Three runs gave yields of 49–61% of isolated, pure product in amounts up to 5.5 g. When product in the forerun and still residue was included, the yield was 69–71%. One run that gave a yield of only 20% was carried out with the flask initially immersed in a Dry Ice bath. When this was done the Grignard reagent did not react until after all of it had been added and the flask was warmed to near room temperature. A large amount of trimethylborane was formed, resulting in a low yield of the desired product. When the reaction was conducted at 0°, an excess of Grignard reagent was avoided, and yields were very good. Dimethylaminodibromoborane reacted equally well.

Dimethylaminodiethylborane was also prepared[9] in 58% yield of distilled, pure product from dimethylaminodibromoborane and an ethyl Grignard reagent.

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Some Reactions of Methyl 2,4,6-Heptatrienoate

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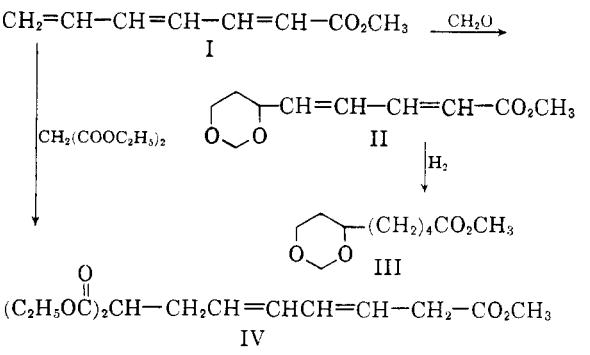
Previous work[1,2] with long conjugated systems has indicated that most reactions yield mixtures of products which are quite difficult to separate. However, we have found that methyl 2,4,6-heptatrienoate[2] (I) reacts with surprising selectivity with formaldehyde in an acid-catalyzed reaction and with diethyl malonate in a base-catalyzed reaction. The products in each case result from attack of the reactive species on the terminal portion of the long conjugated system. There have been few, if any, examples in the literature of this type of selective reaction.

Condensation of methyl 2,4,6-heptatrienoate (I) with formaldehyde in dioxane-sulfuric acid gave methyl 6,8-methylenedioxy-2,4-octadienoate (II) in 35% yield. The structure of II was determined by its ultraviolet spectrum and by hydrogenation to methyl 6,8-methylenedioxyoctanoate (III), a known compound.[3]

The saturated ester III was obtained from I in 48% over-all yield when the ester II was hydrogenated without purification. The identity of III



was further established by its conversion to lipoic acid by a series of known reactions.[3,4]

The base-catalyzed (Michael) addition of diethyl malonate to Compound I occurred predominantly in a 1,8-manner as shown by conversion of methyl 2,4,6-heptatrienoate through the intermediate diolefinic triester (IV) to azelaic acid in 62% yield. The high yield of azelaic acid is remarkable in view of the number of possible side reactions.

EXPERIMENTAL

Azelaic acid. A solution of 0.6 g. of sodium in 6 ml. of absolute methanol was diluted with 10 ml. of dry ether and then mixed with a solution of 20 g. (0.145 mole) of freshly distilled methyl 2,4,6-heptatrienoate in 75 ml. of diethyl malonate. The resulting solution was heated under reflux on a steam bath for 21 hr. and then diluted with 400 ml. of ether. This was washed with 12 ml. of 2*N* hydrochloric acid and two 10-ml. portions of water. The ethereal solution was dried over anhydrous magnesium sulfate, and all material volatile at 0.2 mm. at steam bath temperature was removed. The residual oil was dissolved in 100 ml. of absolute ethanol and hydrogenated in a low pressure apparatus over 10% Pd on carbon. The hydrogen absorption was rapid and stopped sharply. The catalyst was removed and the mother liquor diluted with 200 ml. of 95% ethyl alcohol before 40 g. of potassium hydroxide was added. This mixture was heated under reflux for 2 hr. Water (300 ml.) was then added and the apparatus was arranged for distillation. After all of the alcohol and 100 ml. of water had distilled, the mixture was cooled and carefully treated with 200 ml. of concentrated hydrochloric acid. After refluxing 40 hours, the solution was evaporated to dryness and extracted with ether. The ether was concentrated to give 16.0 g. of crude azelaic acid, m.p. 97–103°. This was recrystallized from water with 85% recovery to give good quality azelaic acid, m.p. 104–106°, which did not depress the melting point of an authentic sample. Infrared comparison confirmed its identity. The ether solution was evaporated to dryness to give an oil which upon distillation yielded (after recrystallization) 0.92 g. of azelaic acid, m.p. 103–107° (total yield 16.92 g., 62%) and 3.5 g. of material distilling at 100–125° (0.2 mm.) which was not identified.

Methyl 6,8-methylenedioxy-2,4-octadienoate (II). A mixture of 55 g. of purified dioxane, 8.0 g. of 96% sulfuric acid, and 6.0 g. (0.20 mole) of paraformaldehyde was stirred briefly and cooled to 0°. Methyl 2,4,6-heptatrienoate, 13.8 g. (0.10 mole), was added slowly, and the mixture was stirred at room temperature for 40 hr. The reaction mixture was cooled in an ice bath and diluted with 300 ml. of ice water. The organic layer was separated, and the water was extracted with three 150-ml. portions of ether. The organic layer and

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the ether extracts were combined and dried with magnesium sulfate, and the ether was distilled. The residue was distilled through a 4-in. Vigreux column to give 4.5 g. (23%) of methyl 6,8-methylenedioxy-2,4-octadienoate, b.p. 108–115° (0.7 mm.), n_D^{25} 1.5180, λ_{max} 257 mμ, ε 23,000.

Anal. Calcd. for $C_{10}H_{14}O_4$: C, 60.59; H, 7.12. Found: C, 60.14; H, 7.34.

A repetition of this experiment using 55.2 g. (0.40 mole) of methyl heptatrienoate afforded 27.4 g. (34.6%) of methyl 6.8-methylenedioxy-2,4-octadienoate, b.p. 95–124° (0.15 mm.), n_D^{25} 1.5178–1.5200.

Methyl 6,8-methylenedioxyoctanoate. A mixture of 4.31 g. (0.0218 mole) of methyl 6,8-methylenedioxy-2,4-octadienoate, 0.5 g. of 10% palladium-on-carbon, and 100 ml. of hexane was placed in a pressure bottle and hydrogenated in a Parr shaker until hydrogenation was complete. The uptake was 112% of the theoretical amount. The solution was filtered to remove the catalyst, and the solvent was distilled. The residue was fractionated in a 4-in. Vigreux column. There was obtained 3.81 g. (87%) of methyl 6,8-methylenedioxyoctanoate, b.p. 78° (0.1 mm.), n_D^{25} 1.4508–1.4518. The *n-m-r* spectrum was consistent with the structure assigned and was confirmed by comparison with *m*-dioxane and methyl valerate references.

Anal. Calcd. for $C_{10}H_{18}O_4$: C, 59.41; H, 8.91. Found: C, 59.24; H, 9.00.

The constants listed for this compound[3] are b.p., 112° (0.01 mm.) and n_D^{22}, 1.4519.

Methyl 6,8-Methylenedioxyoctanoate (direct procedure). A mixture of 19.5 g. (0.65 mole) of paraformaldehyde, 150 ml. of dioxane, and 25 g. of concentrated sulfuric acid was cooled to 0°, and 44.0 g. (0.318 mole) of methyl 2,4,6-heptatrienoate was added. The mixture was stirred at room temperature for 42 hr. and diluted with 300 ml. of ice water. The organic layer was separated, and the water layer was extracted with three 150-ml. portions of ether. The organic layers were combined and dried with magnesium sulfate. The ether was distilled, and the residue was diluted to a volume of 250 ml. with 50% methanol-cyclohexane and hydrogenated using 110 g. of 10% palladium-on-carbon catalyst. Distillation of the product afforded 30.7 g. (48%) of methyl 6,8-methylenedioxyoctanoate, b.p. 94.5° (0.47 mm.) to 102° (0.27 mm.), n_D^{25} 1.4489–1.4498.

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New Route to Carbon-14 Labeled *N*-(1-Hydroxy-2-fluorenyl)acetamide[1]

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The binding of chemical carcinogens or metabolites thereof to cellular proteins is thought to be causally related to the induction of neoplasms.[2] In the case of the carcinogen *N*-2-fluorenylacetamide it has been shown that hydroxylation was required for binding of the compound.[3] Based on this observation the theory has been advanced that the hydroxylated metabolites are further oxidized to quinone imides or imines and that these reactive metabolites combine with proteins.[4] Recently, evidence has been provided that 2-amino-1-fluorenol was oxidized, either by mitochondria and cytochrome c or by cytochrome oxidase and cytochrome c, to the *o*-quinone imine, 1,2-fluorenoquinone-2-imine. In the presence of crystalline bovine serum albumin this oxidation product added rapidly to the protein.[5]

We desired to determine the mechanism of the oxidation of 2-amino-1-fluorenol as well as the site and extent of binding of the oxidation product in the intact cell under physiological conditions. For this purpose, we required 2-amino-1-fluorenol and *N*-(1-hydroxy-2-fluorenyl)acetamide labeled with carbon-14 in the fluorene nucleus. The fluorene system has been shown to be resistant to metabolic attack.[6]

The available chemical synthesis of these compounds[7,8] from fluoranthene does not permit the incorporation of carbon-14 into the molecule. Carbon-14 labeled *N*-(1-hydroxy-2-fluorenyl)-acetamide has been made biosynthetically by feeding *N*-(2-fluorenyl-9-C^{14})acetamide to rats and isolating *N*-(1-hydroxy-2-fluorenyl-9-C^{14})acetamide from the urine, the label here being situated in the stable 9 position.[9] The drawback to this method is that it requires the chromatographic separation of the desired *N*-(1-hydroxy-2-fluorenyl-9-C^{14})acetamide from other labeled hydroxylated metabolites and the careful purification of the isolated material by carrier methods. *N*-(1-hydroxy-2 fluorenyl)acetamide is only a minor urinary metabolite[10] and the method of isolation necessitates further dilution of the label which places limitations on the specific radioactivity of the final product. For these reasons it appeared desirable to work out an alternative route for the chemical synthesis of carbon-14 labeled *N*-(1-hydroxy-2-fluorenyl)acetamide.

A new approach became possible with the development of a synthesis of 1,2,3,4-tetrahydro-

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