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The Conversion of Lanosterol, Cycloartenol and 24-Methylenecycloartanol into Poriferasterol by *Ochromonas malhamensis*

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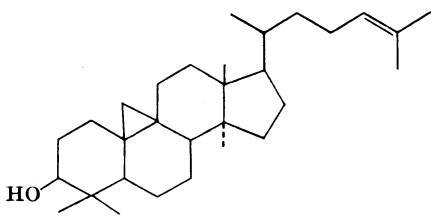
It is postulated that the first cyclized sterol precursor in higher plants and algae is cycloartenol (I) and not lanosterol (II), the known sterol intermediate in animals and fungi (Ardenne, Osske, Schreiber, Steinfelder & Tümmler, 1965; Benveniste, Hirth & Ourisson, 1966; Goad & Goodwin, 1966, 1967, 1969; Goad, 1967; Gershengorn *et al.* 1968). This suggestion is based on the widespread occurrence of cycloartenol (I) and 24-methylenecycloartanol (III) in plants and their rapid labelling from [2-^{14}C]mevalonate and [1-^{14}C]acetate under conditions where phytosterols are being actively synthesized (see references above). To study the involvement of 9β-19-cyclopropane sterols in phytosterol biosynthesis we have now examined the conversion of 24-methylene[2-$^{3}H_2$]cycloartanol, [2 - $^{3}H_2$]cycloartenol, [2 - $^{3}H_2$]cycloartenone and [2-$^{3}H_2$]lanosterol into poriferasterol (IV) by the phytoflagellate *Ochromonas malhamensis*.

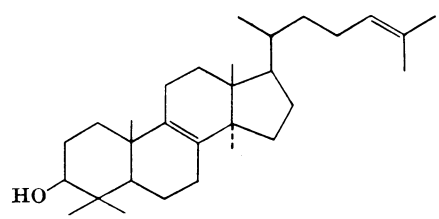
Experimental and results. Lanosterol, cycloartenol and 24-methylenecycloartanol, purified by t.l.c. on $AgNO_3$-impregnated silica gel, were converted into the corresponding 3-ketones by chromic acid oxidation and then labelled with ^{3}H at C-2 by exchange on a basic alumina column that had been deactivated by addition of ^{3}HHO (Klein & Knight, 1965). The ^{3}H-labelled ketones were reduced with $LiAlH_4$ and the resulting 3β-alcohols purified to give [2-$^{3}H_2$]lanosterol (3·4μc/mg.), [2-$^{3}H_2$]cycloartenol (0·5μc/mg.) and 24-methylene-[2-$^{3}H_2$]cycloartanol (1·1μc/mg.). The [2-$^{3}H_2$]-lanosterol and 24-methylene[2-$^{3}H_2$]cycloartanol were shown to be pure by g.l.c., but the [2-$^{3}H_2$]-cycloartenol was found to contain a small amount of impurity that could not be removed by t.l.c. However, because of our limited supply of cycloartenol the experiment was continued with this material. It is not considered that the unidentified impurity in the cycloartenol will have appreciably affected our results.

O. malhamensis was cultured in flasks containing 200ml. of medium as described previously (Smith, Goad, Goodwin & Lederer, 1967; Gershengorn *et al.* 1968). The ^{3}H-labelled sterol substrate was dissolved in 0·3ml. of ethanol, and 0·1ml. of this solution was added to each of three flasks of a 3-day-old culture. After a further 43hr. the contents of the flasks were harvested and the non-saponifiable lipids isolated in the usual manner.

Poriferasterol was isolated by preparative t.l.c.



(I)



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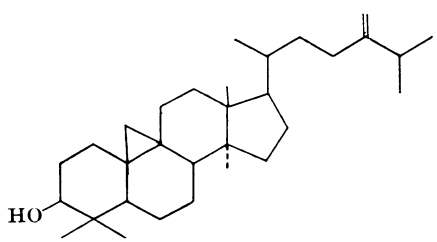
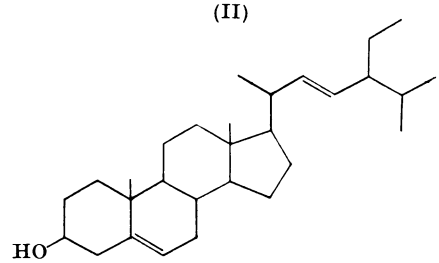


(III)



(IV)

Table 1. *Conversion of possible* ^3H*-labelled sterol precursors into poriferasterol by* O. malhamensis

Substrate	Amount added to incubation (μc)	Sp. radioactivity of poriferasterol (c.p.m./mg.)	Incorporation into poriferasterol (%)
[2-$^3H_2$]Lanosterol	1·9	5480	8·9
[2-$^3H_2$]Cycloartenone	2·3	3090	4·4
[2-$^3H_2$]Cycloartenol	1·1	3430	13·7
24-Methylene[2-$^3H_2$]-cycloartanol	0·85	4670	16·2

on silica gel developed with chloroform followed by t.l.c. on 10% (w/v) $AgNO_3$-impregnated silica gel developed with chloroform. The poriferasterol was finally crystallized to constant specific radioactivity. The results of these experiments are recorded in Table 1. To check that the ^3H was not randomized during the incubation period, the poriferasterol from each incubation, after crystallization, was oxidized by the Oppenauer reaction (Shepherd *et al.* 1955) and the resulting poriferastenone refluxed in methanolic KOH. In all cases the reisolated and purified poriferastenone was completely devoid of ^3H, showing that no randomization of label had occurred.

Discussion. The results presented in Table 1 show that all possible phytosterol precursors tested can be absorbed and converted into poriferasterol with remarkable efficiency by cultures of *O. malhamensis*. In a second similar experiment the incorporations observed were: [2-$^3H_2$]lanosterol, 4%; [2-$^3H_2$]cycloartenol, 5·9%; and 24-methylene-[2-$^3H_2$]cycloartanol, 10·7%. Although lower than in the first experiment, these conversions are again substantial. The differences between the two experiments may perhaps be a result of minor differences in the cultural conditions or of the state of growth of the alga at the time of addition of the substrates. The conversion of [2-$^3H_2$]-cycloartenol, [2-$^3H_2$]cycloartenone and 24-methylene[2-$^3H_2$]cycloartanol into [2-$^3H_2$]poriferasterol establishes that *O. malhamensis* contains an enzyme system that can open the 9β-19-cyclopropane ring of these postulated phytosterol precursors (Goad, Williams & Goodwin, 1967). The involvement of cycloartenol and related compounds in phytosterol biosynthesis by *O. malhamensis* is supported by the demonstration of small amounts of cycloartenol and 24-methylenecycloartanol, coupled with the absence of lanosterol, in this organism (Gershengorn *et al.* 1968). This conclusion also gains compelling support from our recent work with an enzyme preparation from *O. malhamensis* that, under anaerobic conditions, will cyclize squalene 2,3-oxide to cycloartenol without any trace of lanosterol accumulating (H. H. Rees, unpublished work; cf. Rees, Goad & Goodwin, 1968). On this basis the conversion of [2-$^3H_2$]lanosterol into poriferasterol in the present work must be interpreted as a lack of substrate specificity in the subsequent enzyme reactions such as C-24 alkylation (Russell, van Aller & Nes, 1967) and C-4 and C-14 demethylation.

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