Phytochemistry, 1973, Vol. 12, pp. 1135 to 1140. Pergamon Press. Printed in England.

STEROLS OF *XANTHORIA PARIETINA*: EVIDENCE FOR TWO STEROL 'POOLS' AND THE IDENTIFICATION OF A NOVEL C_{28} TRIENE, ERGOSTA-5,8,22-TRIEN-3β-OL

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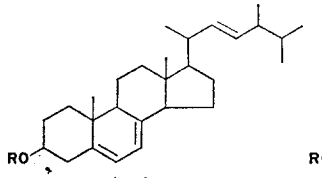
(*Received* 31 *October* 1972. *Accepted* 21 *November* 1972)

Key Word Index—*Xanthoria parietina;* Lichens; sterol pools; novel C_{28} triene; ergosta-5,8,22-trien-3β-ol; lichesterol.

Abstract—Sterols extracted from *Xanthoria parietina* with organic solvents and released by saponification of the residual lichen tissue were analysed by GC-MS. The main components of the solvent-extractable sterols were two C_{28} trienes and those of the more tightly bound sterols were ergost-5-en-3β-ol and two C_{29} compounds. The structures of the C_{28} compounds were shown to be ergosta-5,7,22-trien-3β-ol, Ia (ergosterol) and the previously unreported ergosta-5,8,22-trien-3β-ol, IIa, for which the name lichesterol is proposed. The main C_{29} sterol was identified as (24*R*)-24-ethylcholesta-5,22-dien-3β-ol (poriferasterol).

INTRODUCTION

LICHENS are a symbiotic association of a fungus, usually an Ascomycete, and a green (Chlorophyceae) or blue-green (Cyanophyceae) alga. Several pentacyclic triterpenes have been isolated from lichens[1,2] but have rarely been reported as occurring in free living fungi[3a] or algae.[3b] By comparison, other products of squalene cyclisation, such as sterols, are well established in fungi and algae but have received little attention in lichens.[1,2] A possible hormonal role for terpenoids,[4] produced by, or under the influence of the algal symbiont could be argued in lichens since isolated cultures of the fungi do not undergo sexual reproduction.[5a,b] Also sterols may play an important part in membrane permeability phenomena[6] and perhaps influence the transport of materials from alga to fungus in lichens. In this paper, the nature and distribution of sterols in the common foliose lichen, *Xanthoria parietina* (L.) Th.Fr., is presented as part of an investigation into the metabolism and function of sterols in the lichen association and its isolated symbionts.



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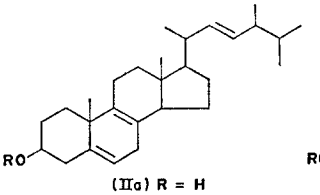

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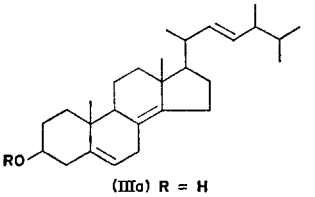

(IIIa) R = H
(IIIb) R = Ac

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RESULTS

Sequential extraction of dried lichen tissue with various solvents revealed that several 'pools' of sterols existed which differed in their ease of extraction from the tissue. Quantitative GLC analysis of the sterols from successive solvent extracts of *X. parietina* showed that although sterols were extracted by refluxing three times with ethanol a greater amount was removed by subsequent acetone extraction (Table 1). The quantity of sterol present in the first $CHCl_3$–MeOH extract was reduced by comparison with the previous extracts and no sterol was detected in the second $CHCl_3$–MeOH extract. Saponification of the lichen tissue after exhaustive solvent extractions released a tightly bound sterol fraction which differed in composition from those of the solvent extracts. Combined GC–MS analysis showed that the solvent-extractable sterols were mainly C_{28} trienes (M^+ at *m/e* 396) whereas the tightly bound sterols released by saponification were ergost-5-en-3β-ol (M^+ at *m/e* 400) and two C_{29} compounds (M^+ at *m/e* 412 and 414 respectively). The predominant C_{29} sterol from the tightly bound fraction was acetylated, separated from the Δ^5 saturated side chain compounds on plates of silica gel G impregnated with $AgNO_3$ using EtOH-free $CHCl_3$ as developing solvent and characterised by GLC, m.p., MS and NMR spectrometry[7] as (24*R*)-24-ethylcholesta-5,22-dien-3β-ol (poriferasterol).

TABLE 1. PERCENTAGE COMPOSITION OF SOLVENT EXTRACTABLE AND TIGHTLY BOUND STEROLS OF *Xanthoria parietina*

Sterol	Relative R_t*	Solvent extractable sterol EtOH (F1)	Acetone (F2)	$CHCl_3$–MeOH (F3)	Tightly bound sterol (F5)
Cholesterol	1·00	1	—	—	—
Brassicasterol	1·12	1	—	—	5
Lichesterol	1·12	55	60	64	17
Ergosterol	1·22	29	34	34	10
Δ^5 Ergostenol	1·30	4	2	—	21
Episterol	1·42	2	3	2	—
Poriferasterol	1·42	6	1	—	42
Clionasterol	1·63	2	—	—	5
Relative yield of sterols		1·00	1·50	0·17	0·67

* 152 cm × 7·6 mm i.d. column 1% SE30, oven 230°, carrier 60 ml min^{-1}.

The principal components of the solvent extracts were ergosterol, a known constituent of several lichens[8,9] and a previously unreported C_{28} triene for which the trivial name lichesterol is proposed. In the NMR spectrum of ergosteryl acetate, Ib, isolated from *X. parietina*, the C-18 and C-19 methyl protons resonated as singlets at δ0·63 and δ0·96 ppm (calc. δ0·64 and δ0·98 ppm[10]) and the olefinic protons as multiplets centred at δ5·20 (2H, C-22, C-23), δ5·38 (1H, C-7) and δ5·56 ppm (1H, C-6) in agreement with an authentic sample. Moreover, the GLC, UV, IR and MS data were all consistent with the identification of ergosterol in *X. parietina*.

In the MS of lichesterol (M^+ at *m/e* 396) the fragmentation ions at *m/e* 271 (M^+-side chain), 253 (M^+-side chain–H_2O) and 211 (M^+-side chain–H_2O-part of ring D) indicated a

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C_{28} triene structure with two nuclear double bonds and a Δ^{22} double bond in the side chain.[11,12] The presence of the base peak at *m/e* 378 (M^+-acetate) in the MS of lichesteryl acetate (M^+ at *m/e* 438) was indicative of a Δ^5 nuclear double bond.[12] Furthermore, the absorption maxima in the IR spectrum were consistent with a *trans* Δ^{22} (965 cm^{-1}) side chain double bond and a Δ^5 (805 cm^{-1}) nuclear double bond. In the MS of the TMS ether derivative of lichesterol (M^+ at *m/e* 468) the fragmentation ion at *m/e* 337 (M^+-part of ring A) suggested a ring B diene system[13] while the UV and ORD curves of the free sterol revealed that the double bonds were unconjugated. Integration of the NMR spectrum of lichesteryl acetate showed only three olefinic protons which appeared as multiplets centred at δ5·44 (1H) and δ5·19 ppm (2H) and were assigned to the protons attached to C-6 and C-22/C-23 respectively.

TABLE 2. COMPARISON OF THE OBSERVED POSITIONS OF THE C-18 AND C-19 METHYL PROTON RESONANCES δ, ppm) IN THE NMR SPECTRUM OF LICHESTEROL WITH VALUES CALCULATED FROM TABLES (VALUES OF CORRESPONDING STERYL ACETATES IN PARENTHESIS)

Compound	C-19	C-18
Ergosta-5,8(9),22-trien-3β-ol*	1·18(1·19)	0·63(0·63)
Ergosta-5,8(14),22-trien-3β-ol*	0·93(0·95)	0·88(0·88)
Lichesterol†	1·19(1·20)	0·67(0·67)

* Calculated from Ref 14. † Observed.

The second nuclear double bond was therefore shown to be *tetra*-substituted and must occupy the $\Delta^{8(9)}$ or $\Delta^{8(14)}$ positions. A clear distinction between the two alternative structures II and III was made by comparison of the observed positions of the C-18 and C-19 methyl proton resonances with the calculated values[14] (Table 2). In lichesterol, IIa, the C-19 methyl protons resonated at comparatively low field strength due to the deshielding effect of the Δ^5 and $\Delta^{8(9)}$ bonds whereas in structures containing a $\Delta^{8(14)}$ bond [10,15] it is the C-18 methyl protons which shift downfield. The unusual $\Delta^{5,8(9)}$ ring B diene system proposed for lichesterol is supported by comparison with NMR spectra reported for adducts prepared from ergosteryl acetate which contain $\Delta^{5,8(14)}$ and $\Delta^{5,8(9)}$ bonds.[16] In the NMR spectrum of lichesteryl acetate, IIb the broad signal at δ2·52 ppm (2H) was assigned to the protons attached to C-7 which are coupled vicinally to the olefinic proton at C-6. The two signals at δ2·36 and δ2·28 ppm, which were deshielded to δ2·42 and δ2·34 ppm in the acetate, were assigned to the 4α (equatorial) and 4β (axial) protons respectively. Doublets centred at δ1·02 ppm (*J* 7 Hz, C-27) and δ0·81 ppm (*J* 7 Hz, C-26) accounted for the side chain methyl proton resonances of lichesteryl acetate and were identical with those of authentic ergosteryl acetate, from which it was concluded that the absolute configuration of lichesterol was (24*R*) the same as that of ergosterol but final proof of this point must await either degradation to a known compound or synthesis by an unambiguous route.

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DISCUSSION

In general C_{28} sterols predominate in fungi[17] while C_{29} sterols are found in algae[18] including the common lichen symbiont *Trebouxia*.[19] From a comparison of the solvent-extractable and tightly bound sterols of dried *X. parietina* (Table 1) it appears that organic solvents, particularly acetone, removed sterols from the mycobiont (fungus) whereas the tightly bound sterols were more characteristic of the phycobiont (alga). A difference in the effectiveness of organic solvents and of the composition of sterols isolated from chloroplasts has previously been demonstrated.[20,21] Recently, highly bound sterols have also been released from aqueous extracts of yeast[22] and *Euglena*[23] by acidic and alkaline hydrolysis. At present there is no evidence to suggest that the tightly bound sterols of *X. parietina* are water-soluble, since they have been released by saponification of the tissue remaining after solvent extraction.

The novel $\Delta^{5,8}$ ring B diene system proposed for lichesterol, the major sterol of *X. parietina*, leads to interesting speculations regarding its origin. It is perhaps conceivable, by analogy with the synthesis of bisergostatrienol,[24] that lichesterol is produced in *X. parietina* by photoisomerization of ergosterol, particularly as lichens are known to be rich sources of dyestuffs and are often exposed to high light intensities.[5b] An enzymic origin for the $\Delta^{5,8}$ diene is, however, suggested by the positive identification of lichesterol and ergosterol in a dark-grown liquid shake culture of the isolated mycobiont (unpublished results). The biosynthetic pathway to lichesterol could involve either a reversible $\Delta^8 \rightleftharpoons \Delta^7$ isomerase[25] at the $\Delta^{5,7}$ level or direct introduction of a Δ^5 bond into a $\Delta^{8(9)}$ sterol, a proposal which is however contrary to the generally accepted scheme for the formation of Δ^5 compounds.[26]

EXPERIMENTAL

M.ps were recorded on a Kofler block and are uncorrected. Specific rotations were recorded on a Bellingham Stanley Polarmatic 62 spectropolarimeter as a 0·3% solution in $CHCl_3$ at 25° and the ORD curve as a 0·05% solution in EtOH at 25° under N_2 flush. UV spectra were recorded in EtOH and IR spectra as KBr discs. 100 MHz NMR spectra were recorded at PCMU, Harwell, on a Varian HA-100D spectrometer in $CDCl_3$ using TMS as internal standard. Sterol samples were analysed in a Pye Series 104 gas chromatograph using 152 cm × 7·6 mm id. glass columns packed with either 1% SE30 or 1% JXR coated on Gaschrom Q, 100–120 mesh (Applied Science Labs.) at an oven temp. of 230° for free sterols (240° for steryl acetates) and a carrier gas flow rate of 60 ml min^{-1}. For GC–MS the effluent from the GLC was passed via a Biemann–Watson separator to an AEI-MS12 mass spectrometer operated at an ionizing energy of 70 eV and an accelerating voltage of 8 kV. Pure sterols were also analysed by direct inlet probe at 180°.

Analysis of solvent extractable and tightly bound sterols of X. parietina *by combined GC–MS. X. parietina* was collected at Minsmere, Suffolk in July 1970, air dried, finely ground (55 g) and extracted successively with EtOH (F1, 3 × 600 ml), acetone (F2, 1 × 600 ml) and $CHCl_3$–MeOH (2:1) (F3, 1 × 600 ml; F4, 1 × 600 ml) each under reflux for 2 hr. The combined F1 extracts were reduced in vol. H_2O added and partitioned with Et_2O (4 × 500 ml). The combined Et_2O layers were partitioned with 5% w/v $NaHCO_3$ (2 × 100 ml) and 5% w/v NaOH (2 × 100 ml) to remove strong (34·4 mg) and weak (311·5 mg) acids which were obtained by adjusting the aq. phases to pH 2·5 with HCl and partitioning with Et_2O (3 × 400 ml). The neutral lipid from F1 (315·1 mg), total F2 (129·6 mg), F3 (123·8 mg), F4 (364·2 mg) and also the residual lichen tissue

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remaining after solvent extraction (F5) were saponfied with 10% w/v KOH plus 1% w/v pyrogallol in 80% EtOH under reflux for 1·5 hr. The saponification mixture was cooled, diluted with H_2O (2 × vol.) and partitioned with Et_2O (3×), dried over Na_2SO_4 and solvent removed to yield the non-saponifiable lipids (F1, 59·0; F2, 23·7; F3, 7·5; F4, 22·0; F5, 243·5 mg). A crude sterol mixture from F5 (22·8 mg) was obtained by column chromatography (20 g, Woelm neutral Al_2O_3, Grade III) eluting with increasing proportions of Et_2O in light petrol. (40–60°). Sterols from each fraction were then purified on plates of silica gel G (0·5 mm thickness) developed 2× in $CHCl_3$, visualized under UV light (366 nm) after spraying with a solution of rhodamine 6G in acetone and eluted with Et_2O (F1, 4·5; F2, 5·3; F3, 0·5; F4, 2·0; F5, 2·0 mg). The two main components of the F1 sterols (RR_t.* 1·12 and 1·22) were shown by GC–MS analysis (4% JXR) to be C_{28} trienes with parent ions at *m/e* 396 and very similar fragmentation ions at *m/e* 378 (M^+-H_2O) 336 (M^+-H_2O-Me), 337 (M^+-part ring A), 271 (M^+-side chain (SC), 253 (M^+-SC-H_2O) and 211 (M^+-SC-H_2O-part ring D). The minor components of F1 were cholesterol (cholest-5-en-3β-ol) *m/e* 386 (M^+), 368 (M^+-H_2O), 353 (M^+-H_2O-Me), 301 (M^+-H_2O-C_5H_7), 275 (M^+-H_2O-C_7H_9), 255 (M^+-SC-H_2O), 213 (M^+-SC-H_2O-part ring D), brassicasterol (ergosta-5,22-dien-3β-ol) *m/e* 398 (M^+), 380 (M^+-H_2O), 300 (M^+-part SC), 271 (M^+-SC-2H), 255 (M^+-SC-H_2O), 213 (M^+-SC-H_2O-part ring D), Δ^5-ergostenol (ergost-5-en-3β-ol) *m/e* 400 (M^+), 385 (M^+-Me), 382 (M^+-H_2O), 367 (M^+-H_2O-Me), 315 (M^+-H_2O-C_5H_7), 289 (M^+-H_2O-C_7H_9), 273 (M^+-SC-H_2O), 213 (M^+-SC-H_2O-part ring D), episterol (ergosta-7,24 (28)-dien-3β-ol) *m/e* 398 (M^+), 383 (M^+-Me), 314 (M^+-84), 271 (M^+-SC-2H), 255 (M^+-SC-H_2O), 213 (M^+-SC-H_2O-part ring D), poriferasterol (24*R*)-24-ethylcholesta-5,22-dien-3β-ol) *m/e* 412 (M^+), 394 (M^+-H_2O), 369 (M^+-H_2O-Me), 351 (M^+-H_2O-43), 300 (M^+-part SC), 271 (M^+-SC-2H), 255 (M^+-SC-H_2O), 213 (M^+-SC-H_2O-part ring D), and clionasterol (24*S*)-24-ethylcholest-5-en-3β-ol) *m/e* 414 (M^+), 396 (M^+-H_2O), 381 (M^+-H_2O-Me), 329 (M^+-H_2O-C_5H_7), 303 (M^+-H_2O-C_7H_9), 273 (M^+-SC), 255 (M^+-SC-H_2O) 213 (M^+-SC-H_2O-part ring D). These assignments were confirmed by resolving the sterols into four bands on 10% w/w/ $AgNO_3$ impregnated silica gel G plates (0·25 mm thickness) using $CHCl_3$ (B.P.) as developing solvent. Band 1 (R_f 0·04–0·09), band 2 (R_f 0·16–0·22)) and band 3 (R_f 0·24–0·30) were shown by direct inlet probe and GLC analysis to be ergosterol (RR_t 1·22), the novel C_{28} triene, lichesterol (RR_t 1·12) and episterol (RR_t 1·42) respectively. Band 4 (R_f 0·32–0·40) was shown by GC–MS to contain mixtures of C_{28}/C_{29} $\Delta^{5,22}$ and $C_{27}/C_{28}/C_{29}$ Δ^5 compounds. F2, F3 and F5 sterols were similarly analysed by GC–MS and resolved by TLC on $AgNO_3$ impregnated silica gel G as described above.

Isolation and identification of ergosterol, lichesterol and poriferasterol from X. parietina. *X. parietina* was collected at Trawsfynnd, Merioneth in May 1971, air-dried, finely ground (550 g) and extracted with acetone (4 × 1·5 l.) under reflux for 3 hr. The combined acetone extracts were reduced in vol. (400 ml), H_2O added (250 ml) and stood overnight in the cold (5°). A rust coloured precipitate (2·55 g) was removed by filtration and washed with Et_2O (400 ml). The filtrate was partitioned with Et_2O (3 × 400 ml) and the combined Et_2O extracts partitioned with 5% w/v $NaHCO_3$ (2 × 200 ml) and 5% w/v NaOH (2 × 200 ml) to remove strong (121·7 mg) and weak (737·6 mg) acids. The neutral lipid (2·78 g) was saponfied (1·29 g) and purified by column chromatography (60 g, Woelm neutral Al_2O_3, Grade III) eluting with increasing proportions of Et_2O in light petrol. The sterol fraction (200·4 mg) was acetylated in pyridine Ac_2O (1:1) overnight at room temp. and the steryl acetates (215·8 mg) resolved on a $AgNO_3$ (14% w/w) impregnated Al_2O_3 column (20 g, Woelm anionotropic) eluting with increasing proportions of Et_2O in light petrol. Lichesteryl acetate (90·5 mg) was eluted before ergosteryl acetate (33·7 mg). The lichen tissue remaining after acetone extraction (446 g dry wt) was saponfied and the non-saponifiable lipid (1·57 g) purified by column chromatography (100 g, Woelm neutral Al_2O_3, Grade III). The crude sterol containing fraction (157·2 mg) was acetylated and the steryl acetates resolved on a $AgNO_3$ (14% w/w) impregnated Al_2O_3 column (20 g, Woelm anionotropic). Poriferasteryl acetate (8·3 mg; R_f 0·40–0·50) was resolved from brassicasteryl acetate (1·4 mg; R_f 0·26–0·36) and Δ^5 steryl acetates (10·6 mg; R_f 0·51–0·58) on $AgNO_3$ (10% w/w) impregnated silica gel G (0·5 mm thickness) plates developed in freshly prepared EtOH-free $CHCl_3$.

Lichesteryl acetate. Crystallized as plates from Et_2O: MeOH, M.p. 125–126°, $[\alpha]_D^{25}$ −51·4° (*c* 0·3, $CHCl_3$). MS. *m/e* 438 (7%, M^+), 378 (100%, M^+-acetate), 363 (86%, M^+-acetate-Me), 253 (41%, M^+-acetate-SC), 211 (18%, M^+-acetate-SC-part ring D). IR: ν_{max}^{KBr} cm^{-1} 805 (trisubstituted double bond), 965 (*trans* disubstituted double bond), 1235, 1265 (acetate), 1740 (acetoxy C=0). UV end absorption: λ_{210}^{EtOH} ϵ 4960. NMR (δ, ppm) 2·02 (*s*, 3H, acetoxy Me), 4·62 (*m*, 3αH), 5·19 (*m*, 2H, C-22/C-23), 5·44 (*m*, C-6H), 2·52 (*m*, 2H, C-7), 2·42 (*m*, 4αH), 2·34 (*m*, 4βH), 1·20 (*s*, 3H, C-19 Me), 0·67 (*s*, 3H, C-18 Me), 1·02 (*d*, *J* 7 Hz, C-21 Me), 0·91 (*d*, *J* 7 Hz, C-28 Me), 0·83 (*d*, *J* 7 Hz, C-27 Me) 0·81 (*d*, *J* 7 Hz, C-26 Me).

Lichesterol was obtained by $LiAlH^4$ reduction of the acetate and crystallized as plates from Et_2O: MeOH, M.p. 114–115°, $[\alpha]_D^{25}$ −26·6° (*c* 0·3, $CHCl_3$). MS. *m/e* 396 (67%, M^+), 363 (100%, M^+-Me-H_2O), 337 (8%, M^+-part ring A), 271 (25%, M^+-SC), 253 (23%, M^+-SC-H_2O), 217 (11%), 211 (12%, M^+-SC-H_2O-part ring D). TMS ether derivative: *m/e* 468 (67%, M^+), 453 (6%, M^+-Me) 378 (12%, M^+-TMS), 363 (100%, M^+-TMS-Me) 337 (40%, M^+-part ring A), 253 (25%, M^+-TMS-SC), 211 (12%, M^+-TMS-SC-part ring D). IR. ν_{max}^{KBr} cm^{-1} 805 (trisubstituted double bond), 965 (*trans* disubstituted double bond), 3400 (−OH).

* Retention times are given relative to cholesterol.

UV end absorption. λ_{210}^{EtOH} ϵ 4950. ORD curve. $[\alpha]_{340}$ −460° (trough), $[\alpha]_{229}$ −6000°, $[\alpha]_{213}$ −3500° (shoulder), $[\alpha]_{207}$ 0° (*c* 0·005, EtOH). NMR (δ, ppm) 3·55 (*m*, 3αH), 5·20 (*m*, 2H, C-22/C-23), 5·43 (*m*, C-6H), 2·52 (*m*, 2H, C-7), 2·36 (*m*, 4αH), 2·28 (*m*, 4βH), 1·19 (*s*, 3H, C-19 Me), 0·67 (*s*, 3H, C-18 Me), 1·02 (*d*, *J* 7 Hz, C-21 Me), 0·91 (*d*, *J* 7 Hz, C-28 Me), 0·83 (*d*, *J* 7 Hz, C-27 Me), 0·81 (*d*, *J* 7 Hz, C-26 Me).

Ergosteryl acetate crystallized as plates from Et_2O. MeOH, M.p. 150–152° decomp., lit. 181°.[27] MS. *m/e* 438 (1%, M^+), 378 (100%, M^+-acetate), 363 (4%, M^+-acetate–Me), 253 (21%, M^+-acetate–SC), 211 (6%, M^+-acetate–SC-part ring D) IR. ν_{max}^{KBr} cm^{-1} 800, 830 (trisubstituted double bonds), 965 (*trans* disubstituted double bond), 1235, 1265 (acetate), 1740 (acetoxy C=O). UV. λ_{max}^{EtOH} 282, 271·5, 294 nm. NMR (δ, ppm) 2·02 (*s*, 3H, acetoxy Me), 4·68 (*m*, 3αH), 5·20 (*m*, 2H, C-22/C-23), 5·38 (*m*, C-7H), 5·56 (*m*, C-6 H), 2·45 (*m*, 4αH), 2·34 (*m*, 4βH), 0·96 (*s*, 3H, C-19 Me), 0·63 (*s*, 3H, C-18 Me), 1·02 (*d*, *J* 7 Hz, C-21 Me), 0·91 (*d*, *J* 7 Hz, C-28 Me), 0·83 (*d*, *J* 7 Hz, C-27 Me), 0·81 (*d*, *J* 7 Hz, C-26 Me).

Poriferasteryl acetate crystallized as plates from Et_2O: MeOH, M.p. 146–147°, lit. 147°.[27] MS. *m/e* 394 (100%, M^+-acetate), 351 (7%, M^+-acetate–43), 255 (14%, M^+-acetate–SC). NMR (δ, ppm) 2·01 (*s*, 3H, acetoxy Me), 4·58 (*m*, 3αH), 5·09 (*m*, 2H, C-22/C-23), 5·35 (*m*, C-6 H), 2·35 (*m*, 4αH) 2·28 (*m*, 4βH), 1·02 (*s*, 3H, C-19 Me), 0·70 (*s*, 3H, C-18 Me), 1·03 (*d*, *J* 6 Hz, Me), 1·03 (*d*, *J* 6 Hz, C-21 Me), 0·82 (*tr*, *J* = 6 Hz, C-29 Me), 0·85 (*d*, *J* 6 Hz, C-26/C-27 Me). Expansion of the side chain methyl proton region (δ 0·60–1·20 ppm) confirmed the assignment of the (24*R*) configuration as a clear distinction between this and the (24*S*) isomer is seen in the region δ 0·80–0·84 ppm.[7]

Acknowledgements—We thank the Science Research Council for financial support. Dr. T. Yoshida and Dr. W. L. F. Amarego for helpful discussions, Dr. Z. A. Wojciechowski for informing us of the use of EtOH-free $CHCl_3$ for resolving poriferasterol from other Δ^5 sterols, Dr. C. Green and Mr. J. R. Green for $[\alpha]_D$ and ORD measurements, Mrs. A. Holcroft and Mr. J. Ireland for MS and the PCMU, Harwell for NMR spectra.

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