

Short Communications

A Method for the Separation of Saturated and Monounsaturated Fatty Acids through Hydroxylation

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In connection with metabolic studies¹ with oleic acid-1-¹⁴C a simple method for the separation of saturated acids from oleic acid was needed to effect a sharper separation than the conventional lead salt fractionation. This separation has earlier also been made by reversed phase partition chromatography^{2,3} followed by hydrogenation of the band containing palmitic and oleic acid and renewed chromatography. The oleic acid originally present is then obtained as stearic acid which is easily separated from the palmitic acid.

We have instead investigated a method involving hydroxylation of the original mixture with subsequent separation of the products formed.

Of the different hydroxylation methods tried (peracetic acid, permanganate, osmium tetroxide) we have found a procedure with performic acid in ethyl formate as described in the experimental part most suitable.

The products formed were transformed into methyl esters with diazomethane and then separated by ordinary chromatography on silicic acid. The esters of the saturated fatty acids were eluted first with pure methylene chloride (A in Table 1) after which the methyl dihydroxystearate was eluted by adding 0.75 % methanol (B) to the eluent. As evidenced in the table the hydroxylation followed by chromatography effected a separation so that a maximum of 2 % contamination is obtained when labelled palmitic or oleic acid is used. The same separation can be effected even more sharply by partition chromatography but we have found this simple method useful for routine work.

A limitation of this hydroxylation reaction is that a highly unsaturated fatty acid such as linoleic acid is not quantitatively transformed into tetrahydroxystearic acid but a number of less polar products is also

Table 1.

Expt. No.	A			B		
	mg	c/m/ mg	c/m total	mg	c/m/ mg	c/m total
Methyl palmitate (21.1 mg) + methyl 1.	18.9	5	94	24.6	280	6 890
oleate-1- ¹⁴ C (21.1 mg) 2.	19.4	0	0	23.7	305	7 250
Methyl palmitate-1- ¹⁴ C (21.1 mg) 3.	19.0	346	6 560	1.7	52	88
Methyl oleate-1- ¹⁴ C 4.	1.9	59	112	23.4	320	7 490
5.	2.0	54	108	24.6	335	8 240

21.1 mg methyl oleate corresponds to 23.4 mg methyl dihydroxystearate.

found. The products do not contaminate the saturated acids but are distributed in both the dihydroxy- and tetrahydroxy band (*cf.*⁴).

In the meantime Savary and Desnuelle⁵ have published a method in which they hydroxylate with permanganate and separate the products by partition chromatography.

Experimental. All the reactions are carried out in a 15 ml round bottom flask with a 15 cm long neck (i.d. 7 mm) so that all reactions and evaporations *in vacuo* can be performed in the same vessel without losing material through spattering.

Hydroxylation of mixture of oleic and palmitic acid. 20 mg of palmitic acid and 20 mg of oleic acid-1-¹⁴C in acetone solution were pipetted into the flask and evaporated to dryness. Ethyl formate (1.5 ml) and 99 % formic acid (1 ml) were added, followed by 0.12 ml of 30 % hydrogen peroxide. The solution was then left for 3 hours in a water bath at 50° and then evaporated to dryness *in vacuo*. The evaporation was repeated twice after addition of 2 ml of toluene. The residue was dissolved in a few drops of methanol and an excess of diazomethane in ether was added and the solvents again evaporated *in vacuo*.

Chromatographic separation of methyl palmitate and dihydroxystearate. Silicic acid (Baker, London) was activated by heating to 120° for 24 hours. 2 g silicic acid and 1 g of Hyflo-Supercel were mixed, stirred in methylene chloride and poured into a chromatographic tube (i. d. 20 mm) on a bed of sand. The mixture of fatty acid esters was dissolved in a few ml of methylene chloride and transferred onto the column.

The unsubstituted esters were eluted with a total of 100 ml of methylene chloride (A). The dihydroxy esters were next eluted with 100 ml of methylene chloride containing 0.75 % (v/v) methanol (B). Tetrahydroxy esters are not eluted with this solvent but require more methanol (2–3 %). The results of a typical set of reactions are shown in Table 1.

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The Decarboxylation of Higher Fatty Acids for Tracer Work

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In connection with work on the intestinal absorption of fat in humans with carbonyl-labelled acids the necessity arose of having at hand a simple method for the decarboxylation of labelled higher fatty acids for the assay of ¹³C in the carboxyl group.

A number of well established methods for the decarboxylation of higher straight-chain fatty acids¹⁻³ are described in the literature but they usually require elaborate apparatus, and we needed a method enabling us to run many decarboxylations simultaneously.

The Schmidt reaction⁴⁻⁶ has been used both for the decarboxylation of short chain labelled fatty acids^{3,7} as well as higher labelled fatty acids^{8,9}.

The present paper describes a simplified procedure for decarboxylation of higher labelled fatty acids by use of the Schmidt reaction. A simple apparatus is described in which the reaction is carried out. The samples are dissolved in benzene and introduced into the reaction vessel which contains sodium azide. Sulphuric acid is added and the carbon dioxide formed is collected in barium hydroxide.

The method has been tested using [1-¹⁴C] palmitic acid and [1-¹³C] oleic acid¹⁰ and was found to give results in good agreement with the theoretical values.

Apparatus and Procedure. The apparatus used for the decarboxylation is shown diagrammatically in Fig. 1. At B is a three way stopcock. Materials: Sodium azide, Merck, p. a. stored in a desiccator; H₂SO₄, Merck, p. a., 97 %; Benzene, dried over KOH, distilled; 0.2 N Ba(OH)₂ stored in waxed vessels.