IDENTIFICATION OF AN UNUSUAL 4,5-DIMETHYLOCTANOIC ACID, IN SUBMERGED MYCELIUM OF *CLAVICEPS PURPUREA*

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Polymethyl-substituted fatty acids are very rare; their examples are 2,4,6,8-tetramethyloctacosan-oic acid from the genus *Mycobacterium* (1) and fatty acids with about 10 carbon atoms from the water fowl preen gland wax (2). The heretofore described branchings of fatty acids are usually interrupted by a methylene group. The precursor is generally assumed to be methylmalonyl-CoA. On the other hand, in the case of sterols the side chain is found to contain vicinal methyl groups one which arises from squalene and the other (or a third one, cf. dinosterol=4,23,24-trimethyl-5α-cholestane-3β-ol) is subsequently incorporated via *S*-adenosylmethionine. In a previous paper (3) the branching of a C_{10} dimethyl fatty acid was not determined from data available.

In this report we used ms, ^{13}C nmr, ^{1}H nmr, and oxidative cleavage to prove the presence of 4,5-dimethyloctanoic acid in *Claviceps* mycelium. To our knowledge 4,5-dimethyloctanoic acid has been obtained only by total synthesis in the 1950s (4). The presence of a vicinal (4,5-dimethyl) octanoic acid is highly unusual, and it points to the possible presence of an enzyme complex with a low specificity whose product is this acid.

In the initial condensation leading to the biosynthesis of this fatty acid, malonyl-CoA condenses with acetyl-CoA. The 4-methyl group can readily be envisaged as arising from the methyl group of methylmalonyl-CoA, whereas the 5-methyl group has a more likely origin in *S*-adenosylmethionine.

EXPERIMENTAL

Methyl esters and trimethylsilyl-derivatives of oxidation fragments were analyzed on a Finnigan 1020 gc/ms instrument with a capillary fused silica column, 50 m×0.22 mm ID×0.25 μm with film phase BP-5. Preparative glc was performed on a 2 m×10 mm ID column with 20 % phase SE-30. ^{1}H-nmr and ^{13}C-nmr spectra were measured on a Brucker AM 300 instrument ($CDCl_3$, ppm/TMS) at 300 or 75 MHz.

Cultivation of *Claviceps purpurea*, strain CP 7/5/35 in a 2-liter fermentor was as described previously (3). Centrifuged mycelium was used to prepare 1.4 g methyl esters (3) which were separated by preparative glc. After 10 injections, we obtained 2.3 mg of condensate with 97 % purity (capillary gc/ms), out of which 0.3 mg was oxidized according to Nicolaides and Fu (5). After oxidation and removal of ketone by distillation, the pH was adjusted to 11, and the mixture was evaporated to 1/10 of original volume. The residue was suspended in pyridine-acetonitrile (1:1) supplemented with a 5-fold amount of BSTFA, and the trimethylsilyl derivatives were analyzed after 30 min at 50° by gc/ms: 2-pentanone: ms *m/z* (rel. int.) 86 (M^+, 100), 71 (64), 58 (57); succinic acid bis-trimethylsilyl ester 262 (M^+, 11), 247 (14), 147 (75), 73 (100); 4-oxopentanoic acid trimethylsilyl ester: 188 (M^+, 21), 145 (34), 75 (100), 73 (86); 4,5-dimethyloctanoic acid methyl ester: ^{1}H nmr 3.631 (3H, s), 1.38-1.24 (br s), 0.871 (t, J=6.7 Hz, 3 H on C_8), 0.851 (d, J=6.7 Hz, 3 H on C_9), 0.845 (d, J=6.5 Hz, 3 H on C_{10}); ^{13}C nmr 175.31 (C_1), 51.37 ($COOCH_3$), 37.29 (C_2), 28.38 (C_3), 31.95 (C_4), 32.47 (C_5), 34.24 (C_6), 22.57 (C_7), 14.11 (C_8), 19.47 (C_9, CH_3 on C_4), 18.46 (C_{10}, CH_3 on C_5); ms *m/z* (rel. int. %) 186 (M^+, 1,4), 168 (M-H_2O, 0.7), 157 (M-C_2H_5, 6), 155 (M-MeO, 16), 143 (M-C_3H_7, 23), 115 (split between C_4-C_5), 111 (143-MeOH, 33), 87 (split between C_3-C_4, 66), 74 (McLafferty rearrangement, 100), 57 (C_4H_9, 35), 55 (C_4H_7, 77), 43 (C_3H_7, 60), 41 (C_3H_5, 63).

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