Photochemical Transformations. Part 34.† Structures of the Toxisterols$_2$

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The isolation and identification of twelve new compounds formed on photolysis of ergosterol in ethanol are described. These include four C_{19} and C_{20} hydrocarbon photofragments derived by loss of ring A, two related vinyl ethers, and six toxisterols$_2$. Three of the previously elusive toxisterols$_2$ have a novel spiro-steroid structure isomeric with ergosterol. The other three are ethers containing the dihydrotachysterol chromophore. Photolysis of ergosterol in cyclohexane also gave a disteroid C_{40} hydrocarbon; a structure is suggested.

CALCIUM metabolism in animals is controlled by 1α,25-dihydroxycholecalciferol (I), a hormone produced by the hydroxylation in the liver and kidneys of ingested cholecalciferol (II).[1] Recognition of the importance of these hydroxylations has renewed interest in structure–activity relationships in the vitamin D field.[2] During the photolytic preparation of ergocalciferol (IVa) from ergosterol (IIIa), several ill-defined compounds have been detected. Over-irradiation of ergosterol (IIIa) has been reported to give compounds characterised by a chromophore at 250 nm,[3] toxicity,[4] and an ability to raise serum calcium levels.[5] Westerhof and Keverling Buisman[6] isolated three crystalline compounds, ' toxisterols$_2$ ‡ A, A', and B, ' but were unable to suggest structures. We have found that the irradiation of ergosterol (IIIa) in quartz apparatus with a high-pressure mercury arc in methanol, ethanol, or propan-2-ol gives a mixture of compounds with intense u.v. absorption at 242, 252, and 262 nm (Figure 1). The yield of this material was increased in the presence of citric acid. However, irradiation in cyclohexane or diethyl ether did not produce the toxisterol absorption centred at 250 nm. Photolysis in anhydrous t-butyl alcohol resembled that in cyclohexane or diethyl ether. In the presence of water the 242, 252, and 260 nm absorption was again obtained.

The crude resin from the photolysis of ergosterol §

† Part 33, D. H. R. Barton, M. Bolton, P. D. Magnus, and P. J. West, *J.C.S. Perkin I*, 1973, 1580.

‡ Subscripts 2 and 3 refer to compounds derived from ergosterol and cholesterol, respectively.

§ Chromatography of ergosterol showed the absence of any hydrocarbons, ethers, or toxisterols.

[1] D. E. M. Lawson, P. W. Williams, and E. Kodicek, *Nature*, 1969, **222**, 171; D. E. M. Lawson, D. R. Fraser, E. Kodicek, H. R. Morris, and D. H. Williams, *ibid.*, 1971, **230**, 228; M. F. Holick, H. K. Shnoes, H. F. DeLuca, T. Suda, and R. J. Cousins, *Biochemistry*, 1971, **10**, 2799; G. Jones, H. K. Schnoes, and H. F. DeLuca, *ibid.*, 1975, **14**, 1250.

[2] W. H. Okamura, A. W. Norman, and R. M. Wing, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 4194; D. Procsal and A. W. Norman, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1975, **34**, 894.

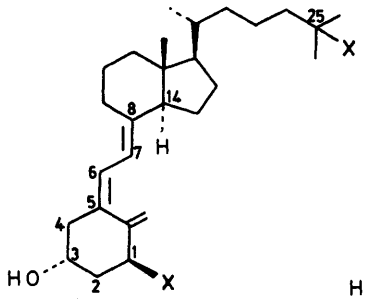
[3] R. A. Morton, I. M. Heilbron, and E. D. Kamm, *J. Chem. Soc.*, 1927, 2000; R. Pohl, *Nachr. Ges. Wiss. Göttingen Mathphysik Klasse III*, 1927, 185.

[4] F. Lacquer and O. Linsert, *Klin. Wochschr.*, 1933, **12**, 753 (*Chem. Abs.*, 1936, **30**, 2326).

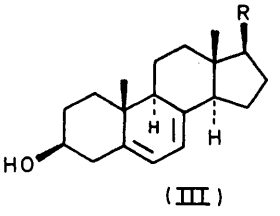
[5] G. M. Sanders, J. Pot, and E. Havinga, *Progr. Chem. Org. Nat. Products*, 1969, **27**, 131.

[6] P. Westerhof and J. A. Keverling Buisman, *Rec. Trav. chim.*, 1956, **75**, 1245.

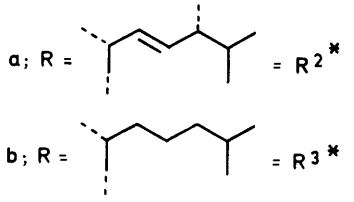
(IIIa) in ethanol was separated by repeated chromatography. The least polar fraction (1) was separated into a hydrocarbon (1.1) ¶ and an ether (1.2) fraction.



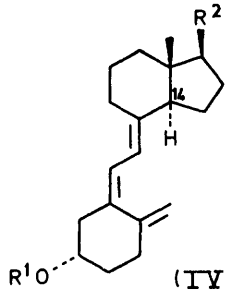
(I) X = OH
(II) X = H



(III)

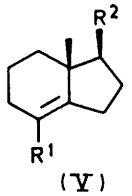


* R^2 subsequently refers to the ergosterol and R^3 to the cholesterol side chain.



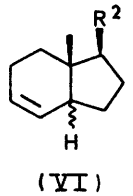
(IV)

a; R^1 = H
b; R^1 = $CO{\cdot}C_6H_3(NO_2)_2$-3,5



(V)

a; R^1 = Me
b; R^1 = H



(VI)

a; 14α-H
b; 14β-H

Chromatography of the hydrocarbon fraction on silver nitrate impregnated silica gave five pure olefins, (1.1.1) and (1.1.3—6). Spectral data showed all the compounds to contain intact rings C and D and side chains. Analysis, mass spectra, and chemical transformations (see below), showed that (1.1.1) had structure (Va), (1.1.3) (Vb), (1.1.4) (VIa), and (1.1.6) (VIIa).[7] Compound (1.1.5) was clearly a C_{20} hydrocarbon but its structure was not elucidated. Fraction (1.1.2) contained C_{21} fragments, but pure compounds were not isolated from it. The structures of the dienes were confirmed by synthesis from ergocalciferol (IVa). Oxidation of ergocalciferol (IVa) with permanganate gave 'Grundmann's ketone' (VIIIa),[8] isolated by chromatography or as the derived semicarbazone (VIIIb). The reaction of methylenetriphenylphosphorane with the ketone (VIIIa) gave the diene (VIIa) (74%), identical with fraction (1.1.6). Acid-catalysed isomerisation of the diene (VIIa) in aqueous tetrahydrofuran gave the more stable tetrasubstituted diene (Va) in quantitative yield. This structure was

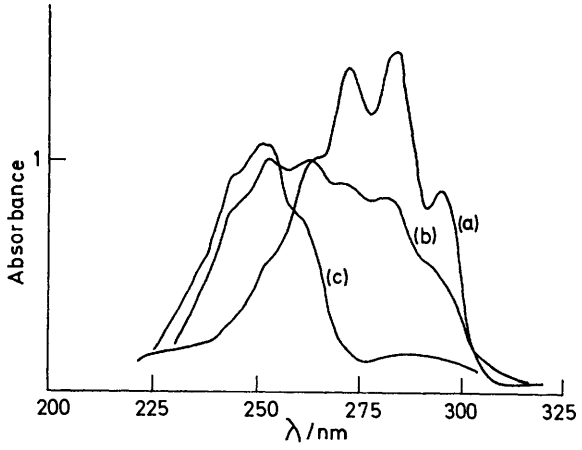


FIGURE 1 Photolysis of ergosterol (IIIa) in ethanol: u.v. spectra (a) before irradiation, (b) after 8 h 40 min, (c) after 26 h 15 min; initial [ergosterol] 1.26×10^{-4}M

assigned on the basis of the absence of vinylic signals in the n.m.r. and i.r. spectra (other than 22- and 23-H), the downfield shift in the n.m.r. spectrum of the 13-methyl signal (τ 9.43 to 9.13) and the presence of a vinyl methyl signal (τ 8.48). The diene (Va) was identical with fraction (1.1.1).

Reduction of the ketone (VIIIa) with sodium borohydride in tetrahydrofuran–dioxan–water gave the expected 8β-alcohol (IXa) as the major (84%) product. The formulation (IXa) was consistent with the shift of the 13-methyl n.m.r. signal to lower field (τ 9.32 to 9.04), the $W_{\frac{1}{2}}$ value of the 8α-proton signal (6 Hz), and conversion into the known 3,5-dinitrobenzoate (IXb).[9] Dehydration of the alcohol (IXa) with methanesulphonic anhydride in pyridine gave the expected diene (Vb), identical with fraction (1.1.3).

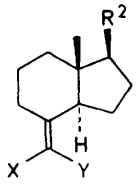
A Chugaev reaction on the dithiocarbonate (IXc) derived from the alcohol (IXa) gave diene (VIa) by a *syn*-elimination. The product was identical with fraction (1.1.4).

Initially fraction (1.1.5) was thought to have structure

¶ Fractions from chromatography are denoted by an integer increasing with polarity on t.l.c. Subfractions are denoted by a second integer, *etc.*

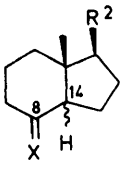
[7] A. G. M. Barrett, D. H. R. Barton, R. A. Russell, and D. A. Widdowson, *J.C.S. Chem. Comm.*, 1975, 102.
[8] A. Windaus and W. Grundmann, *Annalen*, 1936, **524**, 295.
[9] H. H. Inhoffen, G. Quinkert, S. Schültz, G. Friedrich, and E. Tober, *Chem. Ber.*, 1958, **91**, 781.

(VIb), and the synthesis of (VIb) was therefore undertaken. Epimerisation of ' Grundmann's ketone ' (VIIIa) under acidic or basic conditions gave the ketone (VIIIc),[10] reduction of which with sodium borohydride gave two alcohols (IXd and e), characterised as their 3,5-dinitrobenzoates (IXf and g). Since the *cis*-perhydroindane system is flexible, and therefore configuration does not imply conformation, (IXd and e) could not be distinguished by n.m.r. spectroscopy. Dehydration (methanesulphonic anhydride–pyridine–toluene) of the alcohol mixture (IXd and e) gave the diene (Vb) as the major and the diene (VIb) as the minor product.



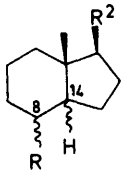
(VII)

a; X = Y = H
b; X = H, Y = OEt
c; X = OEt, Y = H



(VIII)

a; X = O; 14α-H
b; X = N·NH·CO·NH$_2$; 14α-H
c; X = O; 14β-H
d; X = N·NH·SO$_2$·C$_6$H$_4$Me-4; 14α-H
e; X = N·NH·SO$_2$·C$_6$H$_4$Me-4; 14β-H
f; X = N—···(dimer)



(IX)

a; 14α-H, 8β-OH
b; 14α-H, 8β-O$_2$C·C$_6$H$_3$(NO$_2$)$_2$-3,5
c; 14α-H, 8β-O·CS·SMe
d; 14β-H, 8β-OH
e; 14β-H, 8α-OH
f; 14β-H, 8β-O$_2$C·C$_6$H$_3$(NO$_2$)$_2$-3,5
g; 14β-H, 8α-O$_2$C·C$_6$H$_3$(NO$_2$)$_2$-3 5

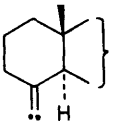
The assignment of structure (VIb) was consistent with the 13-methyl signal at τ 9.13 (*cf.* 14α-epimer, τ 9.30 [11]), the vinylic signals in the n.m.r. (τ 4.47) and i.r. (796 cm^{-1}) spectra, and negative optical rotation. Although fraction (1.1.5) and the diene (VIb) ran concurrently in chromatography, the structures were clearly different.

An attempt to prepare the diene (VIa) by the reaction of butyl-lithium and the 4-tolylsulphonylhydrazone (VIIId) gave the 14β-isomer (VIb) as the major product. Epimerisation must have taken place during the preparation of (VIIId). This was consistent with the variable m.p. and rotation of this compound. The reaction of the epimeric ketone (VIIIc) with 4-tolyl sulphonylhydrazine and subsequently with methyllithium gave the diene (VIb) as the only hydrocarbon. The mass spectra of the 4-tolylsulphonylhydrazones (VIIId and e) showed a variable-intensity peak at *m/e* 548. Accurate mass measurement indicated this to be due to the azine (VIIIf). That the tolylsulphonylhydrazone (VIIId) gave satisfactory analytical figures suggested that disproportionation was occurring in the mass spectrometer.

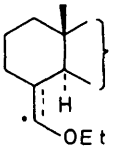
Chromatography of fraction (1.2.2) gave *inter alia* two vinyl ethers, (1.2.2.1) and (1.2.2.2). Although the two isomers could not be distinguished, spectral and analytical data supported structures (VIIb and c). The reaction of ' Grundmann's ketone ' (VIIIa) with ethoxymethylenetriphenylphosphorane gave compounds (VIIb and c), identical with the isolated compounds. Although no other compounds in the ether fraction were completely characterised, fraction (1.2.1.1) was probably a vinyl ether. The intractable compounds remaining all gave yellow colours with 2,4-dinitrophenylhydrazine in methanol–sulphuric acid.

The fragmentation of olefins in vapour phase photolysis has been reported.[12] Although not a major pathway, the mechanism of this process is of interest. The formation of the vinyl ethers (VIIb and c) suggests the intermediacy of species such as the carbene (X), the radical (XI), or the vinyl cation (XII). Photolysis of ergosterol in ethanol under oxygen gave little or no fragmentation (t.l.c.).

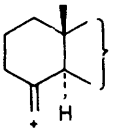
Separation of fraction (2.1) gave two toxisterols$_2$ (D and E) with $\lambda_{max.}$ 243 (ε 22 000), 252 (26 000), and 262 nm (18 000 and 19 000). Both D and E were isomeric with ergosterol (IIIa) (mass spectra and analyses), contained the dihydrotachysterol chromophore (XIII) (u.v. and



(X)



(XI)



(XIII)

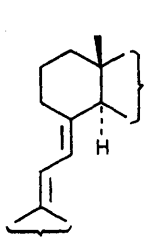
n.m.r. spectra), did not possess a hydroxy-group (polarity on t.l.c. and i.r. spectra), and exhibited a low field 10-methyl n.m.r. signal. Formation of the isomeric cyclic 3,10-ethers (XIV) is consistent with the spectral data.

Toxisterol$_2$ D on reaction with 3-chloroperbenzoic acid gave a crystalline monoepoxide. The constitution and configuration of this epoxide was determined as (XV) by *X*-ray crystallography; [13] hence toxisterols [2] D and E were assigned structures (XIVa and b), respectively.

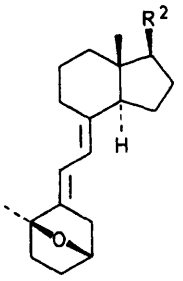
Toxisterols$_2$ A—C were isolated from fractions (2.2—4)

[10] K. Dimroth and H. Jonsson, *Ber.*, 1941, **74**, 520.
[11] L. M. Jackman and S. Sternhell, ' Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon Press, London, 1969, p. 288.
[12] R. Srinivasan, *J. Amer. Chem. Soc.*, 1960, **82**, 5063.
[13] A. G. M. Barrett, D. H. R. Barton, R. A. Russell, P. F. Lindley, and M. H. Mahmoud, *Chem. Comm.*, 1966, 659.

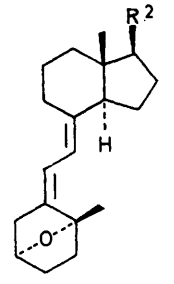
as the 3,5-dinitrobenzoate, free sterol, and benzoate, respectively. Toxisterol$_2$ A 3,5-dinitrobenzoate was identical (m.p.; i.r. and u.v. spectra) with ' toxisterol$_2$ A 3,5-dinitrobenzoate ' isolated by Westerhof and Keverling Buisman.[6] The three toxisterols [2] were alcohols isomeric with ergosterol (mass spectra, analyses, i.r. spectra, and esterification), each with a single $\lambda_{max.}$ [250—253 nm (ε 17 000—22 000)], an intact side chain, and a 13-methyl group (n.m.r. and i.r. spectra). ^{13}C N.m.r. spectra, which will be described elseshere, showed that all three toxisterols [2] contained the heteroannular diene system –CH=CH–C=CMe and each had an ' extra ' quaternary carbon atom (δ 54.11, 52.74, and 53.21, respectively). The formulation of the three spirosteroids as (XVI) differing only in configuration at C-4 and C-8 agreed with all spectral data. The nuclear Overhauser effects between the 7-proton and the 13-methyl group and $J_{3,4}$ values established their respective configurations. Toxisterol$_2$ A 3,5-dinitrobenzoate exhibited a strong Overhauser effect but toxisterols$_2$ B and C benzoate did not. In toxisterol$_2$ A 3,5-dinitrobenzoate the 3α-proton (τ 4.71) was coupled to two *anti*-protons (J 10 Hz) and one *syn*-proton (J 2.5 Hz). The 4-proton (τ 7.00) was coupled to one *anti*-proton (J 10 Hz) and thus was β-oriented. By comparison of the $J_{3\alpha,4}$ and $W_{\frac{1}{2}}$ values, the 4-proton was found to be α in toxisterol$_2$ B and β in toxisterol$_2$ C. Thus the three toxisterols$_2$ were assigned structures (XVIIa), (XVIII), and (XIXa), respectively. An X-ray crystallographic study of toxisterol$_2$ A 3,5-dinitrobenzoate confirmed the structure (XVIIb).[14,15] The configurational assignments at C-8 were confirmed by dehydration with hydrogen chloride in chloroform. Toxisterol$_2$ A (XVIIa) gave an aromatic compound (XX), the structure of which was consistent with spectral and analytical data. Toxisterols$_2$ B (XVIII) and C (XIXa) both gave the isomeric aromatic compound (XXI). A third crystalline hydrocarbon was obtained during ^{13}C n.m.r. measurements on toxisterol$_2$ C benzoate (XIXb). Analysis and the mass spectrum established $C_{28}H_{40}$ as the molecular



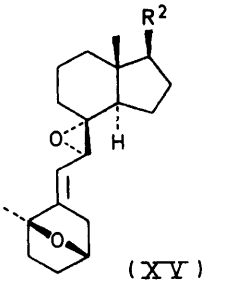
(XIII)



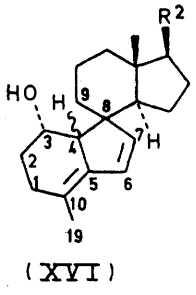
(XIVa)



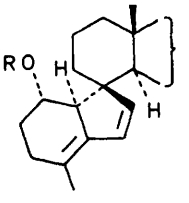
(XIVb)



(XV)

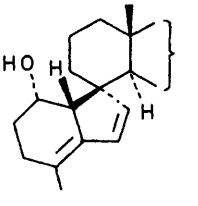


(XVI)

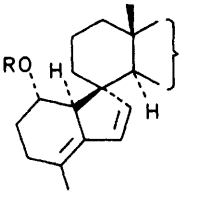


(XVII)

a; R = H
b; R = CO·C$_6$H$_3$(NO$_2$)$_2$-3,5
c; R = CO·C$_6$H$_2$(NO$_2$)$_2$-3,5-Me-4
d; R = COPh
e; R = CO·C$_6$H$_4$·NO$_2$-4
f; R = COMe
g; R = CO·C$_6$H$_4$·N$_2$Ph-4
h; R = CS$_2$Me

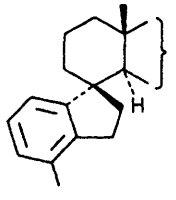


(XVIII)

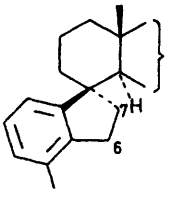


(XIX)

a; R = H
b; R = COPh



(XX)

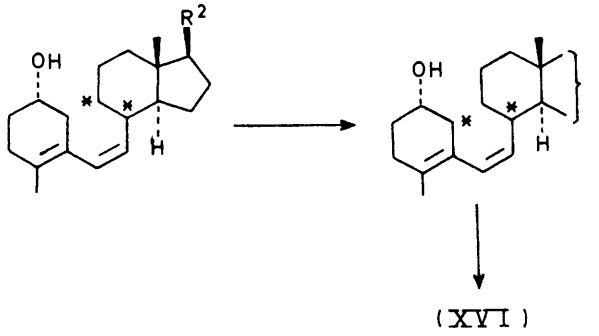


(XXI)

(XXII) 6,7-didehydro

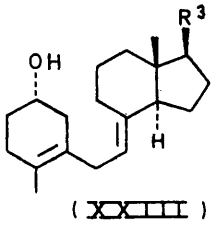
[14] A. G. M. Barrett, D. H. R. Barton, C. H. Carlisle, P. F. Lindley, M. Pendlebury, L. Phillips, R. A. Russell, and D. A. Widdowson, *J.C.S. Chem. Comm.*, 1975, 101.
[15] C. H. Carlisle and P. F. Lindley, *Acta. Cryst.*, in the press.

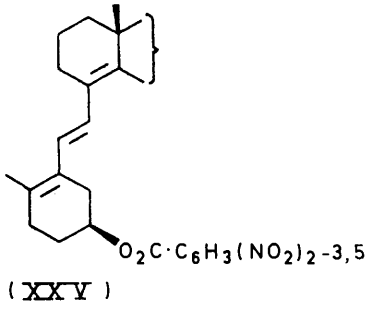
formula and the u.v., i.r., and n.m.r. spectra showed the material to be the substituted indene (XXII). Presumably oxidation accompanied the acid-catalysed dehydration.



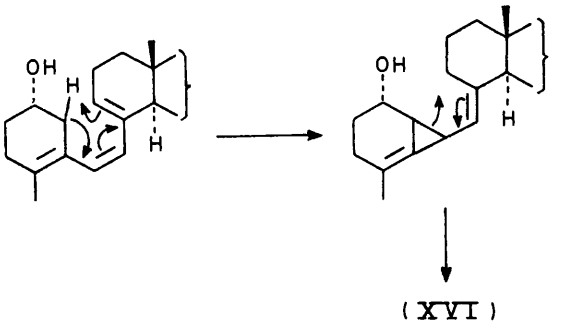
SCHEME 1

Additional crystalline esters of toxisterol$_2$ A (XVIIa) were prepared. In contrast to earlier reports [6] ' toxisterol$_2$-A ' and ' toxisterol$_2$-A' ' were found to be identical.



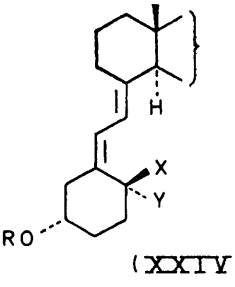


hydrocarbons and sulphur-containing products was obtained.

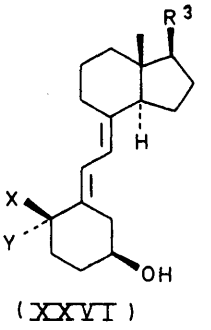


SCHEME 2

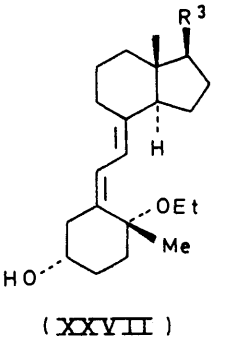
Although it is only speculative, we favour a mechanism of formation of toxisterols A—C which involves a hydrogen atom transfer in an excited pre-ergocalciferol and subsequent cyclisation (Scheme 1). Havinga *et al.*[16] suggested a vinylcyclopropane–cyclopentene rearrangement (Scheme 2).



a; R = OC·C$_6$H$_3$(NO$_2$)$_2$-3,5, X = Me, Y = OEt
b; R = Y = H, X = Me
c; R = OC·C$_6$H$_3$(NO$_2$)$_2$-3,5, X = Me, Y = H
d; R = X = H, Y = Me
e; R = OC·C$_6$H$_3$(NO$_2$)$_2$-3,5, X = H, Y = Me



a; X = Me, Y = OMe
b; X = OMe, Y = Me



In our hands, toxisterol$_2$ A 4-phenylazobenzoate (XVIIg) had m.p. 124.5—126°, in agreement with the reported [6] m.p. of ' toxisterol$_2$-A' 4-phenylazobenzoate '. In an attempt to confirm the configuration at C-4 (prior to *X*-ray crystallography) the pyrolysis of the dithiocarbonate (XVIIh) was examined. An intractable mixture of

Fraction (2.3.1) was isolated as an impure oily benzoate. The material was probably isomeric with ergosterol (IIIa) and may have contained a cyclopropane

[16] F. Boomsma, Ph.D. Thesis, University of Leiden, 1975; F. Boomsma, H. J. C. Jacobs, E. Havinga, and A. van der Gen, *Tetrahedron Letters*, 1975, 427.

ring (τ 9.54 and 9.68). Fraction (2.4) benzoate was also isolated as an oil. Although the compound could not be purified it was probably formed by the addition of ethanol during photolysis [*m/e* 546 (M^+)].

The polar fractions (3—5) were complex mixtures. From these, only ergocalciferol 3,5-dinitrobenzoate (IVb), toxisterol$_2$ F (see further below), and fraction (4.1.2), a compound of dubious purity, were isolated. The mass spectrum of fraction (4.1.2) indicated that it might be a photoreduction product. ' Toxisterol$_3$-R$_1$ ' (XXIII) has recently been described,[16] although no analysis nor mass measurement were reported. Analysis and the mass spectrum indicated toxisterol$_2$ F to be formed by the addition of ethanol to an ergosterol (IIIa) photoisomer. The compound exhibited intense dihydrotachysterol (XIII) u.v. absorption and the n.m.r. spectrum indicated an intact side chain and 13- and 10-methyl (τ 8.63) groups. Chlorotristriphenylphosphine-rhodium-catalysed hydrogenation of ergocalciferol (IVa) gave dihydroergocalciferols II (XXIVb) [17] and IV (XXIVd) [17] as the only products. Comparison of the n.m.r. spectra of the dinitrobenzoates of toxisterol$_2$ F with those of the related compounds (XXIVc and e) supported the identification of toxisterol$_2$ F 3,5-dinitrobenzoate as (XXIVa). Clearly the 3α-proton in toxisterol$_2$ F (XXIVa) (τ 5.07; $W_{\frac{1}{2}}$ 20 Hz) is in the same conformation as that in (XXIVe) (τ 5.07; $W_{\frac{1}{2}}$ 20 Hz) and different from that in (XXIVc) (τ 4.96; $W_{\frac{1}{2}}$ 6.5 Hz). The stereochemical assignment at C-5 and -10 is however tentative. Toxisterol$_2$ F 3,5-dinitrobenzoate (XXIVa) was acid-labile, and rearranged to a single compound (t.l.c.), probably isotachysterol$_2$ 3,5-dinitrobenzoate (XXV).

Other ethanol addition products were certainly present (mass spectrum) but none were isolated pure. The intense u.v. absorption at 242, 252, and 262 nm is clearly the result of alcohol addition and formation of a species with the dihydrotachysterol (XIII) chromophore. Havinga has recently isolated three alcohol addition products, (XXVIa), (XXVIb), and (XXVII), formed on photolysis of 7-dehydrocholesterol (IIIb) in ethanol or methanol.[16] ' Toxisterol$_2$-B ' isolated by Westerhof and Keverling Buisman [6] was probably also an ethanol addition product (analysis). Photochemical addition of an alcohol to an olefin has ample precedent.[18]

Prolonged photolysis of ergosterol (IIIa) in cyclohexane (Figure 2) produced a less complex mixture. Chromatography of this mixture gave all the hydrocarbon photofragments, toxisterols$_2$ A (XVIIa), B (XVIII), D (XIVa), and E (XIVb), suprasterol$_2$ II [isolated as its 3,5-dinitrobenzoate (XXVIIIb)], lumisterol$_2$ [isolated as its 3,5-dinitrobenzoate (XXXb)], and ergocalciferol [isolated as its 3,5-dinitrobenzoate (IVb)]. Two minor, unidentified sterols, possibly photoreduction products (mass spectra) were also obtained. In addition a crystalline hydrocarbon was isolated ($C_{40}H_{64}$ from analysis and mass spectrum). Spectral data showed the presence of an intact side chain, a 13-methyl group with a chemical shift indicative of a 7,8-double bond, a vinyl group (τ 4.64) additional to those of the side chains, and a homoannular diene system. The compound possibly has structure (XXXI), which would be formed by an interesting dimerisation process.

Since the formation of toxisterols from ergocalciferol (IVa) has been reported,[19] we investigated this photolysis. Irradiation of ergocalciferol (IIIa) at −60 °C (to prevent thermal reactions) in pentane gave suprasterol$_2$ II (XXVIIIa), suprasterol$_2$ I (XXIXa), and minor non-toxisterol products, possibly the vinylallenes (XXXII) and vinylcyclobutenes (XXXIII) described by Havinga.[20]

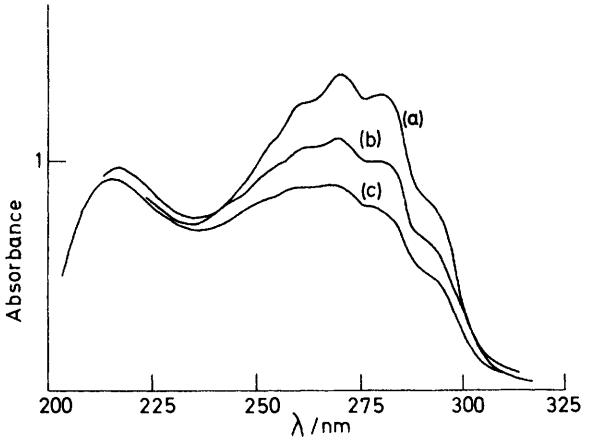


FIGURE 2 Photolysis of ergosterol (IIIa) in cyclohexane: u.v. spectra after (a) 10 h, (b) 30 h, (c) 72 h; initial [ergosterol] 1.62×10^{-4}M

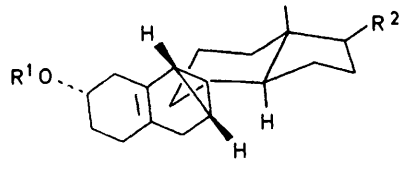
Pfordte [21] described the formation of ' lumicalciferol$_2$ ' on irradiation of ergosterol (IIIa), ergocalciferol (IVa), or lumisterol$_2$ (XXXa) in benzene. The compound formed was thought to be tricyclic with four double bonds. In our opinion ' lumicalciferol$_2$ ' and suprasterol$_2$ I (XXIXa) are identical. Pfordte's tabulated data on ergosterol (IIIa) photoisomers have confused suprasterol$_2$ I (XXIXa) and hexahydrosuprasterol$_2$ I (XXXIV), which was referred to as ' suprastanol$_2$ I$_B$ ' by Windaus.[22] Our samples of suprasterol$_2$ I (XXIXa) and its 3,5-dinitrobenzoate (XXIXb) were identical (m.p. and $[\alpha]_D$) with ' lumicalciferol ' and its 3,5-dinitrobenzoate. Both suprasterol$_2$ I (XXIXa) and its allophanate (XXIXc) have physical properties in agreement with data reported by Setz.[23]

[17] P. Westerhof and J. A. Keverling Buisman, *Rec. Trav. chim.*, 1957, **76**, 683.
[18] P. J. Kropp, *J. Amer. Chem. Soc.*, 1969, **91**, 5783; J. A. Marshall, *Accounts Chem. Res.*, 1969, **2**, 33.
[19] R. M. Moriarty, unpublished results.
[20] S. A. Bakker, J. Lugtenburg, and E. Havinga, *Rec. trav. chim.*, 1972, **91**, 1459.
[21] K. Pfordte, *Pharm. Zentralhalle*, 1967, **106**, 370.
[22] G. Ahrens, E. Fernholz, and W. Stoll, *Annalen*, 1933, **500**, 115.
[23] P. Setz. *Z. physiol. Chem.*, 1933, **214**, 211.

No marked antirachitic activity or toxicity was observed from any of the toxisterols$_2$ or photofragments. The term toxisterol was retained for compounds with $\lambda_{max.}$ 250 for historical reasons.

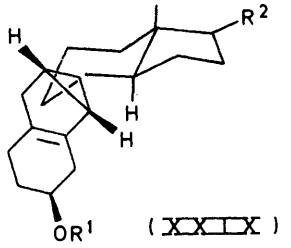
EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Unless otherwise stated u.v. spectra were determined for solutions in cyclohexane, n.m.r. spectra for solutions in acid-free deuteriochloroform, and optical rotation for solutions in chloroform. Preparative (p.l.c.) and analytical t.l.c. were carried out under carbon dioxide with Merck



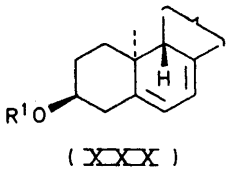
(XXVIII)

a; R^1 = H
b; R^1 = CO·$C_6H_3(NO_2)_2$-3,5
c; R^1 = CO·$C_6H_2(NO_2)_2$-3,5-Me-4



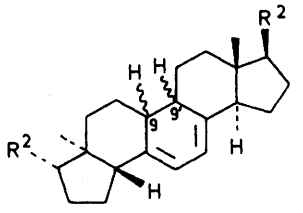
(XXIX)

a; R^1 = H
b; R^1 = CO·$C_6H_3(NO_2)_2$-3,5
c; R^1 = CO·NH·CO·NH_2



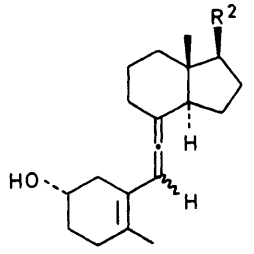
(XXX)

a; R^1 = H
b; R^1 = CO·$C_6H_3(NO_2)_2$-3,5

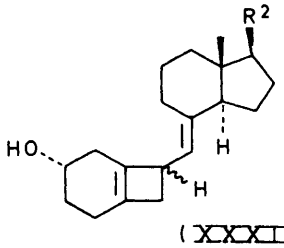


9-H, 9′-H *anti*

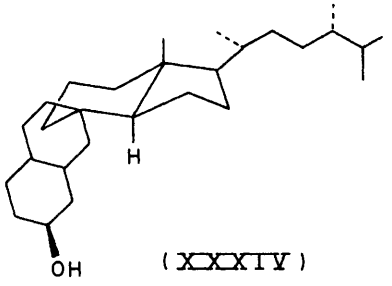
(XXXI)



(XXXII)



(XXXIII)



(XXXIV)

ergosterol (IIIa) (6.0 g) and citric acid (0.9 g) in ethanol (2 280 ml) and water (120 ml) was irradiated until the ergosterol absorption reached a minimum (22—25 h). Sodium carbonate (0.7 g) in water (100 ml) was added and the ethanol removed. The residue from six such photolyses was extracted into diethyl ether; the solution was washed with aqueous 10% sodium carbonate, water, and brine and dried to give a resin (40 g), which was chromatographed over alumina (1 200 g). Elution with light petroleum–diethyl ether (1 : 0, 9 : 1, 4 : 1, and 7 : 3) gave four crude fractions: (1), (2 + 3), (3 + 4), and (5) (in order of increasing polarity).

GF_{254} silica plates; developing solvents are given in parentheses. Spots were located by means of a *p*-anisaldehyde–sulphuric acid–methanol (1 : 1 : 18) spray. Chromatography was carried out on B.D.H. neutral grade III alumina. Light petroleum refers to the fraction b.p. 40—60 °C. Organic extracts were dried over sodium sulphate. Solvents were removed under reduced pressure below 40 °C. Unless otherwise stated all photolyses were carried out in quartz apparatus at room temperature; degassing with nitrogen was carried out prior to and during irradiation with a Phillips HPK 125 W type 57 203 B/00 high pressure arc lamp.

Photolysis of Ergosterol (IIIa) *in Ethanol.*—A solution of

Fraction (1) on p.l.c. (light petroleum–diethyl ether, 197 : 3) gave fractions (1.1) (372 mg) and (1.2) (404 mg). Further p.l.c. (10% silver nitrate–silica plates multiply developed in benzene–light petroleum, 1 : 19—1 : 24) of fraction (1.1) gave (in order of increasing polarity): fraction (1.1.1) [*diene* (Va)] (5 mg), $[\alpha]_D^{25}$ +9.2° (*c* 0.499); fractions (1.1.1 + 1.1.2) (24 mg), τ 4.80 (22- and 23-H), and 8.43, 8.75, 8.92, 9.03, 9.12, 9.22, and 9.32 (methyls), *m/e* 290, 274, 259, 163, 149, and 109; fraction (1.1.3) [*diene* (Vb)] (36 mg), $[\alpha]_D^{24}$ +9.8° (*c* 0.276); fraction (1.1.4) [*diene* (VIa)] (14 mg), $[\alpha]_D^{22}$ +35° (*c* 0.138); fraction (1.1.5) (8 mg), an oil, $[\alpha]_D^{22}$ −106° (*c* 0.49), $\nu_{max.}$ (CS_2) 1 625w, 970s, 890s, and 818s cm^{-1}, τ 4.43 (2 H, m), 4.77 (2 H, m, 22- and 23-H),

8.93, 9.03, 9.13, and 9.23 (12 H, side chain methyls), and 9.13 (3 H, s, 13-Me), *m/e* 272, 267, 229, 201, and 146; fraction (1.1.6) [*diene* (VIIa)] (109 mg), $[\alpha]_D^{23}$ +46° (*c* 0.72); and decomposition product (49 mg).

P.l.c. (light petroleum–diethyl ether, 19 : 1) of fraction (1.2) gave fractions (1.2.1) (78 mg) and (1.2.2) (89 mg) and an intractable mixture of compounds (102 mg), all as oils.

Further p.l.c. of fraction (1.2.1) (10% silver nitrate–silica; benzene–light petroleum, 1 : 19) gave fraction (1.2.1.1) (22 mg) as an oil, τ 4.78 (2 H, m, 22- and 23-H), 6.52 (2 H, q, *J* 7 Hz, O·CH_2·CH_3), 8.30 (3 H, t, *J* 7 Hz, O·CH_2·CH_3), and 8.75, 8.93, 9.03, 9.13, and 9.22 (methyls), *m/e* 318 (M^+). Attempted purification of fraction (1.2.1.1) resulted in decomposition.

P.l.c. of fraction (1.2.2) (multiple development in light petroleum–diethyl ether, 197 : 3) gave (in order of increasing polarity) two minor components; fraction (1.2.2.1) (8 mg), identical (t.l.c. and n.m.r.) with the vinyl ether (VIIb or c); fraction (1.2.2.2) (29 mg) as white plates, m.p. 47—49° (from Me_2CO–MeOH), identical with the vinyl ether (VIIc or b), mixed m.p. 46—48.5°, $[\alpha]_D^{25}$ +56° (*c* 0.203 in cyclohexane); and an intractable mixture (yellow spots with 2,4-dinitrophenylhydrazine in methanol–sulphuric acid spray).

Chromatography of fractions (2 + 3) on alumina gave, on elution with light petroleum–diethyl ether (1 : 0—9 : 1), fractions (2.1—4) and (3) all as oils. Repeated p.l.c. (multiple development in light petroleum–diethyl ether, 47 : 3) of fraction (2.1) gave toxisterol$_2$ D (XIVa) contaminated by a less polar impurity (24 mg), followed by pure *toxisterol*$_2$ D (XIVa) (155 mg) as an oil, $[\alpha]_D^{24}$ +80° (*c* 0.892 in cyclohexane), $\nu_{max.}$ (film) 1 628w, 1 380s, 1 318m, 1 285m, 1 215m, 1 150m, 1 110m, 1 020m, 1 000m, 970s, 950m, 865m, and 795m cm^{-1}. $\lambda_{max.}$ 243 (ε 22 000), 252 (26 000), and 262 nm (19 000), τ (CCl_4) 4.05br, 4.62 (2 H, ABq, *J* 11 Hz, 6- and 7-H), 4.83 (2 H, m, 22- and 23-H), 5.52 (1 H, t, *J* 5.5 Hz, 3α-H), 8.49 (3 H, s, 10-Me), 8.98 and 9.08 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.16 and 9.18 (6 H, 2d, *J* 6.5 Hz, 25-Me_2), and 9.44 (3 H, s, 13-Me), *m/e* 396 (M^+), 381, 353, 271, and 133 (100%) (Found; C, 84.55; H, 11.05. $C_{28}H_{44}O$ requires C, 84.75; H, 11.2%). Further elution gave *toxisterol*$_2$ *E* (XIVb) (54 mg) as an oil, $[\alpha]_D^{24}$ +115° (*c* 0.807 in cyclohexane), $\nu_{max.}$ (film) 1 620w, 1 380s, 1 318m, 1 215m, 1 195m, 1 115m, 975s, 880m, 870m, 855m, and 840m cm^{-1}, $\lambda_{max.}$ 243 (ε 22 000), 252 (26 000), and 262 nm (18 000), τ (CCl_4) 3.91 and 4.35 (2 H, ABq, *J* 12 Hz, 6- and 7-H), 4.84 (2 H, m, 22- and 23-H), 5.55 (1 H, t, *J* 5.5 Hz, 3α-H), 8.58 (3 H, s, 10-Me), 8.98 and 9.09 (6 H, 2d, *J* 6.5 Hz, 20- and 24-Me), 9.17 and 9.18 (6 H, 2d, *J* 6.5 Hz, 25-Me_2), and 9.34 (3 H, s, 13-Me), *m/e* 396 (M^+), 381, 353, 271, and 133 (100%) (Found: C, 84.55; H, 11.1. $C_{28}H_{44}O$ requires C, 84.75; H, 11.2%).

Treatment of fraction (2.2) with 3,5-dinitrobenzoyl chloride in pyridine–toluene gave toxisterol$_2$ A 3,5-dinitrobenzoate (XVIIb) (190 mg) as yellow needles, m.p. 173—174° (lit.,[6] 173—174°) (from Me_2CO–MeOH), $[\alpha]_D^{20}$ −1.3° (*c* 0.989), $\nu_{max.}$ (Nujol) 1 730s, 1 630s, 1 553s, 1 350s, 1 275s, 1 175s, 1 078s, 970s, 765s, 735s, and 725s cm^{-1}, $\lambda_{max.}$ 232 nm (ε 28 000), τ 0.72 and 0.83 (3 H, m, aryl H), 3.68 (2 H, t, *J* 7 Hz, 6- and 7-H), 4.71 (1 H, dt, *J* 10 and 2.5 Hz, 3α-H), 4.81 (1 H, dd, *J* 15.5 and 7.5 Hz, 22- or 23-H), 4.99 (1 H, dd, *J* 15.5 and 8.0 Hz, 23- or 22-H), 7.00 (1 H, d, *J* 10 Hz, 4β-H), 8.28 (3 H, s, 10-Me), 9.06 and 9.13 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.15 and 9.17 (6 H, 2d, *J* 6.5 Hz, 25-Me_2), and 9.10 (3 H, s, 13-Me), *m/e* 590 (M^+), 378 (100%), 253, 159, and 157 (Found: C, 71.2; H, 7.65; N, 4.65. $C_{35}H_{46}N_2O_4$ requires C, 71.15; H, 7.85; N, 4.75%). Although t.l.c. of the mother liquor indicated several components to be present none was isolated pure.

Fractions (2.2 + 2.3) gave *toxisterol*$_2$ *B* (XVIII) (30 mg) as white needles, m.p. 107—109° (from light petroleum), $[\alpha]_D^{19}$ +169° (*c* 0.697), $\nu_{max.}$ (CS_2) 3 550m, 1 210m, 1 175m, 1 090m, 1 065m, 1 010m, 990s, 970s, 860m, 800m, and 760s cm^{-1}, $\lambda_{max.}$ (EtOH) 253 nm (ε 22 000), τ 3.82 and 4.63 (2 H, ABq, *J* 5.5 Hz, 6- and 7-H), 4.83 (2 H, m, 22- and 23-H), 5.39 (1 H, m, $W_{\frac{1}{2}}$ 19 Hz, 3α-H), 7.37 (1 H, m, $W_{\frac{1}{2}}$ 9 Hz, 4α-H), 8.25 (3 H, s, 10-Me), 9.00 and 9.09 (6 H, 2d, *J* 6.5 Hz, 20- and 24-Me), 9.16 and 9.18 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.23 (3 H, s, 13-Me), *m/e* 396 (M^+), 378, and 253 (Found: C, 84.7, H, 11.0. $C_{28}H_{44}O$ requires C, 84.75; H, 11.2%).

Chromatography of fractions (2.3 + 2.4) on silica (Hopkin and Williams M.F.C.; 100 g) on elution with light petroleum–diethyl ether (97 : 3—22 : 3), gave toxisterol$_2$ B (XVIII) (20 mg) and purified fractions (2.3 + 2.4). Benzoylation of the latter (benzoyl chloride–pyridine) and p.l.c. (15% silver nitrate–silica; multiple development in benzene–light petroleum, 7 : 13) gave (in order of increasing polarity) *toxisterol*$_2$ *C benzoate* (XIXb) (411 mg) as an oil, $[\alpha]_D^{22}$ +133° (*c* 0.439); $\nu_{max.}$ (film) 1 725s, 1 600m, 1 582m, 1 275s, 1 115s, 970s, 780s, 760s, 725s, and 690m cm^{-1}, $\lambda_{max.}$ 228 (ε 25 000), 235 (22 000), 250 (17 000), and 279 nm (1 800), τ 1.92 and 2.52 (5 H, m, aryl H), 3.62 and 3.91 (2 H, ABq, *J* 6 Hz, 6- and 7-H), 4.83 (3 H, m, 22-, 23-, and 3α-H), 7.86 (1 H, d, *J* 10 Hz, 4β-H), 8.31 (3 H, s, 10-Me), 9.02 and 9.11 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.20 and 9.22 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.32 (3 H, s, 13-Me), *m/e* 500 (M^+), 378 (100%), and 253; minor impurities at 546 and 424 (Found C, 83.85; H, 9.9. $C_{35}H_{48}O_2$ requires C, 83.95; H, 9.65%); fraction (2.3.1) (165 mg), $\lambda_{max.}$ (EtOH) 231, 250, and 259 nm, τ 2.00 and 2.60 (5 H, m, aryl H), 3.72 and 4.20 (2 H, ABq, *J* 11 Hz), 4.76 (3 H, m, 22-, 23-, and 3α-H), 8.96, 9.02, 9.10, and 9.20 (12 H, side chain methyls), 9.10 (3 H, s, 13-Me), 9.54 (1 H, s), and 9.68 (1 H, s), *m/e* 500, 378, and 253; fraction (2.3.2) (101 mg) as an intractable mixture; and fraction (2.4) (154 mg) as an oil, $\lambda_{max.}$ (EtOH) 235, 241, 250, and 259 nm (shifted by acid to 229, 279, 289, and 301 nm), τ (CCl_4) 2.02 and 2.49 (5 H, m, aryl H), 3.67, 4.27 (2 H, ABq, *J* 12 Hz), 4.80 (3 H, m, 22-, 23-, and 3α-H), 6.68 (2 H, m, O·CH_2·CH_3), 8.87 (3 H, t, *J* 7 Hz, O·CH_2·CH_3), and 8.95, 9.0, 9.1, and 9.2 (15 H, methyls), *m/e* 546 (M^+), 500, 424, 385, 378, 253, 209, 133, 119, and 105 (100%). Neither fraction (2.3.1) nor (2.4) was obtained pure.

Trituration of fractions (3 + 4) (16 g) with ethanol at −20 °C gave ergosterol (IIIa) (2 g). A sample (7 g) of the mother liquor, on chromatography and elution with light petroleum–diethyl ether (9 : 1—7 : 3), gave fractions (3) (1 g), (4.1 + 4.2) (3 g), and (4.2 + 4.3) (3 g). T.l.c. (multiple development in dichloromethane) indicated that fraction (3) contained seven or more components; separation was not achieved.

Reaction of fractions (4.1 + 4.2) with 3,5-dinitrobenzoyl chloride in pyridine–toluene and chromatography [elution by light petroleum–diethyl ether (39 : 1—37 : 3)] gave (in order of increasing polarity) fraction (4.1.1) 3,5-dinitrobenzoate; an intractable mixture of compounds; fraction (4.1.2) 3,5-dinitrobenzoate; and fraction (4.2) 3,5-dinitrobenzoate.

Fraction (4.1.2) 3,5-dinitrobenzoate (43 mg) was obtained as white needles, m.p. 208—209.5° (from C_6H_6–EtOH), $\nu_{max.}$ (Nujol) 1 722s, 1 635m, 1 560s, 1 355s, 1 295s, 990m, and

740s cm^{-1}, u.v. (EtOH) end absorption only, τ 0.80—0.95 (3 H, m, aryl H), 4.85 (3 H, m, 22-, 23-, and 3α-H), and 8.95, 9.05, 9.08, 9.14, 9.21, and 9.45 (methyls), *m/e* 594, 592, and 590. Repeated p.l.c. of the mother liquor gave ergocalciferol 3,5-dinitrobenzoate (IVb), identical with an authentic sample (m.p., mixed m.p., and t.l.c.).

Fraction (4.2) 3,5-dinitrobenzoate gave *toxisterol*$_2$ *F 3,5-dinitrobenzoate* (XXIVa) (720 mg) as a pale yellow gel drying to an amorphous solid (from Me_2CO–MeOH), $[\alpha]_D^{20}$ +47° (*c* 1.42), $\nu_{max.}$ (Nujol) 1 720s, 1 630m, 1 550s, 1 345s, 1 288s, 1 175s, 990m, and 740s cm^{-1}, $\lambda_{max.}$ (Et_2O–EtOH, 1 : 4) 243 (ε 45 000), 250.5 (44 000), and 259 nm (29 000) [shifted by acid to 280 (ε 34 000), 290 (41 000), and 302 nm (30 000)], τ 0.75 and 0.90 (3 H, m, aryl H), 3.58 and 4.12 (2 H, ABq, *J* 11 Hz, 6- and 7-H), 4.82 (2 H, m, 22- and 23-H), 5.07 (1 H, m, $W_{\frac{1}{2}}$ 20 Hz, 3α-H), 6.63 and 6.82 (2 H, dq, *J* 7 Hz, O·CH_2·CH_3), 6.96 and 7.20 (2 H, m, 4-H), 7.65 (1 H, t, *J* 12 Hz), 8.63 (3 H, s, 10-Me), 8.87 (3 H, t, *J* 7 Hz, O·CH_2·CH_3), 8.98 and 9.10 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.17 and 9.19 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.44 (3 H, s, 13-Me), *m/e* 636 (M^+), 621, 590, 466. 465, 425, and 378 (Found: C, 69.7; H, 8.35; N, 4.3. $C_{37}H_{52}N_2O_7$ requires C, 69.75; H, 8.25; N, 4.4%). T.l.c. (multiple development in light petroleum–diethyl ether, 9 : 1) indicated eleven or more additional components in the mother liquor. Fraction (4.2) 3,5-dinitrobenzoate consisted of two or more components of similar R_F (t.l.c.) with *m/e* 634 (M^+), 592, 590, 485, and 363.

T.l.c. (light petroleum–EtOAc, 1 : 1) of fraction (5) indicated a complex mixture of components of high molecular weight (*m/e* 792, 520, 506, 486, 456, and 442).

' Grundmann's Ketone ' (VIIIa).—' Grundmann's ketone ' (VIIIa) (32%), prepared by oxidation of ergocalciferol (IVa) [8] with permanganate and isolated by chromatography by elution with light petroleum–diethyl ether (24 : 1) was obtained as an oil, $\nu_{max.}$ ($CHCl_3$) 1 708s, and 970s cm^{-1}, τ 4.80 (2 H, m, 22- and 23-H), 8.90, 9.02, 9.14, and 9.22 (12 H, side chain methyls), and 9.32 (3 H, s, 13-Me), *m/e* 276 (M^+), 233, and 215. The derived semicarbazone (VIIIb) was obtained as a cream-coloured amorphous solid, m.p. 218—220° (from MeOH) (lit.,[8] 222°), $\nu_{max.}$ ($CHCl_3$) 3 530m, 3 390m, 1 690s, 1 565s, and 970m cm^{-1}, τ 1.83 (1 H, s, rapid exch. D_2O, N–H), 4.47br (2 H, s, exch. D_2O, NH_2), 4.81 (2 H, m, 22- and 23-H), 8.92, 9.00, 9.10, and 9.22 (12 H, side chain methyls), and 9.37 (3 H, s, 13-Me), *m/e* 333 (M^+), 318, 290, 274, 259, 143, and 133 (100%).

The Diene (VIIa).—n-Butyl-lithium (0.73 ml; 2M in hexane) was added to methyltriphenylphosphonium bromide (0.52 g) in tetrahydrofuran (THF) (25 ml) under nitrogen. The mixture was stirred for 4 h at room temperature, the ketone (VIIIa) (88 mg) in THF (5 ml) was added, and stirring was continued overnight. Diethyl ether (30 ml) was added and the solution was washed with aqueous 10% ammonium chloride (3 × 30 ml) and brine (2 × 20 ml), dried, and evaporated. Column chromatography (eluant light petroleum) and subsequent p.l.c. (light petroleum–diethyl ether, 19 : 1) gave the *diene* (VIIa) (65 mg, 74%) as an oil, $[\alpha]_D^{23}$ +48° (*c* 0.628), $\nu_{max.}$ ($CHCl_3$) 1 648m, 973s, and 887s cm^{-1}, τ 4.82 (2 H, m, 22- and 23-H), 5.27 (1 H, d, *J* 2 Hz, 7-H), 5.57 (1 H, d, *J* 2 Hz, 7-H), 8.99 and 9.09 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.16 and 9.18 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.43 (3 H, s, 13-Me), *m/e* 274 (M^+), 259, 231, 176, 175, 161, 149 (100%), and 147 (Found: C, 87.5; H, 12.3. $C_{20}H_{34}$ requires C, 87.5; H, 12.5%).

The Diene (Va).—The diene (VIIa) (21 mg) in THF (5 ml) and aqueous sulphuric acid (30% v/v; 1.5 ml) was heated to reflux for 3¼ h under nitrogen. The mixture was diluted with diethyl ether (20 ml) and saturated aqueous sodium hydrogen carbonate (20 ml) was added dropwise. The organic phase was washed with saturated aqueous sodium hydrogen carbonate (3 × 20 ml) and brine (2 × 10 ml), dried, and evaporated to give the *diene* (Va) (21 mg, 100%), $[\alpha]_D^{23}$ +12.4° (*c* 1.08), $\nu_{max.}$ (CS_2) 970s cm^{-1}, τ 4.77 (2 H, m, 22- and 23-H), 8.48 (3 H, s, 8-Me), 8.93, 9.03, 9.13, and 9.23 (12 H, side chain methyls), and 9.13 (3 H, s, 13-Me), *m/e* 274 (M^+), 259, 245, 231, 203, 189, 175, 162, 161, 149 (100%), and 133. A sample was purified by p.l.c. (10% silver nitrate–silica; benzene–light petroleum, 1 : 19) (Found: C, 87.35; H, 12.3. $C_{20}H_{34}$ requires C, 87.5; H, 12.5%).

Reduction of ' Grundmann's Ketone ' (VIIIa).—Sodium borohydride (108 mg) in water (2 ml) was added to the ketone (VIIIa) (102 mg) in dioxan–THF (2 : 3) (5 ml) at 0 °C. After 6½ h diethyl ether (30 ml) and brine (20 ml) were added. The organic phase was washed with brine (3 × 20 ml), dried, and evaporated. P.l.c. (light petroleum–diethyl ether, 7 : 3) gave the less polar 8β-alcohol (IXa) (86 mg, 84%) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 430m, 1 490s, 1 293m, 1 162m, 1 110—1 040m, and 970s cm^{-1}, τ 4.80 (2 H, m, 22- and 23-H), 5.93 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz, 8α-H), 8.97, 9.03, 9.12, and 9.23 (12 H, side chain methyls), and 9.04 (3 H, s, 13-Me); and an uncharacterised minor product (6 mg). No epimerisation of the ketone (VIIIa) was observed during the reduction. Alternatively the 8β-alcohol (IXa) was obtained as its 3,5-dinitrobenzoate (IXb), pale yellow plates, m.p. 140.5—141.5° (lit.,[9] 146°) (from Me_2CO–MeOH), $[\alpha]_D^{22}$ +70° (*c* 0.289); $\nu_{max.}$ ($CHCl_3$) 1 727s, 1 628m, 1 548s, 1 348s, 1 288m, 1 268m, 1 152m, and 970m cm^{-1}, τ 0.79 (3 H, m, aryl H), 4.49 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz, 8α-H), 4.77 (2 H, m, 22- and 23-H), 8.90, 9.02, 9.10, and 9.20 (12 H, side chain methyls), and 8.87 (3 H, s, 13-Me), *m/e* 472 (M^+), 347, 346, 345, and 260 (Found: C, 65.9; H, 7.55; N, 6.1. Calc. for $C_{26}H_{36}N_2O_6$: C, 66.05; H, 7.7; N, 5.95%). Saponification (KOH–MeOH–Et_2O) gave the 8β-alcohol (IXa) (94%).

The Diene (Vb).—The alcohol (IXa) (47 mg), pyridine (2 ml), and methanesulphonic anhydride (91 mg) were stirred for 2 h at room temperature, 8 h at 60 °C, and 2 h at 80 °C. Water (1 ml) was added and the mixture extracted with diethyl ether (3 × 5 ml). The extract was washed with saturated aqueous sodium hydrogen carbonate (3 × 5 ml) and brine (5 ml) and dried. Evaporation and p.l.c. (light petroleum–diethyl ether, 49 : 1) gave the diene (Vb) (36 mg, 82%), $[\alpha]_D^{22}$ +9.1° (*c* 0.362), $\nu_{max.}$ (film) 980s, 950w, 930w, 928w, 910w, and 800m cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 600w cm^{-1}, τ 4.74 (1 H, m, 8-H), 4.78 (2 H, m, 22- and 23-H), 8.98 and 9.08 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.09 (3 H, s, 13-Me), and 9.16 and 9.18 (6 H, 2d, *J* 7 Hz, 25-Me_2), *m/e* 260 (M^+), 245, 231, 217, 189, 176, 161, 149, and 147 (Found: C, 87.85; H, 12.3. $C_{19}H_{32}$ requires C, 87.6; H, 12.4%).

The Dithiocarbonate (IXc).—Sodium hydride (41 mg; 80% in mineral oil) was washed with light petroleum (10 × 2 ml) and added as a THF slurry (2 × 1 ml) to the alcohol (IXa) (35 mg) and imidazole (4 mg) in THF (5 ml). A pink colour was produced and rapidly discharged. The mixture was heated to reflux under nitrogen (5.5 h). Carbon disulphide (0.5 ml) was added followed, after 1 h, by iodomethane (0.5 ml). After a further 0.5 h the mixture was cooled, diluted with diethyl ether (20 ml), washed with

brine (3 × 10 ml), dried, and evaporated to give a red oil. Chromatography (eluant light petroleum–diethyl ether, 24 : 1) and p.l.c. (light petroleum–diethyl ether, 97 : 3) gave the *dithiocarbonate* (IXc) (39 mg, 84%) as an oil, $\nu_{max.}$ ($CHCl_3$) 1 600w, 1 150s, 1 048s, 980s, and 870m cm^{-1}, τ 4.03 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz, 8α-H), 4.82 (2 H, m, 22- and 23-H), 7.43 (3 H, s, SMe), 8.93, 9.03, 9.13, and 9.23 (12 H, side chain methyls), and 9.03 (3 H, s, 13-Me), *m/e* (inlet 50 °C) (M^+ absent) 260, 60, 48, and 47 (Found: C, 68.5; H, 9.65. $C_{21}H_{36}OS_2$ requires C, 68.4; H, 9.85%).

The Diene (VIa).—The xanthate (IXc) (76 mg) on pyrolysis (250 °C) under argon for 5 h and p.l.c. (10% silver nitrate–silica; multiple development in benzene–light petroleum, 1 : 19) gave the *diene* (VIa) (30 mg, 55%) as an oil, $[\alpha]_D^{21}$ +39° (*c* 0.590), $\nu_{max.}$ (film) 980s, and 675s cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 632w cm^{-1}, τ 4.43 (2 H, m, 8- and 9-H), 4.80 (2 H, m, 22- and 23-H), 8.97 and 9.08 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.16 and 9.18 (6 H, 2d, *J* 6.5 Hz, 25-Me_2), and 9.30 (3 H, s, 13-Me), *m/e* 260 (M^+), 217, 199, 175, 161, 147, 136, 135, 134, and 133 (Found: C, 87.9; H, 12.35. $C_{19}H_{32}$ requires C, 87.6; H, 12.4%). On heating at 220 °C and 0.1 mmHg the xanthate (IXc) distilled over unchanged.

Epimerisation of 'Grundmann's Ketone' (VIIIa).—The ketone (VIIIa) (67 mg) in deuteriochloroform (0.5 ml) containing trifluoroacetic acid (0.02 ml) was epimerised during 3 days at room temperature (n.m.r., t.l.c.). N.m.r. indicated that ketone (VIIIa) (5 mg) in [$^2H_4$]methanol (0.5 ml) was rapidly epimerised by NaOD in D_2O (40%; 1 drop). Both the acidic and basic reaction mixtures were separately neutralised, extracted with diethyl ether and chromatographed (eluant light petroleum–diethyl ether, 24 : 1) to give the ketone (VIIIc) [10] (60 mg, 83%), contaminated by <5% of the ketone (VIIIa); $\nu_{max.}$ ($CHCl_3$) 1 705s, 1 320m, and 975s cm^{-1}, τ 4.80 (2 H, m, 22- and 23-H), 8.93, 9.03, 9.07, 9.13, and 9.23 (12 H, side chain methyls), and 8.93 (3 H, s, 13-Me), *m/e* 276 (M^+), 253, 215, 179, 178, 152, 151, and 133.

The Alcohols (IXd *and* e).—Reduction of the ketone (VIIIc) (85 mg) with sodium borohydride in aqueous THF–dioxan and p.l.c. (light petroleum–diethyl ether, 3 : 1—13 : 7) gave the less polar *alcohol* (IXd or e) (29 mg, 34%) as an oil, τ 4.74 (2 H, m, 22- and 23-H), 6.14 (1 H, m, $W_{\frac{1}{2}}$ 16 Hz, 8-H), 8.97, 9.00, 9.10, and 9.23 (12 H, side chain methyls), and 9.10 (3 H, s, 13-Me); and the more polar alcohol (IXe or d) (36 mg, 42%) as an oil, τ 4.80 (2 H, m, 22- and 23-H), 6.63 (1 H, m, $W_{\frac{1}{2}}$ 16 Hz, 8-H), 8.95, 9.03, 9.10, and 9.23 (12 H, side chain methyls), and 9.10 (3 H, s, 13-Me). On reaction with 3,5-dinitrobenzoyl chloride in pyridine–toluene the less polar alcohol (IXd or e) gave the more polar *ester* (IXf or g) as an oil, $[\alpha]_D^{24}$ −13° (*c* 1.74), τ 0.87 (3 H, m, aryl H), 4.50 (1 H, m, 8-H), 4.67 (2 H, m, 22- and 23-H), and 8.73, 8.87, 8.95, 9.07, 9.15, and 9.23 (15 H, methyls). Similarly the more polar alcohol (IXe or d) gave the less polar *ester* (IXg or f) as an oil, $[\alpha]_D^{24}$ +45° (*c* 0.538), $\nu_{max.}$ ($CHCl_3$) 1 728s, 1 550s, 1 348s, 1 280s, and 975m cm^{-1}, τ 0.87 (3 H, m, aryl H), 4.77 (2 H, m, 22- and 23-H), 4.97 (1 H, m, $W_{\frac{1}{2}}$ 16 Hz, 8-H), 8.90, 9.03, 9.13, and 9.23 (12 H, side chain methyls), and 8.95 (3 H, s, 13-Me). In a subsequent reduction the derived mixture of 3,5-dinitrobenzoates (IXf + g) on p.l.c. (light petroleum–diethyl ether, 9 : 1) was obtained as an oil, $[\alpha]_D^{24}$ +21° (*c* 0.228) (58% dextrorotatory isomer), *m/e* 472 (M^+), 457, 429, 347, 260, 245, 217, and 135 (100%) (Found: C, 65.9; H, 7.85; N, 5.95. $C_{26}H_{36}N_2O_6$ requires C, 65.05; H, 7.7; N, 5.95%). Hydrolysis of the ester mixture (IXf + g) (125 mg) with potassium hydroxide in methanol–ether gave the alcohols (IXd + e) (67 mg, 91%).

The Diene (VIb).—The alcohols (IXd + e) (55 mg) and methanesulphonic anhydride (77 mg) in pyridine (1 ml) were stirred at room temperature for 50 min. Toluene (2 ml) was added and the mixture heated to reflux (3 h). Work-up, chromatography (eluant light petroleum), and p.l.c. (10% silver nitrate–silica; multiple development in benzene–light petroleum, 1 : 19) gave the diene (Vb) (28 mg, 45%), identical (t.l.c.; n.m.r.) with that previously prepared, and the more polar *diene* (VIb) (14 mg, 23%), as an oil, $[\alpha]_D^{22}$ −32° (*c* 0.244), $\nu_{max.}$ (CS_2) 1 650m, 972s, 796m, and 710m cm^{-1}, τ 4.47 (2 H, m, 8- and 9-H), 4.80 (2 H, m, 22- and 23-H), 8.97, 9.07, 9.15, 9.17, and 9.27 (12 H, side chain methyls), and 9.13 (3 H, s, 13-Me), *m/e* 260 (M^+), 245, 217, 189, 161, 136, 135, 134, and 133 (Found: C, 87.3; H, 12.3. $C_{19}H_{32}$ requires C, 87.6; H, 12.4%).

Reactions of the Ketones (VIIIa *and* c) *with* 4-*Tolylsulphonylhydrazine*.—The ketone (VIIIa) (101 mg) and 4-tolylsulphonylhydrazine (80 mg) in methanol (3 ml) were stirred at room temperature for 6 h. Chromatography (eluant light petroleum–diethyl ether, 19 : 1—7 : 3) gave a 4-*tolylsulphonylhydrazone* (151 mg, 93%) as an oil, $[\alpha]_D^{24}$ +25° (*c* 0.824), $\nu_{max.}$ ($CHCl_3$) 3 285m, 3 210m, 1 630s, 1 600m, 1 340s, 1 160s, 1 090m, 970m, and 910m cm^{-1}, τ 2.14 and 2.71 (5 H, ABq, *J* 8 Hz, aryl H and NH), 4.80 (2 H, m, 22- and 23-H), 7.57 (3 H, s, aryl Me), 9.00, 9.10, and 9.20 (15 H, methyls), and 9.60 (minor impurity), *m/e* (inlet temp. 150 °C) 548 [azine (VIIIf)], 444 (M^+), 289 (100%), 260, 245, 217, and 135. A subsequent preparation gave the product as white needles, m.p. 115—117° (from Et_2O–light petroleum), $[\alpha]_D^{24}$ −32° (*c* 0.524), τ (CCl_4) 2.20 and 2.73 (5 H, ABq, *J* 8 Hz, aryl H and NH), 4.83 (2 H, m, 22- and 23-H), 7.57 (3 H, s, aryl Me), and 8.95, 9.03, 9.12, 9.20, and 9.53 (15 H, methyls). Repeated recrystallisation of material from the mother liquor gave white needles, m.p. 102.5—105°, $[\alpha]_D^{22}$ +15 ⟶ 28° (*c* 0.709), *m/e* (inlet temp. 175 °C) 548 (M^+ absent), 288, 162, and 156 (Found: C, 70.05; H, 8.9; N, 6.2; S, 7.45. $C_{26}H_{40}N_2O_2S$ requires C, 70.2; H, 9.05; N, 6.3; S, 7.2%).

Reaction of the ketone (VIIIc) (60 mg) and 4-tolylsulphonylhydrazine (47 mg) gave a product (VIIIe) as white needles, m.p. 100—104° (from Et_2O–light petroleum), $[\alpha]_D^{23}$ +21 ⟶ 29° (*c* 1.048), $\nu_{max.}$ (Nujol) 3 200s, 1 622w, 1 595m, 1 335s, 1 170s, 1 090m, 1 030m, 970m, 927m, 810s, and 680m cm^{-1}, τ 2.13 and 2.70 (5 H, ABq, *J* 8 Hz, aryl H and NH), 4.80 (2 H, m, 22- and 23-H), 7.57 (3 H, s, aryl Me), and 9.02, 9.08, 9.12, and 9.20 (15 H, methyls), *m/e* (inlet temp. 150 °C) 548.508 5, 529, 364, 276, 262, and 246 [Calc. for $C_{38}H_{64}N_2$ (VIIIf): *M*, 548.506 9]. Both laevorotatory and dextrorotatory solids were identical in t.l.c. behaviour (multiple development in $CHCl_3$).

Reaction of the 4-*Tolysulphonylhydrazone* (VIIId *or* e) *with Butyl-lithium*.—n-Butyl-lithium (0.37 ml; 2M in hexane) was added to a stirred solution of the 4-tolylsulphonylhydrazone (VIIId or e) (initial $[\alpha]_D^{22}$ +15°) (83 mg) in diethyl ether (2 ml) under nitrogen. After 10 min, water (5 ml) and diethyl ether (10 ml) were added. The organic phase was washed with water (3 × 10 ml) and brine (10 ml) and dried. Evaporation and p.l.c. [light petroleum–diethyl ether (197 : 3); 10% silver nitrate–silica with benzene–light petroleum, 1 : 19] gave the less polar diene (VIa) (8 mg, 16%) and the diene (VIb) (17 mg, 35%), identical with authentic samples. The reaction of the 4-tolylsulphonylhydrazone

(VIIIe) derived from the ketone (VIIIc) and methyllithium gave only the diene (VIb) (t.l.c.).

Ethoxymethyltriphenylphosphonium Chloride.—The title *salt* (25.3 g, 93%), prepared as for the methoxy-analogue [24] from triphenylphosphine (20 g) and chloromethyl ethyl ether (7.6 g), was obtained as white prisms, m.p. 204—205° (from $CHCl_3$–EtOAc), $\nu_{max.}$ (Nujol) 1 585s, 1 440s, 1 115s, 1 090s, 1 000s, 770s, 752s, 725s, and 695s cm^{-1}, τ 2.24 (15 H, m, aryl H), 4.07 (2 H, d, J 4 Hz, CH_2·P), 6.06 (2 H, q, J 7 Hz, O·CH_2·CH_3), and 8.83 (3 H, t, J 7 Hz, O·CH_2·CH_3) (Found: C, 70.7; H, 6.05. $C_{21}H_{22}ClOP$ requires C, 70.65; H, 6.2%).

The Vinyl Ethers (VIIb *and* c).—n-Butyl-lithium (0.98 ml; 2M in hexane) was added to ethoxymethyltriphenylphosphonium chloride (0.70 g) in THF (10 ml) under nitrogen. After 1 h the ketone (VIIIa) (69 mg) in THF (6 ml) was added to the red ylide. The mixture was stirred overnight and worked up to give a red oil which was extracted with light petroleum (10 × 5 ml). Chromatography (eluant light petroleum), treatment with iodomethane (to remove Ph_3P), and p.l.c. (light petroleum–diethyl ether, 197 : 3) gave the less polar *vinyl ether* (VIIb *or* c) (20 mg, 25%) as an oil, $[\alpha]_D^{22}$ +49° (*c* 0.352 in cyclohexane), $\nu_{max.}$ (film) 1 685s, 1 298m, 1 238m, 1 210m, 1 190m, 1 150s, 1 135s, 1 112m, 1 048m, 970s, and 835m cm^{-1}, τ 4.14 (1 H, s, 7-H), 4.80 (2 H, m, 22- and 23-H), 6.40 (2 H, t, J 7 Hz O·CH_2·CH_3), 8.83 (3 H, t, J 7 Hz, O·CH_2·CH_3), 8.93, 9.03, 9.13, and 9.23 (12 H, side chain methyls), and 9.32 (3 H, s, 13-Me), *m/e* 318 (M^+), 304, 275, 272, 259, 229, 201, 193, 149, and 147 (100%) (Found: C, 83.15; H, 11.8. $C_{22}H_{38}O$ requires C, 82.95; H, 12.05%); and the more polar *vinyl ether* (VIIc *or* b) (20 mg, 25%) as white plates. m.p. 46—48.5° (from Me_2CO–MeOH), $[\alpha]_D^{21}$ +58° (*c* 0.297 in cyclohexane), $\nu_{max.}$ 1 682s, 1 438s, 1 380s, 1 255s, 1 160s, 1 120s, 970s, 840m, and 825m cm^{-1}, τ 4.40 (1 H, s, 7-H), 4.80 (2 H, m, 22- and 23-H), 6.35 (2 H, q, J 7 Hz, O·CH_2·CH_3), 8.80 (3 H, t, J 7 Hz, O·CH_2·CH_3), 8.93, 9.03, 9.13, and 9.23 (12 H, side chain methyls), and 9.43 (3 H, s, 13-Me), *m/e* 318 (M^+). 304, 275, 272, 259, 229, 201, and 147 (100%) (Found: C, 83.15; H, 11.9. $C_{22}H_{38}O$ requires C, 82.95; H, 12.05%).

Toxisterol$_2$ *D Epoxide* (XV).—3-Chloroperbenzoic acid (21 mg), toxisterol$_2$ D (XIVa) (43 mg), diethyl ether (10 ml), and sodium hydrogen carbonate (101 mg) were stirred together for 4 days at 0 °C. The mixture was treated with cyclohexene (1.0 ml) for 1 h, chromatographed (light petroleum–diethyl ether, 4 : 1) and separated by p.l.c. (light petroleum–diethyl ether, 7 : 3) to give (in order of increasing polarity) toxisterol$_2$ D (XIVa) (4 mg): *toxisterol*$_2$ D *epoxide* (XV) (14 mg, 34%) as white needles, m.p. 140.5—142° (from MeOH), $[\alpha]_D^{23}$ +69° (*c* 0.110 in cyclohexane), $\nu_{max.}$ (Nujol) 1 218m, 1 202m, 1 157m, 1 110s, 1 020m, 970s, 950m, 910m, 870s, 860m, and 838s cm^{-1}, τ 4.80 (2 H, m, 22- and 23-H), 4.98 (1 H, d, J 8 Hz, 6-H), 5.55 (1 H, m, 3α-H), 6.72 (1 H, s, J 8 Hz, 7-H), 7.58 (2 H, m, 4-H), 8.50 (3 H, s, 10-Me), 8.93, 9.03, 9.12, and 9.22 (12 H, side chain methyls), and 9.32 (3 H, s, 13-Me), *m/e* 412 (M^+), 397, 394, 384, 383, 369, and 354 (Found: C, 81.35; H, 10.45. $C_{28}H_{44}O_2$ requires C, 81.5; H, 10.75%); and a mixture of three or more compounds (14 mg).

Toxisterol$_2$ *A* (XVIIa).—Potassium hydroxide (115 mg) in methanol (2.0 ml) was added to toxisterol$_2$ A 3,5-dinitrobenzoate (XVIIb) (217 mg) in diethyl ether (20 ml) under nitrogen. The solution became instantly blood red and subsequently purple. After 2 h solid carbon dioxide (1 g) was added followed, after the mixture had warmed to room temperature, by diethyl ether (20 ml). The solution was washed with water (3 × 20 ml) and brine (20 ml) and dried. Evaporation and chromatography (eluant light petroleum–diethyl ether, 5 : 1) gave *toxisterol*$_2$ *A* (XVIIa) (144 mg, 98%), as an oil, $[\alpha]_D^{21}$ −157° (*c* 1.235), $\nu_{max.}$ (film) 3 400m, 1 260m, 1 040m, 1 015m, 990m, 970s, 865m, and 760s cm^{-1}, $\lambda_{max.}$ 250 nm (ε 21 000), τ 3.72 (2 H, s, 6- and 7-H), 4.83 (2 H, m, 22- and 23-H), 6.18 (1 H, m, $W_{\frac{1}{2}}$ 35 Hz, 3α-H), 7.55 (1 H, d, J 10 Hz, 4β-H), 8.33 (3 H, s, 10-Me), 9.00 and 9.09 (6 H, 2d, J 6.5 Hz, 20- and 24-Me), 9.10 (3 H, s, 13-Me), and 9.17 and 9.18 (6 H, 2d, J 7 Hz, 25-Me_2), *m/e* 396 (M^+), 378, 376, 271, and 253 (Found: C, 84.55; H, 11.0. $C_{28}H_{44}O$ requires C, 84.75; H, 11.2%).

Toxisterol$_2$ A (XVIIa) *Esters*.—Esterification of toxisterol$_2$ A (XVIIa) (30 mg) in pyridine with (*a*) 4-methyl-3,5-dinitrobenzoyl chloride, (*b*) benzoyl chloride, (*c*) 4-nitrobenzoyl chloride, (*d*) acetic anhydride in benzene–pyridine, and (*e*) 4-phenylazobenzoyl chloride (4.4 mg) in pyridine gave (*a*) the *4-methyl-3,5-dinitrobenzoate* (XVIIc) (44 mg, 96%) as white needles, m.p. 171.5—172° (from Me_2CO–MeOH), $\lambda_{max.}$ 223 nm (ε 31 000), *m/e* 604 (M^+), 378 (100%), 253, and 157 (Found: C, 71.55; H, 8.25; N, 4.5. $C_{36}H_{48}N_2O_4$ requires C, 71.5; H, 8.0; N, 4.65%), (*b*) the *benzoate* (XVIId) (35 mg, 92%) as white needles, m.p. 106—108° (from Me_2CO–MeOH), $\lambda_{max.}$ 229 (ε 21 000), 235 (21 000), 250 (20 000), and 279 nm (1 100), *m/e* 500 (M^+), 378 (100%), 253, and 157 (Found: C, 83.9; H, 9.55. $C_{35}H_{48}O_2$ requires C, 83.95; H, 9.65%), (*c*) the *4-nitrobenzoate* (XVIIe) (37 mg, 90%) as yellow needles, m.p. 102—103.5° (from Me_2CO–MeOH), *m/e* 545 (M^+), 378 (100%), 253, and 157 (Found: C, 76.9; H, 8.65; N, 2.4. $C_{35}H_{47}NO_4$ requires C, 77.0; H, 8.7; N, 2.55%), (*d*) the *acetate* (XVIIf) (29 mg, 87%) as prisms, m.p. 103.5—105° (from Me_2CO–MeOH), $\lambda_{max.}$ 250 nm (ε 20 000), *m/e* 438 (M^+), 378 (100%), 253, and 157 (Found: C, 82.15; H, 10.45. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%), and (*e*) the 4-phenylazobenzoate (XVIIg) (6.0 mg, 90%), as orange needles, m.p. 124.5—126° (from Me_2CO–MeOH) (lit.,[6] 'toxisterol-A 4-phenylazobenzoate,' 125.5—126.5°).

Toxisterol$_2$ *C* (XIXa).—Saponification of the benzoate (XIXb) (19 mg) (KOH–MeOH–Et_2O) and p.l.c. (light petroleum–diethyl ether, 7 : 3) gave *toxisterol*$_2$ *C* (XIXa) (12 mg, 80%) as an oil, $[\alpha]_D^{21}$ +16.5° (*c* 0.980), $\nu_{max.}$ (film) 3 350s, 1 280m, 1 120m, 1 035m, 970s, 780s, and 760m cm^{-1}, $\lambda_{max.}$ 250 nm (ε 17 000), τ 3.65 and 3.95 (2 H, ABq, J 7 Hz, 6- and 7-H), 4.80 (2 H, m, 22- and 23-H), 6.24 (1 H, m, $W_{\frac{1}{2}}$ 34 Hz, 3α-H), 8.33 (3 H, s. 10-Me), 8.92, 9.02, 9.13, and 9.22 (12 H, side chain methyls), and 9.10 (3 H, s, 13-Me), *m/e* 396 (M^+), 378, 376, 271, and 253 (Found: C, 84.5; H, 10.95. $C_{28}H_{44}O$ requires C, 84.75; H, 11.2%).

Dehydration of Toxisterol$_2$ *A* (XVIIa).—Toxisterol$_2$ A (XVIIa) (16 mg) in chloroform (2 ml) saturated with dry hydrogen chloride was kept overnight under nitrogen (yellow solution). Diethyl ether (20 ml) was added and the solution washed with saturated aqueous sodium hydrogen carbonate (2 × 20 ml) and brine (20 ml) and dried. Evaporation and chromatography (eluant light petroleum) gave the *aromatic compound* (XX) (13 mg, 89%) as white plates, m.p. 85—86.5°, or white needles, m.p. 88—89.5° (from Me_2CO–MeOH), $[\alpha]_D^{23}$ −55° (*c* 0.914), $\nu_{max.}$ (CS_2) 970s, 935m, 778s, 720m, and 710m cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 598m cm^{-1}, $\lambda_{max.}$ 262sh (ε 477), 265.5 (570), and 273.5 nm (585), τ 2.92 (1 H, m, 2-H), 3.07 (2 H, m, 1- and 3-H), 4.81 (2 H, m,

[24] G. Wittig and M. Schlosser, *Chem. Ber.*, 1961, **94**, 1373.

22- and 23-H), 7.22 (2 H, dt, *J* 7 and 1 Hz, 6-H), 7.77 (3 H, s, 10-Me), 8.97 and 9.09 (6 H, 2d, *J* 6.5 Hz, 20- and 24-Me), 9.17 and 9.18 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.17 (3 H, s, 13-Me), *m/e* 378 (M^+), 363, 355, 318, 307, and 157 (100%) (Found: C, 88.6; H, 11.0. $C_{28}H_{42}$ requires C, 88.8; H, 11.2%).

Dehydration of Toxisterol$_2$ C (XIXa).—Hydrogen chloride–chloroform dehydrated toxisterol$_2$ C (XIXa) (42 mg) (solution emerald green) to give, after p.l.c. (light petroleum–diethyl ether, 99 : 1), the *aromatic compound* (XXI) (20 mg, 50%) as white needles, m.p. 67—69.5° (from Me_2CO–MeOH), $[\alpha]_D^{23}$ +6.0° (*c* 0.537), $\nu_{max.}$ (CS_2) 970s, 775s, and 705m cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 598m cm^{-1}, $\lambda_{max.}$ 262sh (ε 612), 265.5 (712), and 273.5 nm (670), τ 2.91 (1 H, m, 2-H), 3.06 (2 H, m, 1-H, 3-H), 4.79 (2 H, m, 22- and 23-H), 7.21 (2 H, t, *J* 7 Hz, 6-H), 7.77 (3 H, s, 10-Me), 8.94 and 9.09 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.05 (3 H, s, 13-Me), and 9.16 and 9.18 (6 H, 2d, *J* 6.5 Hz, 25-Me_2), *m/e* 378 (M^+), 363, 355, 318, 307, and 157 (100%) (Found: C, 88.7; H, 11.1. $C_{28}H_{42}$ requires C, 88.8; H, 11.2%).

Dehydration of Toxisterol$_2$ B (XVIII).—Hydrogen chloride–chloroform dehydrated toxisterol$_2$ B (XVIII) (18 mg) (solution pale blue) to give, after p.l.c., the aromatic compound (XXI) (7 mg, 41%), m.p. 62—64° (from Me_2CO–MeOH), $[\alpha]_D^{22}$ +7.3° (*c* 0.358), identical [i.r., n.m.r. (impurities, τ 8.13, 8.78, 9.28), mass spectra, and t.l.c. (silver nitrate–silica; benzene–light petroleum, 1 : 19)] with the product derived from toxisterol$_2$ C (XIXa).

The Indene (XXII).—The indene (XXII) (15 mg), formed during ^{13}C n.m.r. spectral measurements on toxisterol$_2$ C benzoate (XIXb) (210 mg) in $CDCl_3$ and isolated by p.l.c. (light petroleum–diethyl ether, 91 : 9), was obtained as *prisms*, m.p. 96.5—97° (from Me_2CO–MeOH), $\nu_{max.}$ ($CHCl_3$) 1 600m, 970m, and 910s cm^{-1}, $\lambda_{max.}$ (EtOH) 262 nm (ε 6 900), τ 2.94 (3 H, m, 1-, 2-, and 3-H), 3.19 and 3.27 (2 H, m, 6- and 7-H), 4.79 (2 H, m, 22- and 23-H), 7.60 (3 H, s, 10-Me), 8.73 (3 H, s, 13-Me). and 8.93, 9.03, 9.12, and 9.23 (12 H, side chain methyls), *m/e* 376 (M^+), 361, 333, 305, and 251 (100%) (Found: C, 89.1; H, 10.5. $C_{28}H_{40}$ requires C, 89.3; H, 10.7%).

Toxisterol$_2$ A (S-*Methyl Dithiocarbonate*) (XVIIh).—n-Butyl-lithium (0.20 ml; 2M in hexane) was added to toxisterol$_2$ A (XVIIa) (26 mg) and di-isopropylamine (0.5 ml) in THF (5 ml). After 40 and 110 min carbon disulphide (0.5 ml) and iodomethane (0.5 ml), respectively, were added. Work-up after 3.5 h, chromatography (eluant light petroleum), and p.l.c. (light petroleum–diethyl ether 49 : 1) gave the *dithiocarbonate* (XVIIh) (25 mg, 78%) as a gum, $[\alpha]_D^{21}$ −100° (*c* 0.501), $\nu_{max.}$ (film) 1 250s, 1 218s, 1 055s, 970s, and 765s cm^{-1}, τ 3.68 (2 H, m, 6- and 7-H), 4.28br (1 H, m, 3α-H), 4.80 (2 H, m, 22- and 23-H), 7.43 (3 H, s, SMe), 8.30 (3 H, s, 10-Me), 8.95, 9.03, 9.07, 9.12, and 9.22 (12 H, side chain methyls), and 9.12 (3 H, s, 13-Me), *m/e* 486 (M^+) and 378 (Found: C, 74.15; H, 9.4. $C_{30}H_{46}S_2O$ requires C, 74.0; H, 9.55%). Pyrolysis of the dithiocarbonate (XVIIh) at 220 °C under argon gave an intractable mixture of compounds with *m/e* 426, 378, and 376.

Photolysis of Ergosterol (IIIa) *in Cyclohexane*.—Ergosterol (IIIa) (20 g) in cyclohexane (2.5 l) was irradiated until the intensity of the starting u.v. absorption reached a minimum (72 h). Evaporation, repeated chromatography, and p.l.c. gave *inter alia* (in order of increasing polarity) a hydrocarbon fraction (0.50 g); toxisterols$_2$ D (XIVa), E (XIVb), A (XVIIa), and B (XVIII); suprasterol$_2$ II (XXVIIIa), isolated as its 3,5-dinitrobenzoate (XXVIIIb), m.p. 135—136° (from Me_2CO–MeOH), $[\alpha]_D^{20}$ +108° (*c* 0.345 in acetone); lumisterol$_2$ (XXXa), isolated as its 3,5-dinitrobenzoate (XXXb), m.p. 140—141° (from Me_2CO–MeOH), $[\alpha]_D^{19}$ +20° (*c* 2.755) (lit.,[25] m.p. 140—141°, $[\alpha]_D^{20}$ +24° in benzene), $\lambda_{max.}$ (EtOH), 229 (ε 21 000), 268sh (9 700), 280 (8 600), and 291sh nm (4 700), τ 0.80 (3 H, m, aromatic H), 4.50 (3 H, m, 3α-, 6-, and 7-H), 4.80 (2 H, m, 22- and 23-H), 7.40 (2 H, m, 4-H), 8.93, 9.03, 9.10, and 9.20 (12 H, side chain methyls), 9.17 (3 H, s, 10-Me), and 9.33 (3 H, s, 13-Me), *m/e* 590 (M^+), 465, 378, and 253; an unidentified sterol isolated as its 3,5-dinitrobenzoate, m.p. 208—209.5° (from C_6H_6–EtOH), identical (m.p., t.l.c., u.v., and n.m.r.) with fraction (4.1.2) 3,5-dinitrobenzoate from the ergosterol–ethanol photolysis; ergocalciferol (IVa), isolated as its 3,5-dinitrobenzoate (IVb), m.p. 145.5—147°, identical (mixed m.p., t.l.c., u.v., and n.m.r.) with an authentic sample; a minor unidentified sterol 3,5-dinitrobenzoate as pale yellow needles, m.p. 143—147° (from C_6H_6–EtOH), $[\alpha]_D^{21}$ −27° (*c* 0.415), *m/e* 594, 592, and 590; and a polar intractable resin. Toxisterol$_2$ C (XIXa) was not formed. The light petroleum fraction gave white needles, m.p. 187—217° (from light petroleum). P.l.c. (10% silver nitrate–silica; benzene–light petroleum, 2 : 23) and recrystallisation from C_6H_6–EtOH gave the *diene* (XXXI) (20 mg) as white plates, m.p. 203° (resolidified 205°, to m.p. 210—215°), $[\alpha]_D^{24}$ +210° (*c* 0.308), $\nu_{max.}$ (CS_2) 975s and 850s cm^{-1}, $\lambda_{max.}$ 263.5 (ε 9 000), 273 (12 600), 284 (13 100), and 296 nm (7 600), τ 4.69 (2 H, s, 7- and 7′-H), 4.83 (4 H, m, 22-, 23-, 22′-, and 23′-H), 8.98 and 9.08 (12 H, 2d, 20-, 24-, 20′-, and 24′-Me), 9.15 and 9.16 (12 H, 2d, 25- and 25′-Me_2), and 9.36 (6 H, s, 13- and 13′-Me), *m/e* 544 (M^+), 542, 419, 323, and 293 (Found: C, 88.0; H, 11.8. $C_{40}H_{64}$ requires C, 88.15; H, 11.85%); and a less polar minor solid hydrocarbon (5 mg), τ 4.43 and 4.80 (vinylic), and 8.95, 9.03, 9.12, 9.22, and 9.38 (methyls). T.l.c. (silver nitrate–silica; benzene–light petroleum, 1 : 19) indicated the presence of all the hydrocarbon photofragments in the mother liquors.

Photolysis of Ergocalciferol (IVa).—Ergocalciferol (IVa) (600 mg), in a pentane fraction [b.p. 38—40° (120 ml)] was irradiated at −65 °C. When the starting u.v. absorption had disappeared (4—6 h) the solution was warmed to 0 °C, the pentanes were removed, and the residue was separated by p.l.c. (light petroleum–diethyl ether, 11 : 9) to give (in order of increasing polarity) minor sterols (56 mg, 9%); suprasterol$_2$ II (XXVIIIa) (230 mg, 38%), as white plates, m.p. 110—112° (from Me_2CO) (lit.,[26] 109—110°); and suprasterol$_2$ I (XXIXa) (119 mg, 20%), as white needles, m.p. 101—102° (from Me_2CO) (lit.,[26] m.p. 104°; lit.[21] for 'lumicalciferol' m.p. 102—103°). Esterification gave the derived suprasterol$_2$ II 3,5-dinitrobenzoate (XXVIIIb) as white needles, m.p. 139—141° (from Me_2CO–MeOH) (lit.,[26] 140—141°); suprasterol$_2$ II 3,5-dinitro-4-methylbenzoate (XXVIIIc) as white needles, m.p. 170—171° (from Me_2CO–MeOH) (lit.,[26] 170—172°), $[\alpha]_D^{21}$ +110° (*c* 0.65 in acetone) (lit.,[26] $[\alpha]_D$ 108°); and suprasterol$_2$ I 3,5-dinitrobenzoate (XXIXb) as orange needles, m.p. 147—148° (from Me_2CO–MeOH), $[\alpha]_D^{20}$ −44° (*c* 1.03) (lit.[21] for 'lumicalciferol' 3,5-dinitrobenzoate, m.p. 146—147°, $[\alpha]_D$ −42.7°), *m/e* 590 (M^+), 465, and 378 (Found: C, 71.1; H, 7.8; N, 4.62. Calc. for $C_{35}H_{46}N_2O_6$: C, 71.15; H, 7.85; N, 4.75%).

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Suprasterol$_2$ I Allophanate (XXIXc).—Cyanic acid (from the depolymerisation of cyanuric acid at 400 °C) (0.31 g) in toluene (4 ml) and subsequently pyridine (1 drop) were added to suprasterol$_2$ I (XXIXa) (40 mg) in toluene (1 ml). After 1 h chromatography (eluant EtOAc) gave the allophanate (XXIXc) (45 mg, 92%) as white plates, m.p. 222.5—225° (from EtOAc) (lit.,[26] 219°; lit.,[23] 225—226°; lit.,[6] 223—226°), $[\alpha]_D^{21}$ −58° (*c* 0.259), (lit.,[26] $[\alpha]_D$ −40°; lit.,[23] −58°; lit.,[6] −39°), *m/e* 482 (M^+) and 118 (100%).

Hydrogenation of Ergocalciferol (IVa).—Ergocalciferol (IVa) (306 mg) and chlorotristriphenylphosphinerhodium (79 mg) in benzene (36 ml) were stirred under hydrogen overnight. Evaporation, extraction with light petroleum, filtration through Celite, and p.l.c. (light petroleum–diethyl ether 1 : 1) gave dihydroergocalciferol II (XXIVb) (154 mg, 50%) as an oil and dihydroergocalciferol IV (XXIVd) (82 mg, 27%) as white plates, m.p. 61° (lit.,[17] 61.5—63°) (from MeOH). Esterification gave dihydroergocalciferol II 3,5-dinitrobenzoate (XXIVc) as pale yellow needles, m.p. 164.5—165.5° (lit.,[17] 164—166°) (from Me_2CO–MeOH), τ 0.87 and 0.98 (3 H, m, aryl H), 3.98 and 4.20 (2 H, ABq, *J* 11 Hz, 6- and 7-H), 4.69 (1 H, m, $W_{\frac{1}{2}}$ 6.5 Hz, 3α-H), 4.86 (2 H, m, 22- and 23-H), 6.86 and 7.25 (2 H, m, 4-H), 7.33 (1 H, m), 7.54 (1 H, m), 8.81 (3 H, d, *J* 7.2 Hz, 10-Me), 8.97 and 9.07 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.44 (3 H, s, 13-Me); and dihydroergocalciferol IV 3,5-dinitrobenzoate (XXIVe) as orange prisms, m.p. 174—175° (from Me_2CO–MeOH) (lit.,[17] 175—175.5°), τ 0.86 and 0.90 (3 H, m, aryl H), 3.91 and 4.26 (1 H, ABq, *J* 11 Hz, 6- and 7-H), 4.86 (2 H, m, 22- and 23-H), 5.07 (1 H, m, $W_{\frac{1}{2}}$ 20 Hz, 3α-H), 6.98 and 7.35 (2 H, m, 4-H), 7.43 (1 H, m), 7.52 (1 H, m), 8.83 (3 H, d, *J* 7 Hz, 10-Me), 8.95 and 9.06 (6 H, 2d, *J* 7 Hz, 20- and 24-Me), 9.14 and 9.15 (6 H, 2d, *J* 7 Hz, 25-Me_2), and 9.41 (3 H, s, 13-Me).

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