Phytochemistry, 1971, Vol. 10, pp. 573 to 577. Pergamon Press. Printed in England.

BIOSYNTHESIS OF THE ALKYL GROUP AT C-24 OF PORIFERASTEROL AND Δ^5-ERGOSTENOL IN *CHLORELLA ELLIPSOIDEA**†

YUTAKA TOMITA, ATSUKO UOMORI and EIICHI SAKURAI

Shionogi Research Laboratory, Shionogi & Co. Ltd., Fukushima-ku, Osaka, Japan

(*Received* 26 *May* 1970)

Abstract—Poriferasterol and Δ^5-ergostenol, isolated from *Chlorella ellipsoidea* grown in the presence of (CD_3)-methionine, contain five and three deuterium atoms, respectively. Therefore, poriferasterol is synthesized by a mechanism which does not involve a C-24 ethylidene intermediate and a C-24 methylene derivative is not a precursor for Δ^5-ergostenol. Thus, in conjuction with previously reported work, these results show that even for the same compound, the biosynthetic mechanism for the alkyl group at C-24 is different depending upon the species involved.

INTRODUCTION

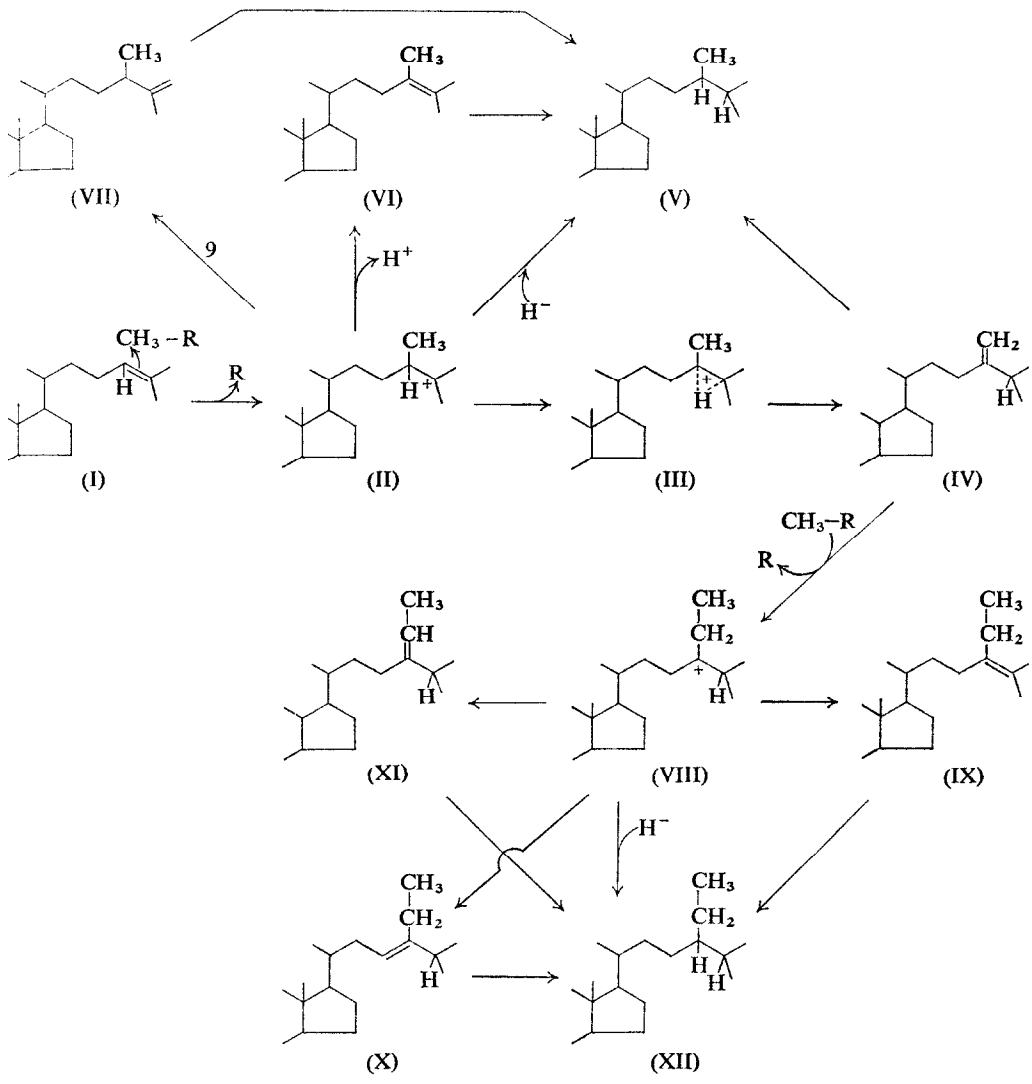
THE EXTRA alkyl group at C-24, characteristic of phytosterols, is derived by transmethylation from adenosylmethionine and the mechanism has been studied by several groups.[1] Lederer *et al.*,[2] Akhtar *et al.*[3] and Barton *et al.*[4] have shown that C-24 methylenelanosterol is an intermediate for ergosterol biosynthesis in yeast (route I → II → III → IV → V) and Pattersson[5] has reported that Δ^5-ergostenol (XIII) in *Chlorella ellipsoidea* might be synthesized by the same route.

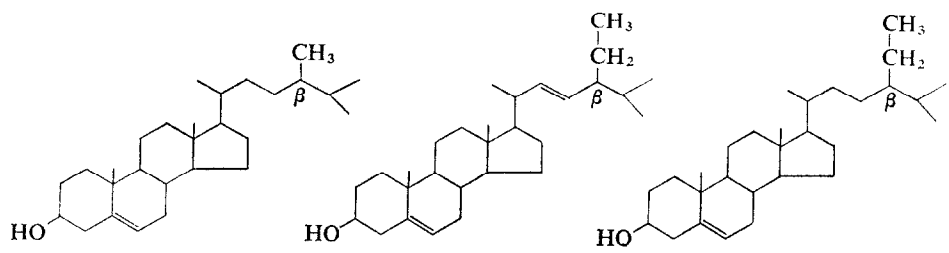
In the biosynthetic studies of the ethyl group at C-24, it has been demonstrated by Smith *et al.*[6] and Pattersson[5] that a C-24 ethylidene derivative is a precursor for poriferastenol (XIV) in *Ochromonas malhamensis* and for clionasterol (XV) in *Chlorella ellipsoidea*. On the other hand, Lenfant, *et al.*[7] showed that a C-24 ethylidene derivative is not an intermediate in the biosynthesis of stigmasta-22-ene 3β-ol in *Dictyostelium discoideum*. In a previous report on the biosynthesis of phytosterols in *C. vulgaris*,[8] we have shown that Δ^7-ergostenol is synthesized by a mechanism which does not involve a C-24 methylene intermediate and that a C-24 ethylidene derivative as an intermediate was excluded from the biosynthesis of chondrillastenol and Δ^7-chondrillastenol. Moreover, we have suggested that a $\Delta^{24(25)}$ precursor, such as compounds (VI) and (IX), might be involved in phytosterol biosynthesis in *C. vulgaris*. In this paper we report on the biosynthesis of C-24 alkyl group in poriferasterol and Δ^5-ergostenol, and compare the results with the mechanism occurring in other species.

* Part II in a projected series entitled "Biosynthesis of Isoprenoids"; for Part I, see *Phytochem.* **9**, 555 (1970).

† Dedicated to Emeritus Professor Munio Kotake on his 77th birthday.

[1] L. LEDERER, *Quart. Rev. Lond.* **24**, 453 (1969).
[2] G. JAUREGUIBERRY, J. H. LAW, J. A. MCCLOSKEY and E. LEDERER, *Biochem.* **4**, 347 (1965).
[3] M. AKHTAR, M. A. PARVEZ and P. E. HUNT, *Biochem. J.* **100**, 38C (1966).
[4] D. H. R. BARTON, D. M. HARRISON and G. P. MOSS, *Chem. Commun.* 595 (1966).
[5] G. W. PATTERSON and E. P. KARLANDER, *Plant Physiol.* **42**, 1651 (1967).
[6] A. R. H. SMITH, L. J. GOAD, T. W. GOODWIN and E. LEDERER, *Biochem. J.* **104**, 56C (1967).
[7] (a) M. LENFANT, E. ZISSMANN and E. LEDERER, *Tetrahedron Letters* 1049 (1967); (b) M. LENFANT, R. ELLOUZ, B. C. DAS, E. ZISSMANN and E. LEDERER, *European J. Biochem.* **7**, 159 (1969).
[8] Y. TOMITA, A. UOMORI and H. MINATO, *Phytochem.* **9**, 555 (1970).





Δ⁵-ergostenol (XIII) poriferasterol (XIV) clionasterol (XV)

RESULTS AND DISCUSSIONS

The presence of poriferasterol, clionasterol and Δ^5-ergostenol in *Chlorella ellipsoidea* has been reported by Pattersson. A mixture of these sterols was isolated by preparative TLC from an unsaponifiable lipid of *Chlorella ellipsoidea* ATCC 11466 grown in the presence of (CD_3)-methionine. After acetylation of the mixture, acetyl poriferasterol and acetyl Δ^5-ergostenol were isolated in pure form by TLC on silver nitrate-silica gel. However, as shown in Fig. 1, acetyl clionasterol was only present in trace amount and could not be isolated in

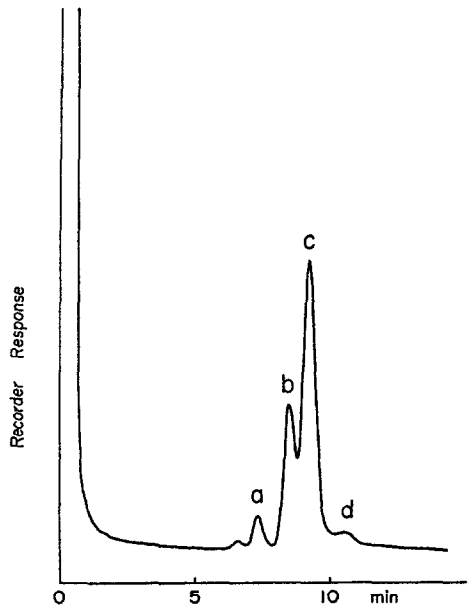
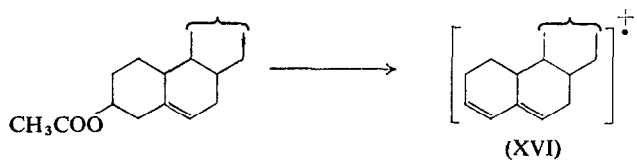


FIG. 1. GAS CHROMATOGRAPHY OF PHYTOSTEROL TRIMETHYL SILYL ETHERS, ISOLATED FROM *C. ellipsoidea*. (a) CHOLESTEROL (b) Δ^5-ERGOSTENOL, (c) PORIFERASTEROL, (d) CLIONASTEROL.

pure. In the mass spectra of acetyl Δ^5-sterols, the molecular ion peak is usually very small but there is a large peak *m/e* (M–60) corresponding to the loss of acetic acid. Therefore, we can establish the number of deuterium atoms in the alkyl group at C-24 from this peak.



(XVI)

In the mass spectrum of acetyl Δ^5-ergostenol, a peak at *m/e* 382 corresponds to the loss of acetic acid from the nondeuterated compound and a large peak at 385 is due to the loss of acetic acid from acetyl Δ^5-ergostenol containing three deuterium atoms per molecule. A peak at *m/e* 367 corresponds to the loss of acetic acid plus methyl.

A peak at *m/e* 261 is probably due to the cleavage of the 7–8 and 9–10 bonds with transfer

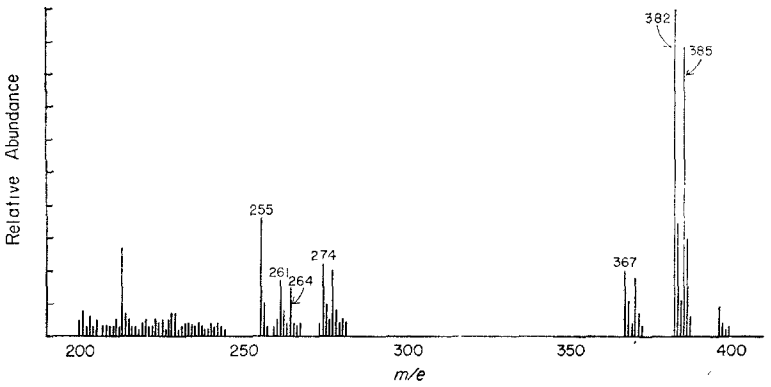
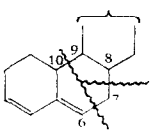


FIG. 2. MASS SPECTRUM OF ACETYL Δ^5-ERGOSTENOL, ISOLATED FROM *C. vulgaris* GROWN IN THE PRESENCE OF (CD_3)-METHIONINE.

of one hydrogen atom and a peak at *m/e* 274 may be due to cleavage of the 6–7 and 9–10 bonds with transfer of two hydrogen atoms.[10] These peaks, due to the ions containing a side chain, are accompanied by ions with *m/e* value +3.



(XVII)

A peak at *m/e* 255, corresponding to the loss of the side chain, was unaccompanied by a higher *m/e* peak. The results show that three deuterium atoms are present in the side chain and therefore a C-24 methylene derivative is not an intermediate in Δ^5-ergostenol biosynthesis.

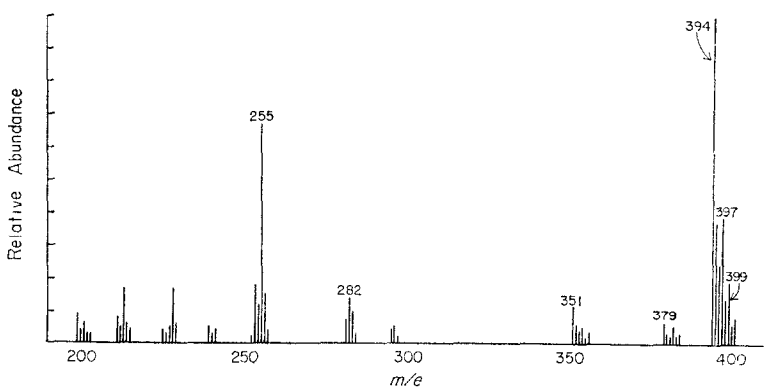
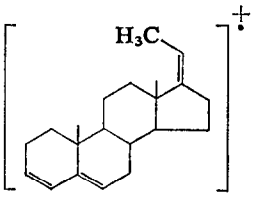


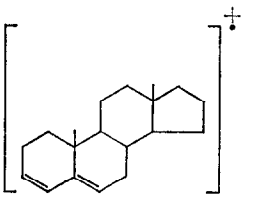
FIG. 3. MASS SPECTRUM OF ACETYL PORIFERASTEROL, ISOLATED FROM *C. vulgaris* GROWN IN THE PRESENCE OF (CD_3)-METHIONINE.

[9] Recently the route (II → VII) has been established in biosynthesis of cyclolaudenol. E. L. GHISALBERTI, N. J. DE SOUZA, H. H. REES, L. J. GOAD and T. W. GOODWIN, *Chem. Commun.* 1401 (1969).
[10] G. GALLI and S. MARONI, *Steroids* **10**, 189 (1967).

In the mass spectrum of acetyl poriferasterol, a peak at *m/e* 394 (M–60) corresponds to the loss of acetic acid from nondeuterated acetyl poriferasterol and the large peaks observed at *m/e* 396, 397 and 399 are due to the loss of acetic acid from acetyl poriferasterol containing two, three and five deuterium atoms per molecule, respectively. The ions observed at *m/e* 379 and 351 correspond to the loss of methyl plus acetic acid and terminal isopropyl plus acetic acid, respectively, and these are both accompanied by ions with *m/e* values +2, +3, and +5. The peaks at *m/e* 282 and *m/e* 255 corresponding to the ions (XVIII) and (XIX), with loss of the side chain, are unaccompanied by higher *m/e* peaks.



(XVIII)



(XIX)

Therefore, all five deuterium atoms are present at the side chain of poriferasterol, and a C-24 ethylidene derivative is excluded as an intermediate in the biosynthesis of poriferasterol in *Chlorella ellipsoidea*. Smith *et al.*[6] found previously that poriferasterol, isolated from *Ochromonas malhamensis* grown in the presence of (CD_3)-methionine, contains four deuterium atoms and suggested that a C-24 ethylidene derivative can act as an intermediate for poriferasterol biosynthesis. Thus, the biosynthesis of the C-24 alkyl group in phytosterols can follow different mechanisms, even in the same compound, depending upon the species involved.

EXPERIMENTAL

Cells of *Chlorella ellipsoidea* ATCC 11466 were grown on basal inorganic medium containing 0·5% glucose and 0·005% (CD_3) methionine for 3 weeks. The cells were harvested and extracted with ethanol for 4 hr (×3). The extracts were evaporated to dryness under reduced pressure and the residue was saponified with 5% KOH in ethanol. After the addition of water the solution were extracted 3 times with petrol and extracts were washed with water, dried and evaporated to dryness.

A mixture of Δ^5-ergostenol and poriferasterol, isolated from the lipids by preparative TLC on silica gel using *n*-hexane–EtOAc–$CHCl_3$ (20:5:5), was acetylated with pyridine and Ac_2O. The mixture of the acetate was applied to $AgNO_3$-silica gel plates and developed with *n*-hexane–$CHCl_3$–HCO_2H (75:25:0·5). Two white zones appeared on the plates by spraying with water. The zone having a lower R_f (0·09) contained mainly poriferasterol acetate, which was obtained in a pure form by repeated TLC on $AgNO_3$-silica gel. The other zone (R_f 0·14) on the TLC plates contained Δ^5-ergostenol acetate.

The mass spectra of these acetates were obtained by Hitachi RMU-6 Single Focus Mass Spectrometer. The gas chromatographic analysis were run on a Shimadzu Gas-Chromatograph GC-4A (PF) instrument fitted with a hydrogen flame ionization detector. A glass U column (4 mm × 1·5 m i.d.) packed with 1% OV-101 on Gas-Chrom Q (100–120 mesh) was operated at 250°. The carrier gas was nitrogen, with a flow rate of 50 ml/min.

Acknowledgement—We wish to thank Dr. H. Minato for helpful discussions.