a. *Preparation of starting materials*

^{15}N-labelled thiourea[16,17] and ethyl 4,4-dimethyl-3-oxovalerate[18] were prepared as described in the literature.
5-Bromo-4-*t*-butyl[1(3)-^{15}N]pyrimidine (1)[19] and its precursors 4-*t*-butyl-2-thioxo[1(3)-^{15}N]pyrimid-6-one[20], 4-*t*-butyl[1(3)-^{15}N]-pyrimid-6-one[19], 5-bromo-4-*t*-butyl[1(3)-^{15}N]pyrimid-6-one[19], 5-bromo-4-*t*-butyl-6-chloro[1(3)-^{15}N]pyrimidine[19] and 5-bromo-4-*t*-butyl-6-hydrazino[1(3)-^{15}N]pyrimidine[19] were prepared according to procedures described for the unlabelled compounds.
The reference compounds 4-amino-6-*t*-butylpyrimidine and 4-*t*-butyl-6-chloropyrimidine were prepared as described in the literature[19], as was 5-bromo-4-*t*-butyl-6-deuteropyrimidine (**4b**) (cf. ref. 5, note d).

b. *Amination of 5-bromo-4-*t*-butyl[1(3)-^{15}N]pyrimidine* (**1**) *with potassium amide in liquid ammonia*[21]

Twenty-five ml of extra-dry liquid ammonia were distilled from potassium. 156 mg of potassium (4 mmol) were added, along with a few crystals of ferric nitrate catalyst. After stirring for 30 min, a solution of 215 mg of 5-bromo-4-*t*-butyl[1(3)-^{15}N]pyrimidine (**1**) (1 mmole) in 2½ ml of absolute ether was run in. The resulting mixture was stirred at −33° for 24 hrs when 500 mg of ammonium chloride were added, after which the ammonia was evaporated. The residue was extracted with 2 × 80 ml of boiling chloroform. After filtration and evaporation of the solvent the oily residue was dissolved in 2 ml of distilled chloroform. Column chromatography of this solution with chloroform as eluent over silica gel (Woelm 02747) gave the unreacted starting material **1** and on changing the eluent to methanol, **2** was obtained. Evaporation of the chloroform gave 120–135 mg of **1**; evaporation of the methanol 45–55 mg of **2**, m.p. 166–170° (lit. 170–171°)[19]. An IR spectrum was identical with that of an authentic sample of **2**.

c. *Conversion of 6-amino-4-*t*-butyl[x-^{15}N]pyrimidine* (**2**) *into 4-*t*-butyl-6-chloro[x-^{15}N]pyrimidine* (**3**)[10]

Fifty mg of 6-amino-4-*t*-butyl[x-^{15}N]pyrimidine (**2**) (0.33 mmoles) were dissolved in 0.5 ml of concentrated hydrochloric acid. A solution of 230 mg of sodium nitrite (3.34 mmol) in 1 ml of water was added dropwise over a period of 20 min, with stirring, maintaining the temperature of the mixture at −10 to −15°. The reaction mixture was stirred for 2½ hrs during which the temperature was allowed to rise to room temperature. N_2 was evolved. Following dilution by the addition of 5 ml of water and adjustment of the pH to 7 through the careful addition of concentrated sodium hydroxide solution, the mixture was extracted with 2 × 30 ml of ether. The ethereal solution was dried over anhydrous $MgSO_4$, the latter was filtered off and the ether was evaporated slowly *in vacuo*, maintaining the bath temperature below 30°. 5–10 mg of **3** were obtained (9–17%). An IR spectrum was identical with that of an authentic specimen.

d. *Calculation of the isotope effect* k_H/k_D

On reacting 215 mg of **4a**, 50 mg of 6-amino-4-*t*-butylpyrimidine (**2**) were obtained, whereas from 215 mg of **4b**, containing 76.1% of deuterium only 22 mg of **2** could be isolated. The isotope effect k_H/k_D was calculated as follows.
From 215 mg of starting material **4b** containing 26.7% of deuterium 137 mg of **4b** containing 32.1% of deuterium was retrieved after the reaction. From these data, utilizing the formula $k_H/k_D = \ln(H/H_o)/\ln(D/D_o)$ the value of 1.9 was obtained.

Acknowledgements

We are indebted to Drs. *C. A. Landheer* and Mr. *W. P. Combé* for the mass spectroscopic data, Mr. *W. Ch. Melger* for advice on the chromatographic analyses and Mr. *A. van Veldhuizen* for recording the IR spectra.

[16] *A. Bendick, J. F. Tinker* and *G. B. Brown*, J. Amer. Chem. Soc. **70**, 3109 (1948).
[17] *A. A. Plentl* and *R. Schoenheimer*, J. Biol. Chem. **153**, 203 (1941).
[18] *R. Levine* and *C. R. Hauser*, J. Amer. Chem. Soc. **66**, 1768 (1944).
[19] *H. C. van der Plas*, Recl. Trav. Chim. Pays-Bas **84**, 1101 (1965).
[20] *G. W. Anderson, I. F. Halverstadt, W. H. Muller* and *R. O. Loblin Jr.*, J. Amer. Chem. Soc. **67**, 2197 (1945).
[21] Precautions should be taken to exclude all traces of water; these lead to several by-products, for example 4-*t*-butylpyrimid-6-one.

The "overirradiation products" of previtamin D and tachysterol: toxisterols[1-3]

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(Received December 17th, 1976)

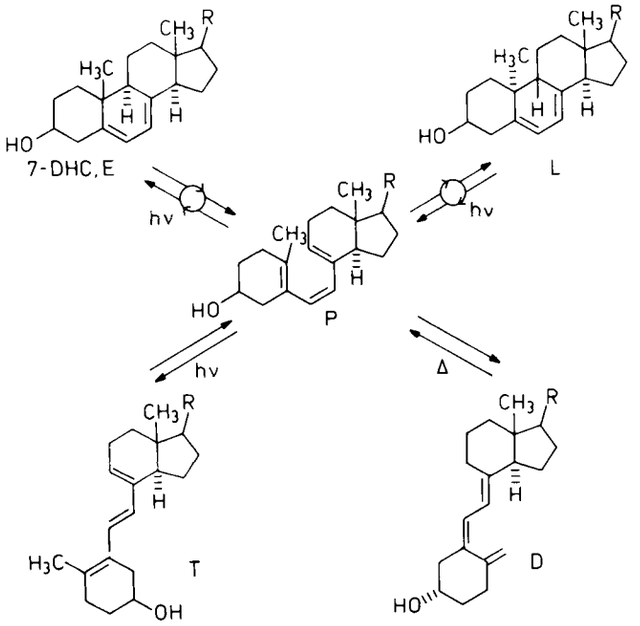
Abstract. The structures of the "toxisterols", products formed upon prolonged irradiation of previtamin D and its photoisomers, have been investigated. Irradiations ($\lambda > 300$ nm) were carried out both in diethyl ether and in alcoholic solvents under conditions where the thermal isomerization to vitamin D is suppressed. From the irradiation mixtures a total of thirteen toxisterols have been isolated. On the basis of structural similarities they have been classified into six categories.
In ethereal solution two bicyclo[3.1.0]hexeno compounds (toxisterols C) constitute the major fraction of the irradiation mixture; in addition, a partly deconjugated 9,10-seco-triene (toxisterol D1), two C-8 spiro compounds (toxisterols A) and a cyclobuteno compound (toxisterol E1) are found. In alcoholic solutions about half of the irradiation mixture consists of three alcohol addition products (toxisterols B) – which, upon chromatography, in part give rise to two conjugated 9,10-seco-trienes (toxisterols D2 and D3) – and a reduction product (toxisterol R1); furthermore, three toxisterols A, two toxisterols C and toxisterol D1 are found. Toxisterols A, B and R represent novel types of products in hexatriene photochemistry.

Introduction

Ultraviolet irradiation of each of the isomers 7-dehydrocholesterol (**7-DHC**) – or ergosterol (**E**) –, previtamin D (**P**), lumisterol (**L**) and tachysterol (**T**) leads to a quasi-photoequilibrium, the composition of which depends upon the ratios of their extinction coefficients at the wavelength of irradiation in conjunction with the quantum yields of the

[1] Paper XXVI in the series, Studies on Vitamin D and Related Compounds. For Paper XXV, see *W. H. Okamura, M. L. Hammond, H. J. C. Jacobs* and *J. van Thuijl*, Tetrahedron Letters 4807 (1976).
[2] Part of this work has been described in a preliminary communication, see *F. Boomsma, H. J. C. Jacobs, E. Havinga* and *A. van der Gen*, Tetrahedron Letters 427 (1975).
[3] A detailed report is given in the Thesis of *F. Boomsma*, Leiden 1975.

various interconversions. When irradiation is carried out at room temperature or higher, the reaction mixture also contains vitamin D (**D**), formed by a reversible thermal isomerization from previtamin D[4]. The scheme of reactions thus obtained is depicted in Figure 1.



7-DHC = 7-Dehydrocholesterol
E = Ergosterol
L = Lumisterol
T = Tachysterol
P = Previtamin D
D = Vitamin D

Fig. 1. Scheme of reversible interconversions from previtamin D; R = C_9H_{17}: Vitamin D_2 series, R = C_8H_{17}: Vitamin D_3 series.

Upon prolonged irradiation these compounds, all showing intense absorption bands between 250 and 300 nm, gradually disappear in favour of a number of so-called "overirradiation products": compounds that are formed in irreversible photoreactions from any of the isomers shown in the scheme. Although the formation of such compounds has already been recognized in the early days of vitamin D chemistry[4], and the isolation of several "overirradiation products" is described in the literature[5], attempts to clarify their structures have met with considerable difficulties. Only fairly recently *Dauben* et al.[6] were able to report the complete structure of suprasterols I and II, both photoproducts originating from vitamin D. Subsequently, *Havinga* and collaborators[7] reported the isolation and structure elucidation of four additional photoproducts[8], thus supposedly completing the family of "overirradiation products" from vitamin D.

The present paper is concerned with an investigation of the products formed upon prolonged irradiation of the dienes and trienes depicted in Fig. 1, under conditions where the thermal isomerization to vitamin D is suppressed. Earlier studies in this laboratory[9] had already indicated that – apart from vitamin D – previtamin D might be a major source of "overirradiation products", although the formation of products from tachysterol and 7-dehydrocholesterol could not be excluded[10]. Since previtamin D is supposed[5f,9] to be the direct precursor of the so-called toxisterols, compounds of alleged toxicity[12] for which no structures had been proposed at the outset of our investigations, this name has been retained for all photoproducts to be described in this paper. A detailed study of their structures and modes of formation was expected not only to enlarge the picture of photoreactions in the vitamin D field, but also to contribute to the understanding of polyene photochemistry in general. In the present article the isolation and structure elucidation of the aforementioned toxisterols is described. In a second, accompanying, article an attempt is made to rationalize the complex photochemical behaviour of conjugated triene systems, such as are present in previtamin D and its 6,7-*trans*-isomer, tachysterol.

Results

7-Dehydrocholesterol was used as starting material. Irradiations were continued until the UV characteristics of **7-DHC**, **L**, **P** and **T** had disappeared. Since some of the "overirradiation products" are reported[12] to show absorption bands around 250 nm, care was taken to prevent photodestruction of these compounds by using radiation of wavelengths longer than 300 nm. Concentrations were chosen such as to avoid formation of bischolestadienols[10]. Solvents used include methanol, ethanol and diethyl ether. The reaction temperature was carefully controlled in order to keep the thermal isomerization to vitamin D_3 to a minimum. As it turned out, formation of vitamin D_3 and its photoproducts, the suprasterols, could be completely avoided in alcoholic solutions, and was small in ether where longer irradiation times were applied.

From the irradiation mixtures a total of thirteen toxisterols were isolated. On the basis of structural similarities they have been classified into six categories: types A through E, and R. The designations A and B correlate with earlier use[4a,5f]. Of the thirteen toxisterols three turned out to possess known structures (apart from the sidechain), toxisterols D2, D3 and R1 being identical with *trans*-isovitamin D[13], isotachysterol[13] and dihydrovitamin D-I[14], respectively. Toxisterol A1 proved to be identical (UV, IR, NMR) with a "toxisterol A" of unknown structure originally isolated in the D_2 series by *Westerhof* and *Keverling*

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[4] For recent reviews, see:
[a] *G. M. Sanders, J. Pot* and *E. Havinga*, Fortschr. Chem. Org. Naturstoffe **27**, 131 (1969);
[b] *E. Havinga*, Experientia **29**, 1181 (1973).

[5] See *e.g.*
[a] *A. Windaus, J. Gaede, J. Köser* and *G. Stein*, Ann. **483**, 17 (1930);
[b] *M. Müller*, Z. physiol. Chem. **233**, 223 (1935);
[c] *F. Laquer* and *O. Linsert*, Klin. Wschr. **12**, 753 (1933);
[d] *O. Linsert*, Chem. Abstr. **30**, 2326 (1936);
[e] *J. Green*, Biochem. J. **49**, 232 (1951);
[f] *P. Westerhof* and *J. A. Keverling Buisman*, Recl. Trav. Chim. Pays-Bas **75**, 1243 (1956);
[g] *K. Pfordte*, Pharm. Zentralhalle **106**, 370 (1967).

[6a] *W. G. Dauben* and *P. Baumann*, Tetrahedron Letters 565 (1961);
[b] *W. G. Dauben*, cited in *R. B. Woodward* and *R. Hoffmann*, The Conservation of Orbital Symmetry, p. 80, Verlag Chemie, Weinheim 1970.

[7] *S. A. Bakker, J. Lugtenburg* and *E. Havinga*, Recl. Trav. Chim. Pays-Bas **91**, 1459 (1972).

[8] One of these probably is identical with the earlier reported "Linsert's compound" (ref. 5d).

[9] *G. M. Sanders*, Thesis, Leiden 1967; see also ref. 4a.

[10] In preliminary experiments (ref. 3) *direct* irradiation of not too diluted solutions of 7-dehydrocholesterol with light of $\lambda >$ 300 nm gave rise to a "dimeric" material which proved to be a mixture of three isomeric bis-cholestadienols, formerly obtained from *sensitized* irradiation of 7-dehydrocholesterol (ref. 11).

[11] *F. Boomsma, H. J. C. Jacobs, E. Havinga* and *A. van der Gen*, Recl. Trav. Chim. Pays-Bas **92**, 1361 (1973).

[12] See ref. 5f and references cited therein.

[13] For the analogous compounds in the vitamin D_2-series, see: *T. Takahashi* and *R. Yamamoto*, Yakugaku Zasshi **89**, 919 (1969) and references cited therein. We thank Dr. *T. Kobayashi* for a translation of this paper.

[14] *J. L. J. van de Vliervoet*, Thesis, Leiden 1956.

Buisman[5f]. The toxisterols of type A and B appear to be the ones mainly responsible for the UV absorption around 250 nm, repeatedly reported[12] for "overirradiation products" (or mixtures of products) from 7-dehydrocholesterol or ergosterol. To our knowledge the types of photoproducts represented by toxisterols A, B and R1 have not been reported previously in hexatriene photochemistry.

The composition of the irradiation mixtures (isolated yields) is given in Table I. The strong solvent dependency will be commented upon in the accompanying paper.

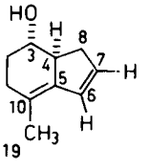
Table I Composition of irradiation mixtures of 7-dehydrocholesterol (isolated yield in % of amount of starting material).

Irradiation in	MeOH (48 hrs)	EtOH (48 hrs)	Ether (48 hrs)	Ether (100 hrs)
Yield / Compound				
Toxisterol A1	2%	2%	4%	8%
Toxisterol A2	5%	5%	–	–
Toxisterol A3	< 1%	< 1%	2%	3%
Toxisterol B1	7%	8%	–	–
Toxisterol B2	11%	12%	–	–
Toxisterol B3	4%	2%	–	–
Toxisterol C1	11%	13%	16%	26%
Toxisterol C2	2%	2%	3%	4%
Toxisterol D1	8%	9%	8%	15%
Toxisterol D2	0–4%	0–4%	–	–
Toxisterol D3	0–4%	0–4%	–	–
Toxisterol E1	–	–	4%	8%
Toxisterol R1	7%	6%	–	–
7-Dehydrocholesterol	–	–	12%	< 1%
Lumisterol	< 1%	< 1%	12%	< 1%
Previtamin D	–	–	13%	< 1%
Tachysterol	–	–	–	–
Suprasterol I	–	–	–	2%
Suprasterol II	–	–	–	4%

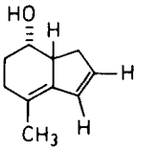
Toxisterols A[15]

Toxisterols A1 and A3 are produced both in alcoholic solvents and in ether; toxisterol A2 is found only after irradiation in alcohols.

Mass spectrometry shows the three toxisterols A to be isomeric with previtamin D_3. Inspection of the ^{1}H-NMR spectra reveals the following characteristics. All three toxisterols A contain a methyl group attached to a double bond, the NMR signal appearing as a doublet with J 2 Hz, and two mutually coupled olefinic protons; the coupling constant (5–6 Hz)[16] indicates *cis*-olefinic protons in a 5-membered ring[17]. In toxisterols A1 and A2 the 3-H signal appears as a doublet (J 3.5 Hz) of triplets (J 11 Hz), attributable to vicinal coupling of an axial proton (3-H) with one equatorial and two axial protons; in toxisterol A3 the narrow 3-H multiplet is indicative of an equatorial proton coupled with one equatorial and two axial protons; the chemical shift values also indicate the hydrogen at C-3 to be axially oriented in A1 and A2, and equatorially in A3. In all three toxisterols A a single proton in the 2–2.5 ppm region is substantially shifted downfield, both upon dinitrobenzoylation and upon Eu(fod)$_3$ addition[3]; it must therefore be situated very close to the hydroxyl group at C-3. In A1 and A2 this proton, apparently 4-H, shows a splitting of 11 Hz, indicating a diaxial vicinal coupling; in A3 the signal apparently arises from equatorial/axial coupling.



I

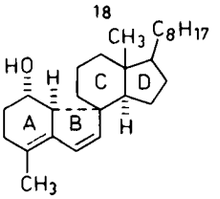


II

From these data we conclude that A1 and A2 have partial structure **I**, and A3 partial structure **II**. This conclusion is confirmed by the UV, IR and CD spectra. The UV absorption spectra of all isomers show λ_{max} 251 nm, ε 15,000; this unusually high wavelength of maximum absorption finds its explanation in the endocyclic position of one of the double bonds in a 5-membered ring[18]. The IR absorption spectra confirm the equatorial (A1 and A2) and axial (A3) orientation of the C–O bonds. The CD curves exhibit identity of sign of the Cotton effects of toxisterols A1 ($\Delta\varepsilon_{248}$ −19.9) and A2 ($\Delta\varepsilon_{249}$ −5.25), as is to be expected if the configuration at C-4 in these compounds is similar.

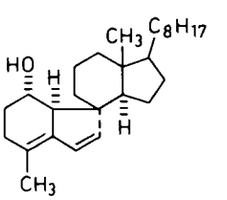
The correctness of these partial structures is established further by double-resonance experiments[3] with A1. In addition to the relationship between 3-H and 4-H these experiments demonstrate that the splitting of the CH_3-19 signal is caused by a homo-allylic long-range coupling with 4-H.

The exclusive coupling of 7-H with 6-H suggests that no hydrogen atoms are attached to C-8. As the ^{13}C-NMR spectra indeed show the appearance of a fourth non-protonated carbon atom (in addition to C-5, C-10 and C-13) we conclude that the C/D part of the steroid molecule is attached to C-8 in a spiro fashion. This leaves structures **1** and **2** (equatorial 3-OH) for toxisterols A1 and A2, and either structure **3** or **4** for A3. Since dinitrobenzoylation of toxisterols A2 and A3 substantially affects the chemical shift of CH_3-18, thus requiring the proximity of the hydroxyl group and the angular methyl group, structure **2** is assigned to A2 and structure **3** to A3. Toxisterol A1 then



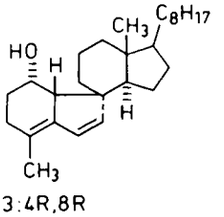
1:4S,8S

A1



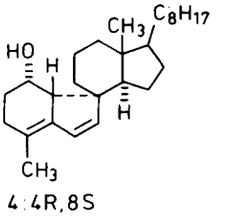
2:4S,8R

A2



3:4R,8R

A3



4:4R,8S

[15] Toxisterols A1, A2 and A3 in the vitamin D_2 series were independently isolated and identified by *Barton* et al.: *A. G. M. Barret, D. H. R. Barton, M. H. Pendlebury, L. Phillips, R. A. Russell, D. A. Widdowson, C. H. Carlisle* and *P. F. Lindley*, J.C.S. Chem. Comm. 101 (1975).

[16] In A1 the two olefinic protons have the same chemical shift. However, addition of Eu(fod)$_3$ shows that they too couple with J 6 Hz.

[17] *P. Laszlo* and *P. Stang*, Organic Spectroscopy, p. 181, Harper and Row, New York 1971.

[18] *J. A. Hirsch*, Concepts in Theoretical Organic Chemistry, p. 40, Allyn and Bacon Inc., Boston 1974.

should have structure **1**, which is confirmed by the upfield shift of CH_3-21 (side chain) on dinitrobenzoylation and by a 12% N.O.E.-enhancement of the 7-H signal upon saturation of the CH_3-18 signal[19].

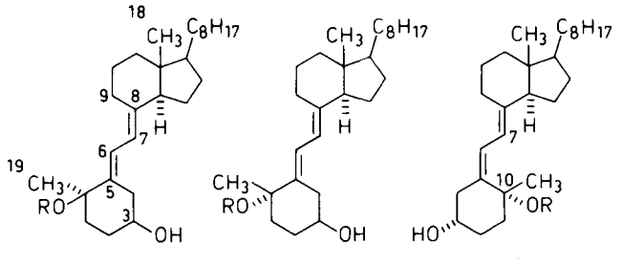
No indications were found for the presence of a fourth isomer, corresponding to structure **4**, in the irradiation mixtures.

Toxisterols B

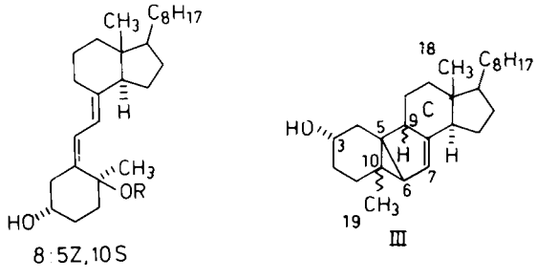
When the irradiations are carried out in alcoholic solutions, mass spectrometry of the resulting mixtures shows the presence of alcohol addition products. From ethanol as well as from methanol solution a set of three such adducts could be isolated. Similarities in chromatographic and spectroscopic behaviour as well as in chemical stability indicate a one-to-one correspondence between the members of these sets.

All adducts show strong UV absorption with a maximum at 251–252 nm and two additional maxima or shoulders at 245 and 260 nm. This absorption pattern, which is identical with that of dihydrotachysterol[5f,20], indicates the presence of s-*trans*-diene moieties in the adducts. The NMR spectra supply further evidence for this feature by showing[21] two olefinic protons coupled with J 11 Hz. The CH_3-18 signals are found at relatively high field (0.56 ppm), as is often the case when a 7–8 double bond is present. The CH_3-19 signals appear at relatively low field (1.30–1.50 ppm) as singlets, suggesting that the alkoxy group is attached to C-10. The methoxy and ethoxy groups exhibit the expected chemical shifts and coupling constants. The CH_2 protons of the ethoxy group are non-equivalent.

These data indicate that all six compounds have a 9,10-seco-5,7-cholestadiene skeleton. Assuming that the 7–8 double bond has the usual[22], more stable, *E* configuration, four isomeric structures (**5**–**8**) are possible. Because of severe steric interaction between the equatorial substituent at C-10 and the hydrogen at C-7, the 5*Z* isomers **7** and **8** are expected to be less stable than the 5*E* isomers **5** and **6**. We therefore ascribe the 5*E* configuration to the more abundant and more stable adducts B1 and B2. Toxisterol B3, which is markedly less stable and is isolated in considerably smaller amounts (Table I), then should have either structure **7** or **8**. In accord with this configurational assignment the UV molar absorptivity of B3 is much lower than that of either B1 or B2, obviously as a result of distortion of the planarity of the diene system in B3[24].



5:5E,10R B1 6:5E,10S B2 7:5Z,10R B3



8:5Z,10S III

The assignment of configuration at the epimeric centre C-10 is complicated by the possible occurrence of ring A conformational equilibria, which appear to be rather common in 9,10-seco-5,7-cholestadienes[25]. In toxisterol B2 however, the chemical shift of the C-3 proton, together with the width of its resonance signal, clearly indicate its axial orientation[26], and thus the equatorial orientation of 3β-OH. In contrast, the chemical shifts of 3-H in toxisterol B1 as well as in B3 indicate an equilibrium mixture of ring-A conformers. Comparison of the IR absorption spectra of toxisterols B in the C–O stretching region confirms the conformational homogeneity of B2 with equatorial C_3–O bond, and the presence of both equatorial and axial C_3–O bonds in B1 and B3. The configuration at C-10 can now be assessed by considering the chemical shift of the C-19 methyl protons. In equatorial orientation the methyl group will be situated in the deshielding region of the 5–6 double bond. The upfield shift (1.29–1.31 ppm) of the methyl protons in B2 relative to those in B1 and B3 (1.47–1.50 ppm) then implies the axial orientation of CH_3-19 in the former compound, and a mixture of axial and equatorial orientations in the latter two toxisterols. In B2 this establishes the *cis* relationship of 3β-OH and CH_3-19, and thus structure **6** corresponds to B2. Toxisterol B1 then must have structure **5**. If we take the near-identity of the relevant IR and NMR spectral properties of B1 and B3 as evidence for the similarity of substitution pattern in ring A, then structure **7** can be assigned to B3.

It should be mentioned that in the methanol irradiation mixtures we have found evidence (TLC, UV) for the presence of a fourth adduct; attempts to isolate this compound, presumably having structure **8**, failed however, due to its lack of stability.

Toxisterols C

Toxisterol C1 is the main product of the irradiations, both in alcoholic solvents and in ether; toxisterol C2 is less stable and is formed in smaller amounts (Table I). Mass spectrometry shows the toxisterols C to be isomeric with previtamin D_3. The UV absorption spectra show maxima at 227 nm (ε 6,600) in the case of C1, and at 229 nm (ε 5,400) in the case of C2, indicative of conjugation between a cyclopropane ring and a double bond[27]. This suggests the presence of a bicyclo-[3.1.0]hexene moiety in both C1 and C2, as pictured in general structure III. Using the rules formulated by *Pete*[27],

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[19] These assignments agree with those recently made by *Barton* et al. (ref. 15), their toxisterols A, B and C being identical (apart from the sidechain) with our toxisterols A1, A3 and A2, respectively (^{13}C-NMR, $[\alpha]_D$). We thank Professor *Barton* for a preprint of the paper concerned, including the ^{13}C-NMR data.

[20a] *J. L. J. van de Vliervoet, P. Westerhof, J. A. Keverling Buisman* and *E. Havinga*, Recl. Trav. Chim. Pays-Bas **75**, 1179 (1956); [b] *R. M. Wing, W. H. Okamura, M. R. Pirio, S. M. Sine* and *A. W. Norman*, Science **186**, 939 (1974).

[21] In the methanol adduct B3 the olefinic protons appear to be isochronous, also after acetylation of the 3-OH group.

[22] *Cf.* Vitamin D, 5*E*-vitamin D, and their 10,19-dihydro derivatives (see ref. 23 for a paper on the dihydro products). The 7*Z* isomers are destabilized by a severe interaction between 6-H and the C-15 methylene group.

[23] *W. H. Okamura, M. L. Hammond, A. Rego, A. W. Norman* and *R. M. Wing*, submitted for publication. We thank Professor *Okamura* for a preprint of this paper.

[24] *H. H. Jaffé* and *M. Orchin*, Theory and Applications of Ultraviolet Spectroscopy, p. 384, Wiley and Sons Inc., New York 1962.

[25] See ref. 23 and references cited therein.

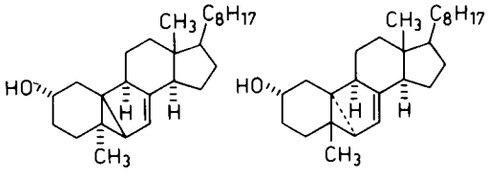
[26] Taking ~4.05 and ~3.50 ppm for the chemical shifts of 3-H in pure axial and equatorial (3β-OH) conformers. Use of the values 4.03 and 3.53 ppm for estimating the A-ring conformational equilibrium in the four 10,19-dihydro derivatives of vitamin D and its 5*E* isomer gives population ratios in good agreement with those determined by *Okamura* et al. (ref. 23) from LIS studies and coupling constants.

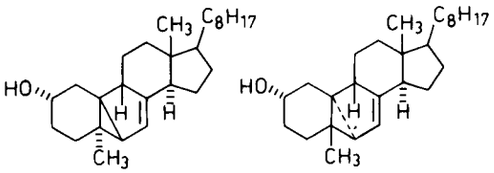
[27] *J.-P. Pete*, Bull. Soc. Chim. France 357 (1967) and references cited therein.

λ_{max} 220–224 nm is calculated for this structure (assuming a dihedral angle of 110 – 90° between the plane of the double bond and the cyclopropane ring), which agrees reasonably well with the observed values for C1 and C2. *Barton* and *Kende*[28] report λ_{max} 222 nm (ε 6,800) for a compound with analogous structure.

In agreement with structure **III** the ^{1}H-NMR spectra of both C1 and C2 show only one olefinic proton (7-H, coupled with 6-H with $J \sim 2$ Hz) and a broad multiplet of an axial 3-H. Toxisterol C1 displays a CH_3-19 signal at 0.81 ppm; the CH_3-18 signal is found at 0.57 ppm, as expected for a compound with a 7–8 double bond. In C2 the CH_3-19 signal appears at 0.92 ppm, while the C-18 protons absorb at 0.73 ppm. The latter value is unusual for a compound with a 7–8 double bond, and indicates some special spatial arrangement around the CH_3-18 group, *e.g.* a boat conformation of ring C or the close proximity of the CH_3-19 group (see below).

The ^{13}C-NMR spectra are in agreement with the proposed general structure **III**.





Excluding the possibility of *trans*-fusion between a cyclopropane ring and a 5- or 6-membered ring, four stereoisomeric structures (**9**–**12**) are possible. Molecular models show that in structures **11** and **12** ring C must have a boat conformation, while in **10** there is considerable steric interaction between the CH_3-18 and CH_3-19 groups. Structure **9**, however, shows none of these destabilizing factors, and should therefore be the thermodynamically most stable isomer. Structures **9** and **12** are formally the result of an overall $[{}_\pi 4_a + {}_\pi 2_a]$ cycloaddition within the triene system of previtamin D, while **10** and **11** are the products to be expected from an overall $[{}_\pi 4_s + {}_\pi 2_a]$ process.

The spectral data obtained did not allow of an unambiguous determination of the stereochemistry of toxisterols C1 and C2, although the chemical shift of CH_3-18 in C1 (0.57 ppm) suggests an essentially undisturbed spatial disposition of the 7–8 double bond with respect to CH_3-18, which, as outlined above, is only to be expected in structure **9**. In view of the interesting mechanistic implications of the stereochemistry of these compounds (see accompanying paper) an X-ray analysis was carried out on the dinitrobenzoate ester of C1. This analysis[29] indeed shows C1 to have structure **9**, *i.e.* the energetically more favourable isomer.

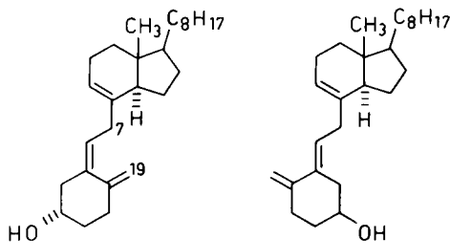
The configuration of C2 can now be inferred from a comparison of the CD and NMR data. While the circular dichroism curve of toxisterol C1 is positive ($\Delta\varepsilon_{233}$ +4.55, shoulder), that of C2 turns out to be negative ($\Delta\varepsilon_{227}$ −7.09). Since the sign of the Cotton effect, associated with the UV absorption band at 227–229 nm, may be expected to reflect the orientation of the cyclopropane ring with respect to the double bond[30], we conclude that in this respect the two compounds display a mirror-image relationship. This rules out structure **11** for toxisterol C2, and leaves only structures **10** and **12** for consideration. We further note that in the ^{1}H-NMR spectra the signals of CH_3-18 and CH_3-19 are both shifted downfield relative to C1. The same holds for the C-18 and C-19 signals in the ^{13}C-NMR spectra. This suggests formula **10** for toxisterol C2, since in this structure a severe steric interaction of the angular methyl groups would be expected, due to their close proximity[31]. We therefore tentatively assign structure **10** to toxisterol C2.

Toxisterols D

Toxisterol D1 is formed by irradiation both in alcoholic solvents and in ether. Toxisterols D2 and D3 are found only after chromatography of the mixtures produced by irradiation in alcoholic solvents. The yields of D2 and D3 vary widely in duplicate experiments. Most likely therefore, the toxisterols D2 and D3 arise by loss of alcohol from the toxisterols B[33]. Mass spectrometry shows the three toxisterols D to be isomeric with previtamin D_3.

The ^{1}H-NMR spectrum of toxisterol D1 presents the signals of four olefinic protons. Two of these are part of a terminal methylene group, as is indicated by their chemical shifts (4.73 and 4.93 ppm), their small coupling constant (J 2 Hz) and the presence of the characteristic IR absorptions at 3075, 1640 and 900 cm^{-1}. The third olefinic proton appears as a multiplet at 5.20 ppm, the fourth at 5.34 ppm as a triplet with J 7 Hz. The latter arises from coupling with two equivalent adjacent protons, which appear at 2.79 ppm as a doublet. The chemical shift of these protons indicates that they are allylic to two double bonds.

These data suggest that toxisterol D1 is either 9,10-seco-5*Z*,8,10(19)-cholestatrien-3β-ol (**13**) or its 5*E* isomer (**14**). In accord with these structures the UV absorption spectrum shows only a shoulder at 217 nm (ε 7,200); (1,2-dimethylenecyclohexane shows[34] λ_{max} 220 nm, ε 5,500).



D1

Evidence for the configuration of the 5–6 double bond was obtained by heating D1 *in vacuo* at 160° for 15 minutes. The UV spectrum now shows the familiar absorption of tachysterol (λ_{max} 280 nm, shoulders at 271 and 290 nm). This transformation can be understood only if D1 has structure **13**, which upon heating can undergo a thermally allowed

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[28] *D. H. R. Barton* and *A. S. Kende*, J. Chem. Soc. 688 (1958).

[29] The X-ray analysis of toxisterol C1 dinitrobenzoate is reported elsewhere; see *A. J. de Kok*, *F. Boomsma* and *C. Romers*, Acta Cryst. *B***32**, 2492 (1976).

[30] *T. Norin*, *S. Strömberg* and *M. Weber*, Acta Chem. Scand. **27**, 15 (1973); see also *P. Crabbé* in *E. Heftmann*, Modern Methods of Steroid Analysis, p. 340, Academic Press, New York 1973.

[31] Although formerly steric interaction has always been associated with upfield shifts in ^{13}C-NMR, more recent studies (ref. 32) report that syn-axial interactions cause a downfield shift ranging up to 3.4 ppm. We believe that an analogous interaction can well explain the downfield shift of C-18 and C-19 in toxisterol C2.

[32a] *F. Khuong-Huu*, *M. Sangare*, *V. M. Chari*, *A. Bekaert*, *M. Devys*, *M. Barbier* and *G. Lukacs*, Tetrahedron Letters 1787 (1975);
[b] *S. H. Grover* and *J. B. Stothers*, Can. J. Chem. **52**, 870 (1974).

[33] The designation of D2 and D3 as toxisterols is probably less appropriate if this label is restricted to *photo*products.

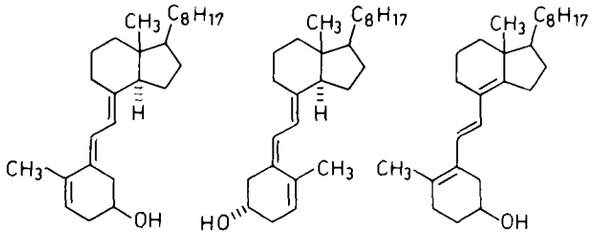
[34a] *A. T. Blomquist* and *D. T. Longone*, J. Amer. Chem. Soc. **79**, 3916 (1957);
[b] *W. G. Dauben*, *J. Rabinowitz*, *N. D. Vietmeyer* and *P. H. Wendschuh*, J. Amer. Chem. Soc. **94**, 4285 (1972).

suprafacial [1,5] hydrogen shift from C-7 to C-19 to form tachysterol.

Toxisterols D2 and D3 both show strong UV absorption with $\lambda_{max} \sim 288$ nm, indicative of an all-*trans* triene system. Though neither was obtained completely pure, the NMR spectra of the 3,5-dinitrobenzoate esters are characteristic enough to allow structure determination.

The NMR spectrum of the dinitrobenzoate ester of D2 shows the CH_3-18 signal at 0.52 ppm (suggesting the presence of a 7–8 double bond), the CH_3-19 signal at 1.96 ppm (indicating that CH_3-19 is attached to a double bond), two olefinic protons at 5.91 and 6.54 ppm as doublets with *J* 12 Hz (indicating a s-*trans* diene) and one other olefinic proton as a multiplet at 5.62 ppm. These data characterize D2 as a 9,10-seco-1(10),5,7-cholestatrien-3β-ol, either the 5*E*-isomer **15** or the 5*Z*-isomer **16**.

Both isomers (*trans*-isovitamin D and *cis*-isovitamin D, respectively) have been described in the vitamin D_2 series by



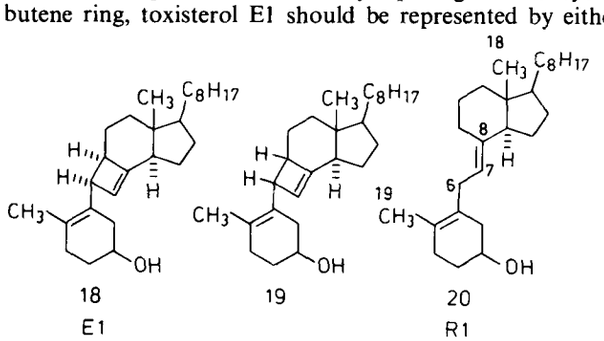
Takahashi and *Yamamoto*[13]. Comparison of their NMR data with those of D2 shows this toxisterol to be the 5*E* isomer **15**.

The NMR spectrum of the 3,5-dinitrobenzoate ester of toxisterol D3 shows the CH_3-18 signal at 0.91 ppm (suggesting the presence of a 8–14 double bond), the CH_3-19 signal at 1.90 ppm (indicating that CH_3-19 is attached to a double bond) and two olefinic protons at 6.30 and 6.54 ppm as doublets with *J* 16 Hz (indicating a *trans*-substituted double bond). These data allow of the identification of toxisterol D3 as isotachysterol **17** (all-*trans*-9,10-seco-5(10),6,8(14)-cholestatrien-3β-ol), described earlier in the vitamin D_2 series[13].

Toxisterol E1

Toxisterol E1 was observed only after irradiation in ether. It appeared to have the same R_F value on TLC and the same gaschromatographic behaviour as previtamin D. Mass spectrometry shows it to be an isomer of previtamin D_3.

Toxisterol E1 shows no UV absorption maximum at wavelengths longer than 210 nm. The NMR spectrum reveals that CH_3-19 is attached to a double bond and that only one olefinic proton is present. Furthermore, the signal of one proton appears at 3.76 ppm, as expected for a proton in allylic position to two double bonds and attached to a 4-membered ring (*cf.* the cyclobutene suprasterols[7]). On heating, toxisterol E1 is converted into previtamin D, as evidenced by its GC behaviour (formation of pyro- and isopyrocalciferol). Since this requires a conrotatory opening of the cyclobutene ring, toxisterol E1 should be represented by either



structure **18** or **19**, both showing a *cis* relationship between the hydrogens attached to C-6 and C-9. We prefer structure **18** for E1 because it can explain the significant upfield shift of the CH_3-18 signal upon dinitrobenzoylation (0.63 to 0.50 ppm).

We note that the thermal conversion of toxisterol E1 to previtamin D indicates its photochemical formation from tachysterol (disrotatory closure). Thus it constitutes a further example of formation of a vinylcyclobutene from a 1,3*E*,5-hexatriene[35].

Toxisterol R1

After prolonged irradiation of 7-dehydrocholesterol in alcoholic solvents the mass spectrum of the reaction mixture shows substantial increase of the *m/e* 386 peak relative to the parent peak (*m/e* 384) of 7-dehydrocholesterol. Analysis of fractions obtained by chromatography indeed showed the presence of at least two reduction products. After several attempts, one of these, toxisterol R1, could be isolated in a pure state.

Toxisterol R1 shows no UV absorption maximum above 210 nm. The NMR spectrum shows one olefinic proton as a triplet with *J* 7 Hz, a methyl group attached to a double bond, and two equivalent protons as a doublet with *J* 7 Hz at 2.80 ppm. The CH_3-18 signal appears at 0.55 ppm, suggesting the presence of a 7–8 double bond. These data are consistent with structure **20**, the 7–8 double bond being assigned the usual, more stable, *E* configuration[22]. This compound, 9,10-seco-5(10), 7*E*-cholestadien-3β-ol, was earlier prepared by *Van de Vliervoet*[14] by reduction of vitamin D_3 with sodium in *n*-propanol, and called dihydrovitamin D_3-I. The coupling between the two protons at C-6 and the olefinic proton at C-7 was demonstrated by double-resonance NMR experiments.

Experimental procedures

Instrumentation

Melting points (uncorrected) were determined in evacuated capillary tubes. UV absorption spectra were measured with a Cary M14 spectrophotometer, IR spectra (in KBr discs or neat) with a Unicam SP 100 instrument. Specific rotations were measured with a Bendix NPL automatic polarimeter 143 D. CD spectra (methanol solution) were obtained using a Roussel-Jouan Dichrographe Mark III. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solutions on a Jeol JNM-PS 100 spectrometer; chemical shifts are reported in ppm downfield from TMS as internal standard. Mass spectra were recorded on an AEI MS 902 mass spectrometer (70 eV, heated direct introductory lock). TLC was carried out using 0.2 mm silicagel plates (Kieselgel G Merck); spots were made visibele by spraying with a solution of *p*-toluenesulfonic acid (40 g in 160 ml of ethanol) and heating for 10 minutes at 100°. GC was performed on a Hewlett-Packard F & M 402 gaschromatograph using a glass column packed with 1% SE-30 on GasChrom Q (80–100 mesh); temperature of injection port and column 280° and 230°, respectively. Elution times are given relative to cholestane.

Materials

7-Dehydrocholesterol of purity >99% (UV, GC) was obtained as a gift from Philips-Duphar (Weesp, the Netherlands). Solvents were distilled before use in chromatography; methanol and diethyl ether of A.R. grade and absolute ethanol were used for the irradiations.

Short-column chromatography

Kieselgel G (Merck) (80 g) was heated at 110° for 24 hrs and suspended in benzene (benzene/acetone 9/1; benzene/hexane 1/1). For

[35a] *J. M. G. Bonfrèr, H. J. C. Jacobs* and *E. Havinga*, Tetrahedron Letters 3741 (1975);
[b] *J. W. J. Gielen, H. J. C. Jacobs* and *E. Havinga*, Tetrahedron Letters 3751 (1976).

Table II Melting points, specific rotations, relative retention times and mass spectral data of toxisterols.

		M.p. (°C)[a]	$[\alpha]_D$[b]	R_F(TLC)[c]	R_{rel}(GC)[d]	MS: m/e
	A1	94.5–96.5°	−100°	0.57 (1)	1.41	384 (M^+)
	A1-DNB			0.65 (3)		
	A2		+ 39°	0.31 (1)	0.98	384 (M^+), 253 (base)
	A2-DNB			0.64 (3)		
	A3		+128°	0.31 (1)	1.31	384 (M^+)
	A3-DNB			0.61 (3)		
MeOH Adducts	B1			0.37 (2)	2.33	
	B1-Ac		+ 28°	0.90 (2)	2.02/2.80/3.27	458 (M^+), 398, 366, 253
	B2			0.41 (2)	2.33	
	B2-Ac		− 10°	0.90 (2)	2.05/2.70/3.27	458 (M^+), 398, 366, 253
	B3			0.48 (2)	1.52/2.15/2.30	
	B3-Ac		+ 8°	0.90 (2)	2.80	458 (M^+), 426, 398, 366, 253
EtOH Adducts	B1			0.42 (2)	1.19/1.80/2.34	
	B1-Ac		+ 27°	0.92 (2)	2.03/2.83/3.28	472 (M^+), 412, 366, 253
	B2			0.46 (2)	2.37	
	B2-Ac		− 24°	0.92 (2)	2.73	472 (M^+), 412, 366, 253
	B3			0.52 (2)	1.39/1.96/2.26	
	B3-Ac		+ 19°	0.94 (2)	1.72/2.60/3.04	472 (M^+), 412, 366, 253
	C1	112°	+141°	0.63 (2)	1.40	384 (M^+), 351 (base)
	C1-DNB	147–148°		0.46 (3)		578 (M^+), 351 (base)
	C2	102–103°	− 69°	0.60 (2)	0.95/1.52/1.60	384 (M^+), 351
	C2-DNB	146–147°		0.49 (3)		
	D1			0.71 (2)	1.69/2.58	384 (M^+)
	D1-DNB	108.5–109.5°	+ 37°	0.76 (1)		
	D2			0.85 (2)	1.39/1.59/2.27	384 (M^+)
	D2-DNB			0.86 (1)		
	D3			0.71 (2)	1.47/1.62/2.36/2.60	384 (M^+)
	D3-DNB			0.69 (3)		
	E1		+155°	0.83 (2)	1.54/1.74	
	E1-DNB			0.66 (3)		578 (M^+), 366 (base)
	R1			0.80 (2)	1.56	386 (M^+)
	R1-DNB		+ 34°	0.47 (3)		

[a] Where m.p.s are not given, the compounds were obtained as colourless oils (alcohols and acetates) or as pale yellow coloured oils (dinitrobenzoates).
[b] Chloroform, 22°C.
[c] Solvent system: (1) benzene; (2) benzene/acetone 9/1; (3) benzene/hexane 1/1.
[d] Retention time relative to cholestane.

2 hrs nitrogen was bubbled through the suspension, which was then poured into a glass tube (length 48 cm, i.d. 3 cm). Elution with benzene (benzene/acetone 9/1; benzene/hexane 1/1) was carried out under nitrogen pressure (7.5 cm Hg). For fractionation an automatic fraction collector (Gilson) was used.

Irradiations

The irradiation apparatus consists of a Philips HPK 150W mercury arc, surrounded by three concentrical vessels made of Pyrex glass. The first vessel may function as a filter compartment; in our case it was filled with distilled water. The second is the reaction vessel, and the third is a cooling compartment, connected to a methanol-filled cryostat. The arc is also surrounded by a cooling coil, connected to a water tap, which keeps the temperature of the water in the inner compartment at about 25°. The cryostat keeps the temperature of the circulating methanol below 0°. As a result, the temperature of the reaction mixture could be kept below 5°. At the start of the irradiations, the reaction vessel is filled with a solution of 1.5 g of 7-dehydrocholesterol in 230 ml of solvent, through which a stream of nitrogen has been allowed to pass for 2 hrs. The progress of the reaction is monitored by measurement of the UV absorption spectrum, and by TLC and GC.

The irradiations are discontinued when the UV absorbance of 20 µl of the reaction mixture in a 1 cm quartz cell (volume 3.8 ml), filled with ether, is

- smaller than 0.1 at 280 nm (irradiations in alcoholic solvents, irradiation time 48–70 hrs), or
- smaller than 0.25 at 280 nm (irradiations in ether, irradiation time 48–70 hrs), or
- smaller than 0.1 at 270 nm (irradiations in ether, irradiation time 100–160 hrs).

a) Irradiations in ethanol

1.5 g of 7-dehydrocholesterol were dissolved in 230 ml of absolute ethanol, nitrogen was bubbled through the solution for 2 hrs, and the solution was poured into the reaction vessel of the immersion unit. After 48 hrs the irradiation was discontinued and the solvent was distilled off under reduced pressure.

Short-column chromatography of the resulting oil (benzene/acetone 9/1) afforded 7 fractions, numbered I to VII in order of increasing retention volume.

Fraction I (160 mg) consisted of toxisterols A1, A2 and A3. Repeated chromatography (benzene) of this fraction afforded 30 mg of A1 and 90 mg of A2, contaminated with some A3. After treatment of the latter with 3,5-dinitrobenzoyl chloride[36] and chromatography (benzene/hexane 1/1) 100 mg of A2-dinitrobenzoate were obtained.

Fraction II (500 mg) contained toxisterols D1, D2, D3 and R1. Repeated chromatography (benzene/acetone 9/1) afforded 70 mg of impure D2, and a mixture of other compounds. Treatment of this mixture with 3,5-dinitrobenzoyl chloride and chromatography (benzene/hexane 1/1) resulted in a fraction IIA containing D1 and D3, and a fraction IIB containing R1. Fraction IIA was dissolved in ether/methanol; after seeding with a few crystals of D1-dinitrobenzoate this compound crystallized at −15° (150 mg). The resulting mother liquor was again chromatographed (benzene/hexane 1/1) affording ~100 mg of impure D3 and 40 mg of D1 as the 3,5-dinitrobenzoate esters. Fraction IIB was saponified[36] and chromatographed (benzene/acetone 9/1). The first fraction obtained (50 mg) consisted of R1, the second fraction (80 mg) con-

[36] Esterification and hydrolysis were carried out in the usual way. For details, see ref. 3.

Table III ^{1}*H-NMR data of toxisterols (δ in ppm, $CDCl_3$, 100 MHz, J between parentheses).*

		Protons attached to							Other protons
		C-3	C-6	C-7	C-18	C-19	C-21	C-26 + C-27	DNB/Ac	
Methanol Adducts	A1	3.85 d(3.5)/t(11)	6.27 s		0.89 s	1.67 d(2)	0.90 d(5)	0.86 d(6)		4-H: 2.51 d(11)/d(~2)
	A1-DNB	5.28 d(3.5)/t(11)	6.31 s		0.87 s	1.73 d(2)	0.78 d(6)	0.85 d(6)	9.07 m	4-H: 3.05 d(11)/d(~2)
	A2	3.81 d(3.5)/t(11)	6.33 d(6)	6.06 d(6)	0.90 s	1.65 d(2)	0.94 d(6)	0.87 d(6)		
	A2-DNB	5.25 d(3.5)/t(11)	6.39 d(6)	6.09 d(6)	0.69 s	1.72 d(2)	0.89 d(5)	0.85 d(6)	9.13 m	4-H: 2.70 d(11)/d(~2)
	A3	4.61 m	6.14 d(5.5)	5.35 d(5.5)	0.75 s	1.76 d(1.5)	0.93 d(6)	0.88 d(6)		
	A3-DNB	6.10 m	6.32 d(5)	5.39 d(5)	0.83 s	1.84 bs	0.92 d(5)	0.88 d(6)	8.88 m	4-H: 2.90 m
	B1	3.77 m	6.32 d(11)	6.15 d(11)	0.56 s	1.47 s	0.94 d(6)	0.88 d(6)		10-OCH_3: 3.15 s
	B1-Ac	4.76 m	6.38 d(11)	6.18 d(11)	0.56 s	1.48 s	0.93 d(6)	0.87 d(6)	2.03 s	10-OCH_3: 3.13 s
	B2	3.52 m	6.33 d(11)	5.90 d(11)	0.56 s	1.29 s	0.92 d(6)	0.87 d(6)		10-OCH_3: 3.07 s
	B2-Ac	4.52 m	6.22 d(11)	5.75 d(11)	0.56 s	1.29 s	0.93 d(6)	0.88 d(6)	2.04 s	10-OCH_3: 3.05 s
	B3	3.74 m	6.26 s*		0.56 s	1.47 s	0.92 d(6)	0.87 d(6)		10-OCH_3: 3.16 s
	B3-Ac	4.80 m	6.21 s		0.56 s	1.47 s	0.93 d(6)	0.88 d(6)	2.05 s	10-OCH_3: 3.18 s
Ethanol Adducts	B1	3.70 m	6.23 s		0.56 s	1.47 s	0.94 d(6)	0.87 d(6)		10-OC_2H_5: 3.32 m/1.17 t(7)
	B1-Ac	4.80 m	6.28 d(11)	6.12 d(11)	0.56 s	1.49 s	0.93 d(6)	0.88 d(6)	2.05 s	10-OC_2H_5: 3.35 m/1.19 t(7)
	B2	3.50 m	6.27 d(11)	5.86 d(11)	0.57 s	1.30 s	0.93 d(6)	0.87 d(6)		10-OC_2H_5: 3.25 m/1.16 t(7)
	B2-Ac	4.57 m	6.35 d(11)	5.87 d(11)	0.57 s	1.31 s	0.94 d(6)	0.87 d(6)	2.04 s	10-OC_2H_5: 3.25 m/1.17 t(7)
	B3	3.96 m	6.24 s		0.57 s	1.50 s	0.93 d(6)	0.87 d(6)		10-OC_2H_5: 3.37 m/1.18 t(7)
	B3-Ac	4.90 m	6.33 d(11)	6.18 d(11)	0.57 s	1.50 s	0.94 d(6)	0.89 d(6)	2.05 s	10-OC_2H_5: 3.40 m/1.19 t(7)
	C1	3.56 m		5.02 d(1.8)	0.57 s	0.81 s	0.90 d(6)	0.86 d(6)		
	C1-DNB	5.10 m		5.10*	0.59 s	0.91 s	0.95 d(6)	0.89 d(6)	9.05 m	
	C2	3.59 m		5.15 d(2)	0.73 s	0.92 s	0.90 d(6)	0.86 d(6)		
	C2-DNB	5.10 m		5.22 d(2)	0.76 s	1.00 s	0.90 d(5)	0.87 d(6)	9.05 m	
	D1	3.92 m	5.34 t(7)	2.79 d(7)	0.67 s	4.73 d(2)/ 4.93 d(2)	0.93 d(7)	0.86 d(6)		9-H: 5.20 m
	D1-DNB	5.28 m	5.42 t(7)	2.87 d(7)	0.66 s	4.86 d(2)/ 5.07 d(2)	0.94 d(6)	0.88 d(6)	9.03 m	9-H: 5.20 m
	D2-DNB	5.42 m	6.54 d(12)	5.91 d(12)	0.52 s	1.96 s	0.93 d(6)	0.87 d(6)	9.12 m	1-H: 5.62 m
	D3-DNB	5.37 m	6.54 d(16)	6.30 d(16)	0.91 s	1.90 s	0.92 d(6)	0.89 d(6)	8.98 m	
	E1	3.90 m	3.76 m	5.57 s	0.63 s	1.58 s	0.94 d(6)	0.88 d(6)		
	E1-DNB	5.40 m	3.83 m	5.56 s	0.50 s	1.65 s	0.92 d(6)	0.87 d(7)	9.06 m	
	R1	3.93 m	2.80 d(7)	4.80 t(7)	0.55 s	1.68 s	0.95 d(6)	0.88 d(6)		
	R1-DNB	5.34 m	2.86 d(7)	4.82 t(7)	0.54 s	1.73 s	0.93 d(6)	0.88 d(6)	8.97 m	

* Splitting pattern not clear because of coincidence with signal of 3-H.

Table IV ^{13}*C-NMR data of toxisterols A1, A2, C1 and C2*[a] *(δ in ppm, $CDCl_3$, 100 MHz).*

C-atom	A1	A2	C1	C2
1	35.2 t	31.4 t	39.7 t	38.4 t
2	33.8 t	34.0 t	32.8 t	33.6 t
3	69.8 d	69.8 d	67.9 d	68.9 d
4	54.0 d	59.7 d	31.5 t	31.7 t
5	122.6 s	120.5 s	34.0 s	37.7 s
6	129.2 d	128.6 d	31.1 d	30.1 d
7	140.6 d	140.8 d	117.7 d	119.7 d
8	54.0 s	53.2 s	148.1 s	148.6 s
9	31.4 t	35.7 t	50.2 d	46.4 d
10	135.7 s	138.1 s	24.5 s	28.3 s
11	20.1 t	19.6 t	22.3 t	19.3 t
12	40.0 t	40.2 t	40.5 t	40.8 t
13	43.9 s	45.0 s	45.4 s	42.6 s
14	55.7 d	48.0 d	51.6 d	48.0 d
15	22.3 t	24.2 t	25.5 t	24.0 t
16	27.2 t	29.8 t	28.6 t	29.1 t
17	57.0 d	54.3 d	55.6 d	57.2 d
18	14.0 q	18.2 q	12.2 q	15.5 q
19	18.4 q	25.5 q	15.2 q	18.4 q
20	35.4 d	36.2 d	36.3 d	36.0 d
21	18.4 q	19.4 q	18.9 q	18.9 q
22	35.9 t	36.8 t	36.3 t	36.0 t
23	23.7 t	23.8 t	23.9 t	24.0 t
24	39.5 t	39.5 t	39.5 t	39.5 t
25	27.9 d	28.0 d	28.0 d	28.0 d
26	22.5 q	22.5 q	22.6 q	22.5 q
27	22.8 q	22.8 q	22.8 q	22.8 q

[a] s, d, t and q denote SFORD multiplicities (s = singlet, d = doublet, t = triplet, q = quartet).

tained more R1 (about half of the fraction), together with two or more unidentified compounds, at least one of which is also a reduction product (MS).

Fraction III (250 mg) consisted of toxisterols C1 and C2. Treatment with dinitrobenzoyl chloride and chromatography (benzene/hexane 1/1) afforded 45 mg of C2 and 300 mg of C1 as the dinitrobenzoate esters. Both were crystallized from ether/methanol at −15°.

Fraction IV (40 mg) consisted of impure (EtOH)toxisterol B3, which was purified by repeated chromatography (benzene/acetone 9/1). Fractions V (150 mg), VI (60 mg) and VII (90 mg) consisted of (EtOH)toxisterol B2, a mixture of about equal amounts of (EtOH)-toxisterols B2 and B1, and (EtOH)toxisterol B1, respectively.

b) Irradiations in methanol

Irradiations in methanol were carried out as described for ethanol, except that a mixture of 180 ml of methanol (A.R. grade) and 50 ml of anhydrous diethyl ether (A.R. grade) was used as solvent (the addition of ether is necessary because of the poor solubility of 7-dehydrocholesterol in methanol). Short-column chromatography of the resulting oil (benzene/acetone 9/1) afforded 6 fractions, numbered I to VI in order of increasing retention volume.

Fractions I (150 mg), II (480 mg) and III (210 mg) consisted of the same compounds as fractions I, II and III from the irradiation in ethanol; compounds were isolated in the same way.

Fractions IV (60 mg), V (160 mg) and VI (110 mg) consisted of (MeOH)toxisterols B3, B2 and B1, respectively.

c) Irradiations in diethyl ether

Irradiations in ether were carried out as described for ethanol, except that anhydrous diethyl ether (A.R. grade) was used as solvent.

1) Irradiation time 48 hrs

Short-column chromatography (benzene/acetone 9/1) of the resulting oil afforded 5 fractions, numbered I to V in order of increasing retention volume.

Table V UV absorption characteristics of toxisterols[a].

	λ_{max} (nm) (ε_{max})			λ_{max} (nm) (ε_{max})
A1	251 (15,000)	MeOH Adducts	B1	252 (27,000), 245 s, 260 s
A2	251 (15,000)		B1-Ac	252 (29,000), 246 s, 260 s
A3	251 (15,000)		B2	251 (32,000), 244, 260
			B2-Ac	251 (30,000), 244, 260
C1	227 (6,600)		B3	252 (15,000), 246 s, 260 s
C2	229 (5,400)		B3-Ac	252 (14,000), 246 s, 260 s
D1	217 s (7,200)	EtOH Adducts	B1	251 (33,000), 245 s, 260 s
D2	287, 276, 298		B1-Ac	251 (28,000), 245 s, 260 s
D3	288, 277, 301		B2	251 (29,000), 244, 260
			B2-Ac	251 (28,000), 244, 260
E1	no absorption maximum at $\lambda > 210$		B3	250 (17,000), 246 s, 259 s
			B3-Ac	250 (17,000), 246 s, 259 s
R1	no absorption maximum at $\lambda > 210$			

[a] Solvent: diethyl ether for toxisterols A3, E1 and R1; ethanol for all other toxisterols; s = shoulder.

Table VI IR frequencies (cm^{-1}) of toxisterols

	A1	3340, 3060, 2950, 2870, 1470, 1380, 1120, 1040, 1020, 990, 930, 860, 760 and 500	[a]
	A1-DNB	3090, 2940, 2920, 2860, 1730, 1630, 1550, 1460, 1340, 1270, 1170, 1075, 970, 920, 770, 730 and 720	[a]
	A2	3390, 3060, 2960, 2940, 2870, 1460, 1370, 1120, 1030, 1015, 990, 775, 755 and 675	[b]
	A2-DNB	3090, 2940, 2920, 2860, 1730, 1630, 1550, 1460, 1340, 1275, 1170, 1075, 970, 920, 780, 730 and 720	[a]
	A3	3430, 3050, 3020, 2930, 2860, 1470, 1380, 1370, 1175, 1090, 1070, 1015, 990, 870, 810 and 770	[b]
	A3-DNB	3090, 2940, 2920, 2860, 1730, 1630, 1550, 1460, 1340, 1275, 1170, 1070, 920, 805, 775, 730, 720 and 680	[b]
MeOH Adducts	B1	3400, 2950, 2930, 2860, 1650, 1610, 1480, 1380, 1370, 1075, 1040, 940, 870 and 835	[a]
	B1-Ac	2950, 2870, 1740, 1650, 1600, 1470, 1380, 1360, 1240, 1170, 1070, 1030, 940, 735 and 680	[b]
	B2	3400, 2950, 2930, 2860, 1660, 1620, 1480, 1390, 1370, 1075, 1035, 940 and 855	[a]
	B2-Ac	2950, 2870, 1740, 1650, 1610, 1460, 1380, 1360, 1250, 1075, 1030, 940, 855, 735 and 680	[b]
	B3	3360, 2940, 2870, 1660, 1480, 1390, 1370, 1075, 1045, 1020, 1000, 970, 950, 865 and 680	[b]
	B3-Ac	2940, 2860, 1740, 1480, 1390, 1370, 1240, 1075, 1035 and 760	[b]
EtOH Adducts	B1	3370, 2960, 2940, 2880, 1660, 1610, 1470, 1380, 1370, 1160, 1070, 1050 and 950	[b]
	B1-Ac	2960, 2940, 2870, 1740, 1640, 1600, 1460, 1380, 1360, 1240, 1160, 1070, 1030, 950 and 735	[b]
	B2	3370, 2960, 2940, 2880, 1660, 1620, 1470, 1380, 1370, 1160, 1125, 1070, 1040, 1000, 950, 850 and 740	[b]
	B2-Ac	2950, 2930, 1740, 1650, 1610, 1460, 1380, 1360, 1240, 1160, 1070, 1030, 950, 900 and 850	[b]
	B3-Ac	2940, 2920, 2860, 1740, 1650, 1610, 1470, 1380, 1370, 1240, 1030 and 760	[b]
	C1	3380, 3060, 2960, 2940, 2880, 1660, 1470, 1380, 1080, 1045, 1030, 1015 and 840	[a]
	C1-DNB	3120, 2960, 2880, 1740, 1640, 1560, 1480, 1350, 1290, 1180, 1080, 925, 780, 735 and 725	[a]
	C2	3360, 3040, 2940, 2860, 1650, 1480, 1390, 1070, 1050, 1020 and 825	[a]
	C2-DNB	3120, 3060, 2960, 2880, 1740, 1640, 1560, 1480, 1400, 1350, 1290, 1178, 1080, 980, 925, 825, 780, 735 and 725	[a]
	D1	3340, 3075, 2950, 2930, 2860, 1640, 1460, 1370, 1050 and 900	[b]
	D1-DNB	3080, 2950, 2880, 1725, 1630, 1540, 1460, 1340, 1290, 1265, 1180, 1080, 980, 960, 930, 910, 780, 735 and 725	[a]
	E1	3420, 2960, 2930, 2870, 1660, 1480, 1390, 1380, 1100, 1040 and 810	[a]
	R1	3330, 3020, 2940, 2920, 2860, 1660, 1470, 1380, 1370, 1040 and 675	[b]
	R1-DNB	3090, 2930, 2860, 1730, 1630, 1600, 1550, 1470, 1350, 1280, 1170, 1075, 970, 920, 820, 775, 730, 720 and 680	[b]

[a] In KBr. [b] Neat.

Fraction I (100 mg) consisted of toxisterols A1 and A3. Repeated chromatography (benzene) afforded 50 mg of A1 and 25 mg of A3.
Fraction II (250 mg) consisted of a mixture of previtamin D and toxisterol E1, which could not be separated.
Fraction III (350 mg) consisted of lumisterol and toxisterol D1. Treatment with 3,5-dinitrobenzoyl chloride and chromatography (benzene/hexane 1/1) yielded 180 mg of D1 and 270 mg of lumisterol as the 3,5-dinitrobenzoate esters. Both were crystallized from ether/methanol at −15°.
Fraction IV (300 mg) consisted of toxisterols C1 and C2. Treatment with 3,5-dinitrobenzoyl chloride and chromatography (benzene/hexane 1/1) afforded 65 mg of C2 and 360 mg of C1 as the 3,5-dinitrobenzoate esters, which were crystallized from ether/methanol at −15°.
Fraction V (175 mg) consisted of 7-dehydrocholesterol.

2) Irradiation time 100 hrs
Short-column chromatography of the resulting oil (benzene/acetone 9/1) afforded 5 fractions, numbered I to V in order of increasing retention volume.
Fraction I (200 mg) consisted of toxisterols A1 and A3. Repeated chromatography (benzene) yielded 100 mg of A1 and 35 mg of A3.
Fraction II (120 mg) consisted of toxisterol E1 and a trace of previtamin D.
Fraction III (300 mg) consisted of toxisterol D1 and suprasterol II. Treatment with 3,5-dinitrobenzoyl chloride and chromatography (benzene/hexane 1/1) yielded 340 mg of D1 and 90 mg of suprasterol II as the 3,5-dinitrobenzoate esters.
Fraction IV (500 mg) consisted of toxisterols C1 and C2. Treatment with 3,5-dinitrobenzoyl chloride and chromatography (benzene/hexane 1/1) afforded 90 mg of C2 and 580 mg of C1 as the 3,5-dinitrobenzoate esters. Both crystallized from ether/methanol at −15°.
Fraction V (25 mg) consisted of suprasterol I.

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