The Conversion of 24-Ethylidene-sterols into Poriferasterol by *Ochromonas malhamensis*

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Summary The 24-ethylidene-sterols fucosterol, 28-isofucosterol and 5α-stigmasta-7,*Z*-24(28)-dien-3β-ol are converted into poriferasterol by *Ochromonas malhamensis*, the efficiency of conversion being dependent on the configuration of the ethylidene group.

IT is now well established that the C-24 ethyl side-chain of phytosterols is derived by successive transmethylations from *S*-adenosylmethionine.[1] A 24-ethylidene intermediate has been implicated in the formation of poriferasterol (I) in *Ochromonas malhamensis*[2] and is suggested[3] in the biosynthesis of sterols in several higher plants. By contrast C_{29} sterol formation in several organisms does not proceed *via* a 24-ethylidene intermediate, and alternative routes have been suggested[4] as indicated in the Scheme. The conversion of fucosterol into clionasterol (II) but not poriferasterol (I) in *Chlorella ellipsoidea* has been observed[5] while recently the conversion of 24-ethylidene-lophenol into poriferasterol (I) was demonstrated in *O. malhamensis*.[6] We now report the conversion of other 24-ethylidene-sterols into poriferasterol (I) using this alga.

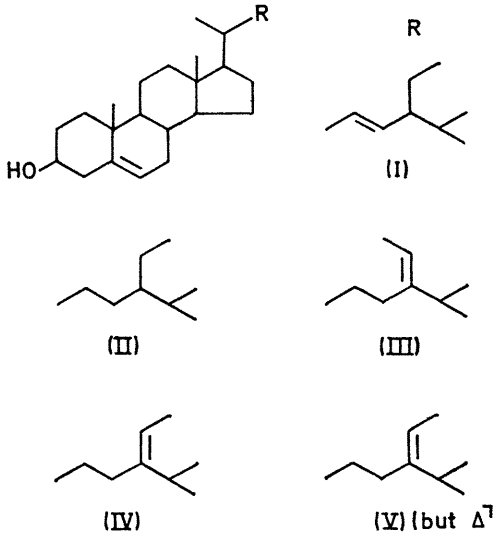
5α-Stigmasta-7, *Z*-24(28)-dien-3β-ol (V),† isolated from *Vernonia anthelmentica* seed oil,[7] was oxidized to 5α-stigmasta-7,*Z*-24(28)-dien-3-one with Jones' reagent. Equilibration of the ketone with tritiated water (1 Ci) in KOH–dioxan followed by sodium borohydride reduction gave [2,2,4,4-$^3H_4$]-5α-stigmasta-7,*Z*-24(28)-dien-3β-ol (60 μCi/mg).

Fucosterol (III), isolated from *Fucus spiralis*, was converted into the tosylate followed by refluxing in acetone–water with potassium acetate[8] to give 3α,5α-cyclostigmast-*E*-24(28)-en-6β-ol. This was oxidized with Jones' reagent to yield 3α,5α-cyclostigmast-*E*-24(28)-en-6-one. Basic equilibration in tritiated water as described above followed by lithium aluminium hydride reduction[9] gave [7,7-$^3H_2$]-3α,5α-cyclostigmast-*E*-24(28)-en-6α-ol which was converted into [7,7-$^3H_2$]fucosteryl acetate (21 μCi/mg) by heating under reflux with fused zinc acetate in acetic acid.[8] 28-Isofucosterol (IV), isolated from *Enteromorpha* species, was converted into [7,7-$^3H_2$]-28-isofucosteryl acetate, (8 μCi/mg) by the method described above for fucosterol. Tritiated fucosterol (III) and 28-isofucosterol (IV) were obtained by saponification of the corresponding acetates. Radiochemical purity of the labelled sterols was established by g.l.c. with sample trapping at one-minute intervals, in each case radioactivity was only associated with the mass peak. Also, after addition of the appropriate carrier sterol there was no drop in specific activity during several crystallizations.

The labelled substrates were fed to cultures of *O. malhamensis* by addition of 0·1 ml of an ethanolic solution. In the first two experiments fucosterol (III) and 28-isofucosterol (IV) were administered as their acetates, in the

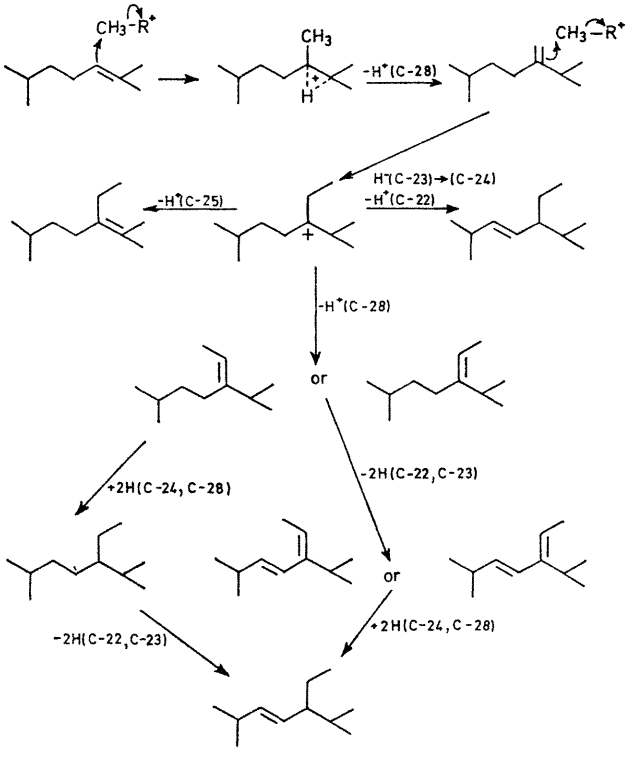
† Compounds were fully characterized by m.p., i.r., u.v., n.m.r., and mass spectrometry. Purity was established by t.l.c. and g.l.c.

third experiment the free sterols were used. After incubation the cells were harvested, the non-saponifiable lipid extracted and the sterols obtained by t.l.c. on silica gel. The distribution of radioactivity in the sterol samples was



determined by g.l.c. with trapping at one-minute intervals.‡ The poriferasterol (I) was then purified by t.l.c. of the remaining sterol on silica gel impregnated with silver nitrate and radiochemical purity established by crystallization with added carrier poriferasterol (I) to constant specific activity. Portions of the labelled poriferasterol (I) from the third [7,7-$^3H_2$]fucosterol (III) and [7,7-$^3H_2$]-28-isofucosterol (IV) incubations were added to carrier poriferasterol, acetylated and oxidized with chromium trioxide[10] to yield 24*R*-24-ethyl-7-oxocholesta-5,22-dien-3β-yl acetate. In each case 92—95% of the radioactivity was lost showing that incorporation with minimal randomization of label had occurred.

5α-Stigmasta-7,*Z*-24(28)-dien-3β-ol (V) and 28-isofucosterol (IV) were incorporated efficiently into poriferasterol (I), (Table). By contrast fucosterol (III) was less readily utilized as a poriferasterol precursor. The preferential incorporation of 28-isofucosterol (IV) compared with fucosterol (III) was substantiated by an experiment in which a mixture of [7,7-$^3H_2$]-28-isofucosterol and [2,7,15,-22,26-$^{14}C_5$]fucosterol§ (^3H:^{14}C = 1·29:1) was incubated with *O. malhamensis* for 4 days. The isolated poriferasterol



SCHEME

TABLE

1st Feedings—3 days	Total fed (d.p.m.)	Total sterol recovered (d.p.m.)	Per cent recovered	Per cent of radioactivity recovered in Precursor	Poriferasterol
5α-Stigmasta-7, *Z*-24(28)-dien-3β-ol ..	8·76 × 10^6	3·22 × 10^5	3·71	36	63
Fucosteryl acetate	9·90 × 10^6	7·39 × 10^5	7·46	70	28
28-Isofucosteryl acetate	8·76 × 10^6	2·50 × 10^5	2·86	31	68
2nd Feedings—6 days					
5α-Stigmasta-7, *Z*-24(28)-dien-3β-ol ..	2·79 × 10^7	2·37 × 10^6	8·14	7	92
Fucosteryl acetate	2·42 × 10^7	6·02 × 10^6	25·0	80	15
28-Isofucosteryl acetate	8·76 × 10^6	1·62 × 10^6	18·5	12	85
3rd Feedings—4 days					
Fucosterol	2·13 × 10^7	5·24 × 10^6	24·7	65	32
28-Isofucosterol	2·13 × 10^7	7·21 × 10^6	33·9	40	57

(I) had a ^3H:^{14}C ratio of 2·35:1 whereas the 28-isofucosterol–fucosterol mixture recovered from the cells had a decreased ^3H:^{14}C ratio of 0·635:1. These results indicate that the conversion of 24-ethylidene-sterols into poriferasterol (I) is somewhat dependent upon the configuration of the 24-ethylidene group. If reduction of the 24-ethylidene group preceeds introduction of the Δ^{22} double bond specificity of the reductase for the configuration of the 24-ethylidene group could be envisaged. The clionasterol

‡ Poriferasterol is the predominant sterol in *O. malhamensis*. For g.l.c. analysis 5α-stigmasta-7, *Z*-24(28)-dien-3β-ol, fucosterol, or 28-isofucosterol were added as appropriate carriers to permit determination of radioactivity remaining in the precursor.

§ Prepared by incubation of *Fucus spiralis* with [2-^{14}C]mevalonic acid.

(II) so formed could then be rapidly transformed into poriferasterol (I) since direct introduction of a Δ^{22} double bond into a saturated sterol side-chain has been demonstrated in several organisms.[11] Alternatively our results could be explained by the intermediary formation of a 22,24(28)-diene system (see Scheme). In this case the orientation of the 24-ethylidene group would be of fundamental importance with regard to steric hindrance at C-22. The proximity of the C-29 methyl group to C-22 in the *E*-24(28) configuration as in fucosterol (III) might be expected to hinder formation of the Δ^{22} double bond. Relevant to such a mechanism, the protozoan *Tetrahymena pyriformis*[11e] transformed 24-methylenecholesterol into ergosta-5,7,22,24(28)-tetraene-3β-ol and 28-isofucosterol (IV) into stigmasta-5,7,22, *Z*-24(28)-tetraen-3β-ol but converted fucosterol (III) only into stigmasta-5,7,*E*-24(28)-trien-3β-ol.

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