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Progress in the field of physiologically active lanosterol compounds

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ABSTRACT. This review correlates the studies (up to 1991) on the isolation, structural determination, biological activity, and synthesis of physiologically active polyoxidised lanosterol derivatives of vegetable (inotodiol, ganoderic acids) and animal (seychellogenin) origin. The cytotoxic, cardiovascular, and other forms of activity of compounds of this type are of considerable interest in relation to their medical use. It is noted that the functionalised side chain (in an open form or containing lactones, lactols, etc.) is generally responsible for the activity exhibited by lanosterol derivatives. Two basic approaches to the derivation of these structures are defined: either by complete reconstruction of the side chain of lanosterol (degradation and rebuilding with oxygen-containing residues) or by progressive functionalisation of the Δ^{24}-side chain of lanosterol. The syntheses of the known anticancer compound "inotodiol", seychellogenins, ganoderic acids, and other compounds are described.

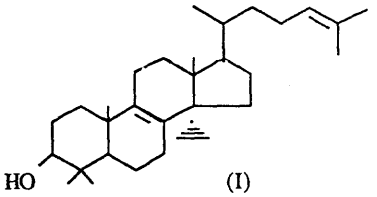
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I. Introduction

Triterpenoids of natural origin with polyfunctional side chains at C(17) and C(20) and with the lanostane skeleton (I) [also known as 4,4,14-trimethylpregn-5α-8(9)-ene or -7,9(11)-diene] have attracted considerable attention[1] from research workers because of their high and varied biological activity.



(I)

Their occurrence in nature, in particular in the plant world and fungi, forms the subject of a number of reviews[2–4] covering the literature up to 1977. It has been established that the main responsibility for their biological activity is borne by the functionalised side chain of lanosterol containing hydroxyl, carbonyl, and other groups, right up to an 18,20-lactone ring, which imparts antifungal and carcinolytic properties to lanostane derivatives. These lactones make up a separate group of triterpenoids — the holothurigenins.[5,6] The regulatory effect on the biosynthesis of cholesterol, when the presence of an O-functional group adjacent to the skeleton [8(9) double bond] is important, constitutes the only exception.[7]

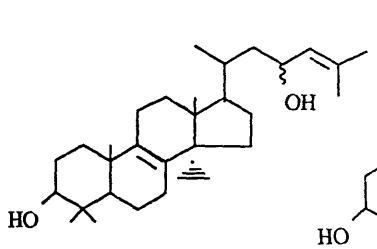
In the present review we have assumed the task of focusing attention on an analysis of the structural features of polyoxidised lanosterol derivatives that have been isolated up to 1991, monitoring the effect of their structure on cytotoxic, antisclerotic, and other types of biological activity, and discussing the most promising synthetic methods. The material has been classified and arranged in an order corresponding to an increase in the degree of oxidation of the side chain of lanosterol. Studies relating to skeletal rearrangements of lanosterol that lead to cucurbitane and fusidic acid have not been covered in this review.[8,9]

II. Natural derivatives of lanosterol

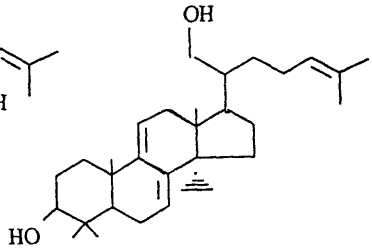
1. Natural derivatives of lanosterol with open side chains containing oxygen functional groups

Of the monohydroxy derivatives of lanosterol, 23ξ-hydroxylanosterol (II) was first isolated in1968 as a metabolite of the fungal mycelium of *Scleroderma aurantium* Pers.[10–12] The absolute configuration at C(23) in natural 23-hydroxylanosterol was determined by correlation of the sign of its molecular rotation with that of known (*R*)(–)- and (*S*)(+)-4-methylpent-3-en-2-ols. The difference between M_D for the natural (23*S*)-23-alcohol and lanosterol is positive while for the synthetic (23*R*)-23-alcohol there is a negative difference.[11] It was subsequently established that this allylic alcohol is unstable and undergoes dehydration during chromatographic separation.[13] The 21-hydroxy derivatives with a

lanosta-7,9(11),24-triene and -8,24-diene skeleton (IIIa, b) were also isolated.[14]

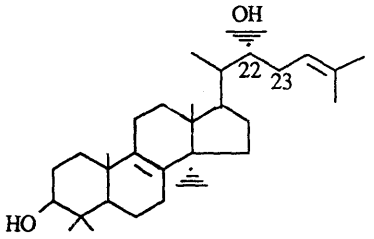


(II)



a: $\Delta^{8(9)}$
b: $\Delta^{7,9(11)}$

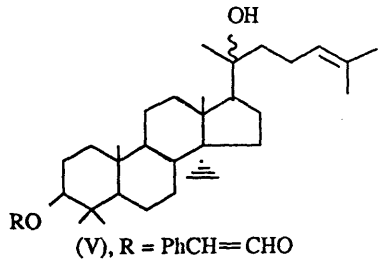
(III)



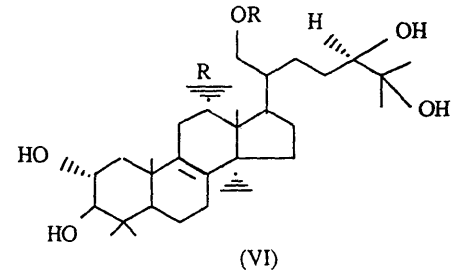
(IV) inotodiol

The 22- and 24-hydroxy derivatives were isolated from the fungus *Inonotus obliquus* and plant *Euphorbius corollatta*,15,16 and the most interesting of these is (22*R*)-hydroxylanosterol (IV)—the so-called inotodiol—a compound which is highly active towards cancer cells grown *in vitro*. It inhibits the growth of sarcoma 180 and carcinoma 755.[17] The 22-hydroxylanosterin derivative with a 4-carboxyl group, which is similar in structure to inotodiol, was isolated from the fungus *Fomes senex*.[18] 25-Hydroxylanosterol was isolated by Japanese researchers.[19] Lanostane with a shortened 22-hydroxy side chain has been isolated from the fungus *Verticillium lecanii*.[20]

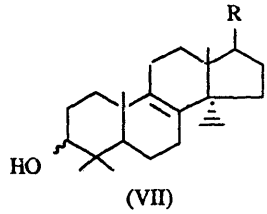
The Indian plant *Euphorbia antiquorum* L has proved to be a very abundant source of 20-hydroxylanosterol (V). Its gum has diuretic, laxative, and toxic properties.[21] It is used in stomatology, in the treatment of nervous disorders, and for treatment of wounds. In the isolation of the active principle, the cinnamic ester of 20-hydroxylanosterol (V), known as anticulol A, was obtained. Its mass spectrum did not exhibit a peak due to the molecular ion but a peak from the dehydrated form with *m/z* 556 occurred. The position of the 20-hydroxy group was confirmed by the ^{13}C NMR spectroscopic data (δ 75.4 ppm). In the PMR spectrum, in addition to the chemical shifts (δ) corresponding to the protons of cinnamic acid, signals from the 21-CH_3 group on C(20)—OH (δ 1.15 ppm) and from C(18)—CH_3 (0.98 ppm) were recorded. The skeletal structure of 8(9)-dihydrolanostane was confirmed by analysis of the ^{13}C and ^1H NMR spectra. Japanese scientists isolated 2α,3β,21,24,25-pentahydroxy derivatives of lanosterol (VI)—the so-called fasciculols, which are novel plant growth inhibitors—from the plant *Naemotoloma fasciculara*.[22] The 22,24-, 22,25-, and 22,23-dihydroxy derivatives of lanosterol (VIIa—d) were isolated from *Pisolithus tinctorius* fungi.[23] These compounds exhibited appreciable cytotoxic activity on hepatoma and tinoma cells *in vitro*. The structure of the side chains, in particular the position of the OH groups has been demonstrated by a combination of known phyiscal methods, including the nuclear Overhauser effect (NOE) and the formation of derivatives.



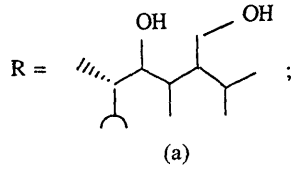
(V), R = PhCH=CHO



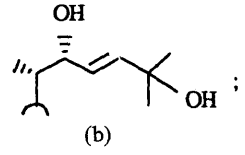
(VI)



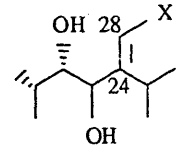
(VII)



(a)



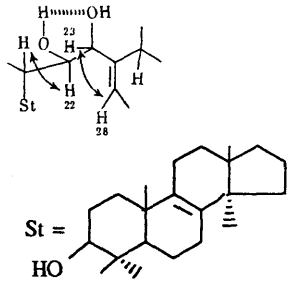
(b)



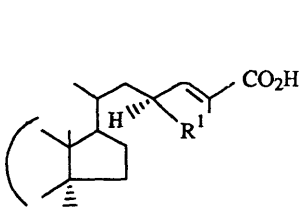
, X = H (c) CH_3 (d)

Thus, the presence of the C—CH=CX group (where X = CH_3) was confirmed from the signals at δ 1.47 ppm (3H, d, *J* 6 Hz) and δ 5.63 ppm (1H, q, *J* 6 Hz) while the secondary OH groups were identified from the multiplets at δ 3.98 ppm (1H, *J* 9 Hz) and δ 3.62 ppm (1H, *J* Hz) due to the protons attached to the carbon atoms bearing these OH groups. Their 22-acetates give chemical shifts at lower field (δ 5.2 and 4.2 ppm). The mass spectra were characterised by fragments corresponding to loss of a single proton from the side chain of the molecule, which indicates that all of the compounds differ only in the degree of substitution of the steroid side chain. The double bond in the side chain was confirmed by oxidation of glycol (VIId) to the 22-aldehyde and 3-methyl-2-methylenebutanal isolated as its dinitrophenylhydrazine derivative. The stereochemistry of the substituents in the side chain of the steroid was demonstrated by means of NOE experiments in the PMR spectra. Thus, a *Z*-configuration was assigned to the 24(28)-olefin because of the interaction of H(28) with H(22) and H(23) rather than with H(25).

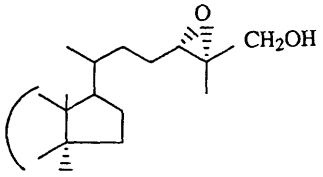
The existence of an intramolecular hydrogen bond was demonstrated.



Extracts from the fungal mycelium of *Ganoderma lucidum* proved extremely interesting in terms of their biological properties.[24] A pharmacological study demonstrated their effect on the cardiovascular system (decrease in blood pressure). Lanostane alcohols and acids—24,25,26-triols and 23-hydroxy-26-acids[25]—were isolated from the mycelium and fruit body of this fungus, and an (*S*)-configuration of the 23-centre was established for the latter by measurement of the Cotton effect (CE) of the 23-monodimethyl-*p*-aminobenzoate.



(VIII), R^1 = OH,$OCOC_6H_4NH_2$,

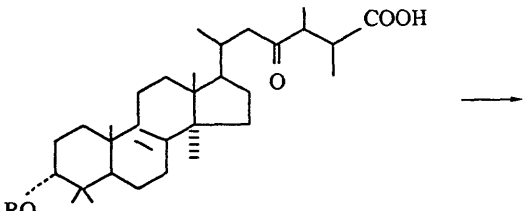


(IX)

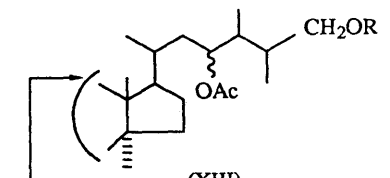
The 24,25-epoxy derivative of the 26-alcohol (IX) was isolated from the same fungus,[26] and the configuration of its oxide ring was established from NOE data and by a method described by Dillon and Nakanishi,[27] which involved the reduction of the α-epoxide with lithium aluminium hydride and determination of the sign of the Cotton effect in the circular dichroism spectrum in the presence of $Pr(dpm)_3$ according to the empirical rule of Nakanishi.[27]

The 21-carboxy derivatives of lanosterol (X), known as trametenolic acids, were isolated by several groups of scientists[28,29] from *Polyporaceae Fomes Hartigii* Allescher fungi; some of these were subsequently called eburicoic acids.[4] The lanosta-7(8),9(11),24-triene-21-acid[30] and lanosta-17(20),24-diene-21-acid substituted at C(6), C(7), and C(11) [31] were also isolated. The 26-acids, so-called polyporenic acids (XI), occur widely in nature.[4] The novel 23-keto-26-acid (XII), known as carboxaquercinic acid, was isolated as its 3-malonate from fungal metabolites of *Daedalea quercina*.[32]

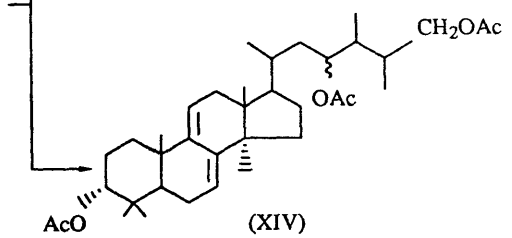
Its structure was demonstrated by a combination of PMR and mass spectrometry data as well as by chemical conversions to the dihydroxy derivatives (XIII) and (XIV).



(XII), R = $COCH_2COOH$

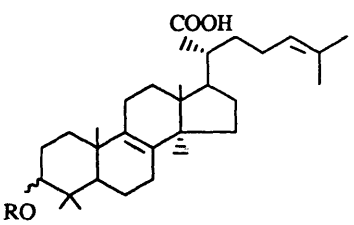


(XIII)

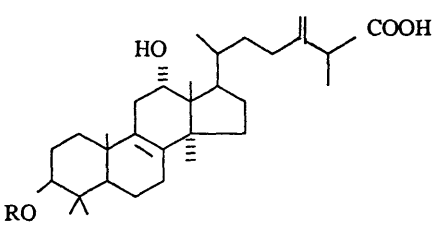


(XIV)

Lanostane 26-acids differing in the configuration of substituents at C(3) have been isolated from Chinese[33] and Mexican[34] plants. A large group of triterpenoids isolated from the fungal mycelium of *Ganoderma lucidum* was made up by 26-acids with a functionalised lanostane skeleton (XV).[35] The useful biological properties of the "ganoderic" acids, in particular their cytotoxicity towards hepatoma cells[36–40] acted as a stimulus for detailed studies on the extracts from fungi used in Chinese, Indian, and Korean popular medicine as anodynes. To date more than 30 studies[35] in which the isolation of ganoderic and ganoderenic acids (labelled with the letters of the alphabet from A to T) is reported to have been published. They have been isolated from a chloroform–ethanol extract by chromatography on silica gel columns. In some cases separation is more effective after conversion of the acid mixtures to their methyl esters. Ganoderic acids differ in the degree of oxidation of the side chain. Thus, the distinctive group fo the 23-keto-26-acid[40,43] and 22-hydroxy-26-acid[40] has been detected. The $\Delta^{20(22)}$-23-keto-26-acid (XV) and 20-hydroxy-23-keto-26-acid (XVI) with the same

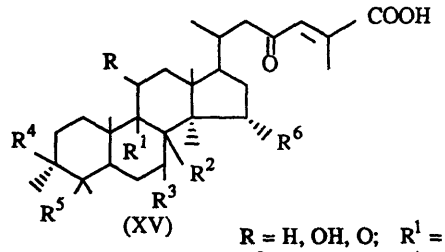


3α– or 3β–(X)



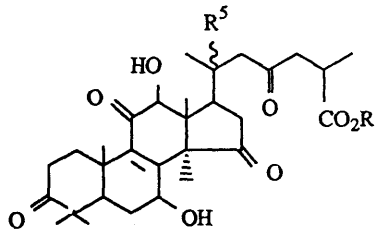
(XI)

functionality in the skeleton, known as ganoderenic acids[44,45] and 21,26-acids,[46] have been isolated from *Ganoderma lucidum* Karst. The amide of the 26-acid (XVII) has also been isolated.[47]

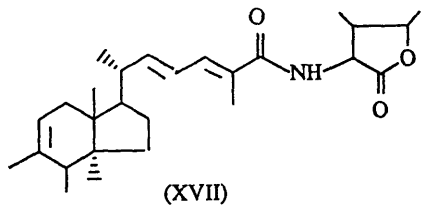


(XV)

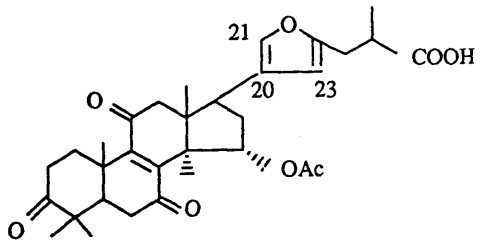
R = H, OH, O; R^1 = H; R^1, R^2 = Δ; R^3 = H, α-OH; R^4, R^5 = O; R^4 = OH; R^5 = H, OH; R^6 = H, OH.



(XVI), R^5 = H, OH; $\Delta^{20(22)}$



(XVII)

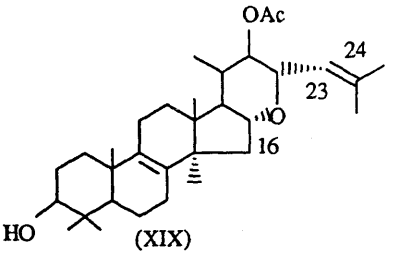


(XVIII)

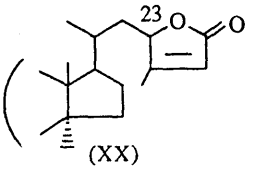
The furanoganoderic acid (XVIII) was isolated by Nishitoba et al.[35] The identification and structural assignment of ganoderic acids was based on an analysis of their ^{13}C and ^{1}H NMR spectra and the fragmentation in their mass spectra. A structural feature of their skeleton is the presence of the cross-conjugated 7,9(11)-diketo system, in some cases a 7β-hydroxy group and 3-carbonyl, and in certain instances a variable configuration of the 3-hydroxy group. It was noted by Lobo et al.[23] that the variation in the configuration of the substituent at C(3) in ganoderic acids is dependent on the source from which they are isolated — the fruit body of the fungus (3β-hydroxy) or from a culture of mycelium (3α-hydroxy). This finding is interesting in relation to the possible biosynthetic pathways of triterpenoids in *Ganoderma lucidum*. By studying the biosynthesis of ganoderic acids in a culture of mycelium using labelled squalene (1,2-^{13}C)-acetate, Tiasen and Meifen[33] found that ganoderic acid T, for example, is formed from (3*S*)-oxidosqualene via the 3-keto compound by the same route as that which occurs in higher plants with formation of the 3α-hydroxy substituent. All of the ganoderic acids have signals corresponding to the 4,4,14,18,19-CH_3 groups. The presence of double bonds is reflected in the appearance of signals from olefinic protons in the region of 5–6 ppm. Conjugated keto groups are demonstrated by the absorption in the UV spectra. In some cases the presence of a double bond in the molecule of a natural acid can be confirmed by ozonisation and identification of the resulting products. The configuration of the hydroxide at C(3) is determined from the PMR spectroscopic data; 3β-OH appears as two doublets at δ 3.5 ppm with *J* 10.5 Hz while 3α-OH has a lower spin–spin coupling constant. In the PMR spectrum of furanoganoderic acid (XVIII) there was no signal at 2.2 ppm due to a 21-CH_3 group but two signals due to olefinic protons appeared as singlets at δ 6.90 and 5.77 ppm. From these data together with the signals in the ^{13}C NMR spectrum at δ 107.7 and 138.4 ppm (from two methine olefinic carbons) and δ 153.2, 153.6, 150.9, and 124.9 ppm (from four tertiary olefinic carbons), De Bernardi et al.[53] could establish the presence of a 2,4-substituted furan ring in the side chain of this novel ganoderic acid. The structure of furanoganoderic acid — 21,23-epoxy-15α-hydroxy-3,7,11-triketo-5α-lanosta-8,20(21),22-trien-26-oic acid (XVIII) — corresponds to its biogenesis.

2. Natural lanosterol derivatives with lactone, lactol, and furan rings in the side chain

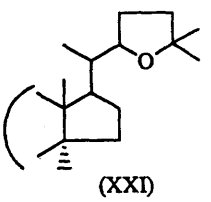
Compounds with a lanostane skeleton and a heterocyclic structure in their side chains have been found amongst natural polyhydroxytriterpenoids.[18,48–51] It has been argued, however, that they may be secondary products formed during isolation as a result of the condensation of hydroxy and keto acids.[32] Echinodol (XIX) [49] and abieslactone (XX) [51] are amongst such compounds. Echinodol was isolated from *Echinodontium tsugicola*[52] fungi and from plants having anticancer activity. The structure of (XIX) was demonstrated by a sequence of reactions, including dehydration, acid hydrolysis, Jones oxidation, and Wolff–Kishner reduction, giving lanosterol, and it was also supported by the PMR spectroscopic data.[52] The structure of abieslactone (XX) was established by X-ray diffraction analysis. It is (23*R*)-3α-methoxy-5α,9β-lanosta-7,24-dien-23,26-olide. Senexenol (XXI) isolated from *Fomes senex* fungi contains a 22,25-tetrahydrofuran residue.[18]



(XIX)



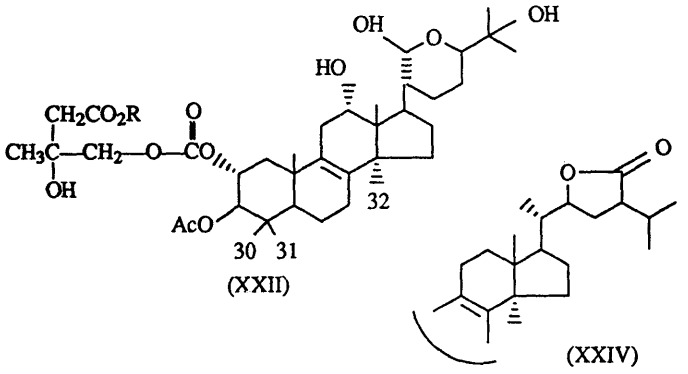
(XX)



(XXI)

3β-Acetyl-2α-(3′-hydroxy-3′-methyl)glutarylcrustulinol (XXII), which is active against leukaemia, was isolated from *Hebeloma species* fungi.[53] The molecular structure of (XXII) was determined

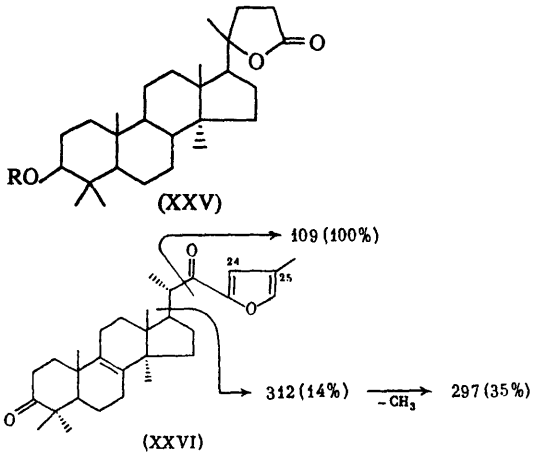
from its ^{13}C NMR data. The three acetyl carbons give signals in the region of δ 170–174 ppm; the methines attached to two oxygen atoms appear at δ 93.2 and 5.48 ppm. The tetrasubstituted 8(9)-double bond gives signals at δ 135.8 and 132.7 ppm. Oxidation of the hemiacetal (XXII) with Fetizon reagent—silver acetate on Celite—gave a δ-lactone (XXIII). The conformation of the tetrahydropyran ring in (XXII) existing in the boat form was established from PMR spectroscopic data. The anomeric OH group and isopropyl group have a pseudoaxial conformation. The γ-lactone (XXIV), which is similar in structure to hemiacetal (XXII), was isolated from the fungus *Pisolithus*,[23] and it also exhibited appreciable cytotoxic activity. Lobo et al.[54] noted that this was the first natural example of a triterpene carboxylic acid derivative. Its structure was demonstrated by elemental analysis and the data of NMR and mass spectrometry. Thus, 4 signals (δ 0.72, 0.97, 0.81, and 0.88 ppm) are attributed to those from the CH_3 groups at C(18), C(19), C(30), and C(31). The groups at C(21), C(26), and C(27) appear as singlets at δ 1.66, 1.02, and 0.94 ppm. The mass spectrum is characterised by a fragment with *m/z* 155 ($C_9H_{15}O_2$), which corresponds to cleavage between the steroid skeleton and side chain. The structure and configuration of (XXIV) was confirmed conclusively by X-ray diffraction analysis.



(XXII) (XXIV)

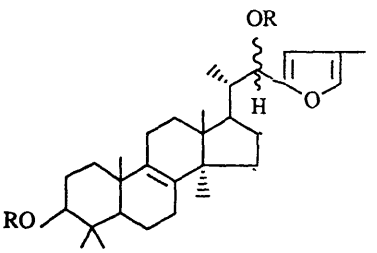
A γ-lactone on C(20) of the side chain of lanostane was isolated[55] from the leaves of the Indian plant *Lansium* qn Bedd and was given the name lansilactone. The structure of (XXV) was established from its ^{13}C and ^{1}H NMR data, and the point of attachment of the lactone ring was determined from the nature of fragmentation in the mass spectra.[55]

The novel furanoid triterpenoid pomacerone (XXVI) [56] was isolated from the fungus *Phellinus pomaceus*.

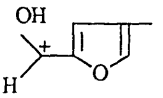


(XXVI)

The presence of a furan residue was confirmed from the absorption in the UV spectrum with λ_{max} at 234, 245, and 292 nm and by the absorptions in the IR spectrum at 1660 and 1600 cm^{-1}. The main fragment in the mass spectrum corresponds to cleavage of the C(20)–C(22) bond (*m/z* 109, 100%). The PMR spectrum of (XXVI) is characterised by signals due to the angular CH_3 groups at δ 0.81, 0.92, 1.05, 1.07, and 1.1 ppm and the one secondary CH_3 group as a doublet at δ 1.17 ppm, which is characteristic of a CH_3 group at C(21). The side chain exhibits the following signals: δ 2.07, 3.22 (1H dq., *J* 6.8, 10.5 Hz), 7.04 (1H, br. s.), and 7.35 (1H, br. s.), corresponding to the protons on C(27), C(20), C(24), and C(26). The signals that appear between δ 2.50 and 0.90 ppm correspond to the two methine protons and eight methylene protons (one of which is at an α-position relative to the carbonyl group at δ 2.45 ppm). With double resonance the symmetric quartet due to H(17) is converted to a triplet by irradiation on the H(20) nuclei at δ 3.22 ppm. This suggests that the coupling constant between H on C(16) and C(17) is of the same order as that between H on C(17) and C(20) and is consistent with the Karplus equation for structure (XXVI). At the same time the *J* values between the protons on C(20) and C(21) (6.8 Hz), C(24) and C(26) (9.9 Hz), C(16) and C(17) (10.0 Hz), C(17) and C(20) (10.5 Hz), and C(26) and C(27) (1.5 Hz) were determined. Thus, on the one hand, interaction between the protons on C(24), C(26), and C(27) is confirmed as is that between the proton on C(20) and the methine proton on H(17) and the protons on H(21), on the other hand. Compound (XXVI) was reduced with lithium aluminium hydride. The resulting 3,22-tetrahydropomacerone (XXVII) displayed signals in its PMR spectrum due to the furan protons at δ 6.10 and 7.15 ppm and at δ 3.20 and 4.72 ppm from the H(3) and H(22) protons that are geminal relative to both OH groups. These signals do not appear when lithium aluminium deuteride is used for reduction. Acetylation of derivative (XXVII) gave 3,22-diacetoxy-23,26-oxidolanosta-8(9),23,25-triene (XXVIII), in whose PMR spectrum the signals due to the H(24) and H(26) furan protons are shifted to δ 6.14 and 7.15 ppm while the signals from H(3) and H(22) occur at δ 4.55 and 5.84 ppm. The mass spectrum of (XXVIII) has a fragment with *m/z* 111 ($C_6H_7O_2$).



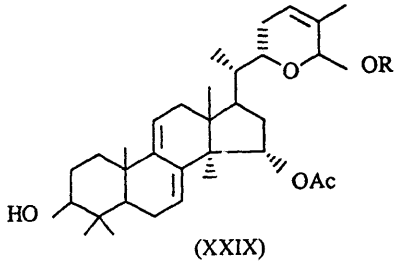
(XXVII), R = H;
(XXVIII), R = Ac



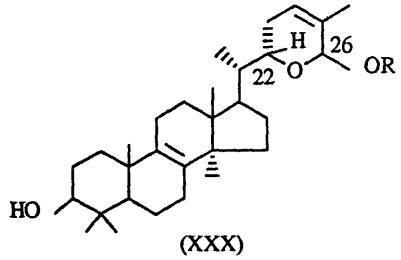
m/z 111

Triterpenoids with a six-membered hemiacetal residue in the side chain—the so-called perenniporiols—were first isolated from the mycelium of the higher fungi *Perennipria ochroleuca*. These compounds with the same structural characteristics as the group of nautral vitanolides[57–60] are of interest because of their high diverse biological activity. Vitanolides with a lanostane skeleton have been isolated in small quantities.[61,62] The structure and configuration of the diaxially interacting (22*S*,26*S*)-sites in (XXIX) have been established by comparision of the ^{13}C NMR spectroscopic data with the results of X-ray diffraction analysis, and the compound has been

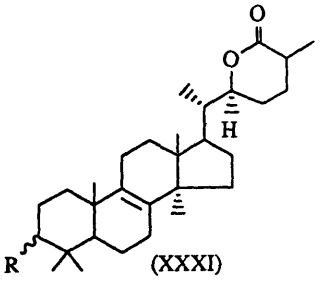
identified as (22*S*,26*S*)-15α-acetoxy-22,26-epoxy-3,26-dihydroxylanosta-7,9(11),24-triene (perenniporiol). The 15-desacetoxy-7,11-dihydroperenniporiol (XXX) was also isolated. The configuration of the lactol rings in both compounds was confirmed by comparison of the PMR spectroscopic data—the *J* values and position of the signals due to H(22) at δ 3.95 ppm (dd. *J* 12 and 2.5 Hz) and δ 5.16 ppm (d. *J* 4.9 Hz).



(XXIX)



(XXX)

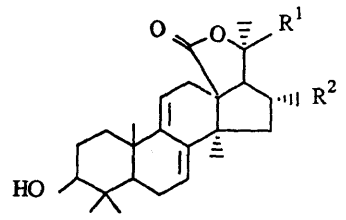


(XXXI)

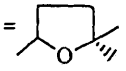
R = α-H, β-OH (a)
β-H; α-OH (b)

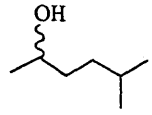
The δ-lactone astrahygrol (XXXI), which has a similar structure, was isolated from the fruit body of the fungus *Astraeus hygrometricus* (Pers) Morgan (*Gasteromycetes*), familiar in Chinese popular medicine as a haemostatic agent.[62] The presence of two secondary and five tertiary CH_3 groups in the PMR spectrum of the molecule of (XXXI) provided support for a lanostane skeleton, and the fragment with *m*/*z* 314, corresponding to loss of the side chain from the molecular ion in the mass spectrum confirmed the presence of a δ-lactone ring in the side chain. The ^{13}C NMR data attested to the presence of a double bond between rings. The structure of the side chain as a δ-lactone was also clearly demonstrated by the respective signals in the PMR spectrum—a single-proton doublet of doublets at δ 4.3 ppm with *J* 11.6 and 3.0 Hz due to the proton on C(22) proton, a single-proton doublet of quartets at δ 2.61 ppm with *J* 10 and 7 Hz due to H(25), and a single-proton doublet at δ 0.99 ppm with *J* 6.3 Hz due to H(21).

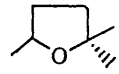
Triterpenoids with a 18,20-γ-lactone ring and a double bond at the 8(9)-position or double bonds at the 7(8)- and 9(11)-positions have been isolated from sea cucumbers (holothurians) as glycosides and consequently have been called holothurigenins or seychellogenins. In some cases they contain oxygen substituents at C(16), C(23), and other positions.[7,63–67] These compounds act as powerful growth inhibitors of pathogenic fungi and have significant carcinostatic activity.

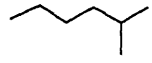


(XXXII)

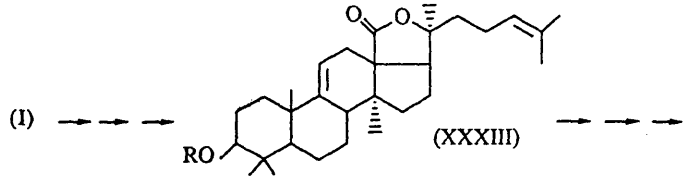
R^1 =  , R^2 = H, 17-desoxy-22,25-oxidoholothurinogenin;

R^1 =  , R^2 = OH, griseogenin;

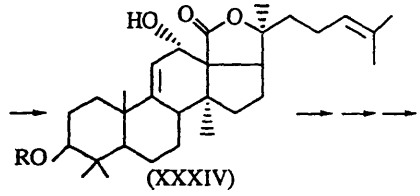
R^1 =  , R^2 = OH 22,25-oxidoholothurinogenin;

R^1 =  , R^2 = H seychellogenin

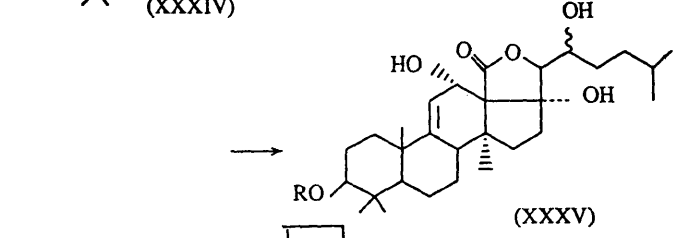
It is assumed that holothurinogenins are formed as a result of bioconversions involving the oxidation of lanosterol according to the scheme shown below. In the majority of cases the 8(9)-double bond of the precursors migrates to the 7(8)-position. Subsequent hydroxylation, acetylation, and saturation of the 24(25)-double bond gives a compound with the general formula (XXXIII). Oxidation of the side chain leads to derivatives (XXXIV)–(XXXVI), etc.



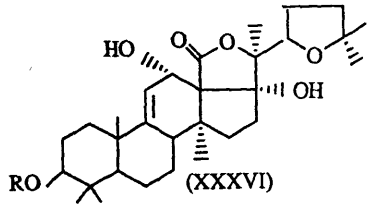
(XXXIII)



(XXXIV)



(XXXV)



(XXXVI)

Thus, it can be seen that natural sources provide a substantial number of compounds that are functionalised both on the lanostane skeleton and in the side chain of the molecule. The existing, generally fragmentary data on their biological activity, in particular on the medicinal properties of the plants and fungi containing these compounds that are frequently used in popular medicine, especially in countries of the East, suggest the possibility of finding interesting and promising drugs amongst them and their analogues. However, the "active principles" are present in extremely small quantities and sometimes undergo unwanted conversions during isolation. Therefore, the synthesis of these complex structures and their biological study have been and remain an important task.

III. Synthetic approaches to lanostane derivatives with a functionally substituted side chain

One of the possible courses in the modification of lanosterol aimed at yielding biologically active derivatives is the introduction of oxygen-containing substituents into the side chain. There are a number of examples in the literature on this type of approach.

The introduction of oxygen substituents into the lanosterol molecule has been carried out without disruption of the C(18)-side chain using the existing 24(25)-double bond of 3β-hydroxylanosta-8,24-diene (I). An alternative route is degradation of the side chain to C_2-C_4 and its reconstruction.

1. Partial conversions of the side chain of lanosterol

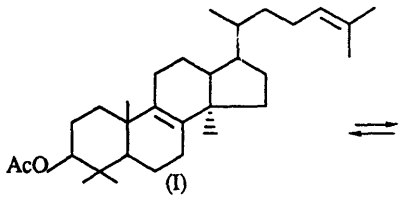
Oxidised sterols are potential inhibitors of the demethylation of lanosterol to cholesterol. It has been proposed that lanosterol derivatives with an oxidised side chain may be converted to metabolites that have the ability to inhibit the biosynthesis of cholesterol.[5,68] In order to study this problem, mono- and poly-oxidised derivatives of lanostane—22ξ-hydroxy-, 24-hydroxy-, 25-hydroxy-, and 26-hydroxy-lanosterols were synthesised and the following series of decreasing activity was drawn up: 26-hydroxy- > 25-hydroxy- > (24*R*)- and (24*S*)-hydroxy-24,25-dihydrolanosterol > (22*R*)- and (22*S*)-hydroxylanosterol.[5,68]

The synthesis of 25-alcohols is the simplest to carry out.[69] Several methods, such as direct hydration of the Δ^{24}-bond and the use of an intermediate 24,25-epoxide, have been developed.

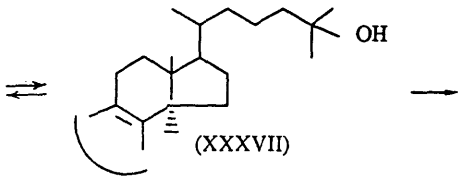
Hydration of the Δ^{24}-bond leading to 25-hydroxydihydrolanosterol was used by Rodewald and Jagodzinski[70–74] as a method for the synthesis of 25-alcohols and for selective protection of this bond for the isolation of analytically pure lanosterol from a mixture also containing dihydrolanoserol and dihydroagnosterol in a commercial product.

In fact, all methods for the isolation of pure lanosterol that were developed in the 1960s involve the selective addition of bromine to the side chain, crystallisation of the mxiture of bromides, and reduction of the resulting 24ξ,25-dibromo derivative with zinc in acetic acid to the 24-ene. These yield the required starting material in low yield. A method for selective hydration using a hydroxymercuration—demercuration reaction has been proposed. For this purpose a mixture was treated with mercuric acetate in a water–THF mixture[70] and the resulting organomercury compound was reduced with sodium borohydride. Separation on a silica gel column gave the analytically pure 25-alcohol (XXXVII) in quantitative yield. The Δ^{24}-bond may be regenerated as required by refluxing in a mixture of acetic anhydride and acetic acid. Thus, the total yield of pure 8,24-diene is more than 70% based on the commercial product. The same 25-alcohol (XXXVII) was obtained by low-temperature epoxidation of the Δ^{24}-bond, reduction with lithium aluminium hydride, and acetylation of the 3β-hydroxy group.

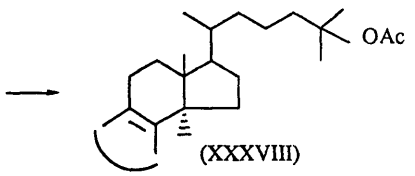
25-Hydroxylanosterol is itself of interest as a possible precursor of vitamin D analogue. The use of 25-hydroxylation as a means of protecting the double bond was investigated in the oxidation of the 8(9)-double bond of lanosterol to give seco derivatives.[71] It was shown that the alcohol is stable during oxidation with chromium trioxide and during alkali treatment. However, under acidic conditions dehydration in the side chain occurs. In order to prevent the occurrence of side reactions, the 25-hydroxy group in (XXXVIII) is acetylated with isoprenyl acetate. The 25-acetate is stable towards elimination in an acid medium.



(I)



(XXXVII)

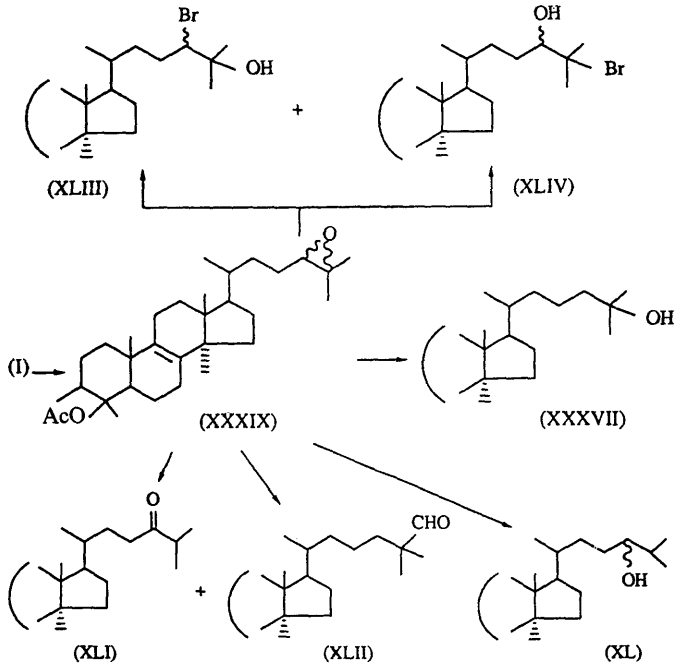


(XXXVIII)

The 25-alcohol is also formed on opening of the 24,25-epoxide (XXXIX) with HBr followed by removal of bromine. The absolute configuration of 24ξ,25-epoxylanostanes was established by converting them to the corresponding 24ξ-alcohols (XL) through reduction with diborane oxide and sodium borohydride, without affecting the C(24)–O bond. The configuration of the 24-alcohols was established by the method of Horeau.[75,76]

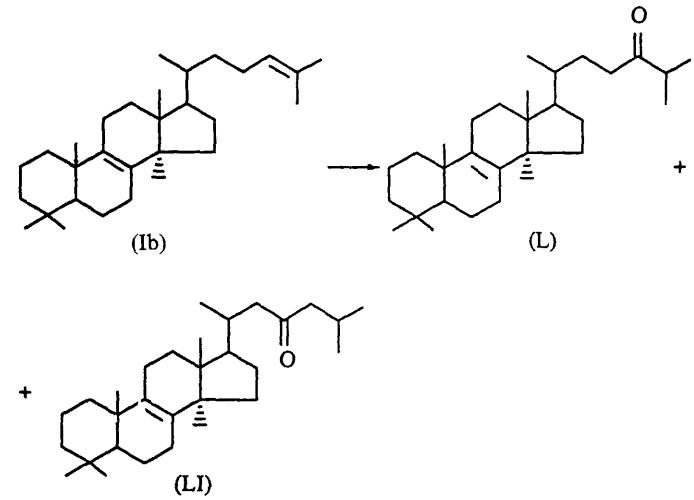
24-Hydroxy derivatives may also be obtained by isomerisation of the 24,25-epoxide on treatment with boron trifluoride etherate followed by reduction of the corresponding ketone (XLI).[77] However, in this reaction isomerisation of the oxide (XXXIX) to the

24-aldehyde (XLII) occurs together with formation of the 24-ketone (XLI).[77,78]

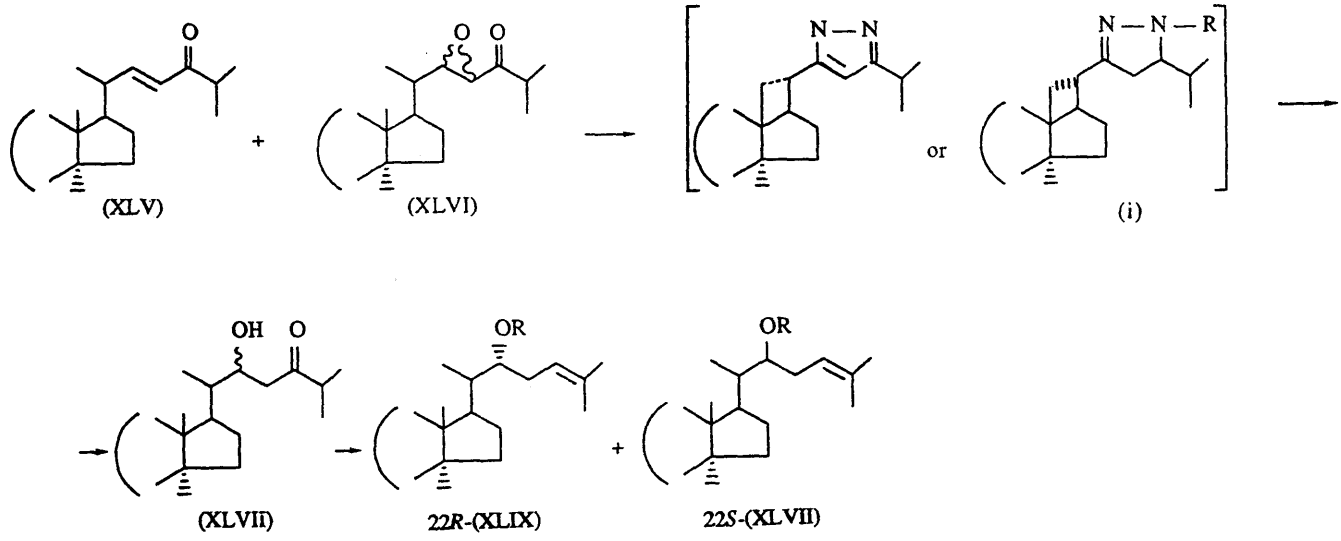


On dehydrogenation of the 24-ketone (XLI) using selenium dioxide,[77] the α,β-unsaturated 24-ketone (XLV) was obtained, albeit in low yield. It was epoxidised with hydrogen peroxide in an alkaline medium. The resulting predominant isomer of the 22,23-epoxyketone (XLVI), gave an intermediate of the type (i) when a Wolff–Kishner reduction was attempted, and this was then converted by decomposition of the diimide with alkali to give a mixture of C(22)-isomers of 22-hydroxy-24-ketosteroids (XLVII). Subsequent acetylation, reduction of the 24-keto group with sodium borohydride, dehydration on treatment with $POCl_3$, and hydrolysis gave (22*S*)- and (22*R*)-hydroxylanosterols (XLVIII) and (XLIX) in the ratio 2:1.[79]

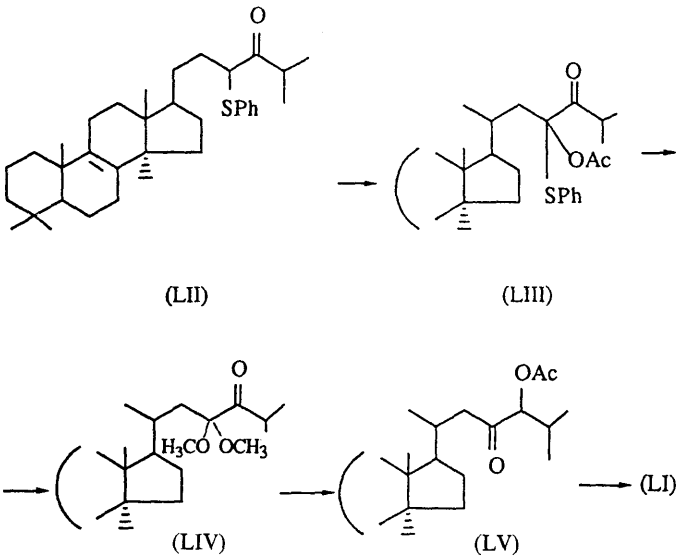
The 24-ketone of 3-desoxylanostane (L) was synthesised by Yamashita et al.[80] by hydroboration of the Δ^{24}-bond followed by oxidation of the resulting 24-alcohol. The authors found that, depending on the reaction conditions (hydroboration directly with diborane or with sodium borohydride in the presence of boron trifluoride etherate), very small quantities of the 23-derivative (LI) were formed together with the expected product. The mechanism of formation of 23-ketodihydro-3-desoxylanosterol (LI) is not clear; it was assumed to be a rearrangement product and it was detected using high-resolution liquid chromatography.



The presence of additional methyl groups at C(4) and C(14) in the lanostane skeleton compared to steroids results in a considerable steric effect on the reactivity of the α-carbon atoms relative to the 24-ketone in the side chain. The difficulty in enolisation at the C(23) position and its preferred route on C(25) has been attributed to this.[81] Numerous attempts to functionalise the 23-position of 24-keto-3-desoxylanostane proved unsuccessful. Regioselective sulphenylation at C(23) was achieved[82] in the case of the 24-ketolanostane (L) only under kinetically controlled conditions by the addition of (L), lithium isopropylamide, hexametapol, and THF



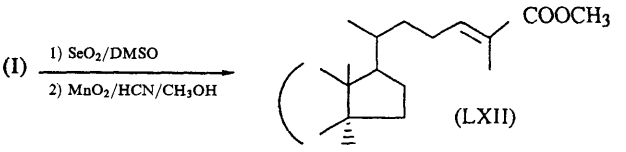
at −78 °C to a solution of diphenyl disulphide. The key sulphur-containing compound (LII) after treatment with lead tetraacetate was converted to a mixture of diastereomers at C(23) (LIII). Subsequent treatment with methanol and iodine gave the 23-dimethylacetal (LIV), and reduction of the 24-carbonyl group, removal of the acetal protecting group, and acetylation yielded the 24-acetoxy-23-ketone (LV). Hydrogenolysis of the 24-acetate using calcium in liquid ammonia gave the 23-ketolanostane (LI).



The successive introduction of hydroxy groups into the C(8)-side chain of lanosterol to obtain 26-hydroxy and 24,25,26-trihydroxy derivatives has been reported by Sato et al.[77,83] The 26-alcohol (LVI) was obtained by the allylic oxidation of lanosterol with selenium dioxide. Epoxidation of its acetoxy derivative (LVII) with peroxyacetic acid in the presence of concentrated sulphuric acid[84] gave the 24ξ,26-diacetoxy-25ξ-hydroxy derivative (LVIII), and hydrolysis of its acetoxy groups led to the formation of 24ξ,25ξ,26-trihydroxylanosterol (LIX). 24ξ,25-dihydroxylanosterol (LX) was obtained by reduction fo the 25-hydroxy-24-ketone (LXI) with sodium borohydride. The latter ketone was detected[77] amongst the products of the oxidation of lanosterol with the oxide system $KMnO_4-NaIO_4$. These polyoxidised lanosterol derivatives exhibited considerable anticholesterolemic activity.

The vicinal 24,25-diol (LX) is a convenient starting compound for the preparation of the 24-aldehyde, which is used to extend the side chain by the Wittig reaction.[85]

By applying more severe conditions in the allylic oxidation of lanosterol it is possible to obtain 26-acids of the polyporenic type (LXII).[4]



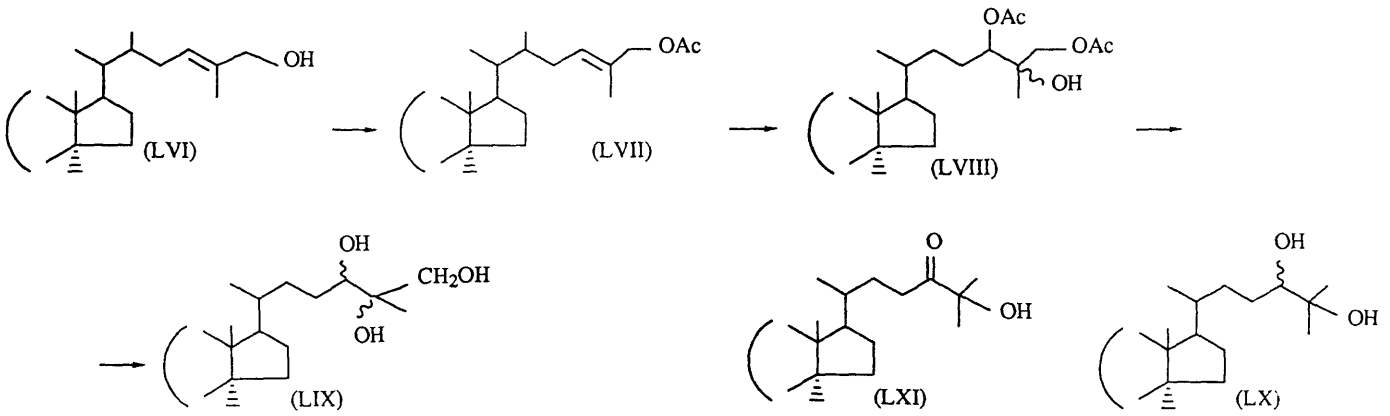
2. Chemical methods for degradation of the side chain of lanosterol

All the methods described in the literature for degradation of the side chain of lanosterol are essentially based on preparation of key 17(20)-, 20(22)-, and 22(23)-olefins. Their successive epoxidation, hydroxylation or direct ozonisation can give the required or intermediate epoxides, aldehydes or ketones (with a shortened side chain).

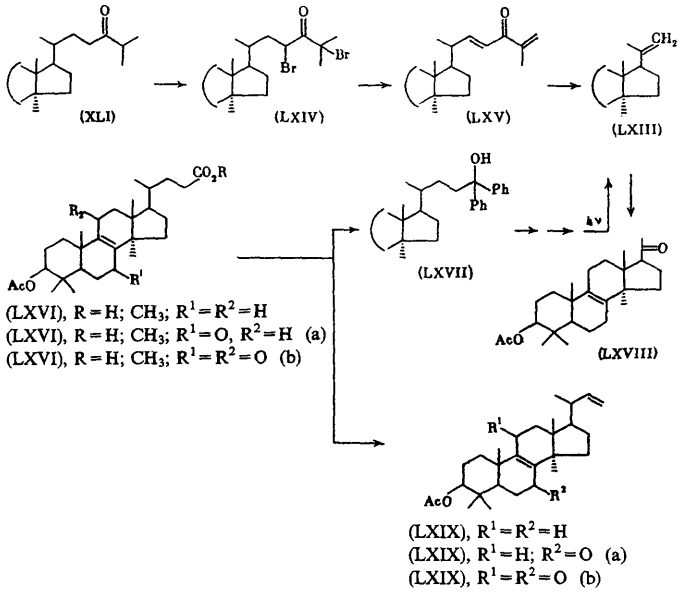
It should be noted straight away that degradation of the side chain of 3β-hydroxylanosta-8,24-diene is not always simple since by-products that are highly oxidised in the skeleton are often formed.

Several schemes for degradation of the side chain of lanosterol using chemical methods have been developed. These are all generally multistage processes and differ in the number of stages and chosen sequence of reactions, and the yields are low.

Thus, three main methods for the preparation of the 20(22)-olefin (LXIII) have been reported. The first (with the greatest number of stages and the least effective)[78] involves the bromination of the above-mentioned 24-ketolanosterol (XLI) to the dibromide (LXIV), its dehydrobromination, and subsequent irradiation. Another scheme[87] involves cleavage of the Δ^{24}-bond in lanosterol to the 24-acid (LXVI). Conversion of (LXVI) with phenylmagnesium bromide to the tertiary alcohol (LXVII), its subsequent isomerisation, and irradiation of the keto derivative afford (LXIII). The third,[88] most effective method of preparing the 20(22)-olefin (LXIII) involves the treatment of the 24-acid (LXVI) with carbonyldiimidazole followed by irradiation at λ_{max} 220 nm.[89]



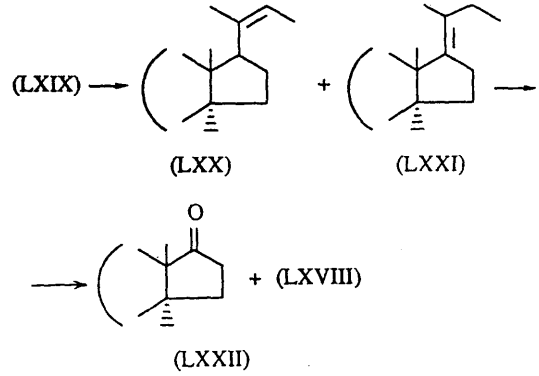
Hydroxylation of olefin (LXIII) accompanied by oxidation with periodic acid, gives the pregnenolone analogue (LXVIII) with a lanostane skeleton.[87]



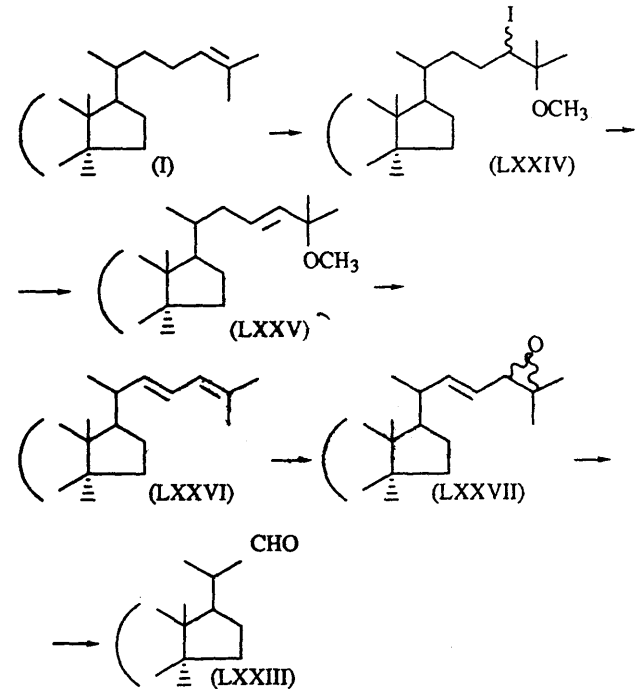
Key lanostane olefins with the double bond at a position other than 20(22) have been obtained by decarboxylation of the 24-acids. It was shown by Reshetova et al.[90] that the Δ^8-7-keto acid (LXVIa) and Δ^8-7,11-diketo acid (LXVIb) were formed in addition to the Δ^8-acid (LXVI) on oxidation of lanosterol by the method of Lumé–von Rudolf. Decarboxylation of these keto acids gave 24,25,26,27-tetranor-Δ^8-7-keto- and 24,25,26,27-tetranor-Δ^8-7,11-diketo-lanosta-8,22(23)-dienes (LXIX)–(LXIXb), which act as intermediates in the stereospecific introduction of oxygen functions into the lanosterol molecule.[91]

In two studies[77,92] the initially formed terminal 22(23)-olefin (LXIX) was isomerised on treatment with lithium in ethylenediamine to the 20(22)-olefin (LXX) and 17(20)-olefin (LXXI).

In most studies all of the olefins described were primarily used for preparing 17- or 20-ketolanostanes or aldehydes. However, ozonisation generally has low selectivity and gives mixtures of highly oxidised analogues of 17-ketones, 17-acids, etc.[77,92]



A more effective method than that of Sato and Sonoda[77] was degradation of the side chain of lanosterol to obtain the 22-aldehyde (LXXIII) by means of the electrophilic addition of methoxyiodide to the Δ^{24}-bond.[93] The subsequent elimination of hydriodic acid from (LXXIV) using potassium t-butoxide accompanied by the elimination of methanol on treatment with acetyl chloride and sodium iodide in a mixture of acetonitrile and methylene dichloride resulted in the formation of the 22,24-diene (LXXVI).[94] In a study of the ozonisation of this diene it was shown that both the C(22)- and C(24)-aldehydes are formed, with the latter in slight excess. Further ozonisation of the 24-aldehyde does not give the required 22-aldehyde. Consequently, the regioselective epoxidation of diene (LXXVI) was carried out with a complex of *m*-chloroperoxybenzoic acid and KF, which gave the 24,25ξ-monoepoxide (LXXVII). Ozonisation of the epoxyene (LXXVII) gave the 22-aldehyde (LXXIII) in high yield (~60%).



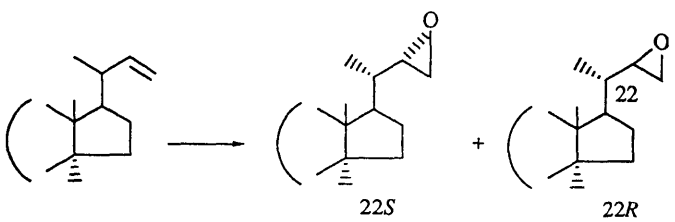
The lanostane derivatives with C_2- and C_3-side chains that are synthesised in this manner have been used in many studies in nucleophilic addition reactions to create novel polyfunctional lanosterol analogues.

3. Formation of oxygen-containing side chains in lanosterol

Many methods have been developed for the stereospecific building of polyfunctional side chains in steroid compounds.[95] However, as demonstrated in compounds simulating the *C/D* rings of lanostane,[96] these cannot be directly transferred and adapted to triterpenoids and 14α-methylsteroids, especially in those cases where the reactions occur in the vicinity of ring *D*, where the α-region is equally or more hindered than the β-region of the molecule. That is probably the reason for the comparatively small number of studies on building up the side chains. There are virtually no studies on the construction of side chains on 17-ketolanostanes

Methods for building the side chains of triterpenoids have been developed with terminal olefins[97] of the type (LXIX) and (LXIII), formed by decarboxylation of 24-acids (as described above), 20-ketones,[98] 22-aldehydes,[9,97] and 24-aldehydes[85,99] as starting materials.

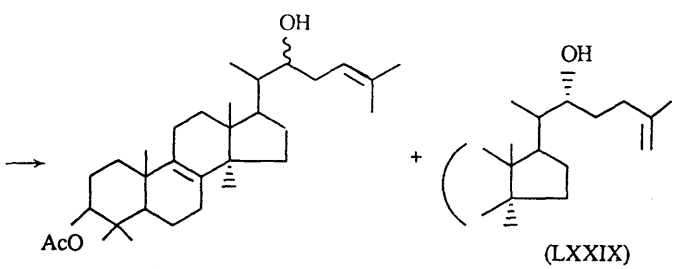
The strategy of Poyser et al.[97] for using 22,23-olefins involved their conversion to the corresponding epoxides and including the latter in a Grignard reaction with a suitable alkyl halide. However, it was demonstrated right at the beginning in studies on this route[97] that epoxidation with peroxyacids under normal conditions usually has an effect on the intraring 8(9)-double bond, with the result that a whole range of reaction by-products is formed. Therefore, the preparation of 22,23-epoxides (LXXVIII) was carried out via bromohydrins followed by dehydrobromination. This procedure, however, gave a mixture of isomeric (22*S*)- and (22*R*)-epoxides.



(LXIX) 22*S* (LXXVIII) 22*R*

Hence a Grignard reaction with isobutenylmagnesium bromide gave a mixture of (22*S*)- and (22*R*)-22-hydroxylanosterols (XLVIII) and (XLIX) that was difficult to separate. Furthermore, the terminal olefin (LXXIX)—a product of isomerisation of the double bond during reaction—was formed. An attempt to oxidise the mixture of 22-alcohols to the ketone followed by reduction with lithium aluminium hydride did not have favourable results.[97]

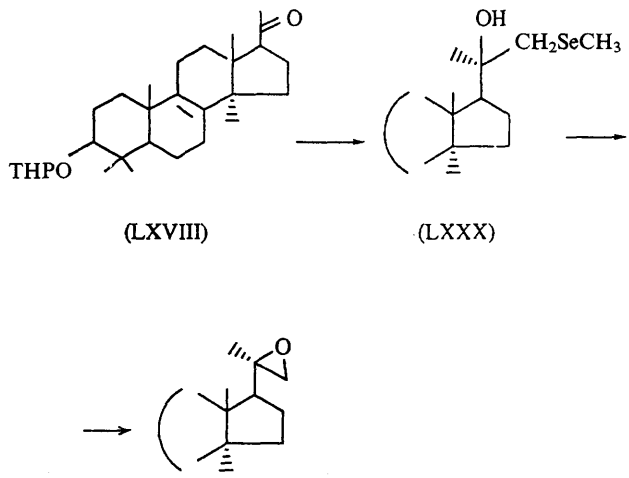
(LXXVIII) →



(XLVIII) + (XLIX) (LXXIX)

Epoxides are known to react with organomagnesium reagents in different ways. Depending on the nature of the reagents and the reaction conditions they give alcohols formed by nucleophilic ring opening (with no change in configuration, as described above) or rearranged alcohols formed by attack of the reagent on an aldehyde intermediate.

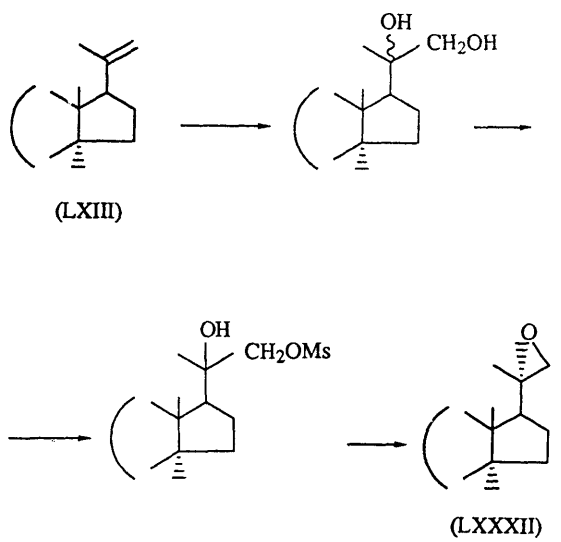
Thus, 22-hydroxy-substituted lanosterols may be obtained from 22-aldehydes that are formed, as we have described above, from a 20(22) olefin or during rearrangement of 20(22)-oxides. At the same time the conditions for stereospecific formation of a chiral centre at C(20) can be selected. The problem of stereoselective synthesis of lanostane 20(22)(*R*)- and (*S*)-epoxides was solved by Schauder and Krief[98] through the use of the reaction of 3-tetrahydropyranyloxy-20-ketolanostane (LXVIII) with methylselenomethyllithium. The resulting 20-alcohol (LXXX) on treatment with base gave the (20*R*)-epoxide (LXXXI) in 60% yield.



(LXVIII) (LXXX)

(LXXXI)

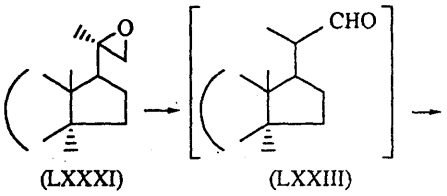
At the same time the (20*S*)-epoxide (LXXXII) was obtained from the 20(22)-olefin (LXIII) by hydroxylation of the latter with osmium tetroxide, mesylation of the 22-hydroxy group, and elimination of methanesulphonic acid on treatment with alkali:



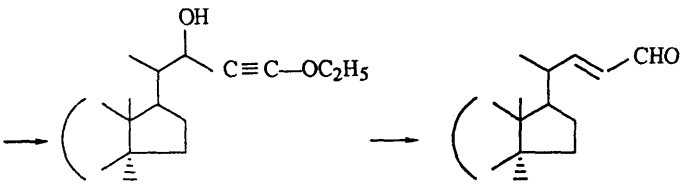
(LXXXII)

Schauder and Krief[98] made a detailed study of the synthesis of 22-alcohols that was stereoselective with respect to C(20) by varying the conditions of a Grignard reaction with ethoxyethynylmagnesium bromide. Moreover, this reagent was used instead of isobutenylmagnesium bromide so as to avoid the formation of regioisomeric by-products (with respect to the position of the double bond in the side chain). It was found that the solvent plays a decisive role in a controlled reaction of the magnesium salt with epoxides.

By conducting the reaction in ether at 20 °C, high yields of the acetylenic alcohol (LXXXIII) (via the 22-aldehyde) were achieved.



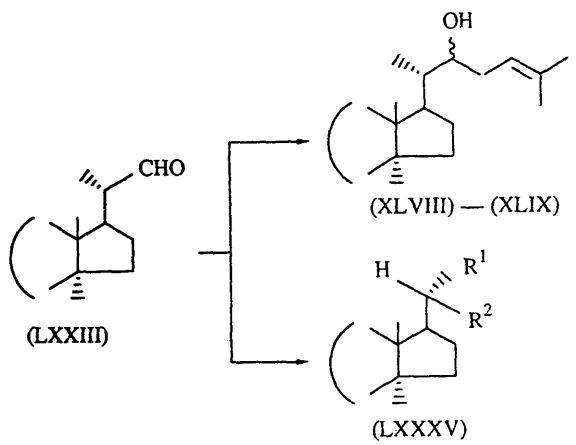
(LXXXI) (LXXIII)



(LXXXIII) (LXXXIV)

Reduction of the ynol ether (LXXXIII) with lithium aluminium hydride and subsequent acid hydrolysis of the resulting γ-hydroxy-enol ether gave the (*E*)-α,β-unsaturated aldehyde (LXXXIV), consisting mainly of the (20*R*)-isomer (98%), which is a precursor in the synthesis[98] of (20*R*)-lanosterol (after hydrogenation of the aldehyde and its Wittig reaction with isopropylidenetriphenylphosphorane). Schauder and Krief[98] obtained (20*S*)-lanosterol with the same sequence of reactions. This scheme appears to have realised its full potential in the synthesis of functional derivatives of (20*R*)- and (20*S*)-lanostane.

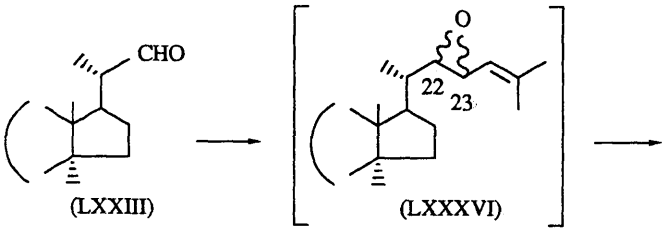
In order to build up the side chain, the 22-aldehyde may be obtained by ozonisation of the olefin or by hydroboration[99] followed by mild oxidation. The use of (LXXIII) in a Wittig reaction[99] or reaction with the Grignard reagent from 4-chloro-2-methylbut-2-ene provided the authors with novel functional lanostane side chains.



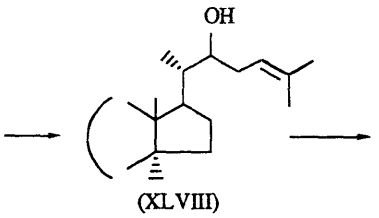
(LXXIII) (XLVIII) — (XLIX) (LXXXV)

$R^1 = CH_3$, $R^2 = C_6H_{11}$, CH_2OH, $CH=CH_2$, $(CH_2)_2CHO$, etc.

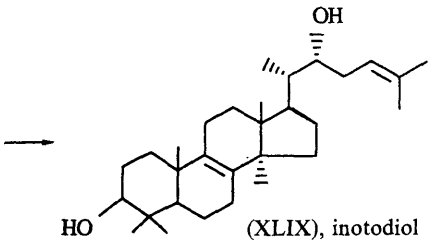
However, a detailed study[93] of the reaction conditions with the 22-aldehyde for formation of a 22-hydroxy-$\Delta^{24(25)}$ residue showed that neither a Grignard reagent nor a sulphur ylide can be used successfully. As was mentioned above, the dimethylallyl residue undergoes rearrangement. If an α-anion of a sulphone is condensed with aldehyde (LXXIII), a sulphinate residue is not eliminated in the latter. Amann et al.[93] solved this problem by using an arsenic ylide obtained from (3-methylbut-2-enyl)triphenylarsonium tetrafluoroborate[100] and n-butyllithium. Condensation of this ylide with the 22-aldehyde (LXXIII) gave the 22,23-epoxy-24(25)-ene intermediate (LXXXVI), and it was reduced with lithium triethylborohydride stereoselectively to give (22*S*)-22-hydroxylanosta-8,24-diene (XLVIII). In order to invert the 22*S* configuration to 22*R*, which is characteristic of the biologically active inotodiol, Amann et al. used the method of Corey—mesylation in pyridine followed by treatment with KO_2 and 18-crown-6 in a mixture of DMF and DMSO. Thus, the overall yield of inotodiol by this synthetic route was about 30%.



(LXXIII) (LXXXVI)



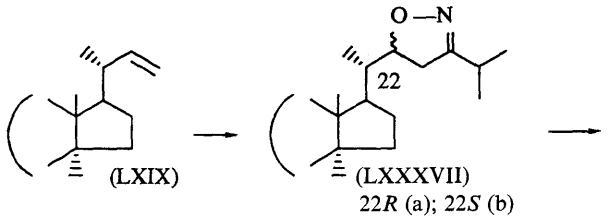
(XLVIII)

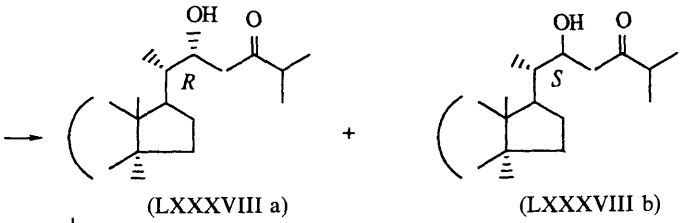


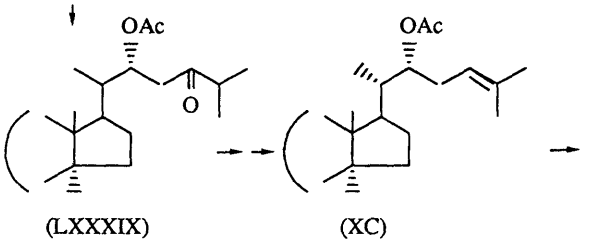
(XLIX), inotodiol

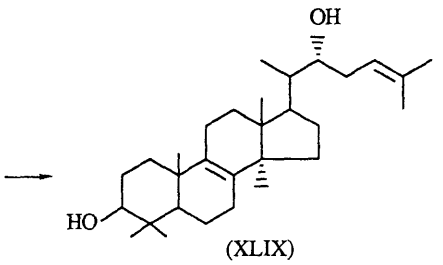
Key intermediates in the stereospecific introduction of oxygen functional groups into the C(22)-position of lanosterol were the 22(23)-terminal olefins (LXIXa, b), obtained by decarboxylation of lanostane 24-acids, by involving them in a 1,3-dipolar cycloaddition reaction.[101,102] Cleavage of the isopropylisoxazolinolanostanes (LXXXVIIa, b) in an atmosphere of hydrogen in the presence of boric acid after resolution of the 22-epimers followed by acetylation provided a route to (22*R*)-3β,22-diacetoxylanost-8-en-24-one (LXXXIX). This isomer was successfully used in a highly effective novel stereoselective synthesis of inotodiol (XLIX),[101–103] which differs from that described above by virtue of its simple operation, greater accessibility of reactants, and lower number of stages. The

yield of required product was about 26% and could be increased if the Corey isomerisation of the (22*S*)-isomer was used.[93]



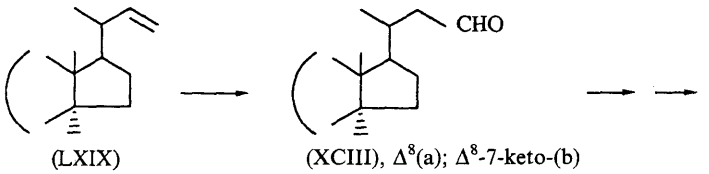


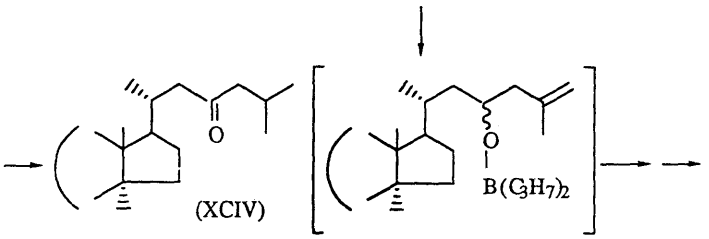


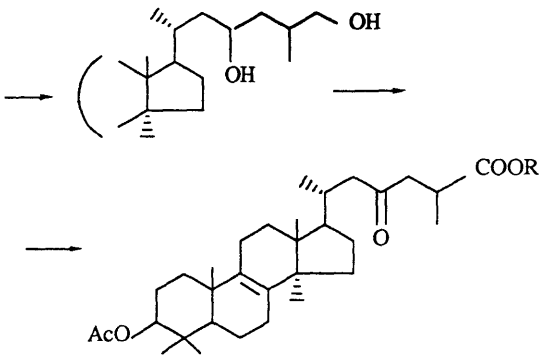


Use of the same diacetate (LXXXIX) in a Reformatsky reaction with ethyl bromoacetate followed by dehydration gave the δ-lactone of (22*R*)-3β-acetoxy-22-hydroxylanosta-8,24(31)-dien-32-oic acid (XCII), which was the first lanostane analogue of the sex hormone of *Achlya* marine fungi[101]

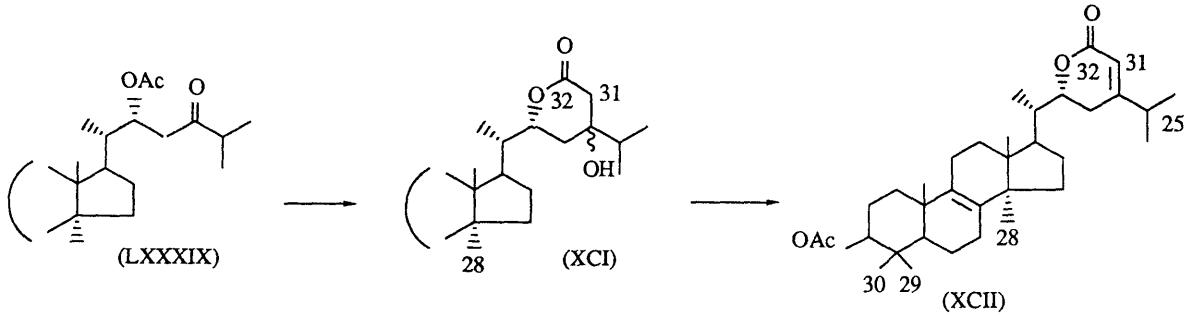
Conversion of 22(23)-olefins to 23-aldehydes (XCIIIa, b) by hydroboration[91] followed by oxidation made the 23-aldehyde (XCIIIa) a convenient starting material for the synthesis of 23-keto-lanostanes (potential cardiotonics) (XCIV).[102] The first two-stage synthesis of 3β-acetoxylanost-8-en-23-keto-24-oic acid (XCV)—a ganoderic acid analogue—was achieved by reaction of allylborane with the 23-aldehyde (XCIIIa) together with the subsequent hydroboration and oxidation in a single stage.[91]





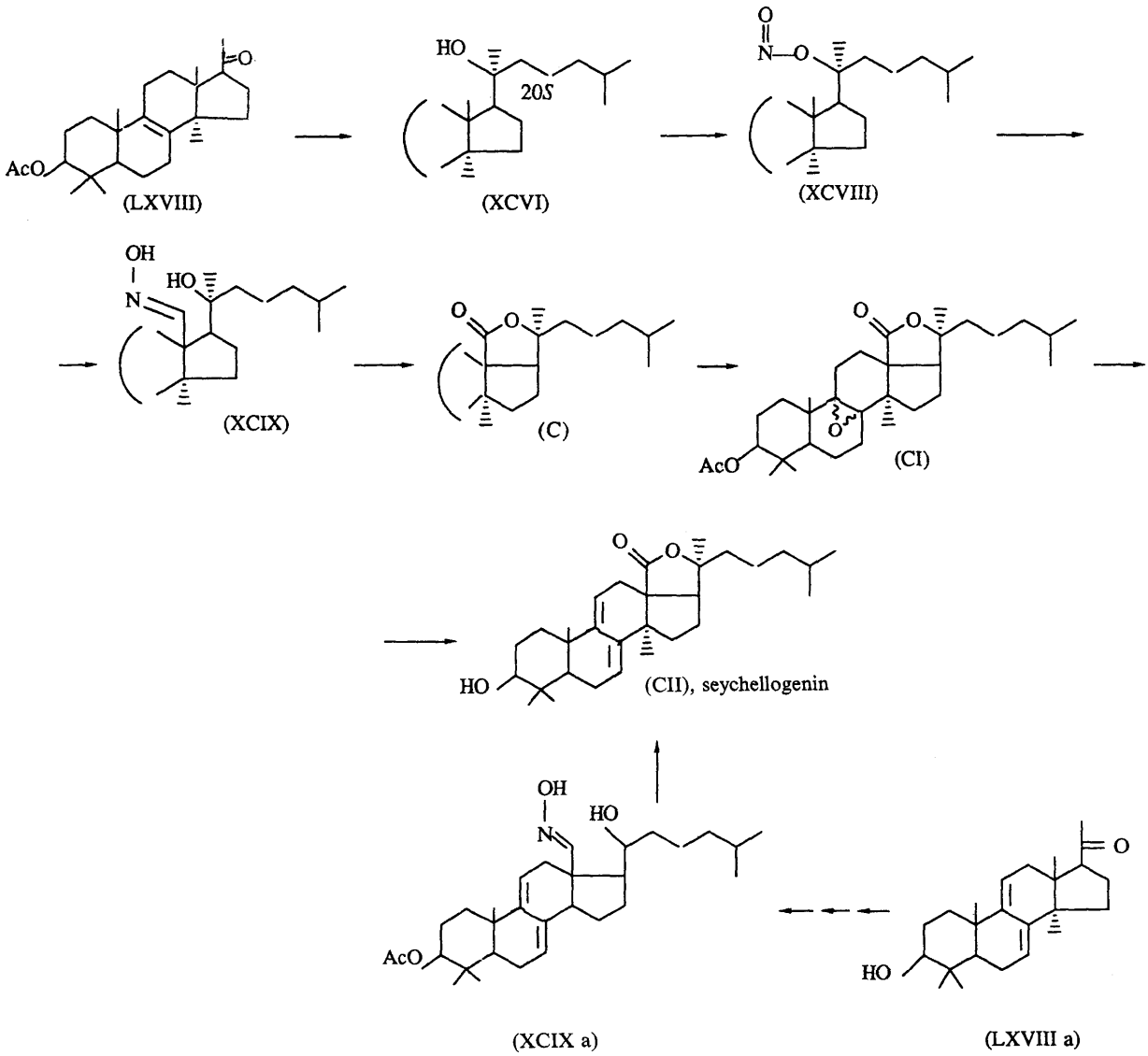


Seychellogenins have various useful biological properties. In view of their limited availability from natural sources, considerable attention has been given to their synthesis. After methods had been developed for dehydrating the side chain of lanosterol and converting it to the 20-ketone (LXVIII),[78] attempts to functionalise the 18-methyl group were initiated. The original scheme for the

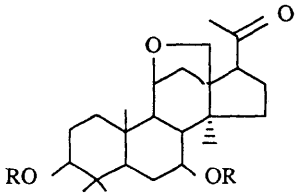


synthesis of seychellogenin[104] involved conversion of the 20-ketone (LXVIII) by a Grignard reaction with isohexylmagnesium bromide to give (20*S*)-dihydrolanost-8-ene, which was converted on treatment with nitrosyl chloride to the nitroso derivative (XCVIII). Photoisomerisation of the latter to the oxime (XCIX) occurred with a yield of 8%, and this governed the low overall yield of (CII) (2%), which included oxidation to the γ-lactone (C), epoxidation of the interring double bond, and acid hydrolysis of the 8,9-epoxide (CI) to the 7(8),9(11)-diene system of (CII). A variant of this scheme used the 20-ketone of 3-hydroxylanosta-7,9-diene (LXVIIIa) as a starting compound.

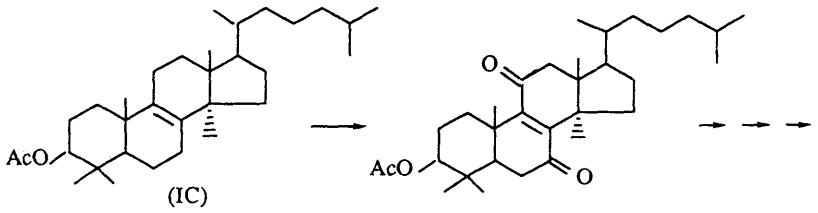
The aim of the second, fundamentally different approach[6,105] was to improve the yield of the required seychellogenin (CII). At the first stage of the synthesis, commercial lanosterol containing a certain amount of 24,25-dihydrolanosterol was hydrated, and pure dihydrolanosterol (IC) was thus obtained. After oxidation of the allylic methylene groups in the rings at C(7) and C(11) with chromium trioxide, the central double bond was reduced using zinc in acetic acid. Further reduction with lithium aluminium hydride followed by selective acetylation gave the 3,7-diacetoxy-11-hydroxylanostane (CIII), which was converted by photoreaction with lead tetraacetate in the presence of iodine to the cyclic ether (CIV). Cleavage of the latter with boron trifluoride etherate in anhydrous acetic acid, hydrolysis with KOH, and hydrogenation of the 9(11)-double bond gave the compound (CVI) with the required *trans* linkage of the *B*/*C* rings. An important subsequent feature was selective acetylation, so that the hydroxyl group at C(18) would remain free. After this problem was solved and (CVII) was obtained, a photoreaction was carried out under the conditions described above to give the cyclic ether (CVIII), oxidation of which with ruthenium tetroxide, hydrolysis, and further selective acetylation gave compound (CX). After dehydration and oxidative introduction of the diene system using mercuric acetate, the acetate of seychellogenin (CII) was obtained in satisfactory yield. The authors of the above synthesis of seychellogenin believe that the 20-ketone having the structure (CXII) would be an excellent precursor in the



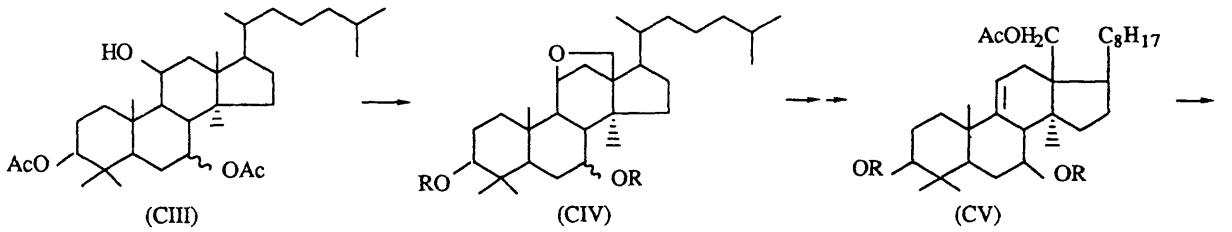
synthesis of other holothurigenins. It is simple to obtain by a combination of the two synthetic methods[104,105] with degradation of the side chain of lanosterol.



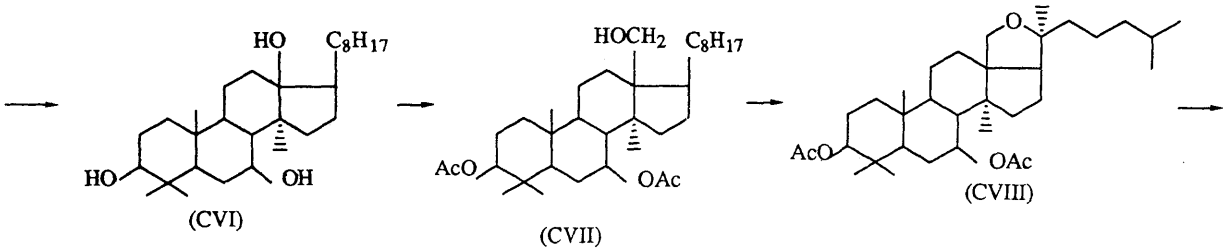
(CXII), R = Ac



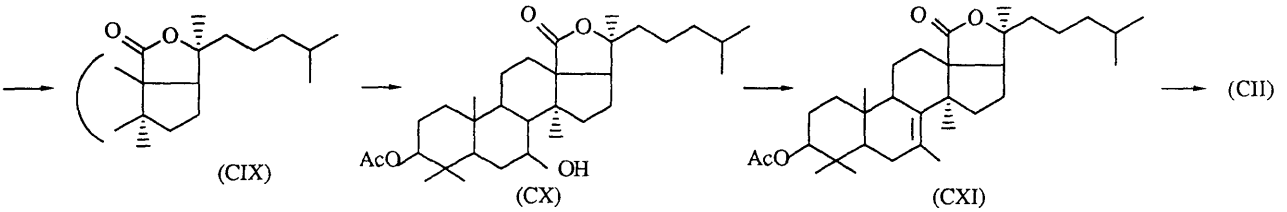
(IC)



(CIII) (CIV) (CV)



(CVI) (CVII) (CVIII)



(CIX) (CX) (CXI) (CII)

The molecule of (CXII) can be converted to the 17α-hydroxy derivative as well as to a compound with a natural 9(11)-double bond after cleavage of the cyclic ether. Although most of the intermediates in these syntheses were converted to hemisuccinates in order to impart greater solubility to these molecules in water, not one of the derivatives exhibited the same degree of activity (toxicity) as the natrual glycosides.[7] Habermehl and Kirsch[5] proposed in future to modify the molecule of natural seychellogenin in this manner in order to study the structure–function relationship.

Thus, it may be concluded from an analysis of the literature that nature has an abundance of various polyfunctional lanosterol derivatives that possess therapeutic properties that are useful for human medicine. Lanosterol itself is an inexpensive, available product that is isolated from the wool "suint" together with cholesterol. This makes it an attractive source in the preparation of synthetic triterpenoids and steroids having hydrophobic alkyl groups in the molecule which play an important part in the interaction with receptors. Therefore considerable opportunities are being opened up in the chemistry of lanosterol for research both into novel reactions and in the application of established methods in steroid chemistry for deriving biologically active polyfunctional structures.

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