SYNTHESIS OF C-14 LABELLED METHYL STERCULATE.

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SUMMARY

Methyl sterculate, a cyclopropenoid fatty acid, has been synthesized with carbon-14 as the methylene carbon in the cyclopropene ring.

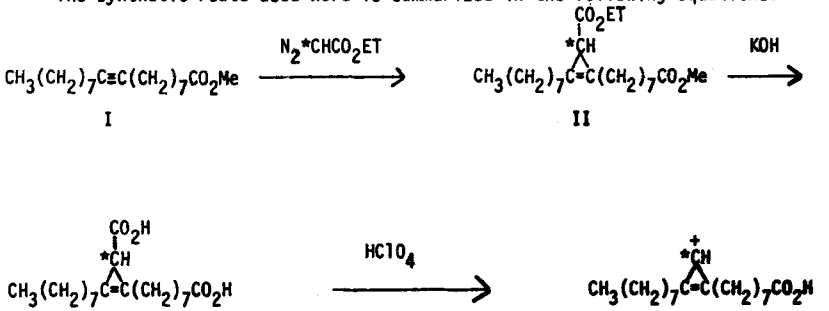
Introduction

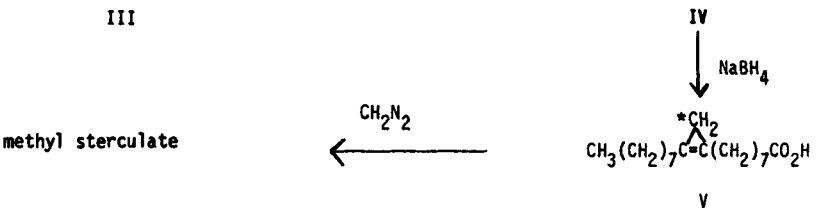
Sterculic and malvalic acids occur in the triglycerides of the commercially important cottonseed and kapokseed oils. These cyclopropenoid fatty acids are held responsible for numerous physiological disorders in farm and laboratory animals (1). More importantly, sterculic acid has been reported as a powerful cocarcinogen (2). Sterculic acid is known to inhibit several fatty acid desaturase systems (3); otherwise its metabolic fate is unknown.

The literature contains several reported syntheses of sterculic and malvalic acids, all of which utilize the particular stability of the cyclopropenum cation (4,5,6). Gensler and co-workers have reported the synthesis of C-14 labeled methyl malvalate using their procedures reported earlier (7). This paper reports the synthesis of C-14 labeled methyl sterculate via another procedure also reported earlier (6).

Results

The synthetic route used here is summarized in the following equations:





The specific activity of the starting material, glycine-2-^{14}C, remained constant throughout the procedure until the decarbonylation-reduction steps III to V. During this stage, the specific activity dropped 10%. The reason is not apparent unless part of the label was on the carboxyl group of the starting glycine.

Loss of the methylene group from the cyclopropene ring accompanies reduction to a small extent. Methyl stearolate produced from this loss is separated on a column and should not affect the specific activity of the product. Gensler has recently discussed this reaction (8).

A side product was also obtained, radioactive sterculyl alcohol, formed by the borohydride reduction of the carboxyl group of sterculic acid. Normally borohydride does not reduce carboxyl groups and usually the amount of reduction during this procedure is small, but variable.

Experimental

Glycine-2-^{14}C (45 mg, 3 millicuries) was dissolved in 50 ml dried absolute ethanol and gaseous HCl bubbled through the solution. After 12 hours, 15 ml of benzene was added and distilled off through a Dean-Stark trap, and the solution was allowed to set another 12 hours. The solution was then warmed slightly to dissolve 3.44 g of inactive, recrystallized glycine ethyl ester hydrochloride. Upon cooling the product, glycine-2-^{14}C ethylester·HCl, was filtered off and the mother liquor evaporated to a small volume and filtered again; yield 98%, specific activity 1.18 x 10^2 mCi/mole.

The above glycine-2-^{14}C ethylester·HCl was dissolved in 18 ml water and added to a solution of 0.64 g potassium acetate and 3.099 g $NaNO_2$ in 18 ml water at 0° C. Two and one-half milliliters of 10% H_2SO_4 was added and the solution extracted with 20 ml of cold ether. This process was repeated twice yielding 1.86 g of product. One gram more of $NaNO_2$ was added, the solution extracted three more times with cold ether yielding 0.74 g, or a total yield of 2.60 g (92.5%) of yellow ethyl diazoacetate-2-^{14}C.

The above diazoacetate was slowly added to 1 g activated copper dust suspended in 10 g neat methyl stearolate at 120° C under a nitrogen atmosphere. Half of this mixture was chromatographed on a 2.5 x 25 cm Merck acid washed alumina column, eluting 75 ml fractions with pentane. In the fifth fraction 10% ether was added and slowly increased to 30% ether by the eighth fraction. By the tenth fraction, all the methyl stearolate had eluted and radioactive methyl 9,10-carbethoxymethano-^{14}C-9-octadecenate II began to elute, specific activity 1.19 x 10^2 mCi/mole; ir(neat) 2935, 2860, 1910, 1755, 1745, 1475, 1450, 1380, 1190 and 1040 cm^{-1}. Examination of this diester by thin layer chromatography (9) demonstrated that all of the radioactivity was with the diester.

Ester hydrolysis on the above substituted cyclopropenoid fatty acid was performed by dissolving it in 12 ml of 1-propanol containing 1.1 g KOH at 105° C under a nitrogen atmosphere for 2 hours. After cooling, diluting,

and neutralizing, the cyclopropenoid diacid III was extracted with ether, dried over Na_2SO_4, and the ether removed under reduced pressure, yielding 1.74 g, 22% based on ethyl diazoacetate; ir(neat) 3400-2400 (broad-OH), 2935, 2870, 1925, 1730, 1484, 1445, 1245 and 955 cm^{-1}.

The above diacid was added to 0.93 g 70% $HClO_4$ in 20 g acetic anhydride at 0° C and allowed to warm to 15° C for 20 minutes. This decarbonylation solution which turned a deep dark red and evolved carbon monoxide, was mixed with 25 ml $CHCl_3$ and 150 ml hexane at -30° C. The dark viscous precipitate was separated. One hundred fifty milliliters more of cold hexane was added to the decarbonylation solution and left one hour at -30° C to separate any additional cyclopropenium ion.

A hydride solution was prepared by warming 10 g $NaBH_4$ to 50° C under nitrogen in 200 ml dimethyl sulfoxide and 60 ml pyridine, then cooling to 5° C. The above dark red, viscous cyclopropenium perchlorate was immediately added to the cold hydride solution with rapid stirring. Any tendency to foam can be suppressed by covering the hydride solution with a layer of pentane. Workup was accomplished by diluting the hydride solution with a large volume of water, neutralization and extraction with pentane. The product was esterified with diazomethane and chromatographed on a 2.5 cm x 25 cm alumina column, yield 390 mg 26% based on the above diacid; specific activity, 1.12 x 10^2 mCi/mole; ratio of the ring methylene resonance in the NMR to the terminal methyl resonance (10) indicated 99% purity; all radioactivity occurs under the methyl sterculate spot on a $AgNO_3$-silica gel thin layer plate (9); ir(neat) 2935, 2870, 1875, 1740, 1475, 1190 and 1005 cm^{-1}. In addition 660 mg of radioactive sterculyl alchohol was eluted off the above chromatography column.

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