 the carbon number of the notional n=alkane whose relative retention time is equal to that of the material being investigated. This value can be determined by interpolation from the retention times of the material and of the n-alkanes eluted before and after it and from the carbon number of the latter. The "steroid number" system is another method with specific application to steroids.[196] In this method, instead of n-alkanes, the standards for comparison are specially selected steranes.

Retention data, recorded by GLC, can also reflect the stereochemistry of organic compounds, thus it has been used extensively for identifying many steroid isomers.[197-9] The GLC technique is also useful for quantitative analysis, for example, by measuring the peak areas of related compounds, usually in comparison with internal standards.

Gas chromatography of bile acid methyl esters was first described by Van DenHeuvel, Sweeley and Horning.[200] Following their work, very extensive studies established the usefulness of the technique in

qualitative as well as in quantitative analysis of bile acids.

During the course of the present project, the GLC technique has been used for both qualitative and quantitative analysis. Tables 4 and 5 show retention times (t_r) and retention indices (I) of some bile acid derivatives which have been studied in this work.

Useful diagnostic information can be obtained, based on the data in Tables 4 and 5, and the differences (ΔI), in the retention index (I) values of structurally similar compounds that result from substitution etc. in individual positions. For instance, under the conditons stated, ΔI for the introduction of 3α-hydroxyl group is + 290 and the 3α-cathyloxy group (EtOC-Ö-) is + 600. (See Tables 6 and 12a). The increment for the conversion of 11, 12-seco-dioic acid dimethyl ester to the corresponding 11, 12-seco-dioic anhydride is + 150. (See Table 7). Tables 6 and 7 summarise ΔI correlations of some sets of compounds.

GLC data recorded for the hecogenin derivatives, used in this work, have also been useful for diagnostic purposes. While Table 10 exhibits the retention times (t_r) and retention index (I) values for these compounds, Table 11, however, illustrates the increments ΔI = + 130 for the acetylation of the 3β-hydroxyl groups.

Table 4: Retention data (t_r and I) (I) of some derivatives of bile acids, having no substitution at C-3.

Column: 6 ft 1% OV-1; Temp. = 240°C

t_r = retention time (min); I = retention index

Retention data values are ± 10

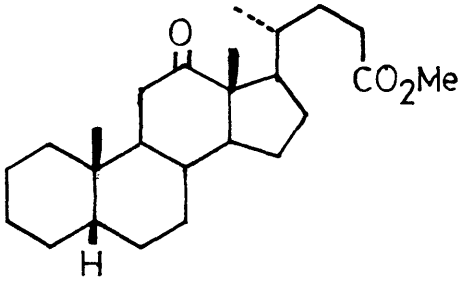
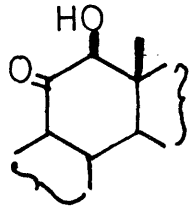
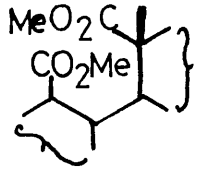
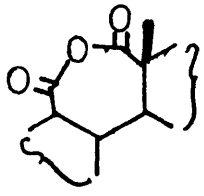
Compound	mol.wt.	t_r (min)	I 1% OV-1 ±240°C
	388	2.20	3010
	404	2.50	3040
	464	2.10	3000
	418	3.50	3150

Table 5: Retention data (t_r and I) of some derivatives of bile acids with substituents at C-3.

Column: 6 ft 1% OV-1; Temp. = 270°C

t_r = retention time (min); I = retention index

Retention data values are ± 10

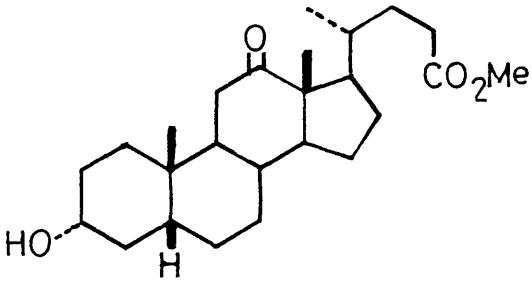
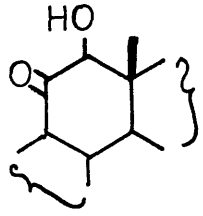
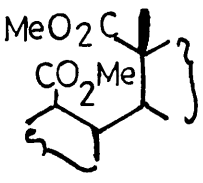
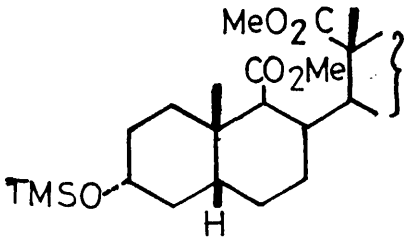
Compound	mol.wt.	t_r (min)	I 1% OV-1 270°C
	404	3.10	3300
	420	3.40	3330
	480	3.00	3290
	552	2.50	3250

Table 5 (contd.)

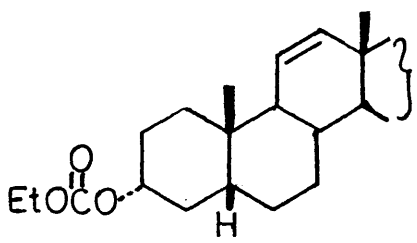
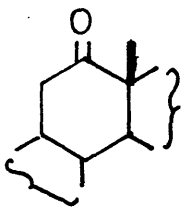
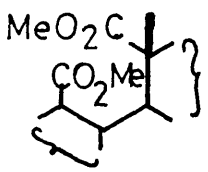
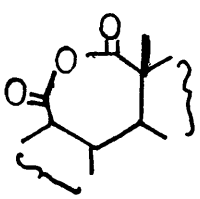
Compound	mol.wt.	t_r (min)	I
	460	4.10	3430
	476	6.10	3610
	552	6.00	3600
	506	8.20	3750

Table 6: ΔI Correlations for Substituents at C-3.

Column: 6 ft 1% OV-1: Temp. = 240°C (270°C in the case of compounds having substituents at C-3).

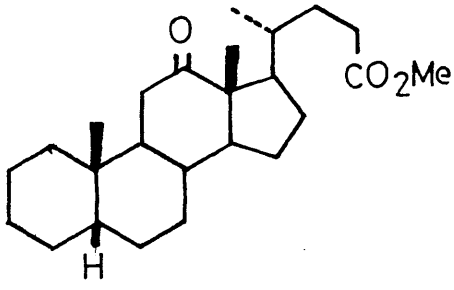
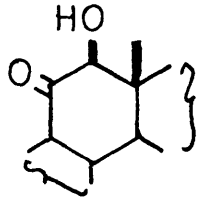
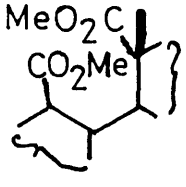
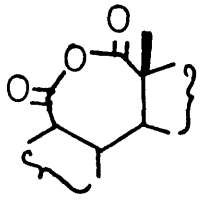
Compound	ΔI for substituents	
	3α-OH	3α-EtOC(=O)-O-
	+ 290	+ 600
	+ 290	-
	+ 290	+ 600
	-	+ 600

Table 7: ΔI Correlation for 11,12-seco-dioic anhydride.
Column: 6 ft 1% OV-1; Temp. = 240°C (270°C in the case of compounds having substituents at C-3).

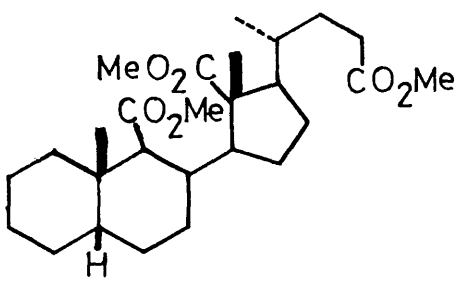
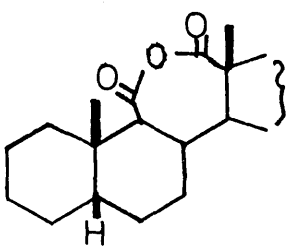
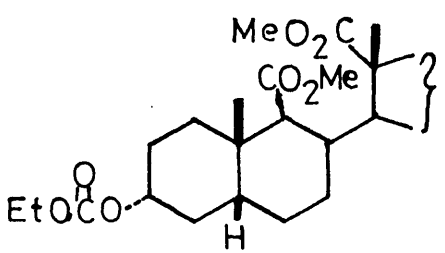
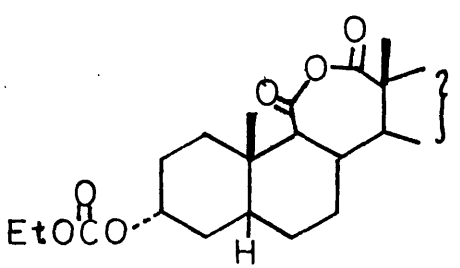
Compound	I	Compound	I	ΔI
	3000		3150	+ 150
	3600		3750	+ 150

Table 10: Gas-Liquid Chromatography (GLC Data for some Hecogenin Derivatives.

(Column: 6 ft 1% OV-1; Temp. 270°C)

t_r = retention time; I = retention index

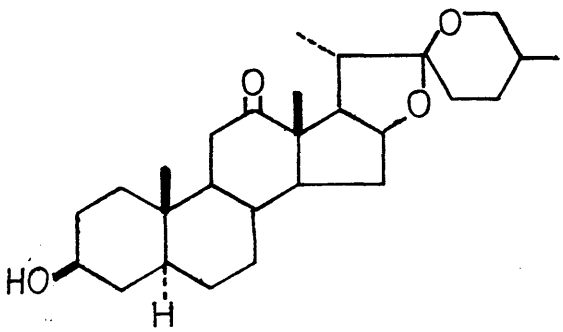
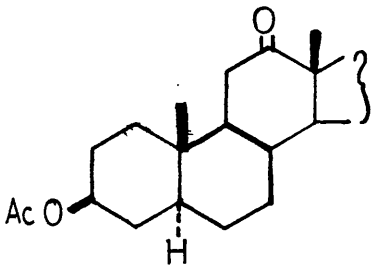
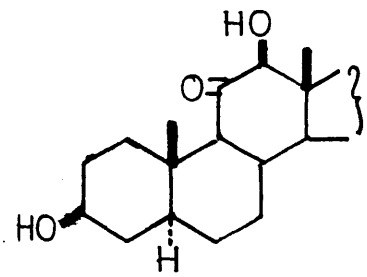
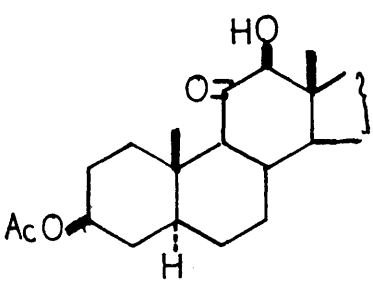
Compound	mol.wt.	t_r (min)	I
	430	2.85	3460
	472	2.75	3590
	446	3.10	3490
	488	4.10	3620

Table 10 (contd.)

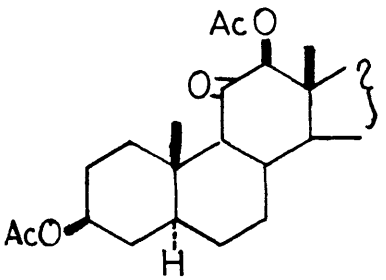
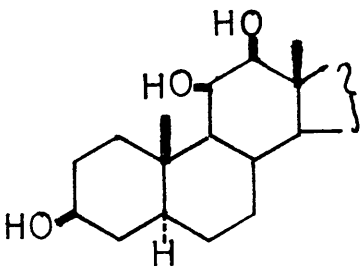
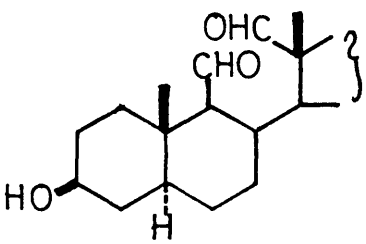
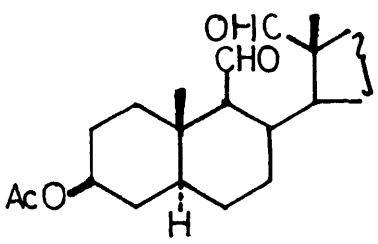
Compound	mol.wt.	t_r (min)	I
	530	4.90	3700
	448	4.05	3615
	446	2.80	3450
	488	3.90	3580

Table 10 (contd.)

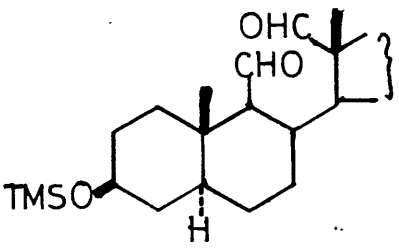
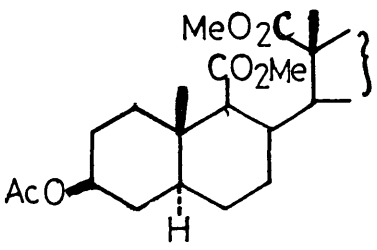
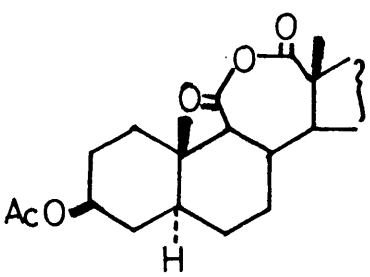
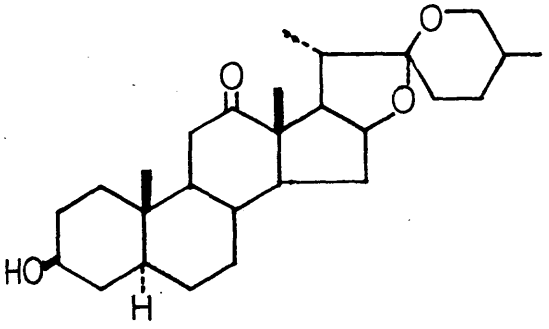
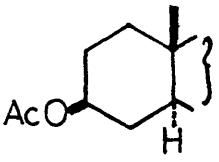
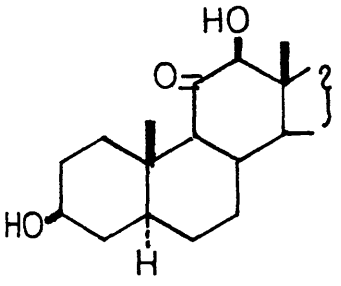
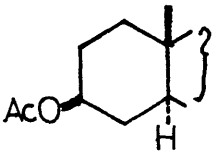
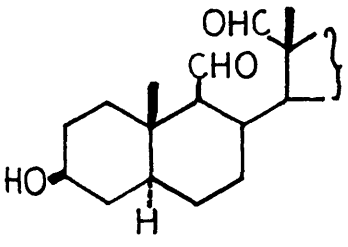
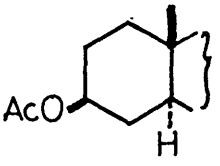
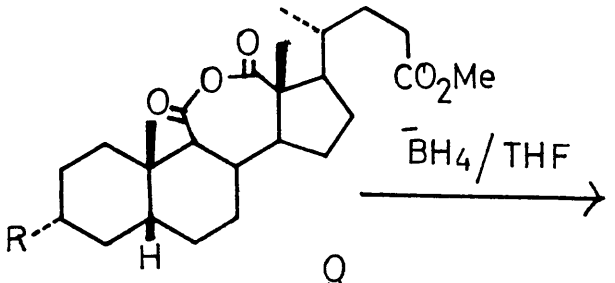
Compound	mol.wt.	t_r (min)	I
	518	3.30	3520
	548	3.70	3560
	502	5.60	3780

Table 11: ΔI Correlations for Acetylation of 3β-OH in Hecogenin Derivatives.

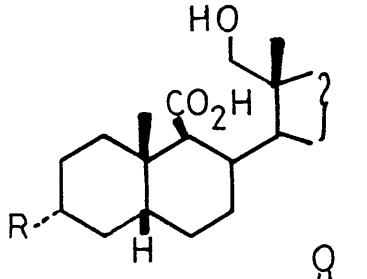
(Column: 6 ft 1% OV-1; Temp. 270°C)

Compound	$I^{1\%\ OV-1}_{270°C}$	Compound	$I^{1\%\ OV-1}_{270°C}$	ΔI
	3460		3590	+ 130
	3490		3620	+ 130
	3450		3580	+ 130



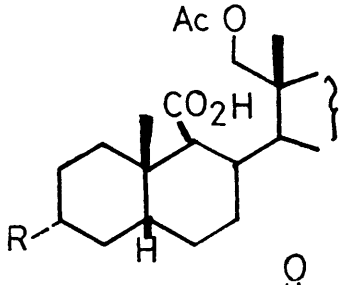
(286) R = EtOCO

(319) R = H



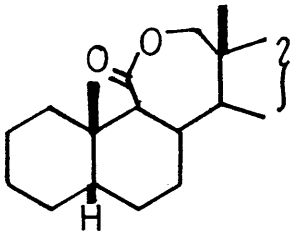
(336) R = EtOCO

(338) R = H

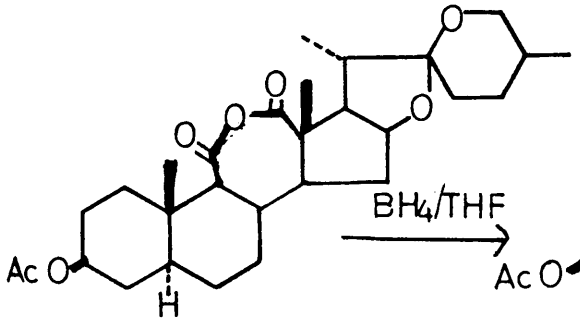


(337) R = EtOCO

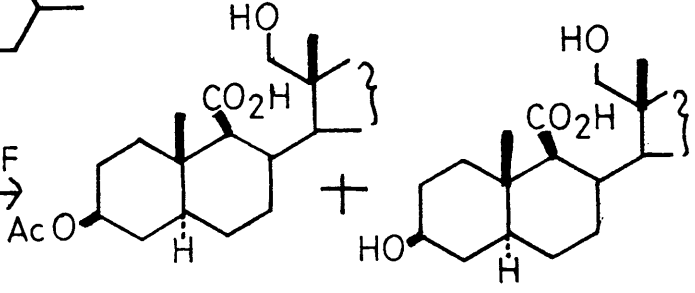
(339) R = H



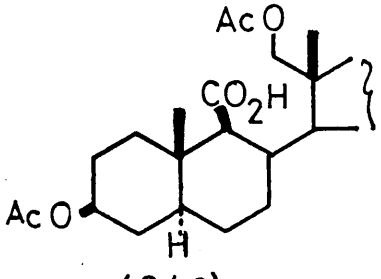
(340)



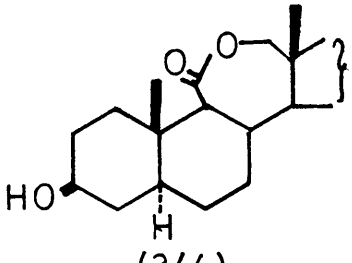
(330)



(341) (342)



(343)



(344)

2.14 Borohydride reduction of cyclic anhydrides

There are extensive reports in the literature describing the reduction of 5- and 6- membered anhydrides to their corresponding lactones; examples are given in the Introduction (sec. 1:8). Unfortunately, very few studies have been made on the reduction of 7-membered ring types of steroidal anhydrides. In fact, we are only aware of two reports[138,172] about the reduction of such systems and both investigations were dealing with ring-B anhydrides. In the present investigation, the following borohydride reductions have been conducted.

(a) Reduction of 2α,3β-diphenylglutaric anhydride (334)

2α,3β-diphenylglutaric anhydride (334) was chosen as a model compound for trial experiments on the borohydride reduction of unsymmetric anhydrides. Therefore, (334) was prepared by refluxing the corresponding dioic acid (2α,3β-diphenylglutaric acid) in acetic anhydride for 1h, m.p. 125-6°C (Lit[206] 126-5-7.0°C). The anhydride (334) was treated with sodium borohydride in dry tetrahydrofuran, at room temperature, for 3h. After the usual work up, a colourless gummy material was collected. GLC analysis showed one peak, tr = 3.15 min.; I = 2220 (1% OV-1; at 200°C), indicating the presence of one compound. This fact was supported by TLC

analysis by exhibiting one spot, R_f = 0.47 (using 8:2 ratio of pet. ether: ethyl acetate). Crystallisation from dilsopropyl ether afforded colourless needles, m.p. 131-2°C. The infrared spectrum showed a band at 1735 cm, attributed to a lactonic carbonyl group. However, the ^{1}H-nmr spectrum confirmed that the anhydride was reduced regioselectively at C-5 to give the corresponding lactone, 3α,4β-diphenyltetrahydropyran-2-one (335), by showing the following chemical shifts: δ7.10-7.30 (m, 10H, 3α-Ph and 4β-Ph); 4.50 (t, 2H, 6-CH_2); 3.79 (d, 1H, 3β-H); 3.25 (1H, double triplets, 4α-H); and 2.18 (m, 2H, 5-CH_2). The mass spectrum showed the molecular ion $M^{+\cdot}$ 252 (27%); in addition to $(M\text{-}CO)^{+\cdot}$, $(M\text{-}CH_2CH_2OC{=}O)^{+\cdot}$, and the base peak $(M\text{-}CH_2CH_2OC{=}O\text{-}C_6H_4)^{+\cdot}$.

(b) Borohydride reduction of 3α-ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12,24-trioic acid-11,12-anhydride 24-methyl ester (286)

Treatment of the 11,12-seco-dioic acid anhydride (286) with sodium borohydride, in dry tetrahydrofuran, under reflux for 3h afforded an oily material which was subsequently sublimed. The product showed one spot on TLC (R_f = 0.14; 3.2 pet:ether:EtOAc). It also gave one

peak on GLC (after treatment with CH_2N_2), I = 3610 (1% OV-1 at 270°C). The mass spectrum showed that the product was the 11,12-seco-12-hydroxy-11,24-dioic acid methyl ester (336), with the expected fragmentation pattern. (see Fig.21). The spectrum exhibited two ions (283 and 277) resulting from cleavage of the bond between C-8 and C-14; in addition to $(M\text{-}EtOCOOH\text{-}CH_3OH)^{+\cdot}$, and $(M\text{-}EtOCOOH\text{-}CH_3OH\text{-side chain})^{+\cdot}$.

Treatment of the obtained hydroxy acid (336) with acetic anhydride[172] led to the corresponding acetoxy acid (337) only, which gave (after methylation) one peak on GLC; I = 3680 (1% OV-1, 270°C). In the mass spectrum, the fragmentation pattern (Fig.22) is in agreement with the assigned structure. The mass spectrum showed, in addition to other ions, $(M\text{-}EtOCOOH)^{+\cdot}$, $(M\text{-}EtOCOOH\text{-}AcOCH_2)^{+\cdot}$, $(M\text{-}EtOCOOH\text{-}AcOCH_2\text{-}MeOH)^{+\cdot}$, and $(M\text{-}EtOCOOH\text{-}AcOCH_2MeO_2CH)^{+\cdot}$. In the ^1H-nmr spectrum, the presence of an isolated AB system represented by a 2H doublet at δ3.54 and 3.92 ppm (J=11.0 Hz), due to two protons at C-12, was evidence that the reduction occurred regioselectively at C-12.

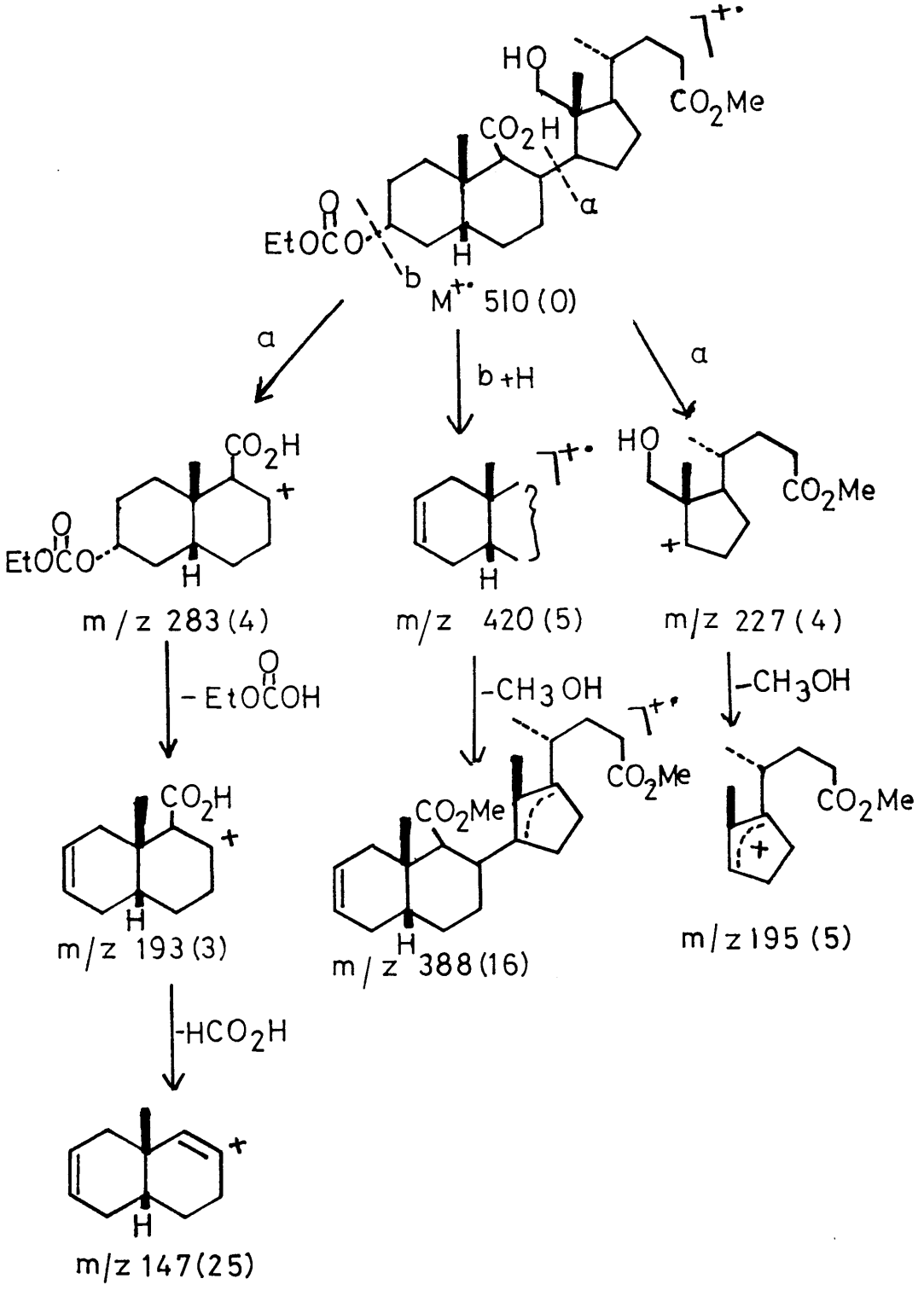

HO
CO2Me
CO2H
a
EtOCO
b
H
M+· 510 (0)
a
b+H
a
CO2H
EtOCO
m/z 283 (4)
m/z 420 (5)
HO
CO2Me
m/z 227 (4)
-EtOCOH
-CH3OH
-CH3OH
CO2H
m/z 193 (3)
CO2Me
CO2Me
m/z 388 (16)
CO2Me
m/z 195 (5)
-HCO2H
m/z 147 (25)

Fig. 21

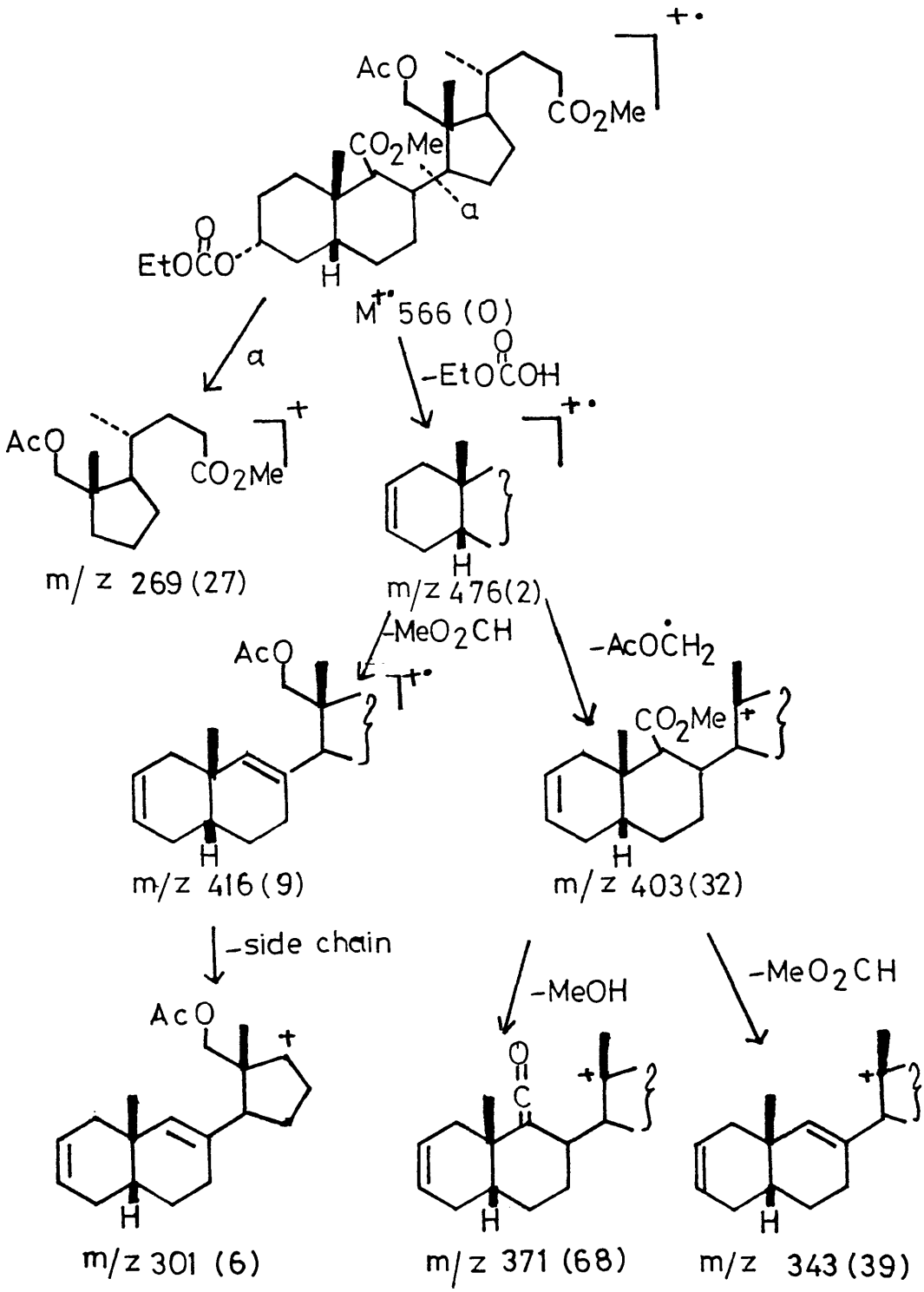

AcO
CO2Me
CO2Me
a
EtOCO
M+· 566 (0)
a
-EtOCOH
AcO
CO2Me
m/z 269 (27)
m/z 476(2)
-MeO2CH
-AcOCH2
AcO
CO2Me
m/z 416 (9)
m/z 403(32)
-side chain
-MeOH
-MeO2CH
AcO
m/z 301 (6)
m/z 371 (68)
m/z 343 (39)

Fig. 22

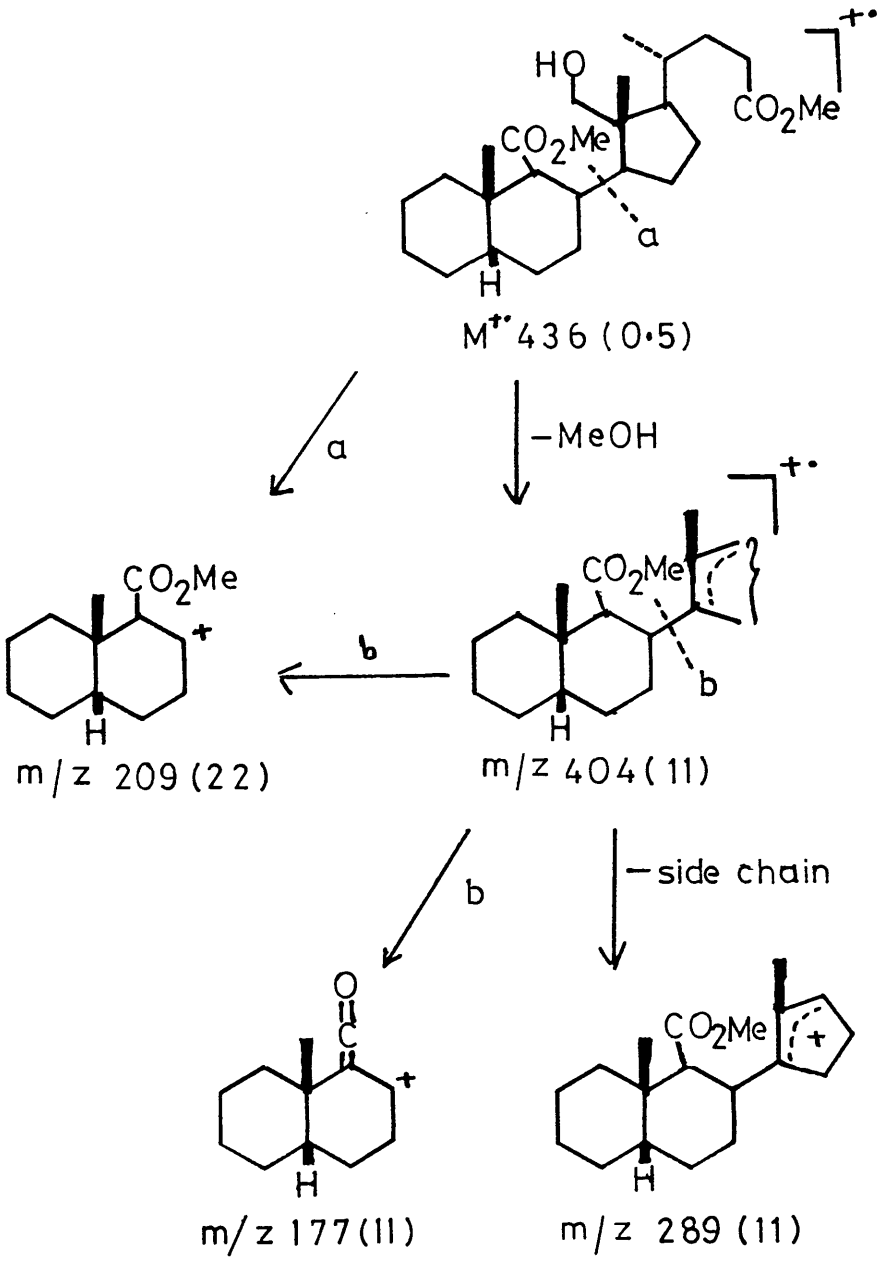

HO
CO2Me
CO2Me
a
H
M+· 436 (0·5)
a
−MeOH
CO2Me
b
CO2Me
b
H
m/z 209 (22)
m/z 404 (11)
b
−side chain
O
C
H
CO2Me
H
m/z 177 (11)
m/z 289 (11)

Fig. 23

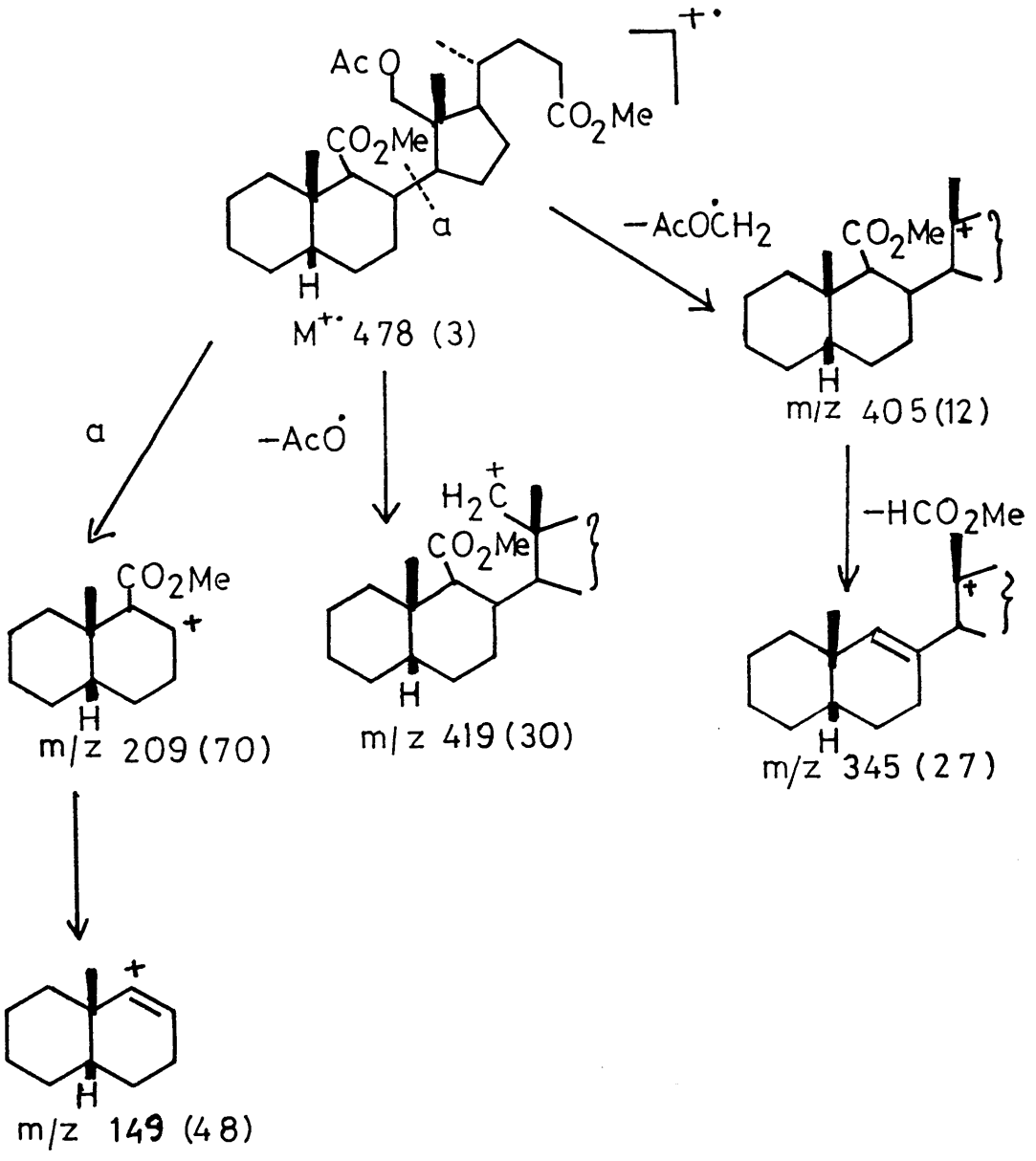

AcO
CO2Me
CO2Me
a
H
M+· 478 (3)
a
−AcO·
−AcOĊH2
CO2Me
H
m/z 405 (12)
−HCO2Me
H2C
CO2Me
H
m/z 419 (30)
CO2Me
H
m/z 209 (70)
H
m/z 345 (27)
H
m/z 149 (48)

Fig. 24

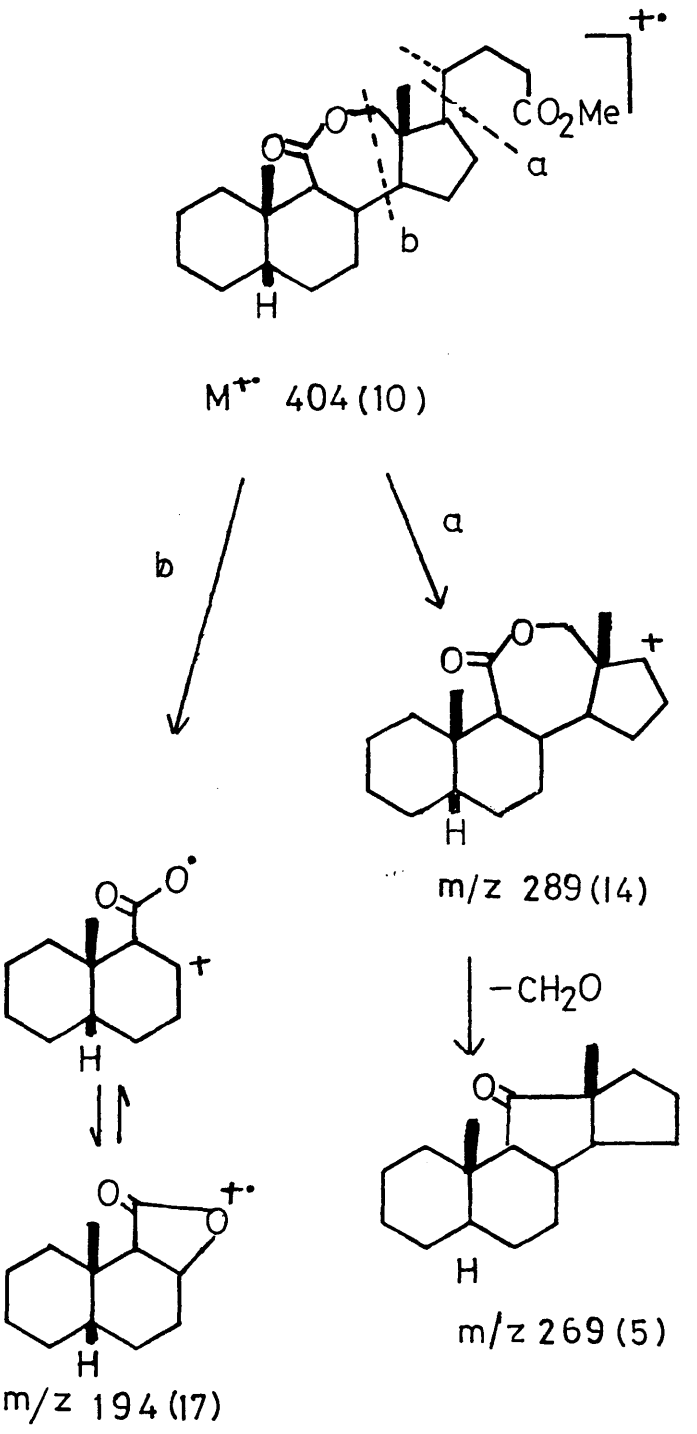


Fig. 25

(c) Borohydride reduction of 11,12-seco-5β-cholane-11,12-24 trioic acid-11,12-anhydride 24-methyl ester (319)

Reduction of the 11,12-seco-anhydride (319) with $NaBH_4$ under conditions similar to those applied in the previous reduction (2.14b) afforded the 11,12-seco-12-hydroxy-11,24-dioic acid (338; p.155). The product showed one spot on TLC (R_f = 0.17; 3:2 pet.ether:EtOAc), and its methyl ester gave one peak on GLC (I = 3035; 1% OV-1, 240°C). The mass spectrum, recorded by GC-MS, of the methyl ester derivative showed the expected molecular ion at 0.5% abundance and the fragmentation pattern (Fig.23) which exhibits: $(M-CH_3OH)^{+\cdot}$, $M-CH_3OH$-side chain$)^{+\cdot}$, as well as other ions.

When the hydroxy acid (338) was subjected to sublimation under reduced pressure, it was recovered unchanged. Treatment with acetic anhydride gave the acetoxy acid (339) as the major product, together with approximately 6% of the corresponding lactone (340) (estimated by GLC). Both compounds were identified by GLC, I (1% OV-1, 240°C) = 3095 and 3100 respectively, and by mass spectra, recorded by GC-MS (see Figs. 24 and 25). The mass spectrum of the methyl ester derivative

of the acetoxy-acid (Fig.24) showed the molecular ion $M^{+\cdot}$ (3%), (M-$AcOCH_2$-$HCO_2Me)^{+\cdot}$, and other fragments. However, the mass spectrum of the lactone (Fig.25) exhibited the molecular ion $M^{+\cdot}$(10%), (M-the bile acid side chain)$^+$, (M-side chain-$CH_2O)^+$; in addition to other ions.

(d) Reduction of 11,12-secotigogenin-11,12-dioic acid anhydride-3β-acetate (330)

Reaction of the 11,12-seco-anhydride (330) with sodium borohydride, as in 2:14b, led to the 11,12-seco-12-hydroxy-11,24-dioic acid via exclusive reduction of the carbonyl function at C-12, with the 3β-acetoxy group partially hydrolysed to the corresponding alcohol. giving a mixture of 341 and 342; (p.155). The components of the crude mixture were characterized by GLC (Table 12) and MS, recorded by GC-MS, of their methyl ester TMS ether derivatives (Figs. 26 and 27). The mass spectrum of the TMS ether derivative of the methyl 3β-acetoxy-11,12-seco-5α : 25R-spirostan-12- hydroxy-11-oate, Fig.26, showed the molecular ion $M^{+\cdot}$592 (8%), (M-$TMSOCH_2)^+$, (M-$TMSOCH_2$-$C_6H_{10}O_2)^+$, and (M-$TMSOCH_2$

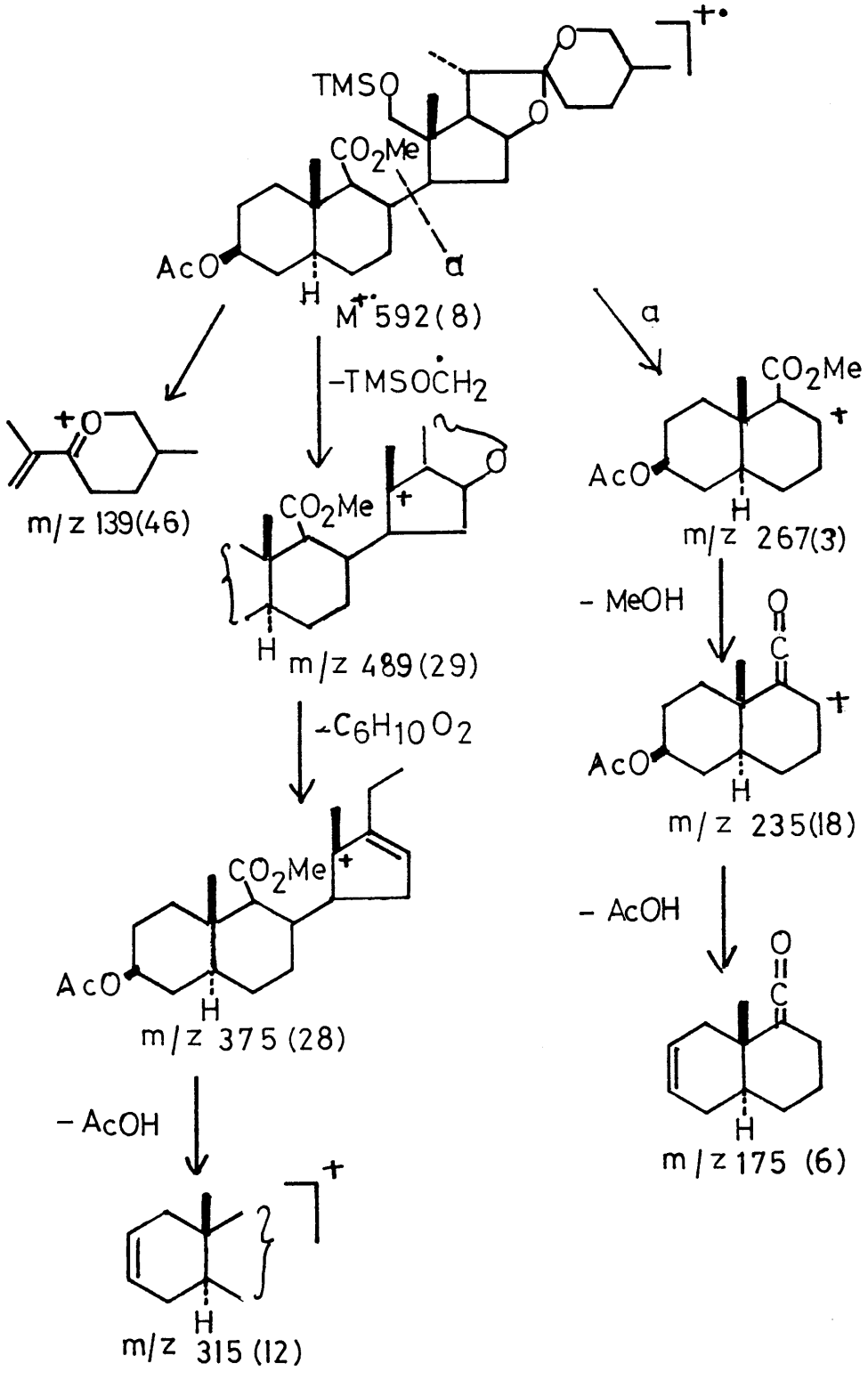

TMSO
CO2Me
AcO
a
M+· 592(8)
-TMSOCH2
m/z 139(46)
CO2Me
m/z 489(29)
-C6H10O2
CO2Me
AcO
m/z 375 (28)
- AcOH
m/z 315 (12)
a
CO2Me
AcO
m/z 267(3)
- MeOH
m/z 235(18)
- AcOH
m/z 175 (6)

Fig. 26

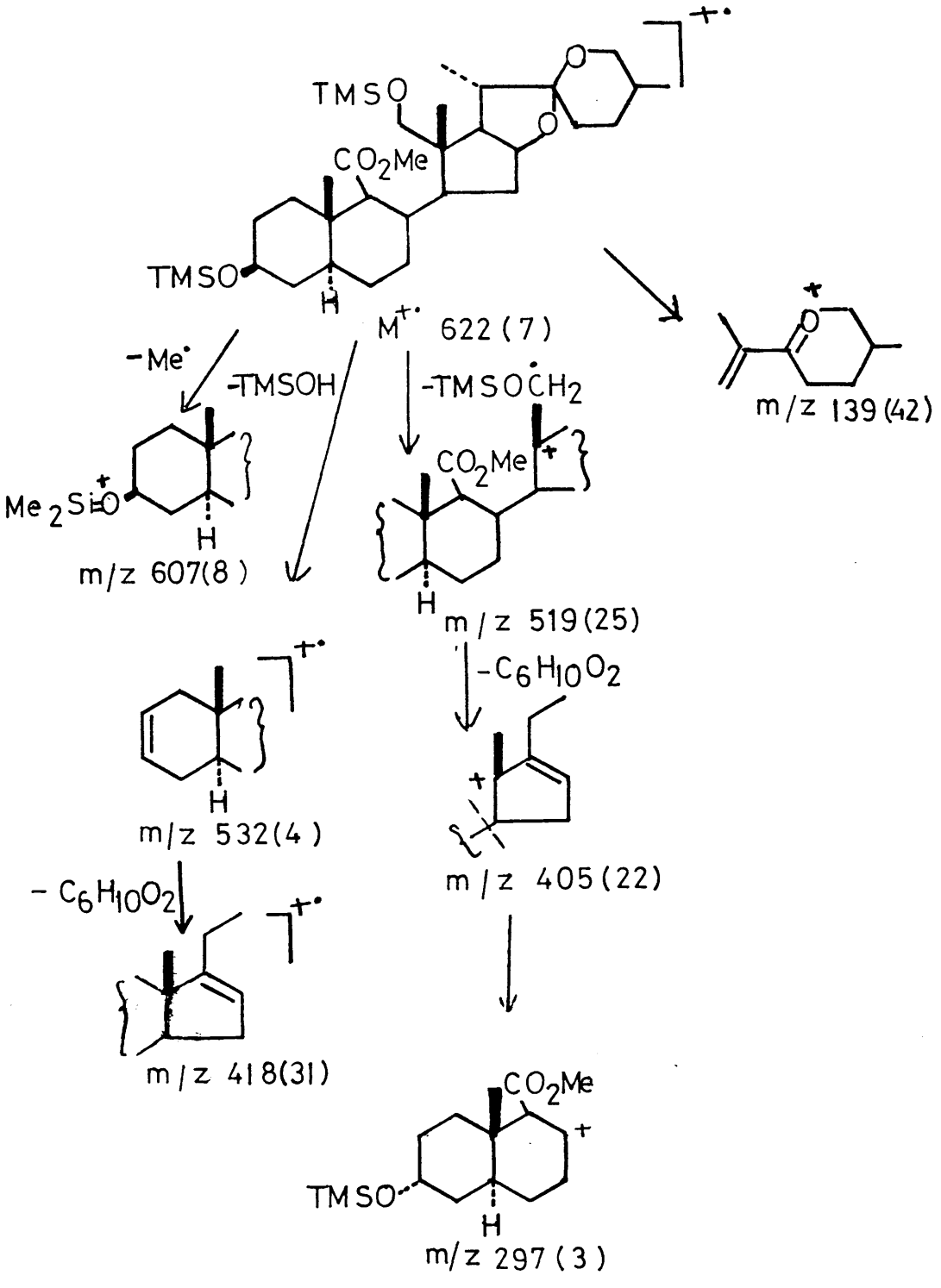

TMSO
CO2Me
TMSO
M+· 622 (7)
-Me·
-TMSOH
-TMSOCH2
m/z 139 (42)
Me2Si=O
m/z 607(8)
CO2Me
m/z 519(25)
-C6H10O2
m/z 532(4)
m/z 405(22)
-C6H10O2
m/z 418(31)
CO2Me
TMSO
m/z 297 (3)

Fig. 27

$-C_6H_{10}O_2-$ AcOH)$^+$. Further, the mass spectrum of the 3,12-di-TMS ether derivative (Fig. 27) exhibited the molecular ion $M^{+\cdot}$ 622 (7%), $(M-TMSOCH_2)^+$, $(M-TMSOCH_2-C_6H_{10}O_2)^+$; as well as other ions.

The crude mixture was hydrolysed to the 3β, 12-dihydroxy-11- oic acid (342). Upon sublimation under reduced pressure, the hydroxy acid (340) was recovered, failing to cyclise to the corresponding lactone. Treatment of (342) with acetic anhydride afforded the 3β, 12-diacetoxy-11-oic acid (343). The mass spectrum of the methyl ester derivative of the 3, 12-diacetoxy-11-oic acid (Fig. 28) showed the molecular ion in a relatively high abundance (25%), the ions $(M-AcOCH_2)^+$, and $(M-AcOCH_2-C_6H_{10}O_2)^+$; in addition to other fragments. In the ^{1}H-nmr spectrum, a double doublet at δ3.63 and 3.97 ppm with the same coupling constant (J=11.0 Hz) is evidence for the presence of 2H at C12, in addition to the other expected signals.

For another lactonisation attempt, the dihydroxy acid (342) was refluxed with p-toluenesulphonic acid in xylene to give a mixture of four components (monitored by GLC). GLC (Table 12) and mass spectral (recorded by GC-MS) analysis of the crude mixture showed the presence of 5% of the lactone (344) (see Fig.29) in addition to three other unidentified

compounds with higher molecular masses.

There are a lot of reports in the literature describing the use of Aldrithiol-2 (2,2'-dipyridyl disulphide) as a cyclizing agent of hydroxy acids; e.g. the conversion of a series of ω-hydroxy acids to the corresponding lactones,[207] and the synthesis of the steroid hydroxylase inhibitor (±) -diplodialide A.[208] Therefore the hydroxy acid (342) was treated with Aldrithiol-2 in dry benzene: it gave recovered starting material in addition to 14% of the desired lactone (344; p.155). The TMS ether derivative of the lactone was identified by means of GLC (Table 12) and mass spectrum, recorded by GC-MS (Fig. 29). The mass spectrum of the TMS ether derivative of the produced lactone, Fig. 29, exhibited the molecular ion in a relatively high abundance (25%), in addition to ($M-C_6H_{10}O_2)^{+\cdot}$, $(M-C_6H_{10}O_2 - CH_2O)^{+\cdot}$, and $(M-C_6H_{10}O_2-CH_2O- TMSOH)^{+\cdot}$.

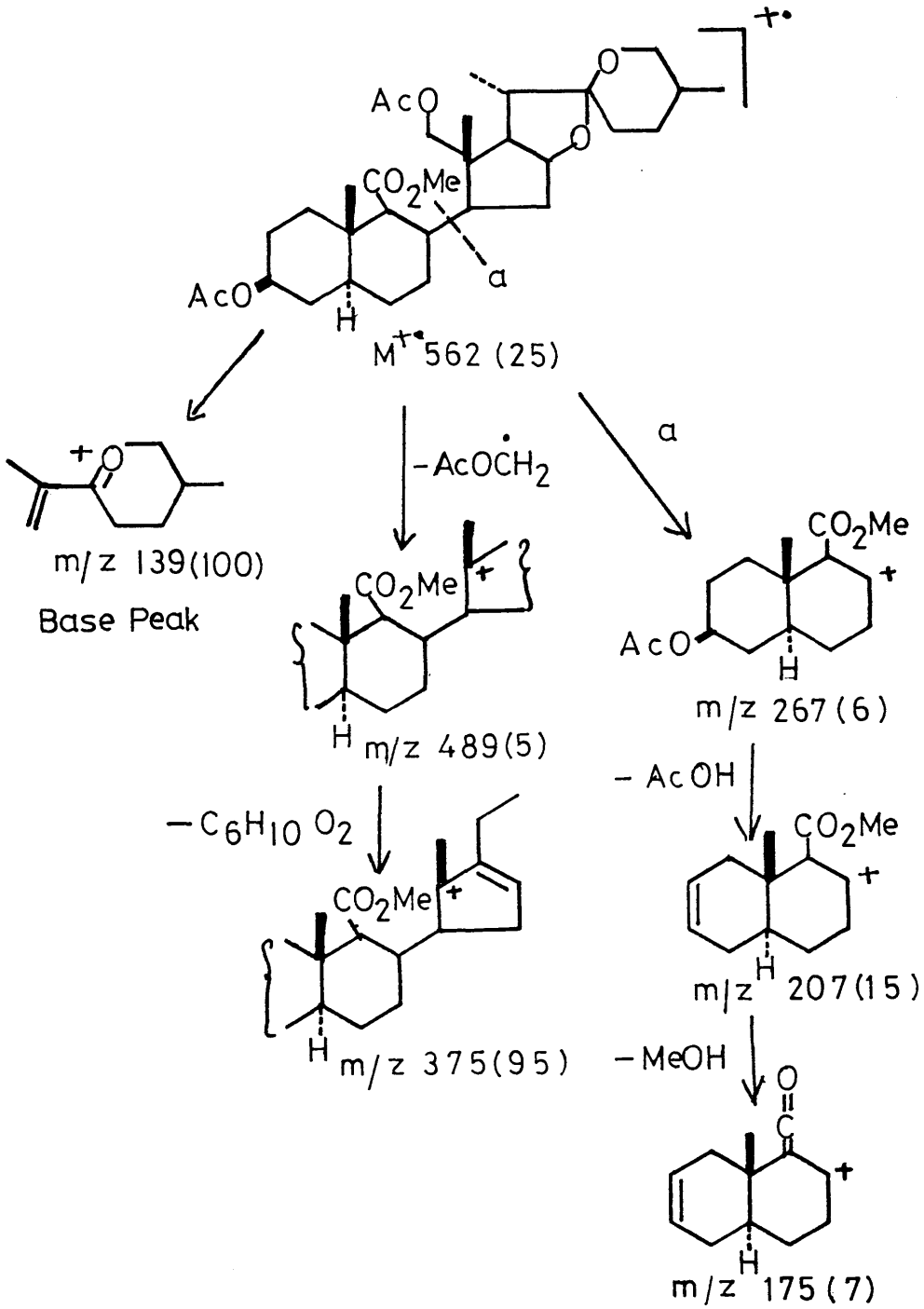

AcO
CO2Me
a
AcO
H
M+• 562 (25)
-AcOCH2
a
m/z 139(100)
Base Peak
CO2Me
H
m/z 489(5)
CO2Me
AcO
H
m/z 267 (6)
- AcOH
-C6H10O2
CO2Me
H
m/z 375(95)
CO2Me
H
m/z 207(15)
-MeOH
O
C
H
m/z 175 (7)

Fig. 28

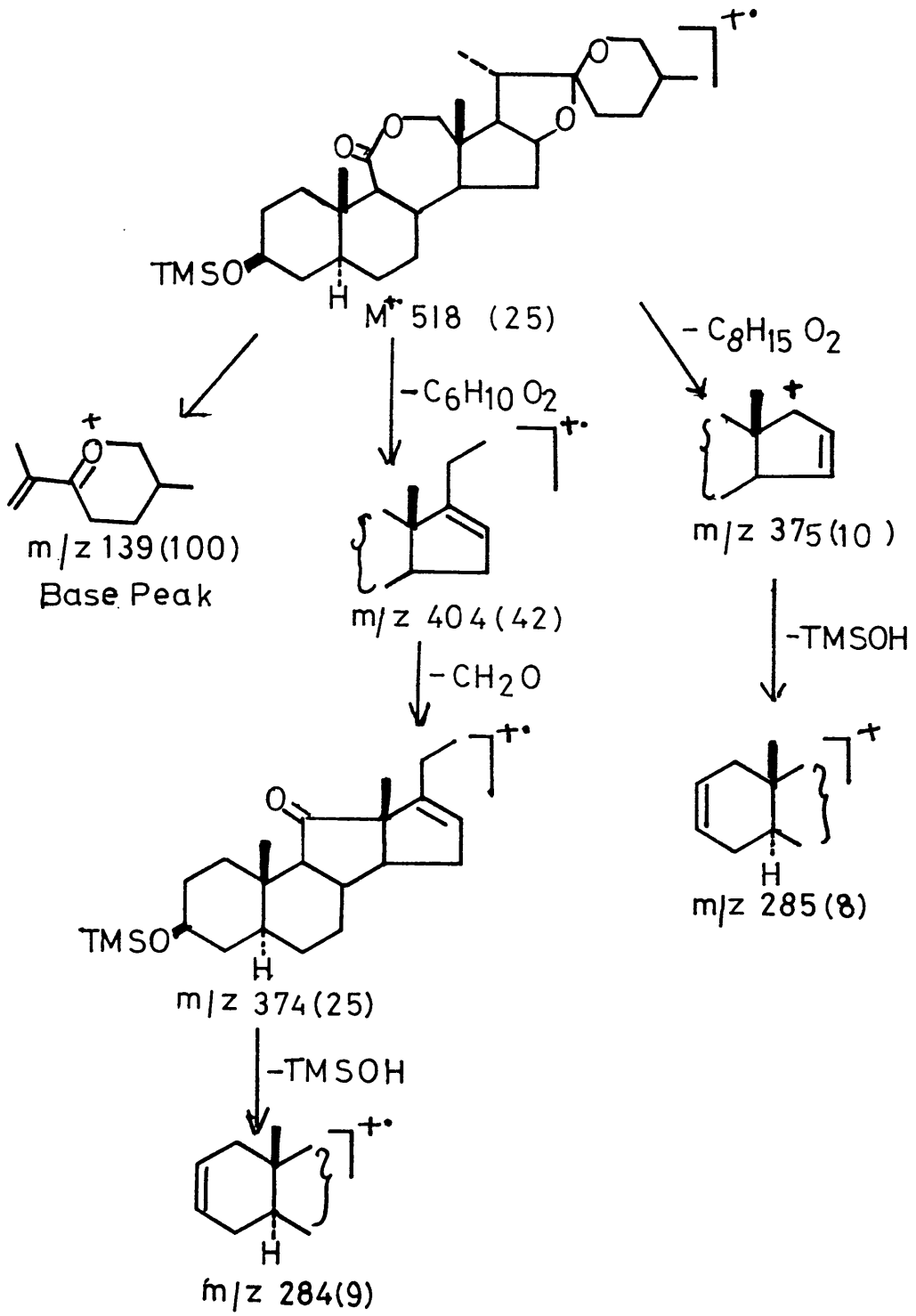

TMSO
M+ 518 (25)
-C8H15O2
-C6H10O2
m/z 139(100)
Base Peak
m/z 404(42)
m/z 375(10)
-TMSOH
-CH2O
m/z 285(8)
TMSO
m/z 374(25)
-TMSOH
m/z 284(9)

Fig.29

Table 12a: Retention data, recorded by GLC, for products from the reduction of anhydrides in the bile acid series.

Column: 6 ft 1% OV-1; Temp. = 240°C (270°C in the case of compounds having substituents at C-3)

Retention values are ± 10

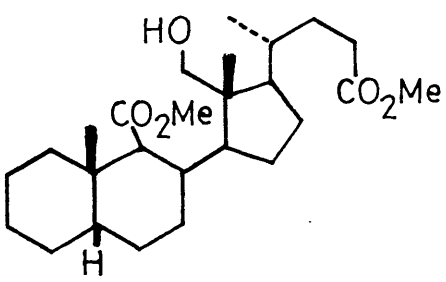
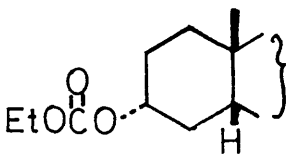
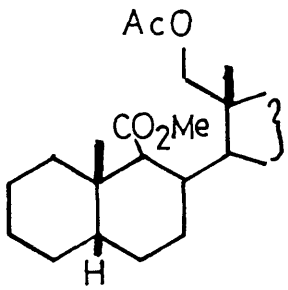
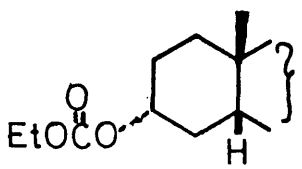
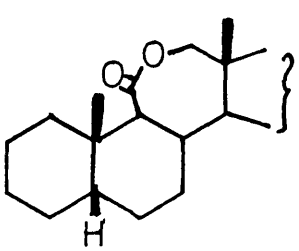
Compound	mol.wt.	I	ΔI for 3α-EtOCO
	436	3030	
	524	3630	+ 600
	478	3090	
	566	3690	+ 600
	404	3100	

Table 12b: Retention data, recorded by GLC, for products from the reduction of anhydrides in the sapogenin series.

Column: 6 ft 1% OV-1; Temp. = 270°C.

Retention values are ± 10

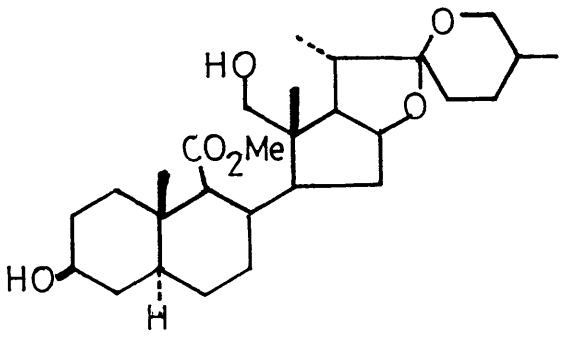
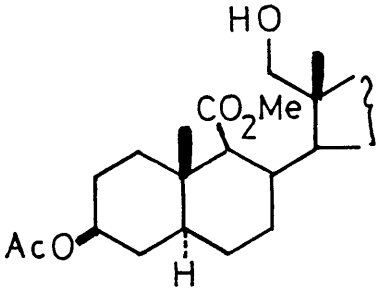
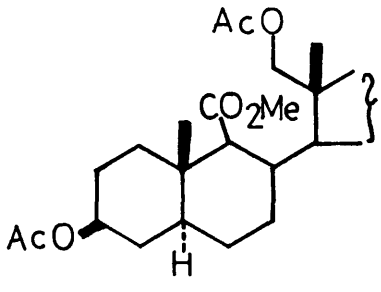
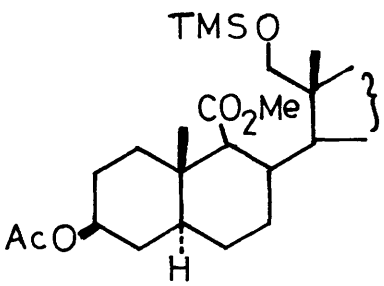
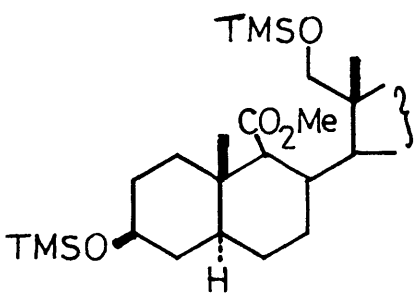
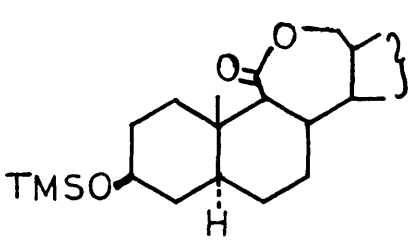
Compound	mol.wt.	I
	478	3490
	520	3610
	562	3690

Table 12b (contd.)

Compound	mol.wt.	I
	592	3530
	622	3450
	518	3630

2.15 Treatment of 11,12-secotigogenin-11,12-dioic acid anhydride 3β-acetate (330) with K-selectride

Since the reduction of unsymmetric cyclic anhydrides with K-selectride (potassium tri-sec-butylborohydride) has been reported[139,141] to give reverse results, in terms of regioselectivity, from that of the borohydride reduction, we decided to try this reagent with steroidal-11,12-seco-dioic anhydrides. Therefore11,12-secotigogenin-11,12- dioic acid anhydride-3β-acetate was treated with K-selectride in dry THF, under nitrogen at low temperature (-50 to 0°C). The low temperature of the reaction medium was reported[140] to be crucial in determining the site of the reduction selectivity. After the work up of the reaction solution, the corresponding 11,12-secotigogenin-11,12-dioic acid-3β-acetate (324) was collected. This result means that the reduction did not take place and the anhydride (330) decomposed to the corresponding dioic acid (329) during the work up process. The reason that no reaction occurred was probably because of steric hindrance of both the anhydride and the selectride molecules. The latter molecule resembles in shape a large and rigid umbrella of which H is a handle. However, it would have been interesting to repeat the experiment under higher temperatures which time did not permit.

2.16 Mechanism of Metal Hydride Reduction of Cyclic Anhydrides

The reduction of unsymmetric cyclic anhydrides by metal hydride has, generally, been shown to occur regioselectively at the carbonyl atom adjacent to the more hindered carbon atom. There have been various attempts to rationalise this regioselectivity in terms of steric and stereoelectronic effects. However, several mechanisms have been put forward, each explaining some but not all experimentally obtained results.

Recently, Kayser et al.[110,209] have conducted an extensive investigation on factors controlling the regioselectivity in the reduction of cyclic anhydrides. They concluded that, in the reduction of unsymmetric cyclic anhydrides, four different factors must be taken into consideration in order to predict, or explain, the course of regioselectivity.

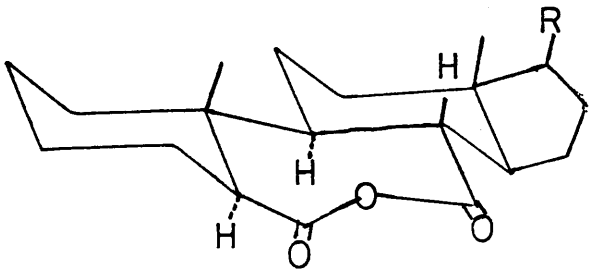
1. The intrinsic reactivity of the two carbonyl functions represented by the size of the LUMO coefficient.
2. The chelating effect: the preferred binding sites for the cation, as the energy-favoured formation of chelates may override any effect due to the intrinsic reactivities of the carbonyl groups involved.
3. The antiperiplanar effect: in systems possessing a certain

flexibility in which therefore some degree of antiperiplanarity may be achieved, attack at the carbonyl function adjacent to the most highly substituted carbon atom should be favoured.

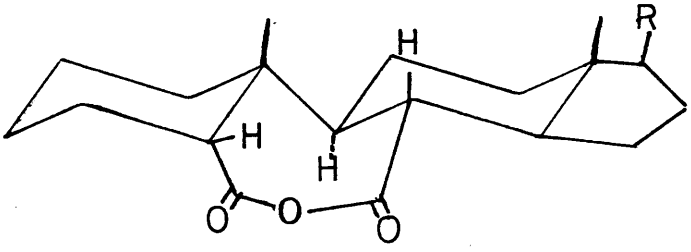
4. The steric congestion: in rigorously rigid systems, preferred attack should occur at the less hindered carbonyl group.

More recently, Brooks and Ekhato[172] have reported the borohydride reduction of 5α-steroid-6,7-dioic anhydrides. Their results showed complete selectivity of the reduction at the C-7 carbonyl group. Dreiding models suggested that these anhydrides are likely to adopt a conformation as shown (345), in which the C-6 and the C-7 carbonyl groups seem about equally hindered on the top face (β-side); the 19-methyl group being close to the postulated paths of nucleophilic attack. On the bottom face (α-side), the C-7 carbonyl function was thought[163] to be slightly more open to a nucleophilic attack, which led to the reduction at this site only.

However in the case of the 5β-steroid-6,7-seco-dioic anhydrides, models suggest a preferred conformation resembling (346) in which the C-6 carbonyl is relatively more open on the β-side to a hydride attack. Accordingly, one such anhydride[138] has been reduced regioselectively at C-6 by $LiAlH_4$.



(345)



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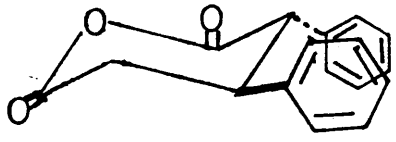

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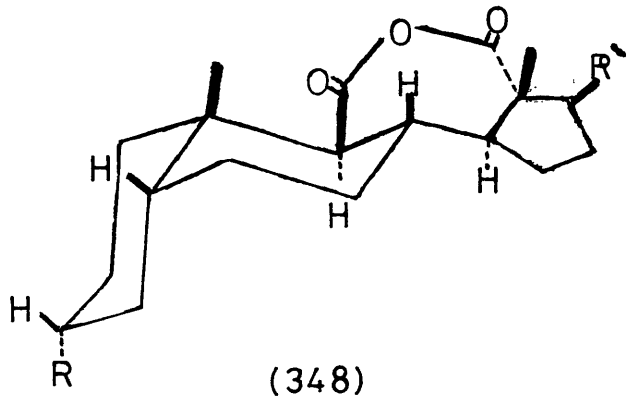

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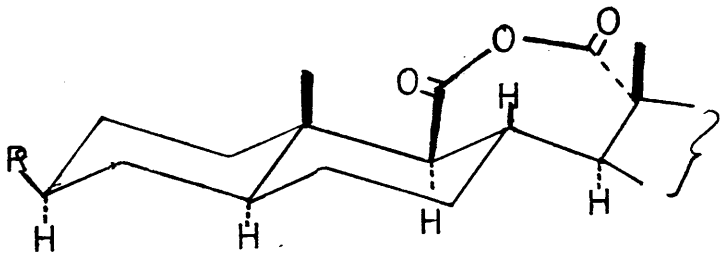
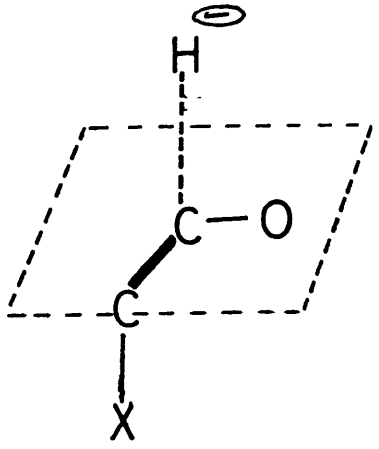


(349)



(350)

In our present investigations of metal hydride reduction of unsymmetric anhydrides, the borohydride reduction of 2α,3β-diphenyl glutaric anhydride afforded exclusively the lactone (335), 3α,4β-diphenyl-tetrahydropyran-2-one. In other words, the reduction occurred in total regioselectivity at the C-5 carbonyl group. Models suggest that the anhydride resembles the conformation shown (347). Both carbonyl groups at C-1 and C-5 seem equally hindered on the top face (β-side) by the 3β-phenyl function. However, on the bottom face (α-side) the carbonyl group at C-5 looks more open to a nucleophile attack than the carbonyl at C-1. Therefore, the reduction occurred selectively at the former (C-5) carbonyl function.

Experimental results of borohydride reduction of 11,12-seco-dioic anhydrides, in both 5α- and 5β-steroidal series, has shown that the reduction occurred, in complete regioselectivity, at the C-12 carbonyl group. Examination of the Dreiding models suggests that these anhydrides are likely to adopt conformations as (348) in the 5β-series and (349) in the 5α- series. It is apparent, from the models, that substituents at C-3, the bile acid side chain (in the deoxycholic acid derivatives), and the spiroketal function attached to ring D (in the hecogenin derivatives) are at a

great distance from the postulated paths of nucleophilic attack, therefore they do not affect the course of regioselectivity in the reduction process. It is also clear that changing the type of A/B ring fusion (from 5α- to 5β-) does not change the degree of the steric hindrance of either of the carbonyl groups (at C-11 and C-12). These facts are in agreement with the experimental results which indicated exclusive selectivity of the reduction at C-12 regardless of the A/B ring fusion or the substitution at C-3. The regioselectivity in the reduction of both 5α- and 5β- 11,12-seco-dioic anhydrides could be rationalised as follows. The nucleophilic attack on the top face (β- side) of the anhydrides is hindered by the β-methyl groups (18-Me and 19-Me). In fact, the carbonyl function at C-11 is much more hindered than the carbonyl group at C-12. However, the bottom face (α-side) of the anhydrides is more open, therefore the nucleophilic attack should occur preferentially on the bottom face of the molecule. Moreover, the two electronic effects promote reduction of the carbonyl group at C-12, since: (a) the more highly substituted carbonyl function should be intrinsically more reactive toward nucleophilic attack[209], and (b) the conformation of the molecule permits attack antiperiplanar to C(13) - CH_3 bond. The antiperiplanar effect has been studied by Kayser and Wipff[110],

and proved to be playing a great part in directing the site of the reduction. These investigations, however, were based on studies conducted by Anh and co-workers[210], which suggested that in the nucleophilic addition to the carbonyl group in ketones, the transition state is more stabilised if there is a neighbouring bond C-X antiperiplanar to the forming partial bond C-H as shown in (350). Moreover, a quantum mechanical study[110] showed that the attack antiperiplanar to the C-CH_3 bond is lower in energy than the attack antiperiplanar to the C-H bond.

The borohydride reduction of the steroidal anhydrides is expected to yield lactones. The postulated mechanism for the reaction is shown in Fig.30. However, the borohydride reduction of the 11,12-seco-dioic anhydrides, as described earlier, afforded only the corresponding 11,12seco-11-hydroxy-12-oic acids. These results indicate that the formed lactones, since they are sensitive to aqueous solutions, have decomposed during the work up process to afford the corresponding hydroxy-acids. Treatment of hydroxy-acids with acetic anhydride is known to give the corresponding lactones. A postulated mechanism for the reaction is shown in Fig.31. However, when this method was applied, the corresponding 11,12-seco-12-acetoxy-11-oic acids were collected, and only in one case the lactone methyl 12-oxa-C-homo-11-oxo-5β-cholanoate, in a very poor

yield (6%). The reason behind this could be due to the speed of the reaction of the hydroxyl group at C-12 with the acetic anhydride, converting it to acetate which is much weaker nucleophile. Therefore, the reaction would proceed as in Fig.32 giving only the corresponding acetoxy-acid.

However, one way of simultaneously activating both the carboxyl and hydroxy groups for mutual reaction would be to utilise a carboxylic derivative which would favour proton transfer from hydroxy to carboxylic oxygen. This idea is illustrated for the specific case of a 2-pyridi- nethiol ester of a hydroxy acid (351) in Fig.33. The proton transfer from hydroxy to carbonyl in 351 is clearly more favourable than for simple esters. The dipolar intermediate (352) (or hydrogen bonded equivalent) generated by internal proton transfer in 351, could reasonably be expected to undergo a facile, electrostatically driven, cyclisation to 353 which then would yield the lactone (354) by elimination of 2-pyridthione (355). This method has been used successfully in the synthesis of a series of -lactones from the corresponding hydroxy-acids.

However, treatment of the 11,12-secotigogenin-12-hydroxy 11-oic acid 3β-acetate with 2,2'-dipyridyl disulphide (Aldrithiol) gave the corresponding lactone in 14% yield. Treatment of hydroxy-acids with other

cyclizing agents, such as 2,2'-dithiobis(4-t-butyl-1-isopropylimidazole, is expected to lead to the corresponding lactones in higher yields,. Unfortunately, time did not permit to apply this reagent on the steroidal 11, 12-seco-12-hydroxy-11-acids.

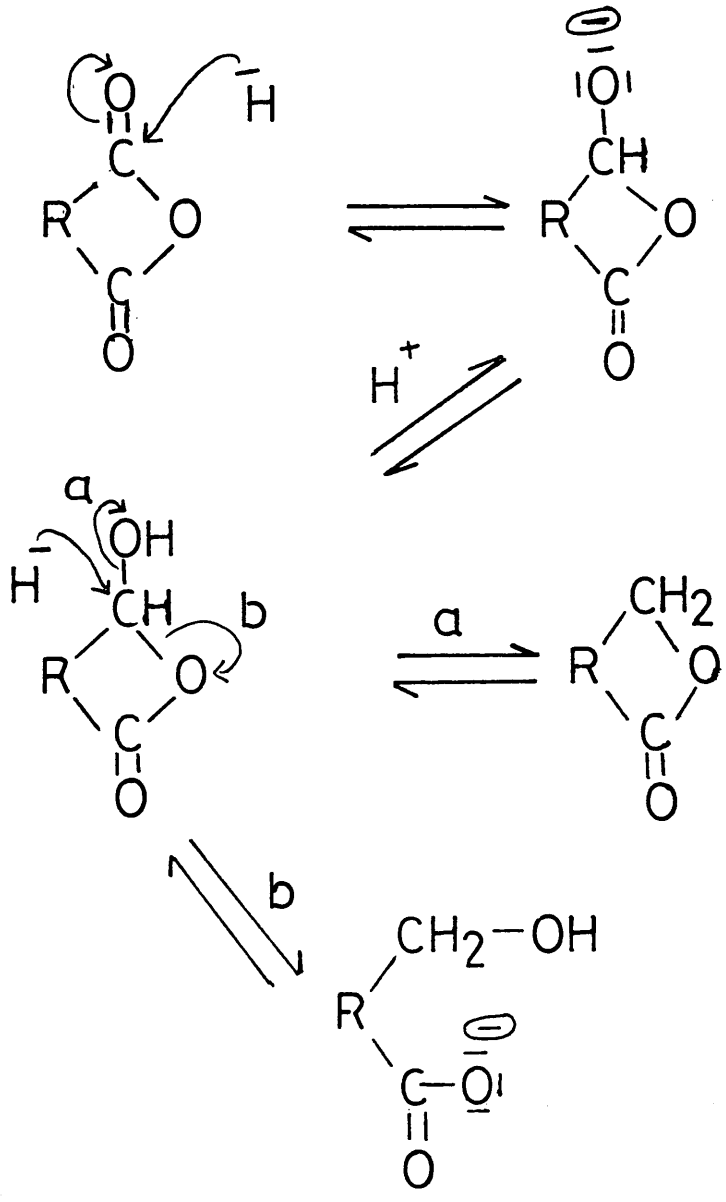


Fig. 30

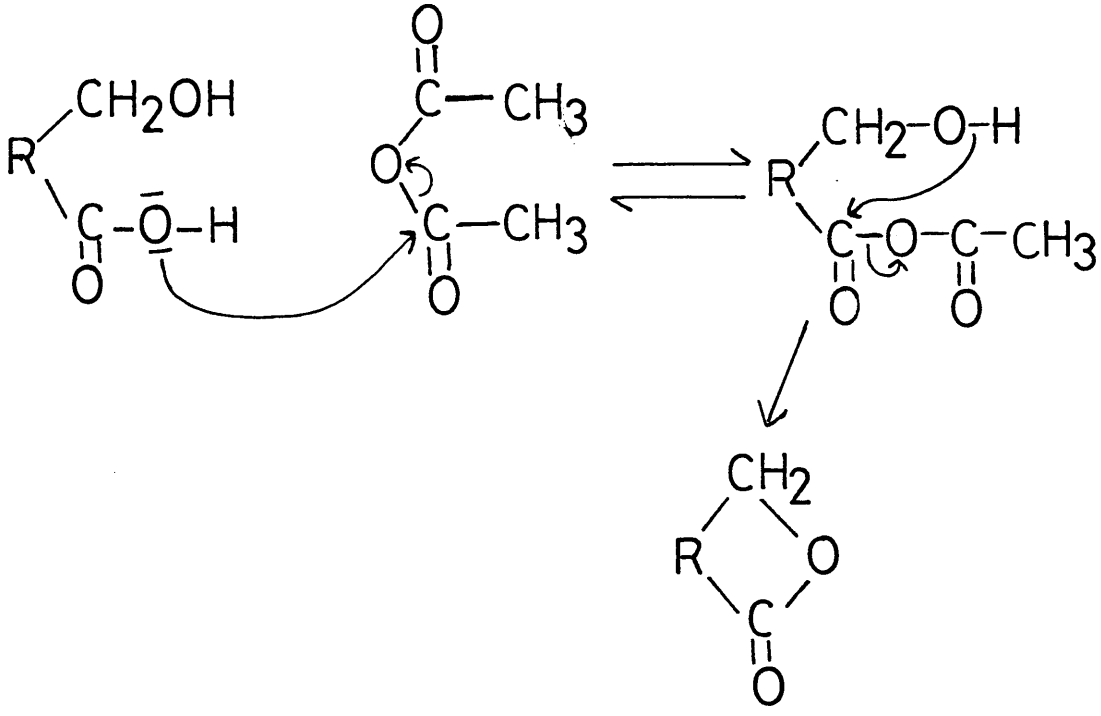


Fig. 31

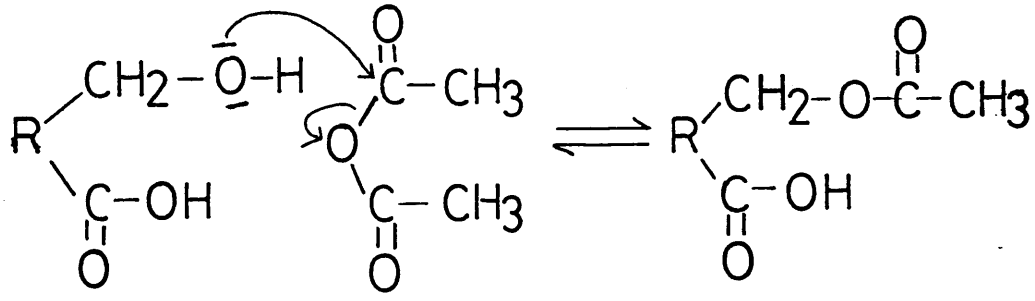


Fig. 32

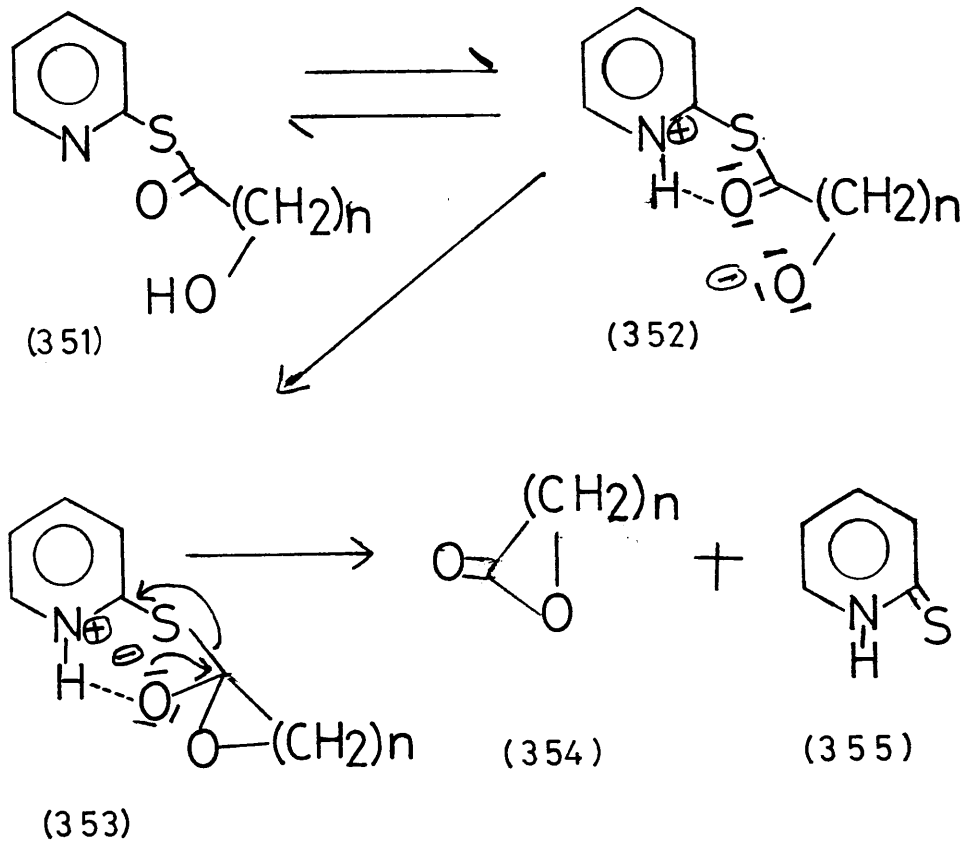


Fig. 33

CHAPTER THREE
EXPERIMENTAL

CHAPTER 3

EXPERIMENTAL

General Analytical Techniques

(i) Thin-Layer Chromatography (TLC)

Glass plates (2.5 x 7.5 cm, 5 x 20 cm, or 20 x 20 cm) were precoated with silica gel GF, with (0.2 mm) thickness; UNIPLATE, ANALTECH INC. ANACHEM.

The mobility of the compounds in TLC, relative to the solvent was indicated as R_f.

Detection methods: compounds were visualised by any of the following methods: UV lamp; iodine vapour; spraying with a solution of 5% (w/v) ceric sulphate in 10% aqueous sulphuric acid followed by heating at 80-120° for a few minutes.

(ii) Gas Liquid Chromatography (GLC)

The instrument used was Perkin-Elmer Gas Chromatograph F 33. The following stationary phase was coated on Gas Chrom Q (100-120 mesh): 1% OV-1 (methyl siloxane polymer). The length and diameter of the coiled glass columns used was 3m x 2 mm i.d. Oxygen-free nitrogen was

used as carrier gas with a flow rate of 28 ml/min unless stated otherwise. Injection temperature was 300° and oven temperature 270°. Samples for GLC were dissolved in ethyl acetate and aliquots (2µl) of 2µg/µl were injected using a 10µl Hamilton syringe. Kováts retention indices "I" were measured with respect to standard n-alkanes.

(iii) Combined Gas Chromatography-Mass Spectrometry (GC-MS)

An LKB 9000 GC-MS insrument was used. This was fitted with a glass column (3m x 2mm, i.d.) of 1% OV-1. The flash heater was set at 260°, and the ion source at 270°. The helium carrier gas flow rate was 30ml/min. The mass spectra were recorded at electron energy 20eV, the trap current was 60µA, filament current 4A, and accelerating voltage 3.5kV.

(iv) Mass Spectrometry (MS)

Mass spectra (direct insertion probe) were measured on a VG MICROMASS 2S8 instrument. Electron energy 70eV.

(v) Infra Red (IR)

IR spectra were measured on a Perkin-Elmer Grating Infra Red Spectrophotometer Model 257.

(vi) Nuclear Magnetic Resonance (NMR)

H-NMR spectra were determined on an R32 90MHz instrument.

(vii) Melting Point Apparatus

Melting points (m.p.) were recorded on a Kofler block with estimated precision of 2°.

PURIFICATION TECHNIQUES

(viii) Column Chromatography

The material used was: silica gel Woelm, for 'Dry-Column Chromatography': Activity 111/30 mm, contains 0.5% inorganic fluorescent indicator. The silica gel was introduced into the columns as a slurry in pet. ether (60-80°), then pre-washed with the solvent (the eluant) before use. Fractions were analysed by TLC.

(ix) Vacuum Sublimation

Analytical samples of some of the compounds were purified by vacuum sublimation/short path distillation using a rotary oil pump. Samples were sublimed at 0.01 torr. in a tube fitted with cold finger condenser, heated in an aluminium block provided with a thermometer.

Block temperatures were selected according to the m.p. of the sublimed materials.

3.1 Methyl Deoxycholate (279)

Dry methanol (300 ml) was placed in a 500 ml conical flask and 5 ml of acetyl chloride added dropwise. Deoxycholic acid (46g) was added to the mixture. The flask was stoppered and the reaction mixture left to stand at room temperature for 48h. Large crystals, formed at the bottom of the flask, were collected and the mother liquor concentrated to give colourless plates. Total yield (42g: 91%); m.p. 77-78°C (Lit[192] 76°C).

3.2 Methyl 3α-ethoxycarbonyl-deoxycholate (280)

Methyl deoxycholate (4g) was dissolved in pyridine (10ml). Ethyl chlorocarbonate (6ml) was added dropwise with cooling in an ice-bath. The mixture was allowed to stand for 1h at room temperature. Dilution with water (100 ml) led to the precipitation of white solid material which was collected, by filtration, washed with water and dried. Recrystallisation from methanol gave 4.1g of colourless plates; m.p. 135-137°C (Lit[173] 136-138°C).

3.3 Methyl 3α-ethoxycarbonyloxy-5β-chol-11-enoate (280)

Methyl 3α-ethoxycarbonyl-deoxycholate (5.0g) was dissolved in pyridine (50ml) and 25.0g (15.1ml) of phosphorus oxychloride

was added. The reaction mixture was left at room temperature for 7 days then poured dropwise, cautiously, into 500ml of iced water. A gummy solid material was formed. This was separated, washed with water and dried. Crystallisation from methanol afforded 2.3g of colourless plates; m.p. 118-125%C. Recrystallisation from methanol raised the melting point to 132-134°C (Lit[173] 134-135°C).

3.4 3α-Ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12, 24-trioic acid 24-methyl ester (282)

Methyl 3α-ethoxycarbonyloxy-5β-chol-11-enoate (500mg) was dissolved in 140 ml of dry chloroform. A slow stream of ozone was bubbled through the solution for 1h at room temperature. An aqueous solution of 5% H_2O_2 was added and the reaction mixture stirred for 5h, then allowed to stand at room temperature overnight. The chloroform layer was separated, washed with water (4 x 100 ml) and extracted with 5% Na_2CO_3. The aqueous extract was acidified immediately with cold dil. HCl, and extracted with chloroform (3 x 50 ml). The combined chloroform extract was washed with water and dried over $MgSO_4$. The solvent was removed to give220 mg of colourless gum. Crystallisation from ethyl acetate-petroleum ether afforded 145 mg of fine colourless plates; m.p.

214-216°C. (Found: C, 64.22; H, 8.43%. Calculated for $C_{28}H_{44}O$: C, 64.10; H, 8.45%).

3.5 Treatment of methyl 3α-ethoxycarbonyloxy-5β-chol-11-enoate with $NaIO_4$ -$KMnO_4$

Methyl 3α-ethoxycxarbonyloxychol-11-enoate (100 mg) was dissolved in 10 ml of t-butanol-water azeotrop (a mixture of 8.82 ml of t-butanol and 1.18 ml of water). This solution was placed in a 500 ml 3-neck flash provided with magnetic stirrer. A solution of potassium carbonate (90 mg) in 2.5 ml of water was added with vigorous stirring before the addition of 2 ml of a solution of 1.20 g of sodium periodate ($NaIO_4$) in 15 ml of water. Then, 0.5 ml of 1% aqueous solution of potassium permanganate ($KMnO_4$) was added. The remainder of the ($NaIO_4$) solution was added dropwise during a period of 2h. More aqueous solution of $KMnO_4$ (0.5 ml; 1%) was added and the reaction mixture was stirred for a further 2h. Sodium bisulphite was added to destroy excess $KMnO_4$ and after the work up in the usual manner, a colourless gummy material (96 mg) was collected. TLC and GLC analysis showed that the product obtained was identical with the starting material.

3.6 Treatment of Methyl 3α-Ethoxycarbonyloxy-5β-chol-11-enoate with RuO_2-$NaIO_4$

Methyl 3α-ethoxycarbonyloxy-5β-chol-11-enoate (50 mg) and 10 mg of ruthenium dioxide (RuO_2) were added to 2 ml of chloroform. The mixture was stirred, with magnetic stirrer, while a solution of 200 mg of $NaIO_4$ in 2 ml of water was added dropwise. The stirring was continued at room temperature, with occasional shaking for four days. Equal volumes of water and chloroform were added. After the usual work up, the starting material was recovered (proved by TLC, GLC and m.p.).

3.7 3α-Ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12,24-trioic acid-11,12-anhydride 24-methyl ester (286)

3α-ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12,24-trioic acid 24-methyl ester (80 mg) was refluxed in acetic anhydride (4 ml) for 1h. The oil obtained upon evaporation crystallised from ethyl acetate-petroleum ether to give colourless needles (28 mg); m.p. 152-154°C. (Found C, 66.16; H, 8.40%. Calculated for $C_{28}H_{42}O_8$: C, 66.38; H, 8.35%).

3.8 Methyl 3α-Ethoxycarbonyloxy-12-oxo-5β-cholanoate (287)

Methyl 3α-ethoxycarbonyloxy-deoxycholate (6.0g) was dissolved in 120 ml of glacial acetic acid. The solution was cooled in an ice bath and while stirring 8.0 ml of Jones' reagent was added dropwise during 5 min. The reaction mixture was removed from the ice bath, the flask was stoppered, and the mixture left to stir at room temperature for 30 min. The dark green solution was poured into ice water. The mixture was left to stand for 20 min. then filtered and the white precipitate washed several times with water then dried. Recrystallisation from methanol afforded (5.2g) of colourless needles; m.p. 158-159°C (Lit [209] 157-158°C).

3.9 Methyl 11α-bromo-3α-ethoxycarbonyloxy-12-oxo-5β-cholanoate (288)

Methyl 3α-ethoxycarbonyloxy-12-oxo-5β-cholanoate (5g) was dissolved in 50 ml of glacial acetic acid and a solution of 2.5 ml of bromine in 40 ml of acetic acid was added dropwise, during 3h, at 50-60°C. The mixture was left to stir overnight maintaining the temperature at 50-60°C, then allowed to cool to room temperature before 300 ml of water was added gradually with stirring. A reddish-yellow gum was formed, separated and washed once with a mixture of 35 ml ethanol, 245 ml water, and 1-6 ml acetic acid and then washed twice with 250 ml of water. The

gummy product was taken up by chloroform, dried over $MgSO_4$, then the solvent was evaporated to dryness to leave 5.8g of a yellow gum. This was remethylated, as the ester was partially hydrolysed, monitored by analytical TLC, by treatment with diazomethane, then passed through a column of silica gel to give 4.250g of crystalline material. This was recrystallised from ethyl acetate-petroleum ether to afford pure bromo-ketone; m.p. 110-112°C. (Found: C, 60.41; H, 7.61; Br, 14.23%. Calculated for $C_{28}H_{43}BrO_6$: C, 60.54; H, 7.46; Br, 14.34%).

3.10 Methyl 3,12-dibenzoyl-deoxycholate

Methyl deoxycholate (1.0g) was dissolved in 3 ml of pyridine. Benzoyl chloride (1.0 ml) was added and the suspension was left aside at room temperature overnight. The suspension was poured into water, and the residue was collected by filteration. This was dissolved in ethyl acetate and washed twice with 2MHCl, 3 times with water and dried before the solvent was removed under reduced pressure to give 1-2g of gummy oil. Crystallisation from methanol afforded 920g of methyl 3,12-di- benzoyl-deoxycholate, m.p. 142-3°C (Lit. 143-143.5°C).[203]

3.11 Methyl 3-benzoyl-deoxycholate (289)

Methyl deoxycholate (4.0g) was dissolved in dry benzene (20 ml) and 10 ml of the benzene were boiled off at atmospheric pressure,

to remove traces of methanol. Dry benzene (5 ml), pyridine (1 ml), and benzoyl chloride (1.25 ml) were added and the flask was set aside at room temperature overnight. (Note: after a few minutes from the addition of benzoyl chloride, the clear solution became turbid and a white precipitate deposited at the bottom of the flask). Ethyl acetate (20 ml) was added and the mixture washed with dilute HCl, a solution of $NaHCO_3$, water (3 times) and brine. The organic extract was dried over $MgSO_4$ before the solvent was evaporated to dryness to give 4.2g of colourless gum which crystallised from ethyl acetate-petroleum ether to give 3.9% of colourless needles; m.p. 84-86°C (Lit[200] 90-95°C).

3.12 Methyl 3α-benzoyloxy-12β-hydroxy-5β-cholanoate (290)

Methyl 3α-benzoyl-deoxycholate (1.0g) was dissolved in 20 ml of acetic acid in a 25 ml conical flask provided with a magnetic stirring bar. This solution was cooled in an ice bath, then 1.4 ml of Jones' reagent was added dropwise. The reaction flask was removed from the ice bath, stoppered and stirred at room temperature for 30 min. The solution which became dark green was poured into ice water. The greenish-yellow solution was filtered and the white solid material was washed several times with water, dissolved in ether and washed with water, then dried

over $MgSO_4$. The ether was evaporated to give a white gummy solid material (0.92g). Recrystallisation from methanol gave colourless needles (0.86g); m.p. 95-95°C (Lit[210]94-95°C).

3.13 Methyl 3α-benzoyloxy-11α-bromo-12-oxo-5β-cholanoate (281)

Methyl 3α-benzoyloxy-12-keto-5β-cholanoate (291) was dissolved in 10 ml of glacial acetic acid. This was treated with 0.2 ml of bromine in 8 ml of acetic acid, dropwise during 1h at 50-60°C. The flask was stoppered and the reaction mixture was stirred overnight while the temperature was maintained at 50-60°C. The solvent was removed and the residue treated with diazomethane to give a yellowish gum (1.2g) which failed to crystallize. A sample was taken for ^{1}H-nmr analysis (see Table 2) which confirmed the structure of the desired compound.

3.14 Treatment of methyl 3α-benzoyloxy-11α-bromo-12-oxo-5β-cholanoate with KO_2

Methyl 3α-benzoyloxy-11α-bromo-12-keto-5β-cholanoate (100 mg; 17 mmol) was added to a stirred mixture of powdered potassium superoxide (KO_2) (100 mg; 1.41 mmol), Aliquat 336 (0.2g; 0.495 mmol) and dry benzene (10 ml). The flask was stoppered and the reaction mixture

was stirred at room temperature for 48h. The mixture was poured into water and the layers were separated. The aqueous layer was acidified (dil. HCl) and extracted with ether. Washing with water, drying (Na_2SO_4) and evaporation afforded a colourless gum (74 mg) which showed three spots on TLC. This was chromatographed on a column of silica gel. Elution with 9:1 ethyl acetate:petroleum ether gave in 25.5% yield, 3α-hydroxy-11,12-seco- 12-nor-5β–cholane-11,24-dioic acid (306). (Found: C,70.30, H, 0.45%. Calculated for $C_{23}H_{38}O_5$: C, 70.05; H, 9.65%).

The elution was continued with the same solvent system to afford 3α, 13ζ-dihydroxy-11,12-seco-12-nor-5β-cholane-11,24-dioic acid (307) as a crystalline material (32.4%) which was recrystallised from ethyl acetate-petroleum ether to give colourless prisms; m.p. 243.5°C. (Found: C,67.51; H, 9.14%. Calculated for $C_{23}H_{38}O_6$: C, 67.32; H, 9.27%).

The third component, collected from the silica gel column, was the 3α-hydroxy-11,12-seco-5β-cholane-11,12,24-trioic acid (305), 42.1%. Recrystallisation from ethyl acetate-petroleum ether afforded colourless needles; m.p. 259.61°C (Lit[174] 259.62°C).

3.15 Methyl 3-tosyl-deoxycholate (312)

(6.0g) of Methyl deoxycholate, dried by repeated

evaporation of solution in toluene was dissolved in 10 ml of dry pyridine and treated with a solution of 3.2g of p-toluene sulphonyl chloride in 5 ml of dry pyridine, dropwise during 5 min at 0°C. The mixture was left at this temperature for 1h then at room temperature overnight. (1.0g) of crushed ice was added to the reaction mixture and stirred for 1/2h, then diluted well with ice and water. The resulting mixture was extracted with ether. The ether extract was washed with cold dil. HCl, a solution of $NaHCO_3$ and water before being dried over Na_2SO_4. The solvent was removed to give 6.2g of colourless solid. Recrystallisation from methanol afforded colourless needles; m.p. 136.8°C (Lit[192] 131.3°C).

3.16 Methyl 12α-hydroxy-5β-chol-3-enoate (313)

Methyl 3-tosyl-deoxycholate (6.0g) was dissolved in (10 ml) of dry pyridine and the mixture was refluxed for 3h. The reaction mixture was then poured into water and extracted with ether. The ethereal solution was washed several times with dil. HCl, washed with a solution of $NaHCO_3$ followed by water before being dried over $MgSO_4$. The solvent was evaporated to leave 4.3g of colourless crystalline material which recrystallised from methanol to afford colourless needles; m.p. 109-110°C (Lit[192] 110-111°C).

3.17 Methyl 12α-hydroxy-5β-cholanoate (314)

Methyl 12α-hydroxy-5β-chol-3-enoate (1.0g) was dissolved in a mixture of 50 ml methanol and 25 ml glacial acid. 30 mg of PtO_2 was then added and the mixture was hydrogenated for 1h. PtO_2 was separated by filtration and the solvent was evaporated. The residue was taken up by ether and washed with a solution of $NaHCO_3$, then with water before being dried over Na_2SO_4. The solvent was removed to give a white crystalline material (1.0g). Recrystallisation from methanol afforded colourless needles; m.p. 116.7°C (Lit[192] 119 °C).

3.18 Methyl 12-oxo-5β-cholanoate (315)

Methyl 12α-hydroxy-5β-cholanoate (1.0g) was dissolved in (10 ml) of glacial acetic acid. The solution was cooled in an ice bath before 1.4 ml of CrO_3 solution was added dropwise, then the mixture was removed from the ice bath and left to stir at room temperature for 30 min. The solution, which became dark green, was poured into iced water. The white precipitate was filtered, washed with water and dried to give 0.89g of a crystalline material. Recrystallisation from methanol afforded colourless needles; m.p. 104.6°C (Lit[192] 106.8 °C).

3.19 11α-Bromo-12-oxo-cholanoic acid (316)

Methyl 12-oxo-5β-cholanoate (400g) was dissolved in 4 ml of glacial acetic acid. Bromine (624 mg; 0.2 ml) in 10 ml of glacial acetic acid was added dropwise during 1h with stirring at 65°C. The reaction mixture was left to stir at this temperature overnight. The mixture was cooled to room temperature, poured into iced water and extracted with ether. The ethereal extract was washed with water and dried over Na_2SO_4. The solvent was evaporated to leave a yellowish gum. This was crystallised from a small amount of ether to afford 320g of colourless needles; m.p. 184-7°C (Lit[192] 180-190 °C).

3.20 Methyl 12β-hydroxy-11-oxo-5β-cholanoate (317)

11α-Bromo-12-keto-cholanoic acid (200 mg) was refluxed in 10 ml of 20% solution of methanolic potassium hydroxide for 2h. The reaction mixture was cooled to room temperature, poured into iced water, acidified with dil. HCl, extracted with ether; the ethereal extract was washed several times with water and dried over Na_2SO_4. The solvent was evaporated to leave 184 mg of colourless gum which was treated with diazomethane and then crystallised from methanol to afford colourless

needles; m.p. 101.3°C (Lit[194] 106.7°C).

3.21 11,12-seco-5β-Cholane-11,12,24-trioic acid 24-methyl ester

Methyl 12β-hydroxy-11-keto-5β-cholanoate (100 mg) in 7 ml of dioxan was treated with sodium periodate (100 mg) in 1 ml of water. The reaction mixture was stirred at room temperature for 48h. The dioxan was evaporated under reduced pressure, and the residue partitioned between water and ether. The ethereal layer was separated and the aqueous phase re-extracted with ether. The combined ethereal extract was dried over Na_2SO_4. The solvent was evaporated, the resulting yellowish oily material dissolved in 2 ml of acetic acid, and a mixture of 100 mg of chromium trioxide, 1 ml of water and 5 ml of acetic acid added in one portion. The reaction mixture was stirred at room temperature for 24h. Excess CrO_3 was destroyed by methanol and the mixture poured into water and extracted with dichloromethane. The dichloromethane extract was washed with water and dried over Na_2SO_4. The solvent was evaporated to give 86 mg of colourless gum. Crystallisation from methanol afforded 54 mg of colourless plates; m.p. 224-6°C. (Found: C,68.62; H, 9.26%. Calculated for $C_{25}H_{40}O_6$: C, 68.81; H, 9.17%).

3.22 11,12-seco-5β-cholane-11,12,24-trioic acid-11, 12-anhydride 24-methyl ester (319)

11,12-seco-5β-Cholane-11,12,24-trioic acid 24-methyl ester (40 mg) was refluxed in acetic anhydride for 2h. The solvent was evaporated under reduced pressure to give a yellowish oil which crystallised from ethyl acetate-petroleum ether to give 14 mg of colourless needles; m.p. 118-120°C. (Lit[164] 120.2°C).

3.23 3β-Acetoxy-5α:25R-spirostan-12-one (Hecogenin acetate) (321)

Hecogenin (10g) was refluxed for 30 min. with 50 ml of pyridine and 50 ml of acetic anhydride. The mixture was cooled to room temperature and water was added. The product was collected by filtration, washed thoroughly with water and dried to give 10.3g. The analytical sample was obtained by crystallisation from chloroform-methanol; m.p. 240-242°C (Lit[62] 243°C).

3.24 3β, 12β-Diacetoxy-5α:25R-spirostan-11-one (325)

A solution of hecogenin acetate (5.0g) in dry benzene (40 ml) was stirred during the addition of bromine (1.3 ml) in benzene (10ml).

After the first ml had been decolourised, the remainder was added in 10 min. The solution was left for a further 30 min. and was then concentrated under reduced pressure at room temperature. The obtained crude 11α:23α -dibromohecogenin acetate (a sample was taken for ^{1}H-nmr analysis) was dissolved in t-butanol (50ml). Sodium hydroxide solution, (2,5g) of NaOH in (50ml) of water, was added and the mixture was refluxed for 3h. Removal of the alcohol under reduced pressure, dilution of the residue with water to 200ml, and filtration, gave the crude crystalline 23-bromo-ketol (4.5g). This was boiled under reflux for 1h with pyridine (37.5ml) and acetic anhydride (37.5 ml). The solution was evaporated to dryness to give the crude product 23-bromo-ketone diacetate, (3.6g; 92%). Debromination of this was carried out by stirring the crude 23-bromo-ketol diacetate with zinc dust (17.0g) and boiling under reflux with acetic acid (50.0ml) for 2h. After cooling, the product was filtered to remove the zinc. Dilution with water gave the crude product (4.0g) which on crystallisation from ether/ethanol gave 3β,12β-diacetoxy-5α:25R-spirostan-11-0ne (3.4g; 81%); m.p. 226-228°C (Lit[201] 224-227°C).

3.25 3β, 12β-Dihydroxy-5α:25R-spirostan-11-one (326)

3β,12β-Diacetoxy-5α:25R-spirostan-11-one (3.0g) was dissolved in (120ml) of methanol. A solution of 6g of KOH in 10ml of water

was added and the mixture was boiled under reflux for 1h. After cooling to room temperature, the mixture was poured into water and extracted with chloroform. The chloroform extract was washed with water and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the crude product which on crystallisation from methanol afforded 3β, 12β-dihydroxy-5α:25R-spirostan-11-one as colourless needles; m.p. 216-218°C (Lit, 212-4°C[201]; 217-8°C[202]).

3.26 3β-Acetoxy-12β-hydroxy-5α: 25R-spirostan-11-one (327)

3β, 12β-Dihydroxy-5α: 25R-spirostan-11-one (2.5g) was dissolved in 25ml of acetic acid and 2.5ml of acetic anhydride was added. The mixture was boiled under reflux for 1h. Water (25ml) was gradually added to the boiling solution and after cooling, the mixture was further diluted with water to 130 ml and filtered. Crystallisation of the solid material from methanol gave the 3-mono-acetate (1.5 g) as colourless prisms; m.p. 217.9°C (Lit[201] 214.8°C).

3.27 3β-Benzoyloxy-12β-hydroxy-5α:25R-spirostan-11-one (328)

3β, 12β-Dihydroxy-5α: 25R-spirostan-11-one (2.0g) was dissolved in 20ml of dry benzene and 10ml of the benzene was boiled off at

atmospheric pressure to remove traces of methanol. Dry benzene (10ml), pyridine (0.50ml), and benzoyl chloride (0.63ml) were added and the flask was set aside at room temperature overnight. Ethyl acetate (10 ml) was added and the mixture was washed with dilute HCl, a solution of $NaHCO_3$, water (3 times) and finally with brine. The organic extract was dried over anhydrous sodium sulphate before the solvent was evaporated to dryness to give a colourless gum which was crystallised from ethyl acetate-petroleum ether to afford (1.6 g) of colourless needles; m.p. 219. 21°C.(Found: C, 74.11 H 8.35%. Calculated for $C_{34}H_{46}O_6$: C, 74.18; H, 8.36%).

3.28 11,12-Secotigogenin-11, 12-dioic acid 3β-acetate (329)

3β-Acetoxy-12β-hydroxy-5α:25R-spirostan-11-one (500mg) in dioxan (25ml) was treated with sodium periodate (500mg) in water (5ml). The reaction mixture was stirred at room temperature for 48h. The dioxan was removed under reduced pressure and the residue partitioned between ether and water. The ethereal portion was separated and the aqueous layer was re-extracted with ether. The combined ethereal extract was dried over anhydrous sodium sulphate and the solvent was removed to leave a colourless gum. This was dissolved in acetic acid (25ml) and a solution of chromium trioxide (500 mg) in water (5 ml) was added. The

mixture was stirred at room temperature for 24h. Excess CrO_3 was destroyed by methanol and the mixture poured into water and extracted with dichloromethane. The organic extract was washed several times with water and dried over Na_2SO_4 before the solvent was removed to leave 455 mg of greenish gum. This was passed through a column of silica gel to give a colourless gummy oil which crystallised from ether to give colourless needles; m.p. 224.6°C. (Found: C, 66.85; H, 8.45%. Calculated for $C_{29}H_{44}O_8$: C, 66.92; H, 8.46%).

3.29 11,12-Secotigogenin-11,12-dioic acid anhydride 3β-acetate (330)

11,12-Secotigogenin-11,12-dioic acid 3β-acetate (100 mg) was refluxed in acetic anhydride for 1h. The solvent was removed under reduced pressure. Traces of acetic anhydride were removed by dissolving the residue in toluene and evaporating the solvent under vacuum. A yellowish gum was obtained which was crystallised from ether to give (36 mg) of colourless needles; m.p. 239-41°C. (Found: C, 69.20; H, 8.42%. Calculated for $C_{29}H_{42}O_7$: C, 69.32; H, 8.37%).

3.30 11,12-Secotigogenin-11,12-dioic acid anhydride 3β-benzoate (333)

3β-Benzoyloxy-12β-hydroxy-5α:25R-spirostan-11-one (50 mg) was oxidised, as in 3.25, to the corresponding 11,12-seco- dioic acid (332), (29 mg), by treatment with sodium periodate followed by reaction with chromium trioxide. The acid (332), m.p. 173.6°C (Lit[71] 172.4°C), was treated with acetic anhydride under reflux to give 11,12-seco-11,12-dioic acid anhydride (333) which was crystallised from ethyl acetate-pet. ether to give colourless needles (11 mg), m.p. 195-97°C. (Found: C, 72.46; H, 7.84%. Calculated for $C_{34}H_{44}O_7$: C, 72.34; H, 7.80%).

3.31 11,12-Secotigogenin-11,12-dialdehyde 3β-acetate

2.20 mmol (22.0 ml; 0.10M) of lead tetraacetate solution in glacial acetic acid was added to as mixture of 5.0 ml of acetic acid, 20.0 of benzene, and 500 mg of 11β,12β-dihydroxytigogenin (which was prepared by reduction of 3β,12β-dihydroxy-5α:25R-spirostan-11-one with $LiAlH_4$ in THF). The mixture was stirred at room temperature for 5 min, then diluted with 20.0 ml of water containing 5.0 g of sodium acetate and 0.20 g of potassium iodide. The resulting colour was discharged by the addition of a saturated solution of sodium thiosulphate (1.0 ml). The solution was

extracted twice with 20.0 ml of ethyl acetate. The combined extract was washed with water, $NaHCO_3$, and water before dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded 500 mg of colourless gum. Crystallisation from aqueous methanol gave 420 g of colourless needles of 11,12-secotigogenin-11,12-dialdehyde m.p. 145-7°C (Lit[71] 146-7°C). GLC (1% OV-1; 270°C) showed: tr = 2.80 min, I = 3450.

^{1}H-nmr exhibited, in addition to the usual signals, δ9.54 (d, 1H, 11-CHO) and δ9.20 (S, 1H, 12-CHO).

Treatment of the dialdehyde with acetic anhydride afforded the corresponding 3β-acetate, m.p. 151-3°C (Lit[71] 152-4°C).

GLC (1% OV-1; 270°C) showed: tr = 3.90 and I = 3580.

3.32 Borohydride Reduction of Cyclic Anhydrides

a) Borohydride Reduction of 2,3-Diphenylglutaric Anhydride

A suspension of crushed $NaBH_4$ (26.60 mg; 0.70 mmol) in 12 ml of dry tetrahydrofuran (THF) was refluxed for 15 min. and then cooled in an ice bath. A solution of 200 mg (1.10 mmol) of 2,3-diphenylglutaric anhydride (prepared by treatment of 2,3-diphenylglutaric acid with acetic anhydride) in 10 ml of dry THF was added dropwise to the stirred, ice-cold suspension of $NaBH_4$. The stirring was continued for 3h during which time

2 drops of 6N HCl were added to the reaction mixture. After quenching with 6N HCl (to pH2), excess water was added and stirring continued overnight. Ether was added and the layers were separated. The aqueous layer was extracted 3 times with ether. The combined ether extract was collected and dried over magnesium sulphate. The solvent was removed under reduced pressure to give colourless gummy material (200 mg). Crystallisation from isopropyl ether afforded 120 mg (the first crop) of the lactone, 2,3-diphenyl-tetrahydropyran-2-one, as colourless needles, m.p. 131-2°C. ^{1}H-nmr spectrum (see 2-14a) confirmed the structure.

(Found: C, 80-86; H, 6.42%. Calculated for $C_{17}H_{16}O_2$: C, 80.95; H, 6.35).

b Borohydride Reduction of 3α-ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12,24-trioic acid-11,12-anhydride 24-methyl ester (286)

3α-Ethoxycarbonyloxy-11,12-seco-5β-cholane-11,12-24-trioic acid-11,12-anhydride 24-methyl ester (10 mg) in 2 ml of dry tetrahydrofuran (THF). The mixture was refluxed for 3 hr, then cooled in an ice-bath and acidified with 6N HCl. This mixture was concentrated and partitioned between ether and water. The aqueous layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the oily residue was transferred to a cold-finger sublimation

tube. Sublimation at 0.02 torr; 120°C afforded 11,12-seco-12-hydroxy-11,24-dioic acid 24-methyl ester (334). Refluxing with acetic anhydride for 1hr gave the corresponding 11,12-seco-12-acetoxy-11,12-dioic acid 24-methyl ester (335) as a gummy oil which failed to crystallise (see data in 2.14b).

c Borohydride Reduction of 11,12-seco-5β-cholane-11,12,24-trioic acid-11,12-anhydride 24-methyl ester (319)

11,12-seco-5β-Cholane-11,12-dioic acid anhydride 24-methyl ester (5 mg) was reduced with $NaBH_4$ under conditions similar to those applied in the previous reduction (3.32b) to give 11,12-seco-12-hydroxy-11,24-dioic acid 24-methyl ester (336), as a gummy oil. The resulting hydroxy-acid had failed to cyclise under sublimation (0.05 torr; 100°C). Treatment with acetic anhydride gave a gummy oil. GLC and MS analysis showed that the crude product is a mixture of 11,12-seco-12-acetoxy-11,12-dioic acid 24-methyl ester (337), as the major product, and approximately 6% of the corresponding lactone (338), (see data in 2.14c).

d Borohydride Reduction of 11,12-secotigogenin-11-12-dioic acid anhydride 3β-acetate

Treatment of 20 mg of 11,12-secotigogenin-11,12-dioic acid

anhydride 3β-acetate with $NaBH_4$ in dry THF, as in 3.32b, afforded a mixture (4:1 ratio) of the 11,12-seco-3β-acetoxy-12-hydroxy-11-oic acid (341) and 11,12-seco-3β-12-dihydroxy-11-oic acid (342), respectively. Hydrolysis of 5 mg of the resulting mixture gave one compound (see GLC and TLC data in 2.14c), the 11,12-seco-3β-12-dihyroxy-11-oic acid (340). This was sublimed at 0.01 torr; 260°C in an attempt to get it cyclised to the corresponding lactone, but the hydroxy-acid was recovered unchanged.

The recovered hydroxy-acid (4 mg) was dissolved in 3 ml of xylene and 0.8 mg of p-toluenesulphonic acid was added. The mixture was refluxed for 15 min. The solvent was removed under reduced pressure. The residue was taken by ether and washed with cold $NaHCO_3$ and then with water. The ethereal extract was dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded 1 mg of colourless gum. GLC analysis showed four main peaks. MS (recorded by GC-MS) of the TMS ether derivative of the crude mixture showed the presence of 5% of the corresponding lactone (344) in addition to three other unidentified compounds with higher molecular masses, (see 2.14d for data). Then, 5 mg of the hydroxy-acid (342) was treated with acetic anhydride at 100°C for 1h. Removal of the solvent under reduced pressure afforded the

corresponding diacetoxy-acid (343), in a quantitative yield. Crystallisation from ethyl acetate-pet. ether gave colourless prisms, m.p. 130.2°C. (Found: C, 67.80; H, 8.65%. Calculated for $C_{31}H_{48}O_8$: C, 67.88; H, 8.76%).

The hydroxy-acid (342) was then treated, for another lactonisation attempt, with Aldrithiol-2 (2,2'-dipyridyl disulphide), as follows: (5.0mg; 0.011 mmol) of the hydroxy-acid, Aldrithiol-2 (3.5mg; 0.016 mmol), and triphenylphosphine (4.20 mg; 0.016 mmol) were dissolved in 2 ml of dry benzene under argon and stirred at room temperature for 5hr. The reaction mixture was diluted with 1 ml of dry benzene and the resulting solution was added slowly by a syringe over 15hr to 10 ml of dry benzene at reflux temperature, under argon. The reaction mixture was kept under reflux for an additional 10hr. The solvent was removed under reduced pressure and the residue was passed through a short column of silica gel. The crude product (4.1 mg; gummy oil) was treated with diazomethane in ether before samples were analysed by GLC and MS (recorded by GC-MS). The obtained data (see 2.14c) showed that the crude product is a mixture of 14% of the corresponding lactone (342) along with recovered hydroxy-acid (340).

3.33 Treatment of 11,12-secotigogenin-11,12-dioic acid anhydride-3β-acetate (330) with K-selectride

11,12-secotigogenin-11,12-dioic acid anhydride-3-acetate (20.0 mg; 0.04 mmol) was dissolved in 2.0 ml of dry tetrahydrofuran. 0.06 mmol of a solution of K-selectride (potassium tri-sec-butylborohydride) in tetra hydrofuran was injected into the anhydride solution at -50°C, under nitrogen. The temperature was allowed to rise to 0°C over a period of 90 min. The reaction mixture was stirred at this temperature for a further 2h. NaOH (4N solution; 0.5 ml) and 0.5 ml of 30% H_2O_2 were added cautiously. After stirring for 20 min, sodium bisulphite was added to destroy the excess of peroxide. The reaction mixture was acidified to pH_2 with 6N HCl and stirred overnight. The layers were separated and the acidic layer saturated with NaCl and extracted with ether and methylene chloride. The combined organic extracts were dried under Na_2SO_4. Removal of the solvent under reduced pressure afforded 18.0 mg of a colourless gummy material. Chromatographic analysis, by means of TLC and GLC, and mass spectrum suggested that the product is the corresponding dioic acid: 11,12-secotigogenin-11,12-dioic acid-3β-acetate (329). This was confirmed by comparing the product with an authentic sample of the dioic acid (329).

APPENDIX

APPENDIX

Mass Spectral Data

Mass spectral studies of bile acid and hecogenin derivatives have revealed common features. The molecular ions ($M^{+\cdot}$) are observed for all compounds, except the 3α-ethoxycarbonyloxy-11,12-seco-12-hydroxy-5β-cholane-11,24-dioic acid 24-methyl ester and its 12-acetoxy derivative. The abundance of the molecular ions varies from 0.5 to 82%, but it is much higher in ketones and ketols than anhydrides, lactones, and other derivatives.

The loss of side chain and the functional group at C-3 (if existed) +H is a common feature in bile acid derivatives, whereas hecogenin derivatives tend to lose the substituents at C-3 +H in addition to fragmentation of the spiroketal function (see Fig.17). In fact, the base peak in the spectrum of hecogenin derivatives, with few exceptions, is always the ion $C_9H_{15}O$; resulting from the fragmentation of the spiroketal function.

The most common feature of anhydrides, in both bile acid and hecogenin derivatives, is the loss of CO_2. However, the corresponding lactones rather tend to lose CH_2O. Whereas, the loss of CH_2OAc and

CH_2OTMS from the hydroxy-acid derivatives is a regular occurrence. Tables of detailed MS data follow.

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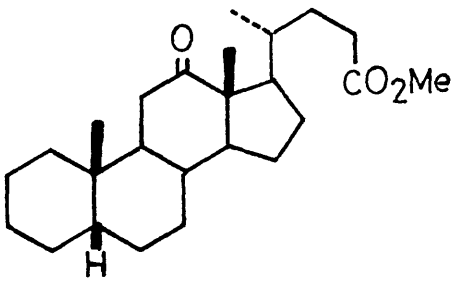
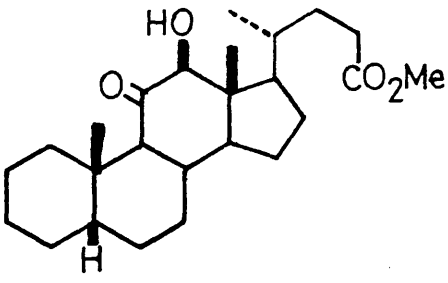
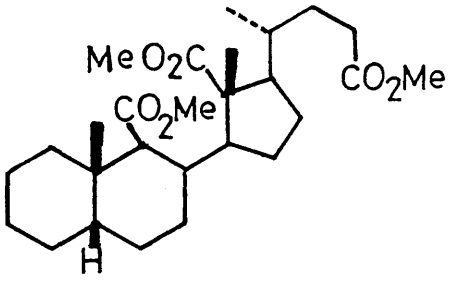
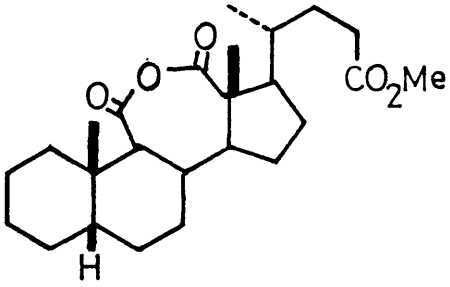
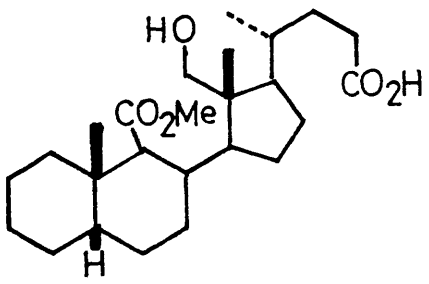
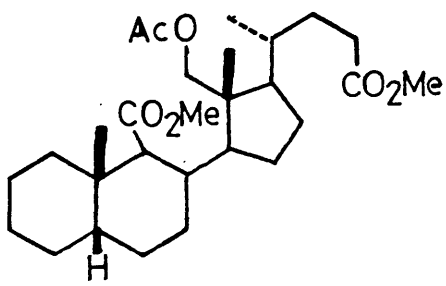
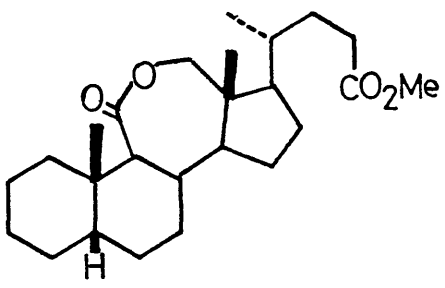
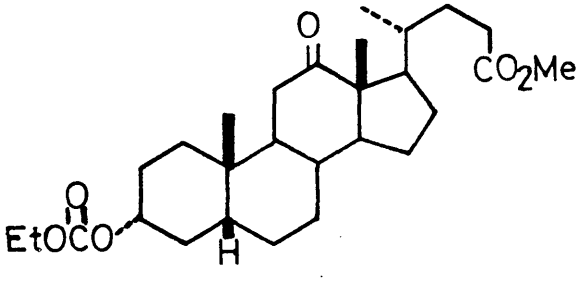
Mass Spectral Data

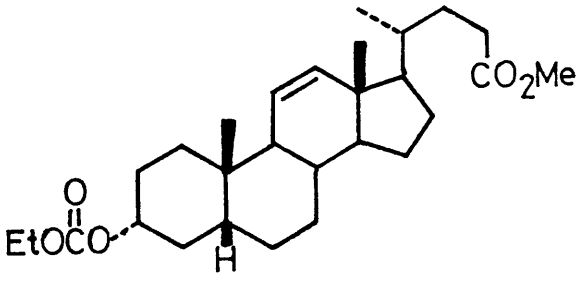
Compound	other ions m/z
 $M^{+\bullet}$ 388(82) Base peak 121	371(9), 370(7), 357(4), 355(5), 339(3), 315(11), 297(2), 290(3), 283(2), 273(13), 271(5), 259(7), 255(11), 246(3), 234(19), 233(98), 229(18), 215(6), 191(5), 190(4), 175(2), 161(5), 149(10), 137(3), 135(6), 122(10), 109(10), 95(5), 83(5), 81(8), 74(1), 69(5), 55(2), 44(4).
 $M^{+\bullet}$ 404(77) Base peak 81	390(9), 338(10), 386(16), 374(4), 373(8), 372(23), 370(3), 358(4), 357(8), 355(8), 344(10), 339(4), 332(7), 331(29), 326(3), 317(2), 315(3), 314(5), 313(17), 211(4), 299(7), 295(3), 290(4), 289(12), 287(2), 278(4), 276(3), 274(1), 273(6), 272(22), 271(14), 261(5), 259(3), 258(3), 257(3), 254(3), 253(10), 249(4), 248(2), 247(3), 246(3), 245(10), 244(3), 243(8), 234(3), 233(12), 232(7), 231(8), 230(2), 229(4), 227(2), 225(5), 220(4), 219(17), 217(4), 216(3), 215(6), 213(3), 209(3), 208(7),

Compound	other ions m/z
	205(3), 203(5), 201(7), 199(3), 197(3), 195(8), 194(4), 193(12), 191(4),190(3), 189(5), 188(3), 187(4), 185(3), 183(2), 179(4), 178(3), 177(17), 176(4), 175(9), 174(2), 173(5), 171(3), 169(1), 167(2), 165(5), 164(3), 163(11), 162(9), 161(15), 160(2), 159(8), 157(4), 155(3), 154(10), 153(2), 151(7), 150(9), 149(45), 148(9), 147(23), 146(3), 145(9), 144(2), 143(4), 141(3), 138(3), 137(9), 136(6), 135(30), 133(17), 132(3), 131(9), 129(4), 128(3), 125(6), 124(6), 123(24), 122(22), 121(41), 120(7), 119(21), 117(8), 116(6), 115(14), 111(13), 110(13), 109(54), 108(17), 107(50), 105(34), 97(19), 96(13), 95(88), 94(20), 93(73), 91(36), 87(8), 85(7), 84(22), 83(30), 82(13), 80(14), 79(58), 77(21), 74(30), 73(25), 71(8), 70(5), 69(25), 68(11), 67(60), 65(6), 59(16), 55(63), 53(10), 43(13), 41(19).

Compound	other ions m/z
 $M^{+\bullet}$ 464(5) Base peak 289	432(60), 414(6), 404(39), 400(17), 390(5), 382(2), 373(20), 372(23), 359(2), 349(8), 345(21), 344(33), 330(2), 317(15), 309(2), 299(1), 285(14), 276(6), 267(2), 257(19), 249(7), 236(9), 235(6), 229(15), 223(22), 215(12), 209(7), 195(36), 191(7), 190(6), 183(10), 175(8), 163(9), 155(8), 154(7), 149(14), 147(5), 135(9), 122(8), 121(6), 115(3), 109(8), 108(6), 107(4), 95(6), 93(6), 81(5), 73(1), 67(2), 55(1), 44(2).
 $M^{+\bullet}$ 418(7) Base peak 81	400(19), 390(2), 374(20), 372(11), 356(7), 344(5), 331(4), 308(13), 303(6), 395(1), 390(4), 385(5), 374(15), 263(10), 259(20), 245(8), 241(7), 231(14), 219(9), 208(15), 195(35), 175(9), 163(8), 154(11), 149(23), 135(13), 123(6), 122(10), 121(16), 115(4), 109(15), 108(9), 107(7), 95(14), 67(3), 55(2), 44(5).

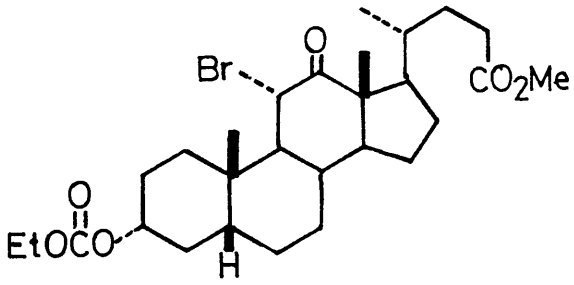
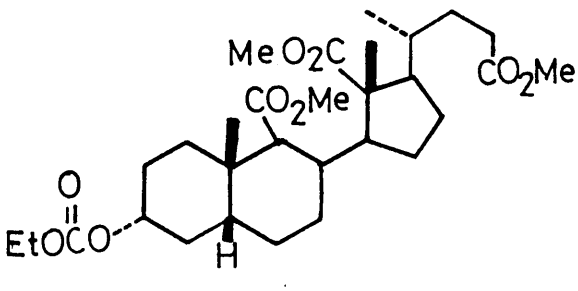
Compound	other ions m/z
 $M^{+\bullet}$ 436(0.5) Base peak 122	418(1), 404(11), 386(12), 374(35), 368(2), 358(8), 345(10), 341(4), 326(2), 320(2), 313(3), 303(4), 291(7), 289(11), 271(12), 257(8), 253(4), 243(7), 231(9), 229(7), 219(3), 209(22), 195(18), 177(11), 175(10), 163(7), 149(25), 135(16), 115(9), 108(31), 95(18), 81(19), 74(4), 67(3), 55(2), 44(2)
 $M^{+\bullet}$ 478(3) Base peak 271	446(5), 419(30), 418(10), 405(12), 387(48), 386(80), 373(34), 368(27), 363(5), 358(80), 345(27), 337(4), 326(8), 321(3), 313(6), 303(22), 299(6), 289(8), 276(7), 270(30), 264(7), 253(27), 243(50), 227(8), 220(10), 209(70), 195(17), 188(10), 177(31), 175(14), 168(13), 163(9), 153(12), 149(48), 147(41), 135(29), 122(28), 115(29), 109(24), 108(16), 107(15), 95(25), 81(19), 74(7), 67(4), 60(2), 43(5).

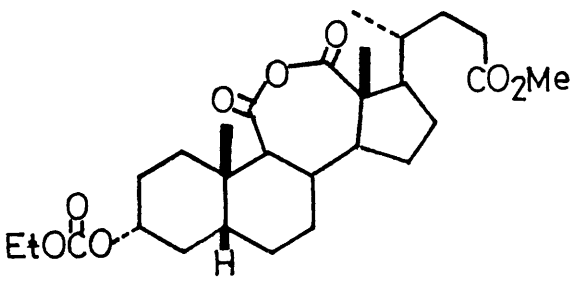
Compound	other ions m/z
 $M^{+\cdot}$ 404(10) Base peak 122	386(7), 373(2), 368(1), 358(4), 345(5), 344(6), 329(1), 313(2), 303(1), 299(1), 289(4), 269(5), 263(2), 253(2), 249(3), 245(4), 231(4), 229(4), 213(2), 209(8), 203(3), 195(6), 194(7), 193(8), 175(9), 161(3), 149(7), 135(7), 134(6), 133(5), 115(7), 108(30), 95(10), 81(10), 74(3), 67(2), 55(1), 44(3).
 $M^{+\cdot}$ 476(30) Base peak 386	403(3), 387(5), 370(5), 369(4), 368(8), 355(10), 354(4), 343(3), 339(3), 337(2), 321(6), 314(8), 313(18), 304(2) 299(3), 290(3), 272(7), 271(26), 263(3), 258(4), 257(11), 254(4), 253(15), 245(6), 244(6), 243(3), 232(6), 231(89), 215(4), 213(5), 199(3), 191(8), 175(5), 173(4), 163(11), 162(3), 161(5), 154(55), 149(12), 135(13), 121(38), 109(14), 108(7), 107(9), 105(5), 95(11), 94(15), 81(12), 69(4), 55(3), 44(6).

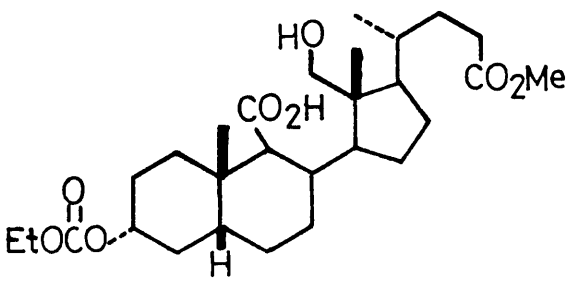
Compound	other ions m/z
	356(6), 355(28), 339(6), 328(10), 323(7), 316(12), 315(14), 314(5), 313(4), 305(2), 301(1), 288(3), 281(6), 276(5), 275(3), 263(2), 262(6), 261(4), 255(76), 242(2), 241(2), 229(14), 223(49), 215(10), 214(24), 213(29), 202(4), 201(16), 200(10), 199(8), 189(3), 188(5), 187(6), 186(2), 185(4), 173(8), 172(3), 171(6), 161(10), 160(3), 159(12), 158(4), 157(7), 147(14), 146(8), 145(14), 133(12), 132(10), 131(14), 120(12), 119(18), 118(13), 109(14), 108(9), 107(10), 106(10), 105(12), 95(10), 94(9), 93(7), 81(8), 74(2), 73(2), 69(2), 67(4), 55(4), 44(5).

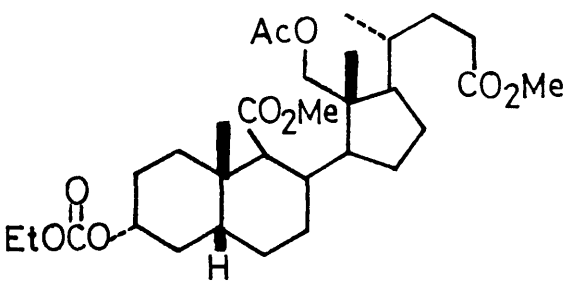
$M^{+\cdot}$ 460(0.5)

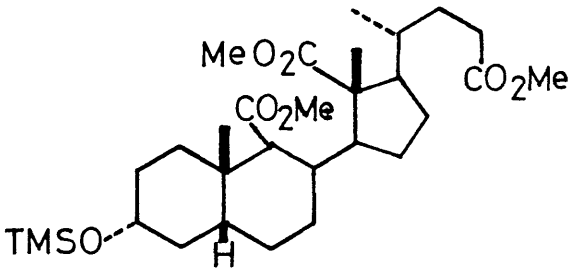
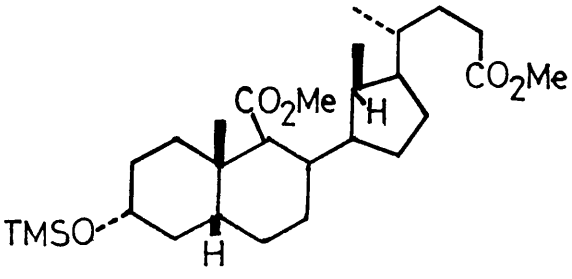
Base peak 370

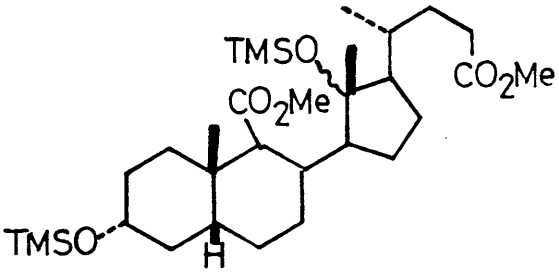
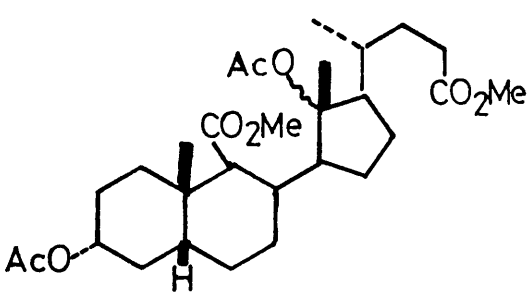
Compound	other ions m/z
 $M^{+\cdot}$ 555(0) Base peak 55	476(6), 475(6), 387(4), 386(12), 385(5), 384(4), 353(3), 313(2), 271(3), 269(1), 244(1), 243(2), 232(2), 231(10), 230(2), 231(5), 213(2), 189(2), 187(2), 177(1), 175(2), 173(3), 163(4), 162(1), 161(5), 159(4), 157(3), 154(4), 149(4), 147(7), 145(6), 143(3), 141(2), 137(3), 136(3), 129(3), 128(2), 123(5), 122(5), 121(16), 117(5), 115(6), 111(3), 109(13), 107(17), 106(6), 105(17), 97(5), 95(19), 93(27), 91(33), 85(5), 83(13), 81(43), 80(10), 79(31), 77(14), 74(14), 73(15), 71(10), 69(20), 67(30), 63(31), 59(15), 57(23), 45(12), 44(7), 43(47), 41(56).
 $M^{+\cdot}$ 552(2) Base peak 287	520(5), 492(16), 462(13), 447(2), 437(8), 430(65), 415(6), 412(8), 402(67), 398(20), 388(6), 377(90), 370(62), 352(8), 343(35), 329(5), 315(29), 307(5), 297(6), 289(10), 288(27), 283(25), 255(86), 248(12), 234(16), 233(6), 227(34), 224(10), 223(54), 224(14), 217(8), 216(12), 215(37), 213(4), 197(10), 196(22),

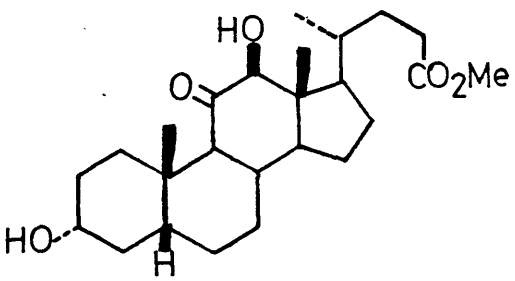
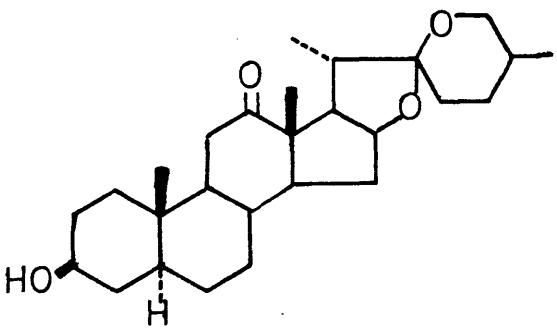
Compound	other ions m/z
	195(96), 194(24), 191(8), 183(18), 182(4), 177(5), 176(4), 175(13), 174(5), 173(9), 165(4), 164(6), 163(14), 155(16), 154(9), 135(7), 134(7), 133(9), 131(5), 122(10), 121(9), 115(10), 108(11), 107(12), 95(5), 94(17), 93(4), 81(9), 69(2), 55(4), 44(13).
 $M^{+\cdot}$ 506(1) Base peak 81	462(3), 444(2), 417(2), 416(5), 339(2), 398(7), 384(2), 380(2), 374(3), 373(4), 372(10), 371(4), 370(7), 358(2), 357(6), 356(2), 355(2), 354(6), 352(4), 347(3), 344(2), 343(4), 342(3), 341(2), 339(4), 338(2), 329(5), 325(2), 321(3), 318(2), 313(2), 311(2), 308(2), 303(2), 301(3), 286(2), 285(2), 283(3), 277(2), 274(2), 273(7), 267(3), 266(2), 265(3), 262(2) 261(4), 258(5), 257(19), 256(2), 255(6), 251(2), 247(3), 245(3), 243(4), 239(13), 237(3), 235(2), 230(5), 229(15), 227(7), 223(4), 221(4), 217(6), 215(4), 213(4), 211(5), 209(6), 208(12), 201(3),

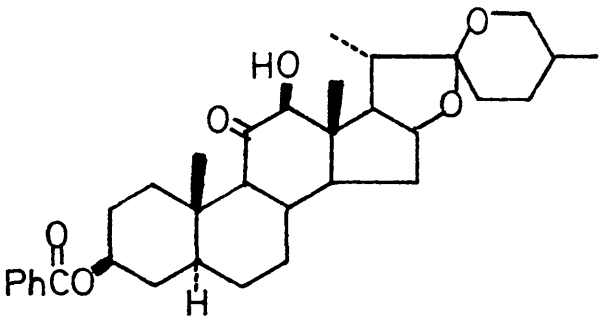
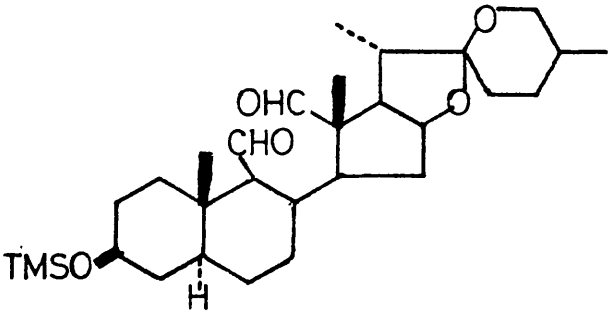
Compound	other ions m/z
	199(5), 198(10), 197(5), 196(7), 195(24), 194(5), 193(5), 191(5), 189(3), 187(4), 185(4), 183(3), 177(5), 175(10), 173(8), 171(4), 165(4), 163(13), 161(6), 159(8), 157(5), 155(4), 154(6), 150(4), 149(25), 147(26), 145(13), 143(6), 135(14), 133(20), 129(5), 123(7), 122(8), 121(25), 120(11), 119(21), 117(9), 115(12), 109(18), 108(10), 107(38), 106(12), 105(38), 97(6), 25(26), 94(16), 93(48), 92(10), 91(49), 87(6), 83(12), 82(11), 79(13), 79(48), 77(18), 74(7), 73(14), 69(14), 67(37), 65(7), 63(16), 59(10), 57(6), 55(51), 53(12), 51(4), 50(3), 44(2).
 $M^{+\cdot}$ 510(0) Base peak 105	492(2), 430(2), 416(3), 398(2), 389(4), 388(16), 386(3), 377(5), 371(11), 370(35), 359(3), 357(7), 356(6), 355(7), 324(2), 316(3), 283(4), 274(2), 273(4), 261(5), 257(7), 256(14), 255(67), 243(2), 241(3), 239(4), 231(4), 229(7), 228(4), 227(4), 221(1), 217(4),

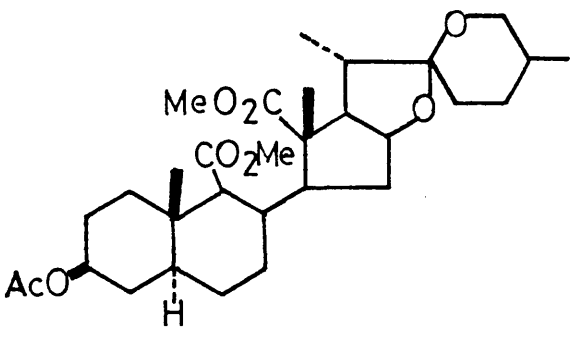
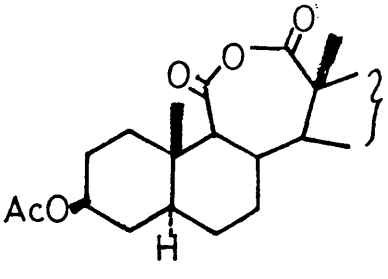
Compound	other ions m/z
	215(5), 209(3), 208(3), 203(4), 201(6), 193(3), 191(4), 189(3), 187(6), 185(4), 177(3), 174(4), 173(10), 171(4), 163(7), 161(11), 157(6), 155(3), 154(10), 149(15), 149(15), 147(25), 145(16), 143(6), 135(12), 134(6), 133(18), 131(14), 129(5), 125(3), 123(26), 122(7), 121(23), 120(16), 119(22), 117(8), 115(7), 107(43), 106(15), 96(6), 95(40), 94(17), 93(51), 91(37), 85(5), 83(15), 82(10), 81(72), 80(10), 79(48), 78(7), 77(50), 74(11), 73(9), 69(37), 68(8), 67(41), 65(6), 59(8), 57(13), 55(62), 53(10), 51(11), 50(5).
 $M^{+\cdot}$ 556(0) Base peak 55	416(9), 404(5), 403(7), 385(21), 384(32), 372(73), 371(68), 370(20), 357(21), 356(15), 354(7), 353(14), 344(20), 343(38), 301(6), 289(4), 287(7), 270(2), 269(27), 257(6), 255(9), 241(7), 229(10), 227(14), 209(15), 208(24), 207(17), 196(3), 195(9), 189(4), 187(6), 177(10), 175(15), 173(7), 163(9), 161(8).

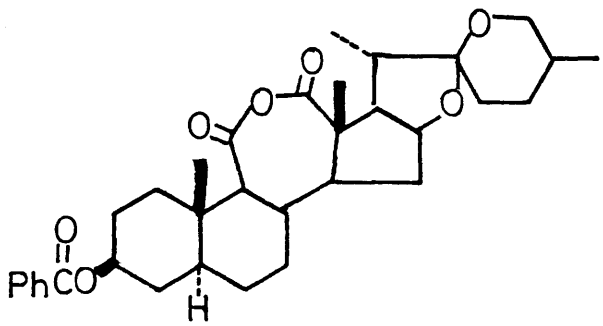
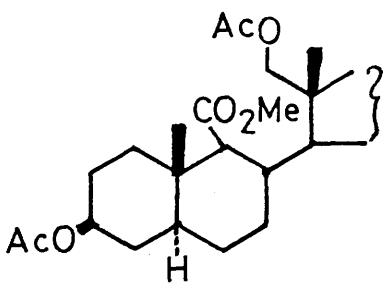
Compound	other ions m/z
	159(15), 149(20), 147(45), 145(15), 135(18), 133(20), 131(10), 123(12), 121(22), 119(25), 111(9), 109(30), 108(15), 107(54), 105(40), 95(36), 81(97), 69(50), 57(30).
 $M^{+\cdot}$ 552 (7) Base peak 377	537(84), 520(27), 492(26), 462(57), 447(13), 437(9), 430(92), 412(17), 402(45), 370(55), 352(26), 343(53), 338(22), 315(28), 298(15), 287(56), 283(40), 274(10), 265(11), 255(83), 234(10), 227(18), 223(57), 215(39), 195(92), 191(14), 183(21), 175(20), 163(17), 155(14), 147(37), 135(14), 129(15), 122(23), 115(9), 108(23), 95(13), 81(15), 75(33), 55(7), 44(25).
 $M^{+\cdot}$ 494(6) Base peak 372	479(35), 462(11), 444(8), 404(45), 373(29), 344(35), 318(12), 299(6), 289(16), 267(7), 257(19), 229(44), 223(24), 207(6), 197(5), 196(11), 175(4), 162(15), 149(58), 147(26), 134(15), 115(10), 109(16), 108(9), 107(14), 94(10), 81(20), 75(20), 44(21).

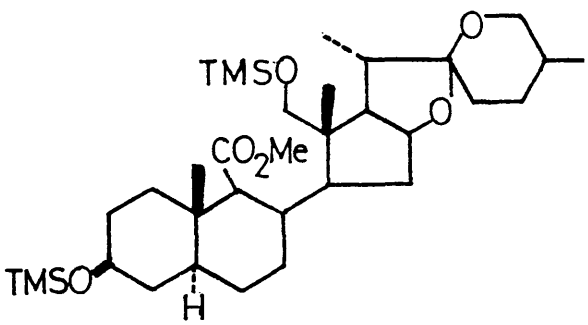
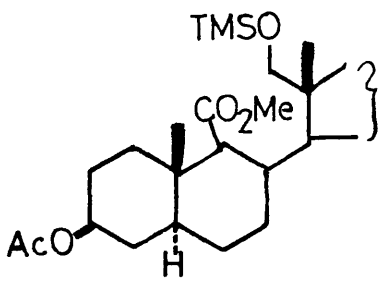
Compound	other ions m/z
 $M^{+\cdot}$ 582 (0.5) Base peak 196	537(1), 524(1), 506(2), 496(12), 492(10), 474(5), 461(1), 446(2), 437(2), 406(5), 402(15), 386(9), 384(10), 374(10), 356(8), 348(8), 347(35), 346(7), 324(16), 311(21), 298(8), 292(24), 279(28), 266(7), 264(7), 256(15), 251(14), 241(13), 224(16), 223(13), 197(16), 195(22), 191(9), 189(7), 179(6), 178(9), 164(20), 161(18), 151(16), 147(11), 137(10), 135(16), 133(21), 129(10), 122(11), 121(9), 115(10), 109(27), 108(95), 95(8), 93(10), 81(22), 75(26), 55(5), 44(12).
 $M^{+\cdot}$ 522 (0.5) Base peak 196	476(1) 462(3), 434(3), 407(4), 402(14), 384(10), 374(8), 347(23), 343(3), 311(21), 298(7), 292(6), 279(30), 266(8), 256(14), 251(16), 241(5), 224(14), 195(25), 189(5), 178(6), 164(16), 163(9), 161(10), 152(4), 151(30), 150(12), 147(13), 137(9), 135(10), 133(12), 122(8), 115(9), 110(10), 107(13), 93(11), 81(23), 80(12), 73(2), 60(6), 55(4), 44(13).

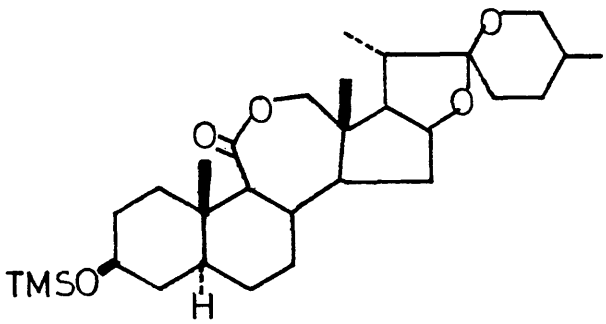
Compound	other ions m/z
 $M^{+\cdot}$ 420(6) Base peak 55	402(6), 387(3), 384(3), 369(4), 342(1), 337(2), 330(4), 329(6), 289(1), 269(4), 257(1), 243(2), 241(3), 229(4), 217(3), 201(2), 199(3), 195(3), 187(3), 175(6), 165(4), 161(6), 151(3), 149(8), 147(14), 145(7), 137(5), 135(10), 133(11), 129(4), 127(3), 121(18), 119(16), 115(8), 109(17), 107(26), 105(24), 97(14), 95(29), 84(85), 81(55), 78(39), 77(20), 69(31), 59(33), 44(6), 41(72).
 $M^{+\cdot}$ 430(12) Base peak 139	412(3), 402(5), 384(1), 371(3), 358(9), 343(3), 340(2), 316(24), 298(7), 288(3), 273(15), 255(4), 221(1), 181(1), 167(3), 126(32), 115(1), 108(2), 93(1), 69(2), 55(1), 43(1), 41(1).

Compound	other ions m/z
 $M^{+\cdot}$ 550(5) Base peak 139	549(1), 534(3), 520(2), 506(1), 491(3), 478(7), 463(3), 448(2), 436(24), 428(1), 418(4), 420(6), 419(5), 418(7), 406(4), 394(1), 378(3), 377(8), 358(2), 332(10), 315(10), 314(27), 298(3), 296(4), 269(3), 268(2), 255(5), 239(3), 193(2), 185(2), 175(4), 173(3), 169(4), 161(3), 159(4), 147(13), 145(6), 137(9), 133(8), 126(23), 122(10), 115(26), 109(13), 105(62), 81(17), 77(25), 69(26), 67(13), 55(26), 43(12), 41(11).
 $M^{+\cdot}$ 518(6) Base peak 126	503(5), 490(4), 472(3), 462(3), 459(3), 449(3), 446(4), 431(3), 404(32), 386(5), 376(25), 352(5), 350(4), 337(4), 322(2), 313(7), 285(2), 275(3), 268(4), 251(10), 187(14), 182(10), 149(11), 139(42), 115(24), 109(25), 108(18), 107(10), 95(5), 75(9), 70(4), 55(4), 44(4), 43(5).

Compound	other ions m/z
 $M^{+\cdot}$ 548(8) Base peak 374	516(3), 488(5), 476(10), 456(2), 447(9), 434(8), 429(6), 419(7), 402(16), 391(3), 387(5), 367(6), 359(17), 342(27), 335(9), 327(3), 315(9), 314(16), 307(3), 299(5), 275(1), 255(4), 247(6), 235(2), 226(12), 225(17), 221(3), 193(3), 182(30), 167(9), 152(3), 147(6), 139(80), 134(4), 126(33), 121(7), 115(10), 110(28), 93(3), 81(1), 73(1), 70(2), 69(2), 55(1), 44(4), 43(4).
 $M^{+\cdot}$ 502(9) Base peak 139	472(1), 458(1), 443(12), 433(10), 430(35), 414(4), 405(3), 396(2), 388(10), 373(2), 360(49), 344(59), 333(2), 327(8), 319(11), 315(8), 314(9), 300(19), 299(14), 284(9), 277(3), 265(4), 259(4), 255(4), 241(3), 237(21), 193(5), 184(6), 173(5), 165(5), 160(5), 147(30), 133(9), 126(96), 115(9), 108(55), 107(22), 93(7), 81(4), 70(8), 55(3), 43(6).

Compound	other ions m/z
 $M^{+\cdot}$ 564(1) Base peak 139	534(1), 520(1), 506(3), 462(6), 447(2), 421(7), 406(3), 392(3), 377(12), 350(1), 342(2), 299(2), 297(2), 280(1), 255(6), 221(1), 199(1), 185(2), 175(2), 173(3), 167(2), 163(2), 147(10), 133(5), 126(37), 108(10), 107(13), 105(47), 93(15), 91(12), 81(11), 79(12), 77(19), 69(23), 55(17), 45(4), 44(2), 43(14), 41(7).
 $M^{+\cdot}$ 562(25) Base peak 139	532(5), 531(4), 503(11), 490(45), 471(3), 461(6), 458(6), 442(2), 433(9), 430(7), 419(12), 401(12), 388(60), 375(95), 356(70), 343(54), 315(47), 313(24), 295(7), 283(5), 268(6), 267(6), 256(5), 255(28), 239(25), 235(11), 223(4), 217(4), 207(15), 191(3), 181(11), 179(11), 175(7), 163(11), 147(18), 126(32), 121(56). 115(28), 109(50), 91(5), 79(4), (71(3), 70(4), 69(5), 60(4), 57(4), 55(7), 44(12), 43(12).

Compound	other ions m/z
 $M^{+\cdot}$ 662(7) Base peak 187	602(8), 550(6), 533(4), 532(4), 519(25), 515(5), 501(4), 487(9), 459(5), 448(4), 431(3), 418(31), 405(22), 388(4), 377(4), 369(13), 358(6), 345(4), 325(3), 313(3), 255(11), 246(3), 239(8), 223(7), 217(17), 211(9), 181(10), 173(2), 167(5), 147(12), 139(42), 126(23), 121(32), 115(5), 109(9), 103(7), 93(6), 81(4), 73(6), 70(5), 56(2), 44(13), 40(14).
 $M^{+\cdot}$ 592 (8) Base peak 187	577(6), 533(3), 520(10), 502(4), 489(29), 471(3), 457)8), 429(13), 418(3), 401(4), 388(44), 375(28), 369(8), 356(8), 343(5), 328(13), 327(3), 326(3), 325(1), 315(12), 299(3), 269(5), 268(4), 267(3), 255(13), 239(12), 235(18), 217(14), 211(15), 181(9), 175(6), 165(6), 147(8), 139(46), 135(15), 126(36), 121(38), 115(6), 109(14), 103(11), 97(3), 81(4), 75(12), 73(4), 60(3), 57(3), 44(7), 43(6), 40(9).

Compound	other ions m/z
 $M^{+\cdot}$ 518(25) Base peak 139	503(5), 488(5), 462(10), 459(13), 449(16), 446(40), 431(10), 414(4), 404(42), 487(8), 486(9), 375(10), 374(25), 369(4), 359(7), 341(5), 313(27), 300(3), 301(6), 295(3), 285(8), 284(9), 267(6), 257(3), 239(5), 223(60), 194(7), 193(8), 187(32), 180(13), 173(5), 166(7), 159(6), 147(13), 126(40), 121(50), 115(30), 108(18), 93(8), 83(5), 75(7), 70(7), 55(5), 44(9), 43(7).

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