FATTY ACID METABOLISM IN SERRATIA MARCESCENS.

II. THE OCCURRENCE OF HYDROXY ACIDS.

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Scheuerbrandt et al. (1961) have recently reported that the biosynthesis of unsaturated fatty acids in certain micro-organisms involves hydroxy fatty acids of medium chain length. The occurrence of one such acid, 3-hydroxydecanoic acid, in compound form has been recorded in several species of micro-organisms (Asselineau and Lederer, 1960); a compound with L-serine (serratamic acid) in Serratia, with L-rhamnose in Pseudomonas and with a polypeptide (viscosine) in P. viscosa.

During a study of the fatty acids present in a typical strain of Serratia marcescens, 3-hydroxydecanoic, 3-hydroxydodecanoic and 3-hydroxy-5-dodecenoic acids have been identified.

EXPERIMENTAL

Cells of S. marcescens (NCTC No. 1377) were grown at 30°C for 48 hours on Bunting's medium in glass dishes as previously described (Bishop and Still, 1962). The cells were extracted four times for 60 minutes each with ethanol:ether, 3:1, at room temperature. The combined extracts were evaporated to dryness under reduced pressure and the residue taken up in a small amount

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of chloroform. The chloroform solution was evaporated to dryness and the residue saponified in 1N KOH in methanol by refluxing for 6 hours. After removal of nonsaponifiable material the acids were extracted into ether and methylated with boron trifluoride reagent (Metcalfe and Schmitz, 1961). The methyl esters were fractionated by gas-liquid chromatography (GLC) using columns (1m x 12.5mm) packed with 25% Apiezon L on 40-60 mesh acid-washed Celite 545. The column was operated at 220°C using helium as carrier gas and the methyl esters collected in a tube cooled to -50°C. Individual fractions were tentatively identified on the basis of their retention volumes relative to methyl myristate on columns (1m x 4mm) containing Apiezon L or polyethylene-glycol-adipate as stationary phase.

RESULTS AND DISCUSSION

The presence of an hydroxyl group in a fatty acid leads to a greatly increased retention volume relative to the unsubstituted acid. Using this criterion, the presence of three monohydroxy acids in the mixture was established. One of these was recognized by comparison with the retention volume of an authentic specimen of methyl 3-hydroxydecanoate (Delta Chemical Co.). Retention data showed that the remaining two acids each contained twelve carbon atoms. The presence of the hydroxyl group in each acid was confirmed by infrared spectroscopy, the characteristic band in the region of 2.9 μ being present in all three compounds.

Structure of hydroxydecanoic acid. The methyl ester of the hydroxydecanoic acid did not undergo hydrogenation in methanol in the presence of platinum oxide. When hydrogenation was attempted in ethanol it was found that transesterification occurred giving

rise to an ethyl ester. Methyl hydroxydecanoate slowly took up bromine, the reaction requiring 24 hours for completion. This reaction is characteristic of hydroxy acids. The position of the hydroxyl group was established by oxidation of the methyl ester with potassium permanganate (James and Webb, 1957). The degradation products were methylated with diazomethane and analyzed by GLC. The predominant product was octanoic acid with traces of heptanoic, hexanoic and pentanoic acids. Degradation of a sample of authentic methyl 3-hydroxydecanoate gave an identical result. Finally, the infra-red spectra of the isolated and authentic esters were identical. The structure of the acid is thus 3-hydroxydecanoic acid.

Structure of the hydroxydodecanoic acids. Catalytic hydrogenation of the methyl esters of the two twelve carbon acids showed that they differed only in the presence of one double bond, one acid being mono-unsaturated, the other saturated.

The mono-unsaturated ester was hydrogenated and the saturated product oxidized with potassium permanganate. Analysis by GLC of the breakdown products after methylation showed that the predominant product was decanoic acid with traces of shorter chain acids. By analogy with the oxidation products of 3-hydroxydecanoic acid the hydroxyl group is shown to be on carbon atom 3. The main product after oxidation of the mono-unsaturated ester was found to be heptanoic acid. This suggests that the double bond in the molecule is between carbon atoms 5 and 6. Degradation of 3-hydroxy-5-dodecenoic acid would give rise to heptanoic acid, propionic acid and acetic acid. As the latter two products would not be observed by GLC under the conditions used the only product expected would be heptanoic acid. The proposed structure of the unsaturated hydroxy acid is 3-hydroxy-5-dodecenoic acid.

Oxidation of the saturated hydroxydodecanoic acid gave rise to decanoic acid with traces of shorter chain acids. It is suggested therefore that this acid has the structure 3-hydroxydodecanoic acid.

Of the three hydroxy acids found in S. marcescens, two have not previously been reported to occur in micro-organisms. 3-hydroxydecanoic acid comprises 5.2%, 3-hydroxy-5-dodecenoic acid 1.5% and 3-hydroxydodecanoic acid 2.5% of the extracted fatty acids. This investigation has not yet been concerned with the form in which they occur in the cell. 3-hydroxydecanoic and 3-hydroxy-dodecanoic acids have been postulated by Scheuerbrandt et al. (1961) as intermediates in the biosynthesis of mono-unsaturated acids in micro-organisms and the closely related 5-dodecenoic acid has been shown to have a biotin sparing effect on lactobacilli (Hofmann et al., 1959). It is possible that 3-hydroxy-5-dodecenoic might serve if required, as a precursor of a di-unsaturated acid. However, an eighteen carbon acid derived from 3-hydroxy-5-dodecenoic acid would have its double bonds in the 9,11 position and some isomerization of the double bond would be necessary to produce linoleic acid. As yet this investigation has not revealed the presence of any di-unsaturated fatty acid in the lipids of S. marcescens.

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