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Inversion of the 4β-Hydrogen during the Conversion of the Sterol Obtusifoliol into Poriferasterol by *Ochromonas malhamensis*

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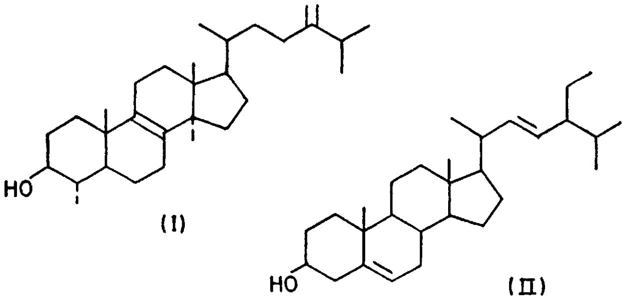
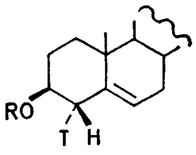
Summary The conversion of [2,2,4-$^3H_3$]obtusifoliol into poriferasterol by the alga *Ochromonas malhamensis* has been demonstrated and proceeds with retention of at least 30% of the axial 4β-hydrogen which is inverted into the equatorial 4α-position of poriferasterol.

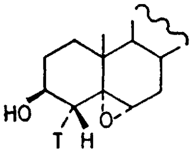


(I)

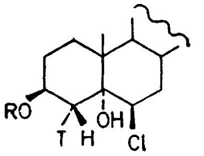
(II)



(III)



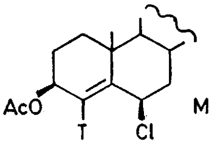
(IV)



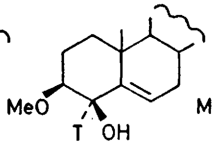
(V)

(III) a; R = H
b; R = Me

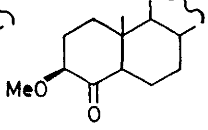
(V) a; R = H
b; R = Ac



(VI)



(VII)



(VIII)

DEMETHYLATION of 4,4-dimethyl sterols in animals[1] and plants[2] proceeds by loss of the 4α-methyl group with the 4β-methyl group epimerising into the 4α-position of the product 4-monomethyl sterol. In the subsequent demethylation of the 4α-methyl sterol the fate of the 4β-hydrogen is unknown but the preparation of tritium-labelled obtusifoliol (I), a suggested phytosterol precursor,[3] and its ready conversion into poriferasterol (II) by *Ochromonas malhamensis* has now permitted a study of this aspect of the C-4 demethylation.

[2,2,4-$^3H_3$]Obtusifoliol (29·8 mCi/mmol) was prepared from unlabelled obtusifoliol by the methods described previously.[4] The [2,2,4-$^3H_3$]obtusifoliol (30 μCi) was incubated with *O. malhamensis* for six days and the sterol (2% incorporation) isolated and analysed by g.l.c. with sample trapping which revealed that 95% of the radioactivity was present in the major component[5] poriferasterol (II). To facilitate the determination of the fate of the C-4 tritium the tritiated poriferasterol was mixed with [1,7,15,22,25-$^{14}C_5$]poriferasterol‡ and the mixture crystallised to constant specific radioactivity and $^3H:^{14}C$ ratio of 9·18 : 1. A sample of the poriferasterol (partial structure IIIa) was epoxidised to give the derivatives§ (IV), $^3H:^{14}C$ ratio 9·03 : 1, which was converted into the chlorohydrin[6,7] (Va), $^3H:^{14}C$ ratio

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‡ [1,7,15,22,26-$^{14}C_5$]Poriferasterol was kindly donated by Dr. G. H. Beastall and was obtained by the culture of *O. malhamensis* in the presence of [2-^{14}C]mevalonic acid.

§ All compounds were fully characterised by m.p., i.r., u.v., n.m.r., and mass spectrometry. Purity was established by t.l.c. and where possible also by g.l.c.

9·02 : 1. Formation of the monoacetate (Vb) followed by dehydration with $SOCl_2$ in pyridine[7] gave the acetate (VI), $^{3}H : ^{14}C$ ratio 9·00 : 1, which was then reduced with $LiAlH_4$[8,9] to produce the dienol (IIIa, poriferasterol), $^{3}H : ^{14}C$ ratio 9·11 : 1. It is established that the dehydration of alcohols of type (Vb) occurs *trans*-diaxially[10] and this was verified in the present work by taking (24*S*)-24-ethyl-4β-[$^{2}H_1$]-cholesta-5,22-dien-3β-ol through the above sequence of reactions with a resulting loss of deuterium.

Since no changes in the $^{3}H : ^{14}C$ ratios were observed the above results established that either tritium was in the 4α-position in the poriferasterol or alternatively no tritium was located at C-4. To check the latter possibility a portion of the dual labelled poriferasterol was converted into the tosylate and then heated under reflux in anhydrous methanol[10] to give the methyl ether (IIIb), $^{3}H : ^{14}C$ ratio 8·82 : 1. The methyl ether (IIIb) was then refluxed in acetic acid–benzene with SeO_2[11] to produce (VII), $^{3}H : ^{14}C$ ratio 8·95 : 1. The retention of all the tritium in this allylic oxidation would be predicted if the tritium was in the equatorial 4α-position and the reaction occurred with retention of configuration. The configuration of the 4β-hydroxy-group was established by the n.m.r. spectrum [τ 5·76 (d, *J* 4 Hz, *eq*-4-H) and 4·33 (deshielded m, olefinic 6-H)]. Further confirmation of this point was obtained by preparation of samples of (24*S*)-24-ethylcholesta-5,22-dien-3β,4α-diol by SeO_2 oxidation of the corresponding 3β-monol, and by basic hydrolysis of 4β-acetoxy-(24*S*)-24-ethylcholesta-5,22-dien-3β-ol obtained by Br_2–silver acetate treatment[12] of (24*S*)-24-ethylcholesta-5,22-dien-3β-ol. The two compounds were identical and formed the same acetonide, which could only occur with a *trans*-diequatorial or a *cis*-diol. Since the n.m.r. spectrum revealed an equatorial C-4 proton the C-4 hydroxy-group must have been β-orientated and this established the stereochemistry of the SeO_2 oxidation.

To determine the presence of tritium at C-4 the radioactive 4β-alcohol (VII) was reduced[11] to 3β-methoxy-(24*S*)-24-ethylcholestan-4β-ol, $^{3}H : ^{14}C$ ratio 8·86 : 1. Oxidation with Jones' reagent then gave the ketone (VIII), $^{3}H : ^{14}C$ ratio 7·87 : 1, with a loss of 10·5% of tritium. In a duplicate degradation, production of (VIII) resulted in a loss of 12·3% of tritium whilst labelled poriferasterol obtained by incubation of 24-ethylidene[2,2,4-$^{3}H_3$]lophenol with *O. malhamensis*[4] lost 16% of tritium upon conversion into (VIII).

It can therefore be concluded that tritium was located at C-4 of the poriferasterol (II) and that it was exclusively in the 4α-position. Thus demethylation of the 4α-monomethyl precursor (I) must have proceeded by a mechanism in which at least 30% of the original 4β-hydrogen was retained and inverted to the 4α-position. The fact that only about 10—12% of the tritium content of the poriferasterol was in the 4α-position compared with a possible maximum of 33% may indicate that some exchange of the C-4 proton occurs during 4α-demethylation, for example by enolisation of the 3-oxo-compound which is implicated[13] as an intermediate. Alternatively it may be a consequence of the basic enolisation process employed[4] to prepare the [2,2,4-$^{3}H_3$]obtusifoliol which may favour enolisation towards the less substituted C-2 with the result that much less tritium is incorporated into the 4β- than the 2α- and 2β-positions. A final decision between these two explanations awaits further investigation.

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