266. *A High-yield Synthesis of Fatty Acids Labelled at* $C_{(9)}$.

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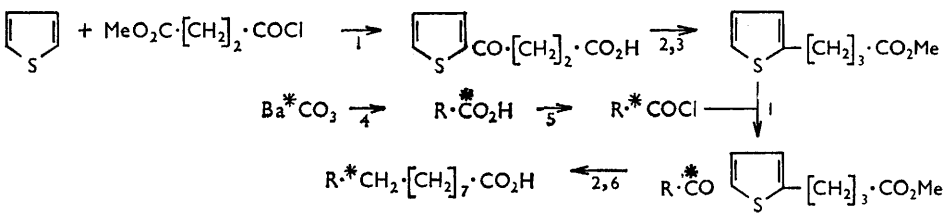
A convenient route to [9-^{14}C]-stearic, -palmitic, and -decanoic acid has been developed, based on the acylation of methyl γ-2-thienylbutyrate.

Although ^{14}C-labelled fatty acids have been much used in studies of lipid metabolism nearly all the work relates to the use of acids labelled in the carboxyl group or at $C_{(2)}$, which are easily prepared from readily available starting materials and indeed are commercially available. Because of the metabolic lability of this labelling in many circumstances, acids with labelling more remote from the carboxyl group have a special attraction, and syntheses of certain acids of this type have been described.[1] One especially useful class is that of fatty acids labelled at $C_{(9)}$, since in the majority of natural fatty acids this carbon atom is at a point of unsaturation and can readily be isolated in oxidative degradations.

After preliminary studies [2] in which [9,10-$^{14}C_2$]-stearolic and -stearic acid were prepared from [$^{14}C_2$]acetylene in 12% radiochemical yield, essentially by Hüber's route,[3] a more convenient route has been developed which now makes possible the synthesis of straight-chain [9-^{14}C]-fatty acids in 85% radiochemical yield from barium carbonate. The method is based upon the use of thiophen derivatives, a variation of a general route described by Badger *et al.*; [4] it is summarised in the chart; the starting materials are methyl γ-2-thienylbutyrate, which can be prepared on a normal scale, and the readily available [1-^{14}C]acid

[1] Dauben, *J. Amer. Chem. Soc.*, 1948, **70**, 1376.
[2] Ridyard, Ph.D. Thesis, Manchester, 1958.
[3] Hüber, *J. Amer. Chem. Soc.*, 1951, **73**, 2730.
[4] Badger, Rodda, and Sasse, *J.*, 1954, 4162.

chlorides. We have applied this synthesis on a 5-millimolar scale to preparations of [9-^{14}C]-stearic, -palmitic, and -decanoic acid, and as a typical procedure the preparation of [9-^{14}C]stearic acid is described in the Experimental part. We have little doubt that the



Reagents: 1, $SnCl_4$. 2, N_2H_4–NaOH. 3, CH_2N_2. 4, RMgBr. 5, $(COCl)_2$. 6, Ni.

method could be readily adapted for work on a still smaller scale with higher specific activities, and obvious modifications can be used for syntheses of other acids.

Experimental

2-β-*Carboxypropionylthiophen.*—The requisite 3-methoxycarbonylpropionyl chloride, b. p. 35—37°/0·1 mm., 100—105°/20 mm., was readily prepared from monomethyl succinate [5] and thionyl chloride; the succinoylation was effected by a modification of the method of Papa *et al.*[6] Anhydrous stannic chloride (45 ml.) was added slowly in 1 hr. at 0° to a stirred solution of purified thiophen (25 ml.) and 3-methoxycarbonylpropionyl chloride (40 g.) in dry benzene (300 ml.). After being stirred at room temperature for a further 1 hr. the mixture was poured on ice (300 g.) and concentrated hydrochloric acid (250 ml.); benzene and thiophen were then removed in a current of steam during 1 hr. in which ester hydrolysis was complete. When the clear solution was cooled to 0°, 2-β-carboxypropionylthiophen, m. p. 118—120°, crystallised in 96% yield.

Methyl γ-2-Thienylbutyrate.—The above keto-acid (32 g.) was reduced with hydrazine in alkaline diethylene glycol,[7] to give γ-2-thienylbutyric acid (67%), b. p. 106—112°/0·2 mm., converted by diazomethane into the required ester (93%), b. p. 100—102°/3·5 mm.

[1-^{14}C]*Acyl Halides.*—[1-^{14}C]-Octanoyl chloride and -decanoyl chloride were prepared in 90% radiochemical yield by carboxylation of appropriate Grignard reagents [8] and reaction of the labelled acids with oxalyl chloride.

Acylation of Methyl γ-2-Thienylbutyrate.—The second acylation step was carried out essentially as the first, with appropriate modifications for the reduced scale. Anhydrous stannic chloride (3·5 ml. in 10 ml. of dry benzene) was slowly added at −5° to the thienyl ester (3·7 g.) and [1-^{14}C]decanoyl chloride (3·8 g.) in dry benzene (50 ml.); the mixture was stirred for 1 hr. at −5° and a further 2·5 hr. at 25°. The mixture, and washings from the flask, was poured on ice (40 g.) and concentrated hydrochloric acid (20 ml.); after steam-distillation to remove unchanged decanoic acid (0·04 g.), the aqueous phase was extracted with benzene (3 × 5 ml.), and the combined extracts were dried and evaporated, to give crude methyl γ-(5-decanoyl-2-thienyl)butyrate (98%); recrystallisation of unlabelled material gave a product with m. p. 44—45°; saponification afforded the parent keto-acid, m. p. 61—65° (equiv., 320. Theor., 324).

Reduction of the Keto-ester.—The above (crude) keto-ester (1·53 g.) with potassium hydroxide (0·86 g.) and 60% aqueous hydrazine hydrate (1·6 ml.) in diethylene glycol (25 ml.) was heated under reflux (*ca.* 160°) for 30 min.; the excess of hydrazine and water were then distilled off, and when the temperature of the mixture reached *ca.* 200° heating under reflux was resumed for a further 4 hr. The mixture was then cooled, diluted with water (50 ml.), acidified with concentrated hydrochloric acid, and extracted with ether (3 × 30 ml.); the combined extracts were dried and evaporated, giving crude γ-(5-decyl-2-thienyl)butyric acid (98·5%). Unlabelled material, recrystallised from light petroleum at −25°, had m. p. 31—32°.

[5] Cason, *J. Amer. Chem. Soc.*, 1942, **64**, 1106.
[6] Papa, Schwenk, and Hankin, *J. Amer. Chem. Soc.*, 1947, **69**, 3018.
[7] Huang-Minlon, *J. Amer. Chem. Soc.*, 1946, **68**, 2487.
[8] Dauben, Reid, and Yankwich, *Analyt. Chem.*, 1947, **19**, 828.

Desulphurisation; [9-^{14}C]*Stearic Acid.*—For desulphurisation the method of Badger *et al.*[4] was adapted; freshly prepared Raney nickel (from 20 g. of alloy) was added in water (100 ml.) to the crude γ-(5-decyl-2-thienyl)butyric acid (1·31 g.) in saturated aqueous potassium carbonate (30 ml.), and the mixture stirred for 2 hr. at 80—90°. The catalyst was filtered from the warm solution and washed with further potassium carbonate solution (60 ml.). The combined alkaline solutions were acidified and continuously extracted with light petroleum (b. p. 30—40°); the catalyst was dissolved in hydrochloric acid, the product added, without filtration, to the aqueous residues, and extraction continued. The petroleum extract was then evaporated and the product chromatographed in light petroleum (b. p. 30—40°) on a column of silica gel previously washed with dry ether and light petroleum. Elution with gradually increasing proportions of ether in light petroleum (b. p. 30—40°) gave (with 3% of ether) pure [9-^{14}C]-stearic acid, colourless, m. p. 69·4—70·5°. The yield based on [1-^{14}C]decanoic acid was 90%.

Essentially similar procedures were used for preparations of [9-^{14}C]-palmitic and -decanoic acid, save that in the last case the desulphurisation catalyst was simply washed with hot ethanol. When 4 mc of barium carbonate were used for the C_{16} and C_{18} acids the products had activities of *ca.* 2 mc/g.

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