THE CONVERSION OF CIS-2-OCTENOIC ACID TO LINOLEIC ACID[1]

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Scheuerbrandt et al. (1961) have reported the anaerobic biosynthesis of oleic acid from octanoate by Clostridium butyricum. At the same time, it was reported from this laboratory that 1-C^{14}-cis-2-octenoic acid may be converted into linoleic acid by the laying hen (Reiser et al. 1961, 1962). The synthetic steps suggested by Scheuerbrandt et al. (1961) may explain both syntheses, as diagrammed below in the discussion.

In the present study, unlabeled cis-2-octenoic acid was fed and the hen injected with 1-C^{14} acetate. More direct and rigorous techniques than in the earlier work were used for the location of the labeled carbon atoms in the resultant linoleic acid.

EXPERIMENTAL

Cis-2-octenoic acid was prepared by the method of Knight and Diamond (1959) and converted to its methyl ester by diazomethane. The composition of this preparation was found by gas chromatography to be 52.4% octanoate, 44.7% cis-2-octenoate and 2.9% octynoate.

A white leghorn hen was placed on a low fat diet (Table 1) for three weeks, at which time the linoleic acid of the egg has been shown to be reduced almost to minimum levels (Reiser, 1950 and 1951).

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Table 1

Composition of basal low fat diet

Ingredient	Amount in g/kg diet
Cerelose	542.61
Drackett[1]	225.00
Woodpulp	30.00
Mineral Mix[2]	35.08
Vitamin Mix[3]	14.47
$CaHPO_4$	52.66
Choline	2.00

[1] Drackett Assay Protein C-1 obtained from Archer-Daniels-Midland and Company, Cincinnati, Ohio.

[2] Oyster shell, 15.00 g; sodium chloride, 5.00 g; magnesium sulfate, 5.27 g; manganese sulfate, 0.15 g; ferrous sulfate, 0.011 g; cupric sulfate, 0.011 g; zinc chloride, 0.106 g; potassium iodate, 0.0129 g; cobalt chloride 0.0016 g; potassium chloride, 7.63 g; and sodium molybdate, 0.0076 g.

[3] Thiamin HCl, 10 mg; calcium pentathenate, 20 mg; pyridoxine, 6 mg; para amino benzoic acid, 20 mg; inositol, 500 mg; biotin, 0.2 mg; vitamin A. (10,000 IU/g), 10 g; vitamin D_3 (200,000 IU/g), 10 mg; niacin, 75 mg; folic acid, 4 mg; aureomycin HCl, 10 mg; BHT (anti-oxidant), 133 mg; menadione, 5 mg; riboflavin, 10 mg; myomix, 167 g; methionine, 7.50 g; glycine, 3.5 g; and vitamin B_{12}, 20 mg of 0.1%.

After three weeks on the basal diet 5% of cis-2-octenoate was added and the hen administered, intraperitoneally, 0.2 mc of 1-C^{14} acetate daily at 1:00 PM. On the third day 5% of a triglyceride made up of a 1:1 mixture of lauric and myristic acids was added to the diet on the possibility that these acids would spare the octenoic acid for conversion.

On the fourth day the hen quit laying and was, therefore, sacrificed on the eighth day.

Isolation of Linoleic Acid

Eggs were laid on the second and third days only. The fat was extracted from the yolks of these eggs and from the developing eggs in the ovary (Reiser et al. 1962) as well as from the liver and adipose tissue. The

specific activity of the mixed lipids or the first egg was not significantly above background. That of the second egg was 749 dpm/mg, of the ovary 3785 dpm/mg, of the liver 2258 dpm/mg and of the body fat 410 dpm/mg.

The ovary triglycerides were separated from the phospholipid on silicic acid and saponified. The tetrabromide was prepared from a part of these acids as previously described (mp 114-115°C) and found to have a specific activity of 1045 dpm/mg.

The remainder of the mixed acids of the ovary triglycerides were converted to methyl esters by diazomethane and eight 250 mg batches separated with a preparative gas chromatograph apparatus[4] (Reiser et al. 1962).

68.5 mg of methyl linoleate, containing 4.3% methyl oleate and 1.2% methyl palmitate, were obtained. The mixed acids had a specific activity of 3693 dpm/mg, those through oleic acid 3818 dpm/mg, and the linoleate fraction 1293 dpm/mg.

Degradation of Linoleic Acid

67.0 mg of the linoleate fraction was oxidized with potassium permanganate and acetic acid under conditions to yield caproic acid, azelaic acid and three moles of carbon dioxide:

The linoleate was transferred to a small round bottom flask containing a magnetic stirrer and 2 ml of glacial acetic acid. The flask was fitted with an exit tube leading to a test tube containing 10 ml of 10%, carbon dioxide free, sodium hydroxide solution, with phenolphtalein, and inlet tubes for nitrogen and for the addition of solid $KMnO_4$.

The system was filled with nitrogen and the temperature held at 40-45°C. 250 mg of powdered $KMnO_4$ were added over a period of half an hour with slow stirring. During the reaction a slow stream of nitrogen flushed the CO_2 formed into the alkaline solution.

After five hours a 10% solution of $BaCl_2$ was added to the alkaline solution which was then neutralized to phenolphtalein with dilute HCl. The

[4] Megachrom, Beckman Instrument Company, Fullerton, California.

$BaCO_3$ formed was filtered and washed thoroughly with water to remove all barium soaps of any possible contaminating volatile fatty acids. A blank experiment yielded no $BaCO_3$.

131.7 mg of $BaCO_3$ were obtained from the 67 mg of methyl linoleate, which is a yield of 97.8%.

The reaction mixture was diluted with water, made alkaline with sodium hydroxide, the manganese salts reduced with sodium bisulfate, and the soaps finally decomposed with 2N H_2SO_4. The volatile acids were steam distilled with the aid of a slow stream of nitrogen. The volatile fatty acids were collected in 10 ml of 10% sodium hydroxide solution. The water was evaporated from the sodium hydroxide solution and the dried salt mixture slurried with ether. This was acidified (phenolphtalein) with a few mg of powdered metabisulfite and a few drops of water. The acidified slurry was triturated several times with ether. The combined ether extracts were evaporated to a small volume at room temperature in a slow stream of nitrogen. The fatty acids in the ether solution were converted into methyl esters with diazomethane. Analysis by gas chromatography (10% high vacuum grease on Chromosorb W at 130°C) demonstrated it to be almost 100% caproic acid.

The non-volatile acids in the residue were extracted with ether, converted to methyl esters and analyzed by gas chromatography. It was found to consist of 90% azelaic, 9% suberic and 1% adipic acids.

One mg of this mixture was fractionated on a 10% silicone grease, 60-80 mesh Chromosorb W column at 170°C and a flow rate of 40 ml of argon per minute.

The methyl azelaate and methyl suberate were collected independently.

The specific activity of 7.7 mg of the $BaCO_3$ was determined by scintillation spectrometry and found to be inactive. The methyl caproate was also inactive. The dimethyl azelaate had an activity of 1731 dpm/mg and the dimethyl suberate 1539 dpm/mg.

It is concluded that the inactive cis-2-octenoic acid was converted into linoleic acid by the addition of the labeled acetate and subsequent

dehydrogenation. Scheuerbrandt et al. (1961) have recently described the anaerobic synthesis of oleic acid from octanoic by Clostridium butyricum. The synthesis of linoleic acid from cis-2-octenoic acid my be explained by the same mechanism, as follows:

Oleic acid synthesis (Scheuerbrandt et al. 1961)	Linoleic acid synthesis
$CH_3(CH_2)_4CH_2CH_2COOH$	$CH_3(CH_2)_4CH=CHCOOH$
$\downarrow AcO^-$	$\downarrow AcO^-$
$CH_3(CH_2)_4CH_2CH_2CH_2CH_2COOH$	$CH_3(CH_2)_4CH=CHCH_2CH_2COOH$
$\downarrow AcO^-$	$\downarrow AcO^-$
$CH_3(CH_2)_4CH_2CH_2CH_2CH_2CHOHCH_2COOH$	$CH_3(CH_2)_4CH=CHCH_2CH_2CHOHCH_2COOH$
$\downarrow -H_2O$	$\downarrow -H_2O$
$CH_3(CH_2)_4CH_2CH_2CH_2CH=CHCH_2COOH$	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2COOH$
$\downarrow 3\ AcO^-$	$\downarrow 3\ AcO^-$
Oleic acid	Linoleic acid

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