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AN IMPROVED SYNTHESIS OF METHYL-LABELED FATTY ACIDS

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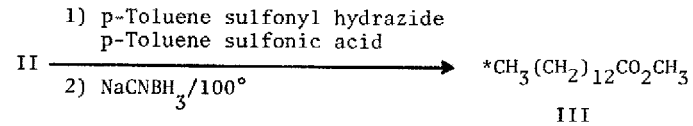
For ongoing studies of lipid-apoprotein interactions in this laboratory, a homologous series of fatty acids labeled at the methyl terminus were requried as precursors for the corresponding labeled phospholipids. Our synthetic scheme for these methyl labeled fatty acids utilized the corresponding methyl keto esters which, upon reduction and hydrolysis, would give the desired acid. In one report (1) of the synthesis of methyl keto esters, dimethyl cadmium was coupled to the half acyl halide of a dicarboxylic acid mono ester to give the desired keto ester. Using this procedure, we obtained low yields of methyl keto ester and many side products. The obvious disadvantages of this procedure in isotopic labeling are: 1) the requirement for excess labeled methyl iodide, 2) loss of the volatile as well as hazardous dimethyl cadmium during the removal of ether which is replaced with benzene, and 3) low yields of methyl keto ester. We, therefore, investigated the use of methyl cadmium chloride and found that it gives high yields of methyl keto esters as illustrated in the scheme below for the synthesis of tetradecanoic-14-^{13}C acid (* indicates ^{13}C label).

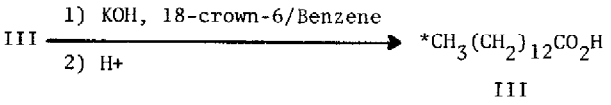
$$*CH_3I + Mg \xrightarrow{\text{ether}} *CH_3MgI \xrightarrow{CdCl_2} *CH_3CdCl$$

I

$$I + Cl\text{-}CO(CH_2)_{11}CO_2CH_3 \xrightarrow{\text{Benzene}} *CH_3\text{-}CO(CH_2)_{11}\text{-}CO_2CH_3$$

II





The labeled methyl cadmium chloride (I) is formed from the Grigard reagent in diethyl ether by the addition of an equimolar amount of anhydrous cadmium chloride. After evaporating the ether and replacing it with dry benzene, an equimolar quantity of the half acyl chloride of a dicarboxylic acid mono methyl ester was added, and upon workup the desired methyl ketoester (II) was obtained in excellent yield. Reduction of the ketoester (II) by p-toluene-sulfonyl hydrazide and sodium cyanoborohydride (2) in DMF/sulfolane (1:1) gave the fatty acid ester (III), which was hydrolyzed in benzene to the potassium salt of the fatty acid using powdered KOH and 18-crown-6 as a catalyst (3).

This modified procedure has also been applied successfully to the synthesis of other ketoesters in excellent yield (4). The detailed procedure given below for a ^{13}C derivative is typical of that used for similar terminal ^{2}H, ^{3}H, and ^{14}C methyl labeling.

Experimental

Methyl 13-Oxo-tetradecanoate-14-^{13}C (II). One gram (7 mmole) of methyl iodide (90% ^{13}C, Koch Isotopes) in 15 ml of a Na-dried diethyl ether was added to 0.17 g (.007 g-atom) of magnesium turnings in 25 ml of dry diethyl ether under a dry N_2 atmosphere. The reaction started immediately and after 30 minutes the Mg was consumed. The methyl magnesium iodide was cooled in an ice bath and 1.29 g (7 mmole) of anhydrous powdered cadmium chloride was added; the ice bath was removed and after one hour, the diethyl ether was completely removed by distillation; 40 ml dry benzene was added and 20 ml of benzene distilled to ensure complete removal of the diethyl ether. The reaction mixture was cooled with an ice bath and 2.05 g (7 mmole) methyl 12-(chloroformyl)-dodecanoate (5) in 10 ml of dry benzene was added over a two minute period and the reaction mixture was then stirred 6 hours at room temperature; after which 2% sulfuric acid was added until two distinct phases formed. The benzene layer

was collected and dried over anhydrous magnesium sulfate.

The benzene was removed under reduced pressure and the product purified by column chromatography on silica gel; the methyl 13-oxo-tetradecanoate was eluted by 8:2 hexane-ethyl acetate, yield 1.68 g (85%); m.p. 22°-24°C; ir bands at 1735 cm^{-1} (ester); 1715 cm^{-1} (keto); ^{13}C NMR for $*\underline{C}H_3$, 29.18 ppm(s); $CH_3-\underset{\underset{O}{\|}}{\underline{C}}-$, 208-207 ppm(q); $\underline{C}O_2CH_3$, 173 ppm(s), from internal TMS.

Methyl Tetradecanoate-14-^{13}C (III). Methyl 13-oxo-tetradecanoate (II) (1.6 g, 7.5 mmole) was reacted with 1.4 g (7.5 mmole) of p-toluenesulfonylhydrazide in 20 ml of 1:1 DMF/ sulfolane containing 50 mg of p-toluenesulfonic acid. After one hour 1.5g (24 mmole) of sodium cyanoborohydride (Aldrich) was added and the reaction mixture maintained at 100°-110°C for 5 hours. The reaction was diluted with 20 ml cold water, extracted with hexane, 4 X 25 ml; and the organic extract dried over anhydrous magnesium sulfate. The hexane was evaporated under reduced pressure and the crude ester purified by column chromatography on silica gel. The ester was eluted with 9:1 hexane:ethyl acetate yielding 1.3 g (86%) of the methyl tetradecanoate (6) as an oil which crystallized when stored at 4°, lit. (6) m.p. 19°. The ir spectrum showed the disappearance of the band at 1715 cm^{-1} (keto) and displacement of the ester band to 1740 cm^{-1}.

Tetradecanoic-14-^{13}C Acid (IV). Methyl tetradecanoate (1.3 g, 5 mmole), powdered potassium hydroxide (1.12 g, 20 mmole) and 18-crown-6 (0.4 g, 1.5 mmole) were mixed in 20 ml dry benzene and stirred for 12 hours at room temperature after which the reaction mixture was acidified with 6N hydrochloric acid and extracted with 3 X 20 ml of benzene which was then dried over anhydrous sodium sulfate. Rotary evaporation of the solvent gave 1.0 g (86% yield) of tetradecanoic acid m.p. 52°-54°C, lit. (7) m.p. 54°-55°; C^{13} NMR, 13.89 ppm(s) for $^{13}CH_3$ and 179.92 ppm(s) for COOH relative to internal TMS.

Similarly, using perdeutero and tritium labeled methyl iodide, 14-$^{2}H_3$ and 14-^{3}H tetradecanoic acids were synthesized in excellent yield. All fatty acids showed satisfactory physical constants and were 99% pure by thin layer and gas chromatographic analysis of the acid and its methyl ester, respectively.

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4. Unpublished results from this laboratory.
5. Methyl 12-(chloroformyl)-dodecanoate was synthesized from the half ester of 1,11-undecane dicarboxylic acid by treatment with thionyl chloride for 6 hours, and distillation at 120-125°C/0.05 mm.
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7. Beil, 2, 911.