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THE CONVERSION OF 24-ETHYLIDENE STEROLS INTO PORIFERASTEROL BY THE ALGA *OCHROMONAS MALHAMENSIS*

F. F. KNAPP*, L. J. GOAD and T. W. GOODWIN

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, England

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Key Word Index—*Ochromonas malhamensis*; Chrysophyceae; sterol biosynthesis; poriferasterol; fucosterol; 28-isofucosterol.

Abstract—A convenient method is described for the preparation of fucosterol-[7-$^3H_2$] and 28-isofucosterol-[7-$^3H_2$]. Both of these 24-ethylidene sterols, as well as 5α-stigmasta-7,*Z*-24(28)-diene-3β-ol-[2,4-$^3H_4$], were converted into the 24β-ethyl sterol, poriferasterol, by cultures of the chrysophyte alga *Ochromonas malhamensis*. However, fucosterol-[7-$^3H_2$] was not so efficiently incorporated as the other two compounds thus indicating that the configuration of the 24-ethylidene group is of some importance. It is suggested that a 24-ethylidene sterol of the *Z*-configuration is produced in *de novo* poriferasterol synthesis and that a $\Delta^{22,24(28)}$-diene may be an important subsequent intermediate.

INTRODUCTION

The typical plant sterols are characterised by the presence of alkyl substituents at C-24 which arise by transfer of methyl groups from *S*-adenosylmethionine to an olefinic precursor sterol [1–3]. The substrate for the first transmethylation is a $\Delta^{24(25)}$-compound (**1**, Scheme 1) and the reaction can give either a 24-methylene (**2**) or a 24-methyl-25-methylene (**3**) product [4]. For the formation of C_{29} sterols the 24-methylene intermediate (**2**) acts as a substrate for a second transmethylation step [2, 3, 5–7] to give a carbonium ion which, by loss of a proton from C-28, can form either the *E*- or the *Z*-24-ethylidene isomer (**4** or **5** respectively) [8]. With the alga *Ochromonas malhamensis* the incorporation of four deuterium atoms from methionine-[methyl-$^2H_3$] into the major sterol, poriferasterol (**9**) indicates that a 24-ethylidene intermediate is formed as outlined in Scheme 1 and then reduced to give the 24β-ethyl group of poriferasterol (**9**) [9]. In support of this mechanism is the incorporation of 24-ethylidene lophenol-[2, 4-$^3H_3$] into poriferasterol (**9**) by *Ochromonas malhamensis* [10] and the demonstration of the hydrogen migration from C-24 to C-25 required by the proposed biosynthetic route [11, 12]. However a 24-ethylidene sterol has not yet been isolated from *O. malhamensis* and therefore the configuration of the precursor 24-ethylidene sterol produced is not known. To gain further information on sterol biosynthesis in *O. malhamensis* we have developed a method for the tritium labelling of the isomeric 24-ethylidene sterols, fucosterol (**10**) and 28-isofucosterol (**11**) and examined their incorporation into poriferasterol (**9**). A preliminary communication on some of this work has been published previously [13].

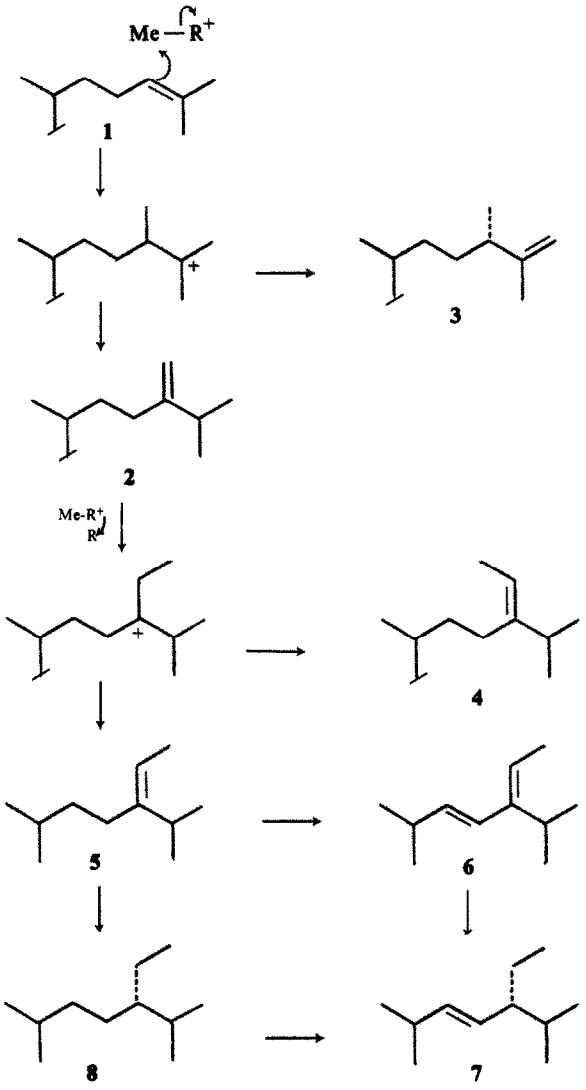
RESULTS AND DISCUSSION

A method for the labelling of Δ^5-sterols with tritium at the C-2 and C-4 positions has been described previously [14]. However, for the present work a new method was developed for the preparation of C-7 tritiated fucosterol and 28-isofucosterol. The Δ^5-sterol (**10** or **11**) was first converted to the *i*-sterol (**12**) [15–17] which was then oxidised to the 6-ketone (**13**) followed by basic equilibrium in the presence of tritiated water to introduce tritium into the C-7 position. Reduction to restore a 6α-hydroxyl group and rearrangement then gave the labelled acetate of the parent sterol. 5α-Stigmasta-7,*Z*-24(28)-dien-3β-ol-[2,4-$^3H_2$] (**14**) was prepared by basic exchange of 5α-stigmasta-7,*Z*-24(28)-dien-3-one in the presence of tritiated water as outlined in the Experimental.

The results of feeding the three labelled 24-ethylidene sterols to cultures of *O. malhamensis* are presented in Table 1. After each incubation a portion of the sterol obtained by TLC was mixed with a sample of the unlabelled substrate sterol and analysed by GLC to obtain an estimate of the efficiency of conversion of absorbed precursor sterol into poriferasterol (**9**). The chromatograms from three incubations are shown in Fig. 1. The remaining labelled sterol was then subjected to TLC on $AgNO_3$–silica gel to remove unchanged 24-ethylidene sterol from the poriferasterol. In none of the incubations with 5α-stigmasta-7,*Z*-24(28)-dien-3β-ol (**14**) was there any indication of the accumulation of $\Delta^{7,22}$ or $\Delta^{5,7}$ intermediates. The radiochemical purity of the poriferasterol (**9**) was established by crystallization to constant specific radioactivity after addition of carrier poriferasterol (Table 1). All three 24-ethylidene sterols were converted into poriferasterol by *O. malhamensis* in respectable yields so demonstrating the ability of this organism to reduce both the *E*- and the *Z*- isomers of 24-ethylidene sterols.

The possibility that tritium from the three precursor compounds had been randomised by catabolism of the sterols and then incorporated into newly synthesised poriferasterol was checked. The labelled poriferasterol obtained from the 5α-stigmasta-7,*Z*-24(28)-dien-3β-ol-[2,4-$^3H_4$] feeding was diluted with carrier poriferasterol and converted by Jones oxidation into (24*R*)-24-

* Present address: Oak Ridge National Laboratory, Post Office Box X, Oak Ridge, TE 37830, U.S.A.



Scheme 1. Biosynthetic routes for the production of the phytosterol side chain.

ethylcholesta-4,22-dien-3-one. Basic equilibration of this ketone resulted in the loss of about 85% of the radioactivity as would be anticipated if the tritium were located at the C-2 and C-4 positions as in the parent tritiated precursor sterol. Labelled poriferasterol from the fucosterol-[7-$^3H_2$] and 28-isofucosterol-[7-$^3H_2$] incubations was added to carrier, acetylated and then oxidised to give 3β-acetoxy-(24*R*)-24-ethylcholesta-5, 22-dien-7-one. In both cases this resulted in the loss of 92–95% of the radioactivity so establishing that the tritium of the precursor sterol (**10** or **11**) retained its location at the C-7 position of the product poriferasterol (**9**).

In experiments 1 and 2 (Table 1) the *Z*-isomers, 5α-stigmasta-5,*Z*-24(28)-dien-3β-ol (**14**) and 28-isofucosterol (**11**), were converted with comparable efficiency into poriferasterol (**9**), the better incorporations being obtained, perhaps not surprisingly, in experiment 2 with the longer incubation period where only 10–15% of unmetabolised ethylidene sterol was recovered from the cells. It therefore appears that, once the added 5α-stigmasta-7,*Z*-24(28)-dien-3β-ol (**14**) had been absorbed and reached the site of poriferasterol synthesis, there was a facile transformation of the ring Δ^7 bond to the Δ^5 position.

In experiments 1 and 2 there was apparently a better recovery of total radioactivity in the sterols from the fucosterol-[7-$^3H_2$] (**10**) feeding than with the other two precursors. However the bulk of this radioactivity was in unmetabolised fucosterol-[7-$^3H_2$] (**10**) and in no case did the incorporation into poriferasterol (**9**) approach that observed with the two *Z*-ethylidene sterol substrates (**11** and **14**). These results therefore indicate that a 24-ethylidene sterol with the *Z*-configuration is more efficiently reduced to produce a 24β-ethyl sterol than is the isomer with the *E*-configuration. To establish this point further a mixture of 28-isofucosterol-[7-$^3H_2$] and fucosterol-[2,7,15,22,26-$^{14}C_5$] with a ^3H:^{14}C ratio of 1.29:1 was fed to cultures of *O. malhamensis*. The poriferasterol isolated after a four day culture period was purified by TLC on $AgNO_3$–silica gel and found to have an increased ^3H:^{14}C ratio of 2.35:1. Conversely the recovered unchanged 24-

Table 1. Incorporation of labelled 24-ethylidene sterols into poriferasterol by *Ochromunas malhamensis*

Precursor sterol	5α-Stigmasta-7,Z-24(28) dien-3β-ol-[2,4-$^3H_4$]		28-Isofucosteryl [7-$^3H_2$] acetate		Fucosteryl [7-$^3H_2$] Acetate		28-Iso-fucosterol [7-$^3H_2$]	Fucosterol [7-$^3H_2$]
Experiment number*	1	2	1	2	1	2	3	3
Amount added to incubation (dpm)	8.76 × 10^6	2.79 × 10^7	8.76 × 10^6	8.76 × 10^6	9.90 × 10^6	2.42 × 10^7	2.13 × 10^7	2.13 × 10^7
Non-saponifiable lipid recovered (mg)	32.8	26·6	52.3	16.6	38.0	38.0	84.6	100
Radioactivity of non-saponifiable lipid (dpm)	5.16 × 10^5 (5.9%)	5.20 × 10^6 (18.6%)	6.52 × 10^5 (7.4%)	5.83 × 10^6 (6.6%)	2.87 × 10^6 (28.9%)	1.07 × 10^7 (44%)	—	—
Purified sterol (mg)	10.5	15.4	14.1	14·0	13.0	12.2	24.6	29.8
Purified sterol radioactivity (dpm)	3 22 × 10^5	2.37 × 10^6	2.50 × 10^5	1.62 × 10^6	7.39 × 10^5	6.02 × 10^6	7.21 × 10^6	5.24 × 10^6
% Radioactivity added	3.7	8.2	2.9	18.5	7.5	25.0	33.9	24.7
% Recovered radioactivity in poriferasterol†	63	92	68	85	28	15	57	32
Crystallization with carrier poriferasterol (dpmx10^{-3}/mg)								
1st Crystallisation	4.52	3.63	2.31	4 81	6.76	7.63	5.72	3.72
2nd Crystallisation	4.61	3.51	2.27	4.01	6.34	5.62	5.76	3.58
3rd Crystallisation	4.36	3.41	2.30	3.51	6.47	4.28	5.68	3.76
4th Crystallisation	4.39	3.15	2.18	3 11	6.23	4.24	5.23	3.67
5th Crystallisation	4.27	3.18	—	3.02	—	—	—	—

* Experiment 1: incubation with *O. malhamensis* for 3 days; experiment 2 for 6 days; experiment 3 for 4 days.

† The extent of conversion of the tritiated substrate into poriferasterol was determined by GLC on 3% OV-17 with sample trapping at one minute intervals. The appropriate unlabelled precursor was added prior to analysis.

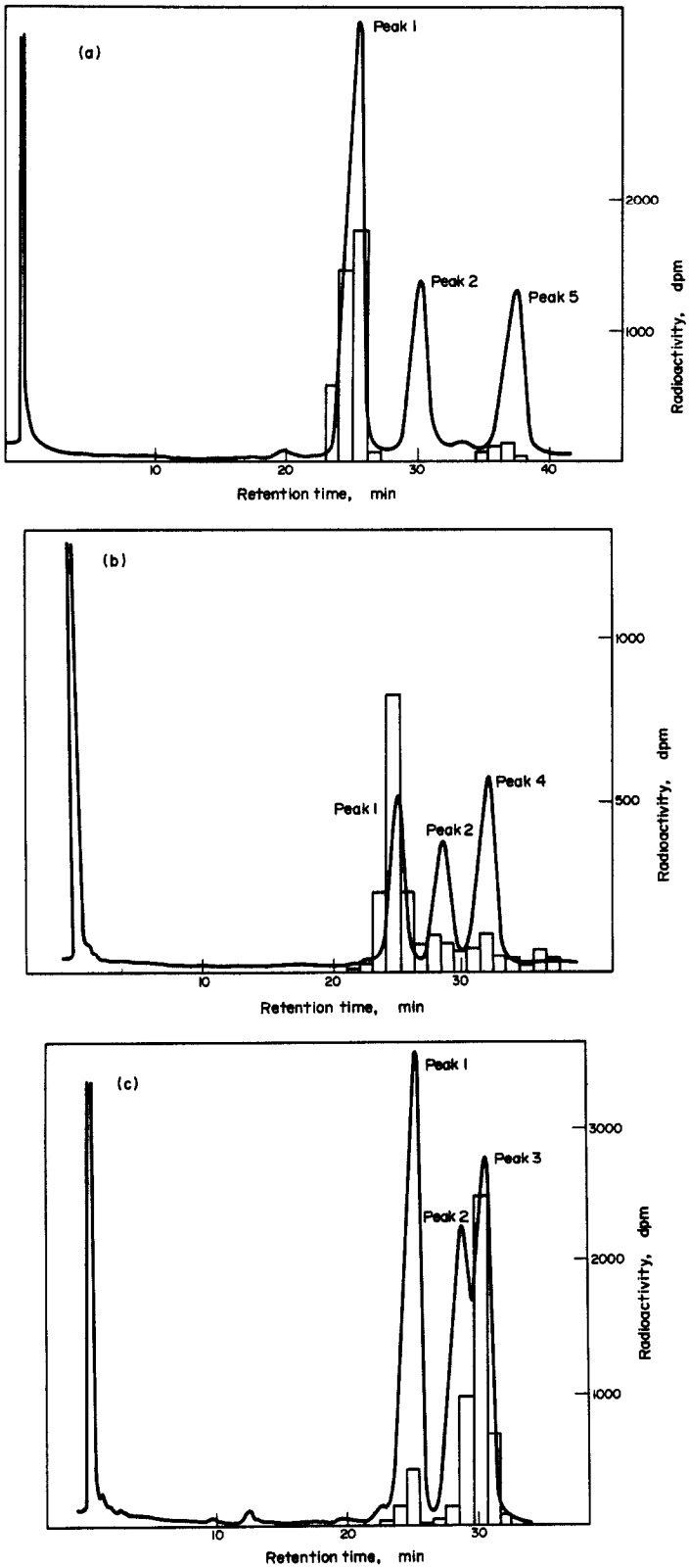
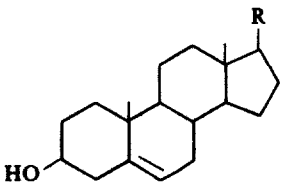
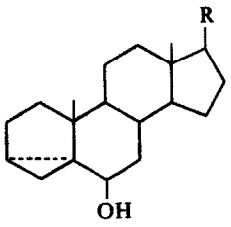


Fig. 1. GLC analysis of the radioactive sterols recovered after the incubation of *O. malhamensis* with (a) 5α-stigmasta-7,Z-24(28) dien-3β-ol-[2,4-$^3H_2$]; (b) 28-isofucosterol-[7-$^3H_2$]; (c) fucosterol-[7-$^2H_2$]. The experimental conditions were those described for experiment 1 in Table 1. Appropriate carrier sterols were added to the radioactive sterol and the GLC analysis performed on a column of 3% OV-17. Samples were trapped at 1 min intervals and assayed for radioactivity. Peak 1: poriferasterol, peak 2: clionasterol; peak 3: fucosterol; peak 4: 28-isofucosterol; peak 5: 5α-stigmasta-7,Z-24(28)-dien-3β-ol.

ethylidene sterol mixture had a decreased $^3H:^{14}C$ ratio of 0.64:1. In a second experiment the $^3H:^{14}C$ ratio of the isolated poriferasterol was only slightly higher than the initial ethylidene sterol ratio possibly due to a longer incubation period permitting more complete utilisation of the substrate mixture. However, the former results are in accord with the results presented in Table 1 and confirm that a 24-ethylidene sterol with the *Z*-configuration is the more efficient precursor of poriferasterol (**9**).



9 R = Side chain **7**
10 R = Side chain **4**
11 R = Side chain **5**
16 R = Side chain **8**



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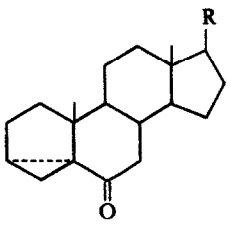

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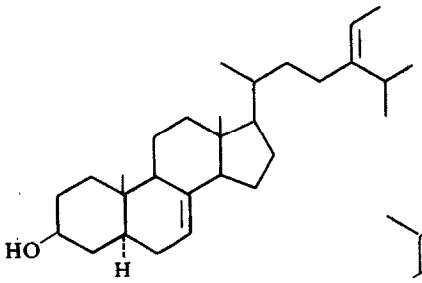

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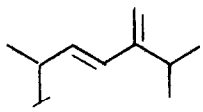

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The preferential conversion of the 24*Z*-ethylidene sterol into poriferasterol (**9**) could result from the stereospecificity exhibited by the $\Delta^{24(28)}$-reductase. However the stereospecificity of this reductase cannot be absolute for 24-ethylidene sterols of the *Z*-configuration since fucosterol (**10**) with the *E*-configuration was also incorporated into poriferasterol (**9**). An alternative explanation for the better utilisation of the *Z*-isomer could be the formation of an intermediate with a $\Delta^{22,24(28)}$-diene side chain (**6** in Scheme 1). Indeed, there is now ample evidence that such a compound with side chain (**15**) is on the major pathway of ergosterol biosynthesis in yeast [18, 19]. The production of a similar diene intermediate in C_{29} sterol elaboration may be dependent upon the configuration of the 24-ethylidene sterol produced by the preceding transmethylation reaction. The proximity of the C-29 methyl group to C-22 and C-23 in the *E*-24(28) ethylidene configuration (**4**), as in fucosterol (**10**), would be expected to hinder sterically the enzymically catalysed formation of the corresponding $\Delta^{22,24(28)}$-diene. Conversely with with the *Z*-configuration (**5**), as in 28-isofucosterol (**11**), the production of the diene (**6**) might be expected to proceed smoothly. Indeed, such a situation has been observed with the protozoan *Tetrahymena pyriformis* which, although incapable of *de novo* sterol synthesis, can modify added sterols [20]. In this organism 28-isofucosterol (**11**) is readily converted into stigmasta-5,7,*E*-22,*Z*-24(28)-tetraen-3β-ol whereas fucosterol (**10**) is converted only into stigmasta-5,7,*E*-24(28)-trien-3β-ol. The formation of a sterol intermediate with side chain (**6**) on the major biosynthetic route to poriferasterol (**9**) in *O. malhamensis* would therefore explain the preferential incorporation of 28-isofucosterol (**11**) observed in the present experiments. The smaller conversion of fucosterol (**10**) into poriferasterol could perhaps result from the steric restrictions being insufficiently rigid to prevent some *E*-22,*E*-24(28)-diene production. However, a more attractive possibility is the prior reduction of the fucosterol to give clionasterol (**16**) followed by introduction of the Δ^{22} bond to produce poriferasterol (**9**).

The present results therefore provide good evidence to support the operation of the biosynthetic route **1** → **2** → **5** → **6** → **7** leading to poriferasterol (**9**) in *O. malhamensis*. However, 24-ethylidene sterols have not yet been reported from this, or closely related algae, therefore a final decision on the involvement of sterols with side chain **5** or **6** must await their isolation.

EXPERIMENTAL

General methods. IR spectra were obtained as KBr discs or in $CHCl_3$. NMR spectra were measured at 60 or 100 MHz in $CDCl_3$ with TMS as the int. stand. Mp's were determined on a Reichert hot stage and are uncorr. TLC was on Si gel developed with $CHCl_3$, for analytical purposes spots were detected by spraying with 10% aq. H_2SO_4 followed by heating at 80°. For preparative work bands were located by spraying with Rhodamine-6G in Me_2CO and examining under UV light; after scraping bands from the plate material was eluted with dry Et_2O. GLC employed a 1.52 m × 0.64 cm column of 3% OV-17 on 100–120 mesh Gas-Chrom Q, the carrier gas was argon (60 ml/min) and the column temp. was 260°. For sample trapping a splitter with a 1:25 split ratio (detector: sample collector) was employed; samples were collected at 1 min intervals in capillary tubes at ambient temp. then transferred with Et_2O to counting vials for radioassay. Radioactivity was determined by liquid scintillation counting using 5% PPO in toluene.

Preparation of 5α-stigmasta-7,Z-24(28)-dien-3β-ol-[2,4-$^3H_4$]. 5α-Stigmasta-7,*Z*-24(28)-dien-3β-ol (**14**) was isolated from the non-saponifiable lipid of *Vernonia anthelmintica* seed oil [21]. The sterol (110 mg) was oxidised with Jones reagent and the product purified by TLC to yield 5α-stigmasta-7,*Z*-24(28)-dien-3-one; plates from MeOH; IR ν_{max}^{KBr} cm^{-1}: 1667 (C=O), 826 (=CHCH$_3$); MS *m/e* (rel. int.): 410 (M^+, 16), 395 (M-Me, 19), 312 (M-part side chain, 92), 269 (M-side chain, 100), 248 (25) and 233 (21). The ketone (7.3 mg) was labelled with deuterium by heating for 30 min at 65° in a mixture of dioxan (4 ml) and D_2O (1 ml) containing a small piece (20 mg) of Na metal. MS of the exchanged ketone showed a parent ion at *m/e* 414 demonstrating the exchange of the four enolizable hydrogens located at C-2 and C-4. Tritiated material was obtained by exchange of the ketone (16 mg) with T_2O (1.0 ml, 1.0 Ci) in dioxan (3.0 ml) by the method of ref. [10]. The labelled product was reduced with $NaBH_4$ and the equatorial alcohol (**14**) purified by TLC and crystallisation from MeOH to yield 5α-stigmasta-7,*Z*-24(28)-dien-3β-ol-[2,4-$^3H_4$] (2.2 mg, sp. act. 63 μCi/mg); mp 138–140°; IR ν_{max}^{KBr} cm^{-1}: 3400, 820; MS *m/e* (rel. int.): 412 (M^+, 22), 397 (M-Me, 15), 394 (M-H_2O, 36), 379 (10), 314 (M-part side chain, 97) 271 (M-side chain, 100), 253 (37), and 213 (18). Crystallisation of a sample with added

carrier sterol from MeOH resulted in no loss of radioactivity (1170, 1170, 1150 dpm/mg for successive crystallisations). GLC analysis with sample trapping indicated that at least 98% of the radioactivity was associated with the mass peak of 5α-stigmasta-7,Z-24(28)-dien-3β-ol (**14**).

Preparation of fucosterol-[7-$^3H_2$]. Fucosterol (**10**) was isolated from fresh *Fucus spiralis* and was fully characterised by mp, GLC, IR, MS and NMR. Fucosterol (200 mg) was dissolved in C_5H_5N (4 ml) containing *p*-toluene sulphonyl chloride (1.5 g, freshly crystallised from petrol) and the mixture left at room temp. for 18 hr. The fucosteryl tosylate was obtained in the usual manner and refluxed with KAc (5 g) in Me_2CO (100 ml) containing H_2O (50 ml) for 6 hr [15–17]. The mixture was partitioned between Et_2O and H_2O, the Et_2O extract dried over dry Na_2SO_4 and the solvent evapd to leave the *i*-sterol, 3α,5α-cyclostigmast-*E*-24(28)-en-6β-ol (**12**, side chain **4**; 120 mg) as a thick oil which could not be crystallised from a number of solvents. MS *m/e* (rel. int.): 412 (24), 397 (14), 394 (8), 379 (4), 357 (12), 314 (100), 299 (18) 296 (29), 271 (13) and 213 (15); IR ν_{max}^{KBr} cm^{-1}: 3400, 819; NMR: complex cyclopropane proton resonances at 0–50 cps. The *i*-sterol (100 mg) was oxidised with Jones reagent to yield 3α, 5α-cyclostigmast-*E*-24(28)-en-6-one (**13**, side chain **4**; 75 mg); needles from MeOH, mp 109–111°; IR ν_{max}^{KBr} cm^{-1}: 1670, 820; UV: end absorption at 208 nm; MS *m/e* (rel. int.): 410 (20), 395 (7), 313 (96), 312 (100) 297 (12) and 269 (5). Basic exchange of the 6-ketone (5 mg) in dioxan-^2H_2O-Na as described above yielded the deuterated compound with a MS parent ion at *m/e* 412 demonstrating the expected exchange of the two C-7 hydrogens. Basic exchange of the 6-ketone (28 mg) with ^3H_2O (1.0 Ci) as described above gave a labelled product which was reduced with $LiAlH_4$ in THF [22] to yield 3α,5α-cyclostigmast-*E*-24(28)-en-6α-ol-[7-$^3H_2$]. Reflux with ZnAc in HOAc [23] yielded fucosteryl-[7-$^3H_2$] acetate which was purified by TLC and crystallised as plates from MeOH (13.5 mg) mp 114–115°; sp. act. 21.6 μCi/mg; IR ν_{max}^{KBr} cm^{-1}: 1768, 840; MS *m/e* (rel. int.): 394 (M^- acetate, 87) 297 (100). There was no loss of radioactivity upon crystallisation of a sample with carrier fucosteryl acetate (2310, 2350, 2240 dpm/mg for successive crystallisations) and only one radioactive peak was observed on GLC with the same retention time as fucosteryl acetate. Fucosteryl-[7-$^3H_2$] acetate was used for some feedings to *O. malhamensis*; for others fucosterol-[7-$^3H_2$] (**10**) was obtained by saponification of the acetate with ethanolic KOH followed by TLC purification.

Preparation of 28-isofucosterol-[7-$^3H_2$]. 28-Isofucosterol (**11**) was isolated from *Enteromorpha intestinalis* [8] and characterised by mp, GLC, MS, IR and NMR. Using identical methods to those outlined above 28-isofucosterol (160 mg) was converted into the tosylate which gave, upon reflux with KOAc–Me_2CO–H_2O, 3α,5α-cyclostigmast-*Z*-24(28)-en-6β-ol (**12**, side chain **5**; 95 mg) as a thick oil; IR ν_{max}^{KBr} cm^{-1}: 3405, 382; MS *m/e* (rel. int.): 412 (M^+, 17), 397 (12), 394 (7), 379 (2), 356 (9), 314 (100), 299 (222), 297 (36), 271 (11) and 213 (12); NMR: 0–50 cps (cyclopropane). The *i*-sterol (85 mg) was oxidised with Jones reagent to give 3α,6α-cyclostigmast-*Z*-24(28)-en-6-one (**13**, side chain **5**; 48 mg), plates from MeOH, mp 108–110°; IR ν_{max}^{KBr} cm^{-1}: 1665, 825, UV: end absorption at 209 nm; MS *m/e* (rel. int.): 410 (M^+, 28), 395 (4), 313 (80), 312 (100), 297 (39) and 269 (9). The *i*-ketone (35 mg) was exchanged with ^3H_2O (1.0 Ci) as described above to give 3α,5α-cyclostigmasta-*Z*-24(28)-en-6-one-[7-$^3H_2$] which was reduced with $LiAlH_4$ in THF to give 3α, 5α-cyclostigmast-*Z*-24(28)-en-6α-ol-[7-$^3H_2$]. Reflux in HOAc–ZnAc then gave 28-isofucosteryl-[7-$^3H_2$] acetate (12.8 mg) purified by TLC and crystallised as plates from MeOH (6 mg) mp 119°; sp. act. 10 μCi/mg; IR ν_{max}^{KBr} cm^{-1}: 1768, 826; MS *m/e* (rel. int.): 394 (M^+-acetate, 80), 297 (100). Crystallisation of a sample with carrier resulted in no loss of radioactivity (8580, 8490, 8360 dpm/mg for successive crystallisations). All the radioactivity chromatographed on GLC with the mass peak of 28-isofucosteryl acetate. 28-Isofucosterol-[7-$^3H_2$] was obtained by ethanolic KOH hydrolysis of the acetate followed by TLC purification.

Preparation of fucosterol-[2,7,15,22,26-$^{14}C_5$]. Fronds (*ca* 5g) of fresh *Fucus spiralis* were chopped into strips (1–3 mm wide) and incubated [24] for 24 hr with mevalonic acid-[2-^{14}C] (10 μCi in 2 ml H_2O). The nonsaponifiable lipid (18 mg) was obtained from the tissue by reflux with ethanolic KOH and Et_2O extraction. Purification by TLC gave labelled fucosterol (**10**, 1.1 mg, 1.41×10^6 dpm). GLC with sample trapping revealed that 85% of the radioactivity co-chromatographed with fucosterol (**10**), the remaining radioactivity had a retention time corresponding to 5α-stigmasta-7,Z-24(28)-dien-3β-ol, an expected precursor of fucosterol.

Administration of radioactive sterols to O. malhamensis. The labelled sterol or steryl acetate was dissolved in 0.2 ml EtOH with slight warming and the soln injected aseptically into flasks (2 × 200 ml) of *O. malhamensis* (see ref. [25] for culture conditions). The cells from duplicate incubations were centrifuged and saponified with 10% KOH–80% aq. EtOH under reflux for 1 hr. The non-saponifiable lipid was extracted with Et_2O and the sterol (95% poriferasterol, 5% brassicasterol) obtained by PLC. A portion of the sterol was analysed by GLC with sample trapping, after addition of the appropriate carrier sterol, to determine the extent of conversion of absorbed precursor into poriferasterol (**9**). The poriferasterol (**9**) was then purified by TLC on 12% $AgNO_3$–Si gel developed with C_6H_6–hexane (1:1). After addition of carrier, the poriferasterol (**9**) was crystallised from MeOH to constant sp. act.

Preparation of 3β-acetoxy-(24R)-24-ethylcholesta-5,22-dien-7-one. In a typical experiment labelled poriferasterol (**9**) from a fucosterol-[7-$^3H_2$] (**10**) feeding was combined with carrier poriferasterol to give 114 mg total (884 dpm/mg) which was acetylated with C_5H_5N–Ac_2O and purified by PLC. The poriferasteryl acetate was dissolved in HOAc (15 ml) and CrO_3 (75 mg) slowly added while the soln was stirred at 52–54° for 2 hr [26]. The mixture was poured into H_2O and the products extracted with Et_2O. The 3β-acetoxy-(24*R*)-24-ethylcholesta-5,22-dien-7-one (23 mg) was obtained by TLC and crystallised as fine needles from MeOH (61 dpm/mg, 92% loss or radioactivity), mp 173–4° IR ν_{max}^{KBr} cm^{-1}: 1740, 1680, 1640, 973; MS *m/e* (rel. int.): 468 (M^+, 14), 408 (59), 393 (2), 365 (70), 267 (100).

Preparation of (24R)-24-ethylcholesta-4,22-dien-3-one. Labelled poriferasterol from the second 5α-stigmasta-7,Z-24(28)-dien-3β-ol-[2,4-$^3H_4$] feeding (Table 1) was combined with carrier poriferasterol (total 28.3 mg, 1320 dpm/mg). The sterol was oxidised with Jones reagent in Me_2CO at 0° for 8 min and the product purified by TLC to give (24*R*)-24-ethylcholesta-4,22-dien-3-one (12.3 mg, 1310 dpm/mg), needles from MeOH, mp 110°; IR ν_{max}^{KBr} cm^{-1}: 1676, 965; UV λ_{max}^{EtOH} nm: 241; MS *m/e* (rel. int.): 410 (M^+, 100), 395 (8), 367 (77), 298 (55), 283 (22), 271 (90), 269 (58), 229 (15), 215 (12), 213 (23).

Nomenclature. Names of sterols used in this paper are as follows. Brassicasterol: ergosta-5,22-dien-3β-ol; clionasterol: (24*S*)-24-ethylcholest-5-en-3β-ol; ergosterol: ergosta-5,7,22-trien-3β-ol; 24-ethylidene lophenol: 4α-methyl-5α-stigmasta-7,Z-24(28)-dien-3β-ol; fucosterol: stigmasta-5,E-24(28)-dien-3β-ol; 28-isofucosterol: stigmasta-5,Z-24(28)-dien-3β-ol; poriferasterol: (24*R*)-24-ethylcholesta-5,22-dien-3β-ol.

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