BIOSYNTHESIS OF CYCLOPROPANE COMPOUNDS VII. SYNTHESIS OF CYCLOPROPANE AND CYCLOPROPENE FATTY ACIDS BY HIBISCUS SEEDLINGS*

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Several plants of the Malvaceae produce cyclopropane fatty acids and the related cyclopropene fatty acids (Smith, Wilson and Mikolajczak, 1961). It is well established that the cyclopropane fatty acids of bacteria are produced by the reaction of the methyl group of S-adenosylmethionine ("active methionine") and an olefinic fatty acid derivative (Liu and Hofmann, 1961; Zalkin, Goldfine and Law, 1963; Chung and Law, 1964). The origin of the methylene bridge of the cyclopropene fatty acids has not been established, although one would predict that it would likewise arise from methionine.

Recently, Smith and Bu'Lock (1964) have established that acetate-1-^{14}C is incorporated into the chains of the cyclopropene fatty acids, but not into the methylene bridges. We wish to report our observations on the incorporation of labeled carbon from methionine-methyl-^{14}C into cyclopropane and cyclopropene fatty acids by seedlings of Hibiscus syriacus.

Seed oils of H. syriacus contain about 3% of 9,10-methylene octadec-9-enoic acid (sterculic acid) and 17% of 8,9-methylene heptadec-8-enoic acid (malvalic acid), along with traces of the corresponding saturated fatty acids (Smith, Wilson and Mikolajczak, 1961). Young seedlings were incubated with solutions of methionine-methyl-^{14}C

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for 1-3 days. The seedlings were ground and extracted with chloroform-methanol, and the fatty acids were isolated from the crude lipid extracts after saponification in the cold. The incorporation of labeled methionine into crude fatty acids varied from 0.2-2%.

The crude ester fraction was subjected to ozonolysis. The cyclopropene esters were converted to β-diketo esters, unsaturated esters were cleaved to aldehydes and aldehydo esters, and saturated esters were unchanged. Silicic acid chromatography separated the diketo ester fraction from the other products. The distribution of radioactive compounds obtained by ozonolysis of the crude fatty acid esters is shown in Table I.

Table I

Silicic Acid Chromatography of Ozonolysis Products

Fraction number (250 ml. each)	Eluate: % ether in pentane	$FeCl_3$ test*	Total Radioactivity (cpm)
1-4	0	-	0
5	0-5	-	150,000
6	5	+++	23,400
7	5	++	17,400
8	5	+	7,600
9	10	-	5,400

* $FeCl_3$ gives a red color with β-diketones

The mixed diketo esters (fraction 6) were diluted with carrier methyl 9,11-diketononadecanoate prepared from sterculic acid (Nunn, 1952), and the mixture was treated with iodine and base to convert the methylene carbon to iodoform:

$$CH_3(CH_2)_7\overset{O}{\overset{\|}{C}}CH_2\overset{O}{\overset{\|}{C}}(CH_2)_7COOCH_3 \xrightarrow[NaOH]{I_2} CH_3(CH_2)_7COONa +$$

$$NaOOC(CH_2)_7COONa + \underline{CHI_3}$$

The specific activity of the mixed diketoester fraction was 37 cpm/mg (12×10^3 cpm/mmole) (based upon the molecular weight of the carrier ester), and that of the resublimed iodoform was 23 cpm/mg (9×10^3 cpm/mmole). This indicates that most of the radiocarbon of the diketo acid esters, and hence the cyclopropene fatty acid esters, was in the methylene bridge.

The fatty acid esters of fraction 5 represent largely unchanged fatty acid esters and aldehydes. An aliquot of fraction 5 was mixed with carrier 9,11-diketononadecanoate and the mixture was treated with I_2 and base. The iodoform was unlabeled, which indicates that no labeled diketo esters were present in this fraction. An aliquot of the esters was subjected to gas-liquid chromatography and the esters were collected and counted. Table II shows the distribution of radioactive compounds. The predominant labeled material is co-chromato-

Table II

Gas-Liquid Chromatography of Fraction 5

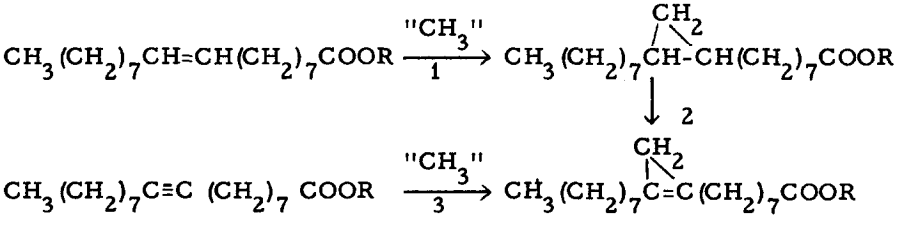
Retention time relative to stearate	Possible identity	Mass %*	% of recovered cpm**
solvent	short chain compounds	2	32
575	palmitate	47	4
1.0	stearate	32	12
1.5	C_{19} cyclopropane	20	45

* by triangulation of peak areas
** collection efficiency: 65%

graphic with the C_{19} cyclopropane fatty acid ester, methyl dihydrosterculate, and represents about 40% of the labeled fatty acids produced by the seedlings. Some short chain compounds were also detected, and these may represent aldehydes formed from unsaturated cyclopropane compounds, where the double bond is outside the ring, or

from other unsaturated branched chain compounds, but their nature remains to be established.

The presence of a large amount of labeled cyclopropane fatty acids and only small amounts of cyclopropene fatty acids when methionine serves as the labeled precursor may indicate that the pathway involves first formation of the cyclopropane fatty acid by a cyclopropane synthetase enzyme system similar to that found in bacteria (Zalkin, Goldfine and Law, 1963; Chung and Law, 1964), followed by introduction of the double bond, either directly into the ring or indirectly by isomerization of a double bond introduced into the chain outside the ring (reactions 1 and 2). Smith and Bu'Lock have proposed an alternate route in which an acetylenic acid would serve as the acceptor of the methylene bridge (reaction 3).



Experiments now in progress are designed to resolve this question.

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