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BIOSYNTHESIS OF THE METHYL AND ETHYL GROUP AT C-24 OF PHYTOSTEROLS IN *CHLORELLA VULGARIS**

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Abstract—Chondrillasterol, Δ^7-chondrillastenol and Δ^7-ergostenol, isolated from *Chlorella vulgaris* grown in the presence of (CD_3)-methionine, contain five, five and three deuterium atoms, respectively. On the basis of these results the mechanism of transmethylation from methionine to a double bond in the phytosterol side-chain is discussed.

INTRODUCTION

THE EXTRA methyl group at C-24 of ergosterol has been shown to arise from methionine[1] and it has been established that the ethyl group at C-24 of β-sitosterol[2] and α-spinasterol[3] and the ethylidene group of fucosterol[4] also arise from methionine by a double transmethylation. A possible mechanism for the introduction of the alkyl group into phytosterols as proposed by Castle, Blondin and Nes[2] is shown in Fig. 1. Lederer *et al.*[5] have shown that ergosterol biosynthesized by *Neurospora crassa* in the presence of (CD_3)-methionine contains only two deuterium atoms, and suggested that the C-24 methylene derivative (I) would be a precursor of C-24 methylsterols (route b). Barton *et al.*[6] and Akhtar *et al.*[7] reported that C-24 methylenedihydrolanosterol can act as a precursor for ergosterol in yeast and for eburicoic acid in *Polyporus sulfureus.*

In biosynthetic studies of the ethyl group, Smith *et al.*[8] found four deuterium atoms in poriferasterol, isolated from *Ochromonas malhamensis* grown in the presence of (CD_3)-methionine, and indicated that fucosterol may be a precursor of C-24 ethylsterols (route d). However, Lenfant, Zissman and Lederer[9] reported that they have isolated Δ^{22}-stigmastenol containing five deuterium atoms from the slime mould *Dictyostelium discoideum.* This would mean that the slime mould synthesizes the C-24 ethyl group by a mechanism which does not involve an ethylidene intermediate (route c). In the present communication, we wish to report on the biosynthetic mechanism of the alkyl group at C-24 of chondrillasterol (IV), Δ^7-chondrillastenol (V) and Δ^7-ergostenol (VI) in *Chlorella vulgaris.*[10]

* Part I in a projected series entitled "Biosynthesis of Isoprenoids".

[1] G. J. ALEXANDER and E. SCHWENK, *J. Biol. Chem.* **232**, 611 (1958).
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[3] S. BADER, L. GUGLIELMETTI and D. ARIGONI, *Proc. Chem. Soc.* 16 (1964).
[4] V. VILLANUEVA, M. BARBIER and E. LEDERER, *Bull. Soc. Chim. France* 1423 (1964).
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[9] (a) M. LENFANT, E. ZISSMANN and E. LEDERER, *Tetrahedron Letter*, **12**, 1049 (1967); (b) M. LENFANT, R. ELLOUZ, B. C. DAS, E. ZISSMANN and E. LEDERER, *European J. Biochem.* **7**, 159 (1969).
[10] G. W. PATTERSON, *Plant physiol.* **42**, 1457 (1967).

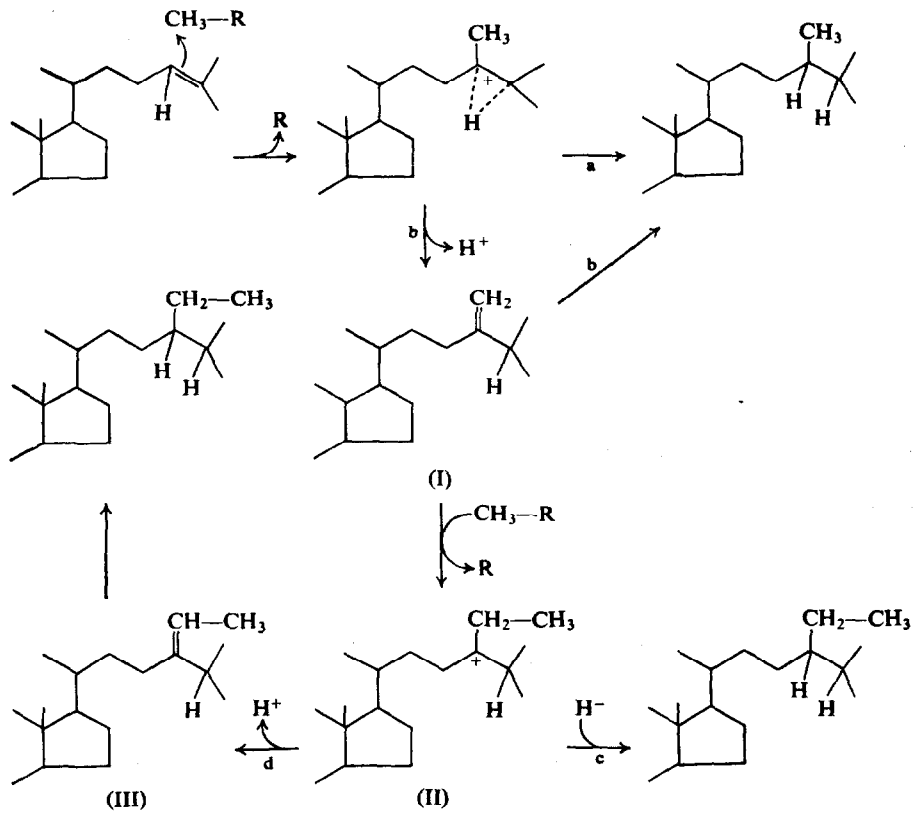
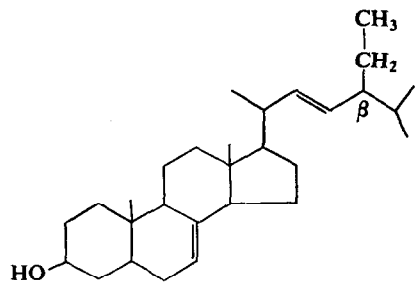
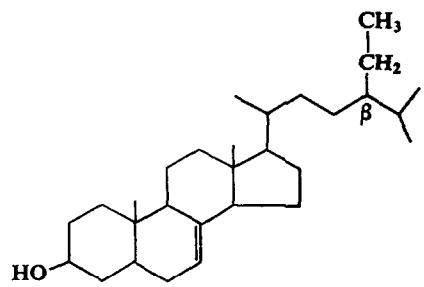


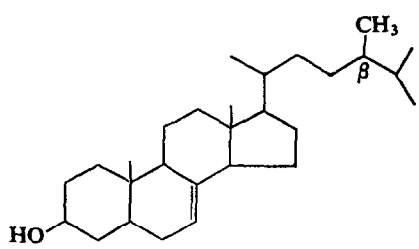
FIG. 1.



Chondrillasterol (IV)



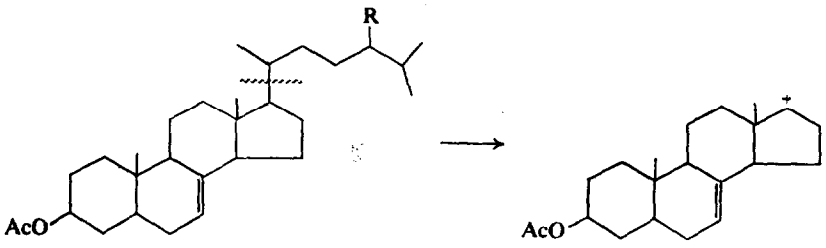
Δ^7-Chondrillastenol (V)



Δ^7-Ergostenol (VI)

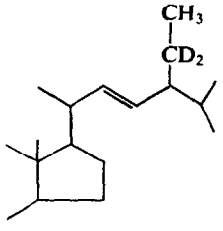
RESULTS AND DISCUSSION

Chlorella vulgaris was grown in the presence of (CD_3)-methionine and a mixture of chondrillasterol, Δ^7-chondrillastenol and Δ^7-ergostenol was isolated from an unsaponifiable lipid by preparative TLC. After acetylation of the mixture, chondrillasterol acetate could be isolated in a pure form by preparative TLC on silver nitrate–silica gel. The mass spectrum of chondrillasterol acetate is shown in Fig. 2. The mass spectra of Δ^7-chondrillastenol acetate and Δ^7-ergostenol acetate were obtained by combined gas chromatography–mass spectrometry. In the mass spectrum of Δ^7-ergostenol acetate, a peak at *m/e* 442 corresponded to the molecular ion peak for the non-deuterated Δ^7-ergostenol acetate. The large peak at *m/e* 445 was that of Δ^7-ergostenol acetate containing three deuterium atoms per molecule. The peaks at *m/e* 427, 382 and 367 corresponded to the loss of methyl, acetic acid and acetic acid plus methyl, respectively. These were accompanied by ions with *m/e* values +3. The peak at 315, corresponding to the ion (VII) with loss of part of the side-chain was unaccompanied by a higher *m/e* peak.

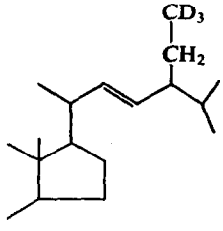


(VII) *m/e* 315

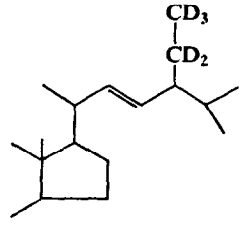
Therefore all three deuterium atoms were present in the side-chain and were derived from methionine. The result shows that C_{24}-methylene derivative (I) as a precursor is not involved in Δ^7-ergostenol biosynthesis. This type of transmethylation is the first example in the biosynthesis of C-24 methylsterols.



(VIII) *m/e* 456



(IX) *m/e* 457



(X) *m/e* 459

In the mass spectrum of chondrillasterol acetate, a peak at 454 corresponded to the molecular ion peak for the non-deuterated chondrillasterol acetate. The large peaks observed at *m/e* 456 (VIII), 457 (IX) and 459 (X) were due to chondrillasterol acetate containing two, three and five deuterium atoms per molecule, respectively. The ions at *m/e* 439, 411, 394, 379 and 351 corresponded to the loss of methyl, terminal isopropyl, acetic acid, acetic acid plus methyl, and acetic acid plus isopropyl, respectively. These were all accompanied by ions with *m/e* value +2, +3 and +5. A peak at *m/e* 342, corresponding to the ion (XI),[9] with loss of part of the side-chain was unaccompanied by higher *m/e* peaks. Therefore, all five deuterium

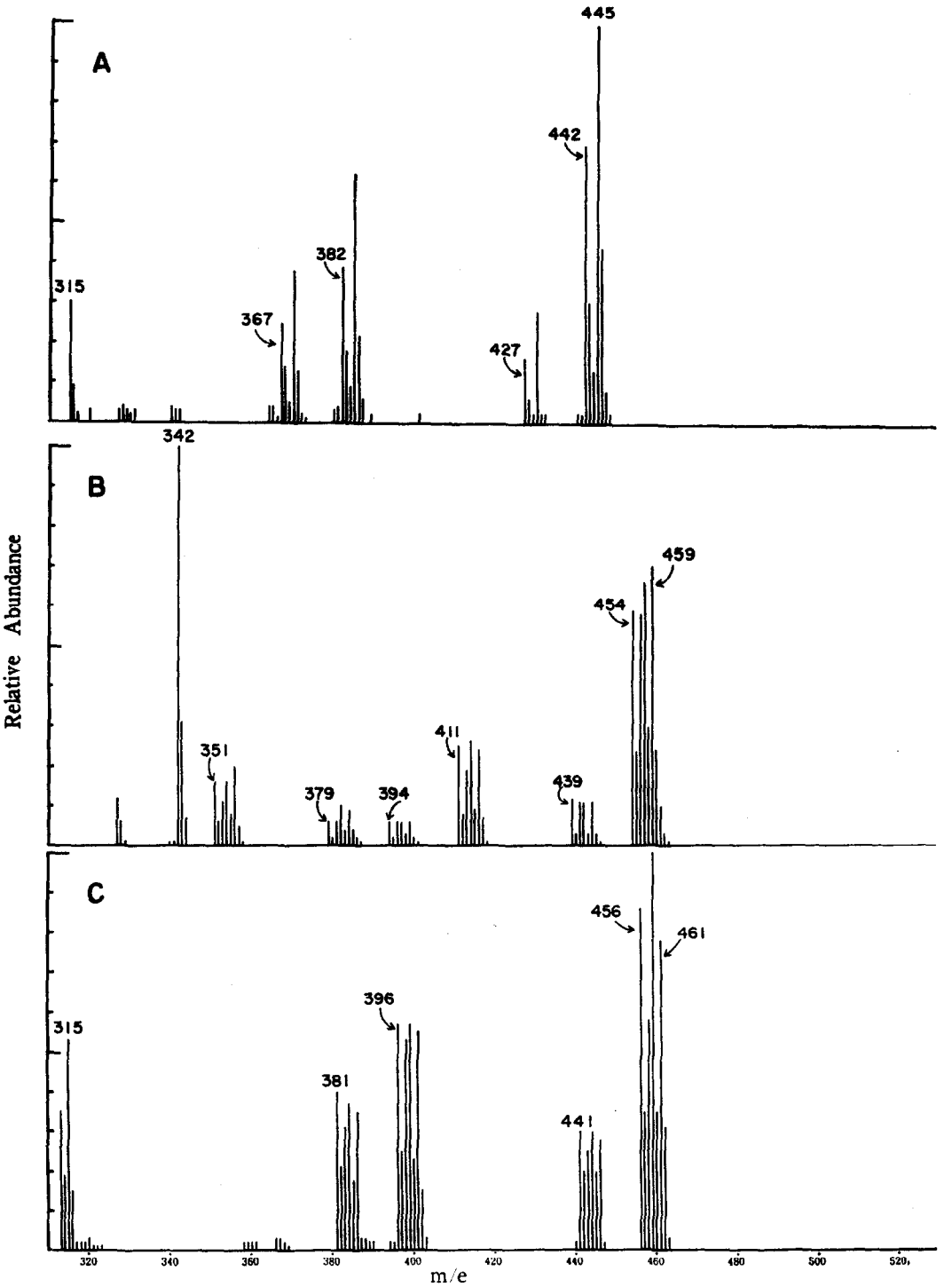
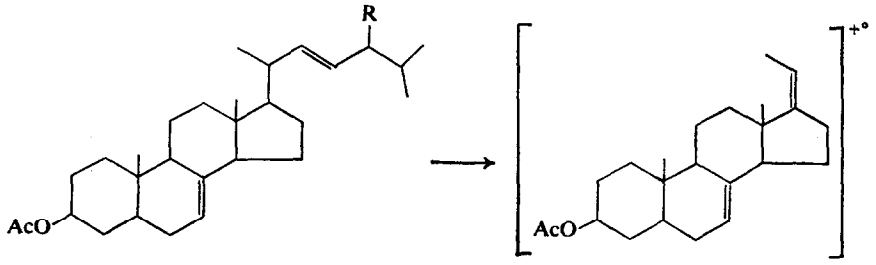
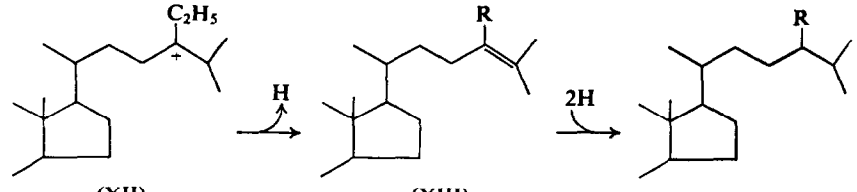


FIG. 2. MASS SPECTRA OF PHYTOSTEROL ACETATES, ISOLATED FROM *C. vulgaris* GROWN IN THE PRESENCE OF (CD_3)-METHIONINE. A: Δ^7-ERGOSTENOL ACETATE; B: CHONDRILLASTEROL ACETATE; C: Δ^7-CHONDRILLASTENOL ACETATE.



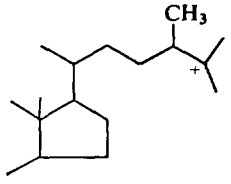
(XI) *m/e* 342

atoms were present in the side-chain of chondrillasterol. In the mass spectrum of Δ^7-chondrillastenol acetate a large peak at *m/e* 456 corresponded to the molecular ion peak for non-deuterated Δ^7-chondrillastenol acetate. The peaks observed at *m/e* 458, 459 and 461 were due to Δ^7-chondrillastenol acetate containing two, three and five deuterium atoms per molecule, respectively. The ions at *m/e* 441, 396 and 381 corresponded to the loss of methyl, acetic acid and acetic acid plus methyl, respectively. These were also accompanied by ions with *m/e* values +2, +3 and +5. A peak at *m/e* 315 with loss of the side-chain was unaccompanied by higher *m/e* peaks. Therefore, all five deuterium atoms were definitely present in the side-chain of Δ^7-chondrillastenol. The mass spectra of deuterated chondrillasterol acetate and Δ^7-chondrillastenol acetate show that all hydrogen atoms of the ethyl group are derived from methionine. Therefore, C-24 ethylidene derivative (III) is not involved as an intermediate in the biosynthesis of these sterols. During biosynthesis of the alkyl group at C-24 of the phytosterol in *C. vulgaris*, the formation of a double bond between C-24 and C-25, such as is found in compound (XIII), would be most plausible in explaining the stabilization of the carbonium ion (XII) or (XII').



(XII) (XIII)

R = C_2H_5 or CH_3



(XII')

On the other hand, the reduction of fucosterol to clinonasterol by *C. ellipsoidea* has been observed.[11] Therefore the mechanism of transmethylation is different, depending upon the species of plants involved.

[11] G. W. Patterson and E. P. Karlander, *Plant physiol.* **42**, 1651 (1967).

EXPERIMENTAL

Cells of *Chlorella vulgaris*** were grown on basal inorganic medium containing 0·5 per cent glucose and 0·01 per cent (CD_3)-methionine for a week in flasks. The cells were harvested and non-saponifiable lipids were isolated in the usual manner. A mixture of chondrillasterol, Δ^7-chondrillastenol and Δ^7-ergostenol, 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 acetates was applied to $AgNO_3$–silica gel thin-layer plates and developed with petroleum ether–$CHCl_3$–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 chondrillasterol acetate, which was obtained in a pure form by repeated TLC on $AgNO_3$–silica gel. The other zone (R_f 0·13) on the TLC plates contained Δ^7-ergostenol acetate and Δ^7-chondrillastenol acetate. The mass spectra of these acetates were obtained by LKB9000 combined gas chromatography–mass spectrometer using an EX-60 column at 270°.

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