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Biosynthesis of the ethyl side chain of stigmasterol derivatives by the slime mould Dictyostelium discoideum

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The ethyl and ethylidene side chains at C-24, characteristic of the phytosterols, are synthesized in vivo by two successive C-methylation steps transferring the carbon atoms of the methyl group of two molecules of methionine onto C-24 of a phytosterol precursor (Castle et al.,1 ; Bader et al.,2 ; Villanueva et al.,3).

Since it has been shown in our laboratory (Jauréguiberry et al.,4) that during the C-methylation leading to ergosterol and to tuberculostearic acid, one of the hydrogen atoms of the methyl of methionine is lost, it seemed interesting to study the fate of the hydrogen atoms of methionine during the double C-methylation reaction mentioned above.

The two alternatives have already been considered in a scheme proposed by Castle et al. (1) in 1963. The first C-methylation step can lead to a methylene compound (I) which could be the substrate for the second C-methylation step ; this can either lead to an ethylidene derivative (III) containing four, or to an ethyl derivative (IV) containing five hydrogen atoms derived from methionine.

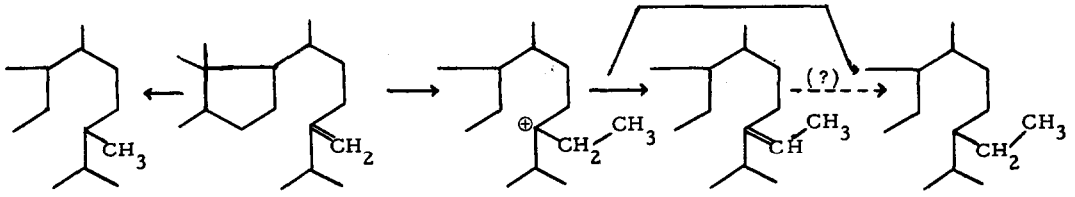
In previous biogenetic schemes (5,6,7,8) ethylidene derivatives (III) such as fucosterol, have generally been considered to be precursors of the ethyl derivatives (IV), in analogy with the methyl derivatives (V) which have been shown recently to originate by saturation of an intermediate methylene derivative (I) (9,10,11).

Mercer (7) and Goad et al. (8) have recently studied the biosynthesis of the phytosterol side chain using (^{14}C, ^{3}H-methyl)-methionine ; they conclude that the ethyl side chain (of sitosterol of maize and larch leaves) contains only four hydrogen atoms derived from methionine, thus indicating the sequence (I) $\longrightarrow$ (II) $\longrightarrow$ (III) $\longrightarrow$ (IV).

As the conclusions drawn from such experiments can be endangered by the strong isotope effect of tritium, we thought that the same problem should be studied using methionine-CD_3. The only difficulty was to choose a biological system permitting a high enough incorporation of the labeled methionine.

In the course of a discussion of this problem, Professor D.Arigoni suggested the use of the slime mould Dictyostelium discoideum, the sterols of which had been studied exten-

sively by Heftmann et al. (12) and Johnson et al. (13). As this slime mould feeds on E. coli, Dr. M. I. Krichevsky made the excellent suggestion that we should first grow a methionine-less mutant of E. coli on methionine-CD_3 and then feed these cells to the slime mould.

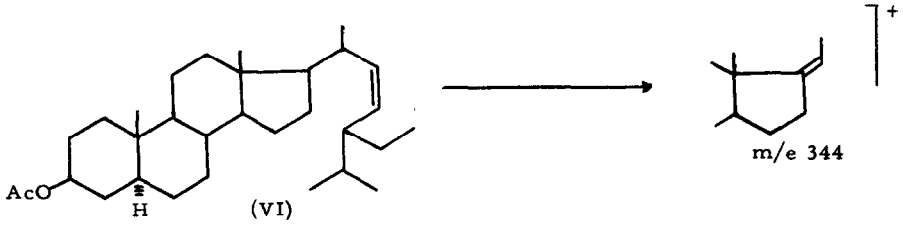


(V) (I) (II) (III) (IV)

[In the above formulae the C-22 unsaturation is not shown because it is not yet clear at what stage it is introduced (14).]

We show in the following that, in fact, a good incorporation of methionine-CD_3 was obtained ; mass spectrometry of the isolated sterol, (stigmast-22-en-3βol, m/e 414) (12) shows without any doubt that five deuterium atoms have been incorporated into the ethyl side chain, excluding an ethylidene derivative as intermediate(*) and indicating that reaction (II)⟶(IV) proceeds either by reduction of (II) by a hydride ion, or by elimination of H^+ (not via III) leading to unsaturated compounds which can then be reduced.

Non deuterated stigmast-22-en-3β-acetate (VI) gave the molecular ion peak at m/e 456 ; among other fragments the mass spectrum showed an intense peak at m/e 344 corresponding to the elimination of a part of the side-chain.



(VI)

There is also a small peak at m/e 427 (M-29) apparently due to the loss of the ethyl group from the side-chain. A peak observed at m/e 413 is considered to be due to the elimination of the terminal isopropyl group by an allylic cleavage.

A purified deuterated sample (VI) from the methionine-CD_3 feeding experiments showed the most intense peak in the molecular ion region at m/e 461 (456 + 5) corresponding to an incorporation of five deuterium atoms in the molecule(**).

(*) In the culture obtained stigmast-22-en-3βol is accompanied by a saturated sterol, probably stigmastan-3β-ol (m/e 416). The molecular ion peak of this compound is also shifted by five mass units in the experiment with deuterated methionine. In both sterols the intensity of the M^++5 peak is approximately three times that of the M^+ peak.

(**) There are also, as expected, peaks at m/e 458 and m/e 459 corresponding probably to molecules of (VI) having two and three deuterium atoms, respectively, in the side-chain.

The peak at m/e 344 remained unshifted in the spectrum of the corresponding deuterated sterol, clearly demonstrating that all five deuterium atoms are in the side-chain. The peak at m/e 413 is shifted to m/e 418 in the spectrum of the deuterated compound ; this shows that the isopropyl group does not contain the incorporated deuterium. The peak at m/e 427 which is apparently due to loss of the ethyl group remains unshifted in the spectrum of the deuterated compound, in agreement with the presence of all five deuterium atoms in the ethyl group.

Our results, which will be confirmed by degradation of the side-chain, are of course only valid for the slime mould studied. It is hoped that analogous experiments using methionine-CD_3 will be possible in higher plants, so as to compare the results obtained with those of Goad et al. (8).

The presence of a CD_3 group in the sterol (VI) is the second case (see 15) proving the transfer of an intact methyl group to a non activated aliphatic double bond. It thus seems that in all cases the $-CH_3$ group of methionine is transferred as a whole ; until now the transfer of the intact methyl group had been shown only for C-methylations of aromatic and other strongly nucleophilic double bonds (15,16,17).

Experimental part - D. discoideum v12/M_1 is grown in 20 Petri dishes (160 mm diameter) on the solid medium of Bonner (18), in presence of living E. coli. At the end of the sporulation stage, the spores are suspended in 10 l of a Sörensen buffer M/60, pH 6 (19) with the centrifugation residue of 20 l of a culture of E. coli 304 D (methionine-less) grown in the presence of 3 g of methionine-CD_3. The aerated (3 l/min) and agitated (200 r.p.m.) culture is incubated at 23° in a fermentor and the cells are harvested at the end of the vegetative stage. 27 g of moist cells are hydrolyzed by dilute HCl (12) ; the neutral fraction (191 mg) is extracted by CH_2Cl_2 and then chromatographed on a column of silicic acid. 50 mg of a 1:4 mixture of the saturated stanol and stigmast-22-en-3βol is thus obtained. After acetylation the acetates are separated by TLC ($AgNO_3$:1, SiO_2:2, hexane-benzene, 5:1). 33 mg of stigmast-22-en-3βol, m.p. 149-151° are obtained.

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