

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

In the meantime Savary and Desnuelle<sup>5</sup> 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-<sup>14</sup>C 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.

This work is part of investigations supported by "Statens Medicinska Forskningsråd" and "Knut och Alice Wallenbergs Stiftelse".

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Received September 2, 1954.

## 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 <sup>13</sup>C in the carboxyl group.

A number of well established methods for the decarboxylation of higher straight-chain fatty acids<sup>1-3</sup> 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 reaction<sup>4-6</sup> has been used both for the decarboxylation of short chain labelled fatty acids<sup>3,7</sup> as well as higher labelled fatty acids<sup>8,9</sup>.

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-<sup>14</sup>C] palmitic acid and [1-<sup>13</sup>C] oleic acid<sup>10</sup> 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; H<sub>2</sub>SO<sub>4</sub>, Merck, p. a., 97 %; Benzene, dried over KOH, distilled; 0.2 N Ba(OH)<sub>2</sub> stored in waxed vessels.

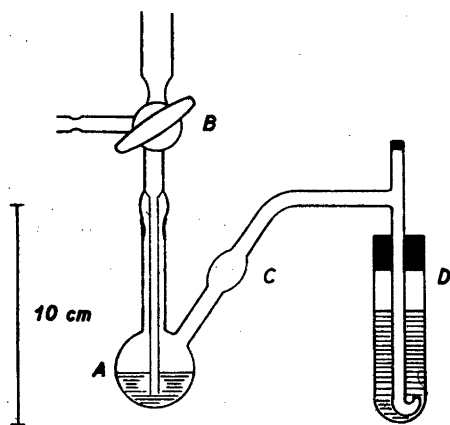


Fig. 1. Decarboxylation apparatus. For description see text.

75 mg sodium azide is introduced into the reaction vessel. The sample to be decarboxylated and containing 0.2 mmole of acid is dissolved in dry benzene and transferred to the reaction vessel with a total volume of 3 ml benzene. With a yield of about 70 % this amount gives 27 mg of barium carbonate. A continuous stream of  $\text{CO}_2$ -free nitrogen is then passed through the apparatus. Conc. sulphuric acid is used as a lubricant at both the joints. 0.5 ml of conc. sulphuric acid is let into the reaction vessel through B and the apparatus is then placed in a bath at  $45^\circ\text{C}$  while it is swept with nitrogen. The effluent gas is bubbled through a trap D with 0.20 N barium hydroxide. Wet cotton wool is used to cool the apparatus at C thus preventing benzene and  $\text{SO}_2$  from the combustion fluid from reaching D. Total wet combustions of samples of fatty acids were carried out according to Borgström<sup>11</sup>. The radioactivity of  $^{14}\text{C}$  samples was measured by a thin mica window G—M tube after plating  $\text{BaCO}_3$  on aluminium planchets in infinite thickness.

**Accuracy of the method.** The method has hitherto been used for decarboxylation of fatty acids. The yields vary from 60 to 90 % of the theoretical.

In order to test the method a comparison of values found by combustion of  $[1-^{14}\text{C}]$  palmitic acid with values found by decarboxylation of the same fatty acid was made. The labelled palmitic acid was diluted with inactive palmitic acid so that the same value after decarboxylation as after combustion of the undiluted fatty acid should be obtained.

Values obtained from wet combustion of $[1-^{14}\text{C}]$ palmitic acid	Values obtained from decarboxylation of diluted $[1-^{14}\text{C}]$ palmitic acid
Specific activity	Specific activity
1 789	1 807
1 828	1 794
1 809	1 826

This work is part of an investigation supported by The Medical Faculty of the University of Lund.

I am greatly indebted to Professor S. Borgström for the labelled fatty acids placed at my disposal.

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Received September 2, 1954.