Biosynthesis of Cyclopropane Compounds

VIII. THE CONVERSION OF OLEATE TO DIHYDROSTERULATE*

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SUMMARY

Oleic acid was converted to dihydrosterculic acid (9,10-methyleneoctadecanoic acid) by Lactobacillus arabinosus cells. This was established by the mass spectra of branched chain esters formed by reductive opening of the cyclopropane ring of the corresponding cyclopropane fatty esters. The mass spectra allowed unambiguous assignment of the ring location to position 9,10.

Oleic acid-9,10-d, was converted to a deuterated cyclopropane acid by L. arabinosus. This finding rules out intermediates in cyclopropane ring formation which have double bonds at carbon atom 9 or 10 after the extra carbon has been added, e.g., a 9,10-cyclopropene compound, 9- or 10-methylene compounds, or 9,10-olefins with a branched methyl group at position 9 or 10.

The details of the process of the conversion of olefinic fatty acids to cyclopropane fatty acids have been the subject of much speculation but remain relatively obscure. Enzymatic systems have been demonstrated in Serratia marcescens and Clostridium butyricum (1) and in Lactobacillus (2) which catalyze a reaction between S-adenosylmethionine and a phospholipid that contains olefinic fatty acids to produce a phospholipid with cyclopropane fatty acids.

Informative experiments with cultures of Lactobacillus were carried out by Hofmann (summarized in Reference 3) and by O'Leary (4). The latter worker has made several interesting proposals which deserve to be tested experimentally. He has suggested (4) that oleic acid, when administered to Lactobacillus cultures, is first isomerized to cis-vaccenic (cis-11-octadecenoic) acid and then converted to lactobacillic acid (11,12-methyleneoctadecanoic acid). This isomerization of an isolated double bond would represent an unprecedented enzymatic transformation, and, indeed, Hofmann has presented reasons for doubting that it occurs (3).

When cultures of Aerobacter aerogenes were fed methionine-methyl-14C, labeled fatty acid esters which were separated from cyclopropane fatty acid esters were detected by gas chromatography and were presumed to be unsaturated precursors of cyclopropane acids. These results have led O'Leary to propose the cyclopropene compound as an intermediate (2, 5). Chung and Law (6) have purified the cyclopropane synthetase enzyme from Clostridium butyricum at least 50-fold and with this preparation were unable to obtain evidence for cofactors or intermediates. However, the enzyme preparation was not of sufficient purity to make this conclusive.

This paper describes experiments which demonstrate that the cyclopropane product has a methylene bridge in the same position as the double bond of the administered fatty acid; i.e., dihydrosterculic acid (9,10-methyleneoctadecanoic acid) is produced from oleic acid. Further, it is shown that the vinyl protons of oleic acid are retained in the dihydrosterculic acid. This rules out unequivocally any intermediates in which hydrogen is lost from carbon atom 9 or 10, e.g., a cyclopropene compound, 9- or 10-methylene compound, or 9- or 10-methyl olefin.

EXPERIMENTAL PROCEDURE

Materials—Stearolic acid (9-octadecenoic acid) was prepared by the method of Khan, Deatherage, and Brown (7).1 Synthetic 9,10-methyleneoctadecanoic acid (dihydrosterculic acid) was prepared from oleic acid (Applied Science Laboratories) by the Simmons and Smith procedure (8). Synthetic 11,12-methyleneoctadecanoic acid (lactobacillic acid) was similarly prepared from cis-vaccenic acid (Hormel Institute). Natural and synthetic cyclopropane acids were reduced to a mixture of the corresponding open chain and branched chain compounds by

1 We are indebted to Mr. Kim Hooper for preparing a sample of this material.
treatment with Adams catalyst and hydrogen in glacial acetic acid (9).

Oleic acid-9,10-d² was prepared by the catalytic reduction of stearolic acid with deuterium gas in the presence of Lindlar's catalyst (10). The product from two separate preparations had about 83% of the dideuterated species (see Table I), as determined by the mass spectra of the methyl esters. The mass spectrum of the acetonide of the 9,10-dihydroxy compound prepared from the deuterated methyl oleate (11) confirmed that nearly all of the deuterium was located on carbon atoms 9 and 10.

Methods—Lactobacillus arabinosus 17-5, ATCC 8014, was grown in the biotin-free medium of Henderson and Snell (12), or that of Cheng et al. (13). Either biotin (10 µg per liter) or Tween 40 (1 g per liter) and appropriate unsaturated fatty acids (20 mg per liter) were added to permit growth of the organisms. Cells were grown for 3 days at 37° and were harvested by centrifugation. The fatty acids were isolated as described previously (14) and were converted to methyl esters by treatment with diazomethane. The C₉ cyclopropane fatty acid esters were isolated by gas chromatography (14).

Mass spectra were obtained with an Atlas-Werke CH-4 mass spectrometer, equipped with a gas chromatographic (1% SE-30) inlet system (15). The transfer system was maintained at 240°, the carrier gas separators at 180°, and the ion source at 250°; ionizing potential was 20 e.v. Spectra were recorded in 4 to 6 sec on the apex of the gas chromatographic peak.

RESULTS AND DISCUSSION

Position of Cyclopropane Ring—Location of the cyclopropane ring was determined from the mass spectra of the mixed branched chain esters obtained through catalytic hydrogenation of the cyclopropane fatty acid esters.

\[
\begin{align*}
\text{CH}_3(\text{CH})_7\text{CH}-\text{CH}(\text{CH}_2)_2\text{COOCH}_3 & \rightarrow \text{H}_2, \\
\text{CH}_3(\text{CH})_7\text{CH}-\text{CH}(\text{CH}_2)_2\text{COOCH}_3 & \rightarrow \text{H}_2,
\end{align*}
\]

Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Undeuterated</th>
<th>Monodeuterated</th>
<th>Dideuterated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl oleate (from reduction of stearolic acid with deuterium gas)</td>
<td>6</td>
<td>11</td>
<td>83</td>
</tr>
<tr>
<td>Methyl dihydrosterculic acid (from L. arabinosus grown with deuterated oleic acid)</td>
<td>9</td>
<td>13</td>
<td>78</td>
</tr>
</tbody>
</table>
The two branched chain esters were not resolved by gas chromatography, but the mixture (usually 80 to 90% of the total reduction products) was completely separated from the straight chain compound and any unreacted cyclopropane ester. The different locations of the methyl branches (and hence of the cyclopropane ring) are reflected in mass spectra intensity differences associated with preferential bond cleavage at points of branching. Thus, the spectrum of the mixture of methyl 11-methylstearate and methyl 12-methylstearate derived from methyl lactobacillate differs distinctly from that of the 9- and 10-methylstearates obtained by reduction of methyl dihydrosterculate, as shown by comparison of Fig. 1 with Fig. 2. In addition to comparison of intensities, location of the points of branching may also be determined from peaks (arrows, Figs. 1 and 2) representing rearranged ions (16) formed by cleavage at the branched carbon atom with abstraction of 1 and 2 hydrogen atoms from the remainder of the molecule. For example, the methyl 10-methylstearate component of the mixture derived from methyl dihydrosterculate would yield m/e 172 and 173 in Fig. 2.

\[
\frac{[\text{CH}_3(CH)(CH(CH)_3)COOCH_3]^{+}}{[\text{H} \text{ and } 2\text{H} + (\text{CH}_3)\text{COOCH})^{+}]
\]

Molecular ion 

\[m/e \ 172 \text{ and } 173\]

Reductive ring opening of the natural cyclopropane acid ester isolated from \textit{L. arabinosus} grown with biotin gave a mixture of compounds identical with that from the synthetic lactobacillate acid ester, while a sample of a C9 cyclopropane acid ester prepared by selective reduction of \textit{Sterculia foetida} fatty acid\(^1\) gave a mixture identical with that from the synthetic dihydrosterculate acid ester, as shown by comparison of their mass spectra. Examination of the mass spectrum of the branched chain esters from the cyclopropane acid isolated from \textit{L. arabinosus} grown on oleic acid (Fig. 3) indicates clearly the identity of the cyclopropane compound as dihydrosterculate (compare Figs. 2 and 3).

The mass spectra of the intact cyclopropane acid esters reveal no characteristic peaks which permit location of the ring. The spectra are in fact virtually identical with those of the corresponding odd chain unsaturated acid esters (17), isomers of which also exhibit very similar mass spectra (16). However, as we have seen, use of the present method with the branched chain compounds provides a simple and unequivocal means of ring location which can be carried out on a few hundred micrograms of sample.

The distribution of deuterium in the oleic acid 9,10-d\(_2\) and in the cyclopropane acid produced by \textit{L. arabinosus} grown on deuterated oleic acid is shown in Table I. These figures are accurate to within several per cent and show that the distributions are very similar. They leave no doubt that the vinyl protons of the olefinic acid are retained during the process of cyclopropane ring formation. Taken together with earlier results (18), the origins of all protons in the cyclopropane ring system are now established.

From methionine methyl

\[
\begin{align*}
\text{CH}_3 & \\
\text{CH}_3(\text{CH})(\text{CH})(\text{CH})(\text{CH})_3 & \rightarrow [\text{H and } 2\text{H} + (\text{CH}_3)\text{COOCH})^{+}]
\end{align*}
\]

From olefinic acid

O’Leary (2) has proposed the following compounds as possible intermediates in the formation of cyclopropane fatty acids.

The formation of any of these compounds would involve loss of protons at position 9 or 10 or both in the conversion of oleic acid to dihydrosterculate, and it is therefore ruled out. Although it is tempting to propose other mechanisms which do not involve discrete intermediates, we feel it more appropriate to await further information.

REFERENCES

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