Synthesis of Rotenone-6a-C^{14} on a Semimicro Scale

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Reaction of 2 mmoles of methyl bromoacetate-1-C^{14} with 3 mmoles of derritol produced 6a,12a-dehydrorotenone-6a-C^{14} in approximately 50% yield. Sodium boron hydride reduction gave the 12-hydroxy-6a-C^{14} intermediate and subsequent Oppenauer oxidation produced rotenone-6a-C^{14}. Yields for conversion of nonradioactive 6a,12a-dehydrorotenone to rotenone were 17 to 25% but, with radioactive material of 2.36 mc. per mmole, the yield was only 7.5%. Other oxidation procedures for the reduction product of 6a,12a-dehydrorotenone are considered, as are possible explanations for the lower yield with the radioactive material.

Rotenoids, in the form of ground derris roots, have been used for insect control and as fish poisons for more than a century. Rotenone, the principal active ingredient, has short residual action and relatively high selectivity as an insecticide. However, it may be very toxic to mammals and produce tissue damage at low dietary levels under certain conditions (*14*, *16*). The toxicity of rotenone has been attributed to its high potency as an inhibitor of glutamic dehydrogenase (*8*) or more specifically of diphosphopyridine nucleotide-flavin-linked electron transport (*6*, *7*, *12*). The biological fate of rotenone is incompletely understood, primarily because of difficulties in specific analysis of micro amounts of the chemical and in the differentiation between rotenone and potential metabolites or degradation products. Future studies on the mode of action and biological fate of rotenone would be greatly facilitated by the availability of the radiotagged compound.

Rotenone-C^{14} has been prepared biosynthetically by foliar application of acetate-C^{14} to *Lonchocarpus nicou* (*11*). The specific activities obtained varied from 0.017 to 0.027 μc. per gram and 0.4 to 1 gram of labeled rotenone was recovered per millicurie of acetate-C^{14} used. Although the biosynthetic procedure might be modified to obtain a product of higher specific activity, the organic synthesis of rotenone-C^{14} appeared to be a more practical way of obtaining rotenone with a specific activity suitable for toxicological studies. Total synthesis of natural rotenone can presumably be achieved by a 17-step procedure, but in an over-all yield of less than 0.01% (*13*). However, the yield for the preparation of rotenone from 6a,12a-dehydrorotenone has been found to be about 22 to 24% (*15*); therefore, utilization of certain fragments from natural rotenone to react with a labeled intermediate appeared to be a practical method for synthesis of radioactive rotenone. The main problems appeared to be potentially low yields under the semimicro synthesis conditions and difficulties in separation of stereoisomers.

An attractive synthetic route was the decomposition of natural rotenone to derritol with the loss of two carbon atoms. These two carbon atoms could be re-introduced by reaction with methyl bromoacetate-1-C^{14} to yield 6a,12a-dehydrorotenone-6a-C^{14}, which could then be converted to rotenone-6a-C^{14}. In addition, derritol was a convenient starting material, as one of the asymmetric carbon atoms, 5′, already has the right configuration and, therefore, the final product is restricted to four isomers of rotenone which may result from the two remaining carbon atoms, 6 and 6a, being re-introduced. This allowed a theoretical yield of twice that of totally synthetic rotenone, which has eight possible isomers because of the three asymmetric carbon atoms that are introduced during synthesis.

Derritol has been converted to 6a,12a-dehydrorotenone by reaction with ethyl bromoacetate to yield derrisic acid ethyl ester, in 17% yield, followed by liberation of derrisic acid (*9*). This acid was converted to 6a,12a-dehydrorotenone in 24% yield (*18*). Purification of such intermediates appeared to be difficult on a semimicro scale. However, in the present study, synthesis conditions were found whereby 6a,12a-dehydrorotenone was made directly from derritol, in approximately 50% yield, without isolation of intermediates. Sodium boron hydride reduction of 6a,12a-dehydrorotenone is known to yield the 12-hydroxy compound, which subsequently can be oxidized to rotenone by the Oppenauer procedure (*15*). After oxidation, Miyano and Matsui (*15*) treated the reaction products with methanol and isolated a material designated as "mutarotenone" which they considered to be a mixture of rotenone and epirotenone. However, on the basis of our studies, their product was not the reported mixture of two isomers but, rather, was a methanol solvate of natural rotenone. In the present investigation, carbon tetrachloride was used for separating rotenone from the reaction mixture because rotenone is the only isomer which forms a crystalline solvate with carbon tetrachloride and this solvate can be converted to rotenone by subsequent crystallization from ethanol.

The reactions involved in this radiosynthesis and the compound nomenclature used are indicated in Figure 1. More complete considerations of the reactions involved are given in recent reviews (*3*, *13*).

Experimental

Chemicals and Methods. Natural rotenone (Aldrich Chemical Co., Milwaukee, Wis.) was recrystallized from ethanol to yield a product with a melting point of 162–63° C. Other rotenone derivatives were prepared as follows: 6a,12a-dehydrorotenone (m.p. 221–22° C.), by treatment of natural rotenone with alkali followed by acid (*19*); derritol (m.p. 163–64° C.), by decomposition of natural rotenone with potassium hydroxide and zinc powder (*10*); and deoxy-12,12a-dehydrorotenone (m.p. 160–61° C.), by reaction of natural rotenone or 6a,12a-dehydrorotenone with sodium boron hydride followed by treatment with 6*N* hydrochloric acid at 0° C. (*13*). All melting points (uncorrected) were determined on microscope cover slips on a hot block by observing single crystals with a microscope. Aluminum isopropoxide (Eastman Organic Chemicals, Rochester, N. Y.) was redistilled, yielding a product

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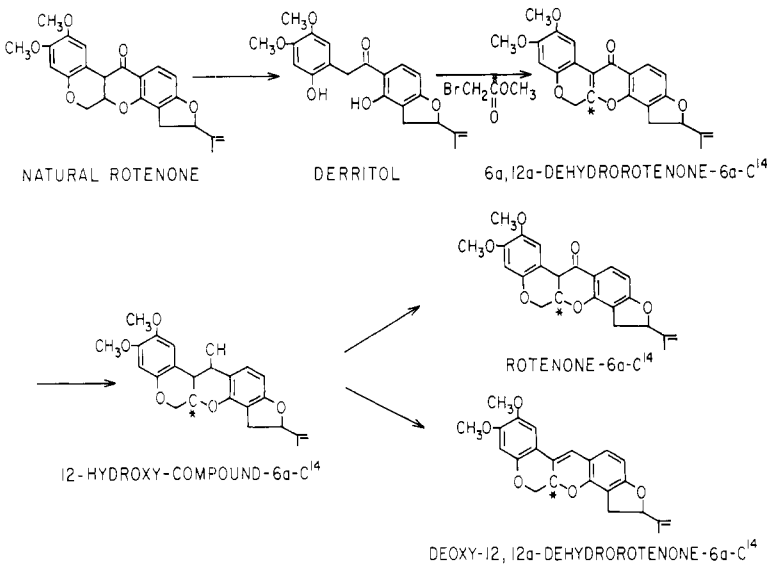

Figure 1. Synthetic pathway for rotenone-6a-C^{14}

Acid degradation of the 12-hydroxy compound formed from sodium boron hydride reduction of 6a,12a-dehydrorotenone yields deoxy-12,12a-dehydrorotenone. This reaction does not necessarily account for the formation of deoxy-12,12a-dehydrorotenone-6a-C^{14} from 6a,12a-dehydrorotenone-6a-C^{14} during the radiosynthesis

boiling at 140° to 150° C. and 12 mm. Sodium boron hydride was obtained from Alfa Inorganics, Inc., Beverly, Mass. Methyl and ethyl bromoacetate-1-C^{14}, each with specific activities of 2.0 to 3.0 mc. per mmole, were obtained from the Volk Radiochemical Co., Burbank, Calif., and New England Nuclear Corp., Boston, Mass. All radioactive measurements were made with the Packard Tri-Carb Model 3003 liquid scintillation spectrometer.

The identity of radiolabeled and nonlabeled compounds (rotenone; 6a,12a-dehydrorotenone; and deoxy-12,12a-dehydrorotenone) was established by mixed melting point determinations and comparison of infrared spectra (10% chloroform solutions, Beckman IR-4 infrared spectrometer). Rotenone-6a-C^{14} and 6a,12a-dehydrorotenone-6a-C^{14} were also cochromatographed with known nonradioactive materials. Paper chromatography utilized Whatman No. 1 filter paper coated with Dow Silicone 550 and development was with dimethylformamide-water (3 to 2), according to Shishido (*17*) (rotenone, R_f = 0.85; 6a,12a-dehydrorotenone, R_f = 0.00). Thin-layer chromatograms (silica gel G, 0.25-mm. thickness) were developed with benzene-methanol (97 to 3) (rotenone, R_f = 0.70; 6a,12a-dehydrorotenone, R_f = 0.59). Radioactive materials were detected photographically, by x-ray film, and known compounds were detected by potassium permanganate on paper chromatograms (*17*) and by sulfuric acid followed by heating on thin-layer chromatograms. Glutamic dehydrogenase inhibition by rotenone and rotenone-6a-C^{14} was measured by a described procedure (*7*), except that mouse liver mitochondria were used instead of insect muscle.

Preparation of 6a,12a-Dehydrorotenone-6a-C^{14} from Derritol and Methyl Bromoacetate-1-C^{14}. Derritol (1.18 grams), methyl bromoacetate-1-C^{14} (2.36 mc. per mmole, 325 mg.), and anhydrous potassium carbonate (426 mg.) in absolute ethanol (4.2 ml.) were heated (100° C.) in a 10-ml. sealed tube containing a glass-covered magnetic stirring bar. In the 4-hour heating and stirring period, a pink precipitate appeared and the solution changed from yellow to dark brown. After the tube had been cooled and opened, the solution was filtered and the precipitate was washed with water and cold absolute ethanol. The product (m.p. 221–22° C.) was identical with authentic 6a,12a-dehydrorotenone as determined by mixed melting point, infrared spectral, and cochromatographic comparisons; therefore, it was 6a,12a - dehydrorotenone - 6a - C^{14}. The yield, based on the radiolabeled intermediate, was 48% and the product was of the anticipated specific activity, 2.36 mc. per mmole.

Yields of 48 to 50% were obtained with either methyl or ethyl bromoacetate as long as the same proportions of reactants and alcohol volume were maintained; the specific activities were from 0 to 3 mc. per mmole and the preparation utilized 1 to 2 mmoles of bromoacetate ester. Yields were not increased by doubling the amount of potassium carbonate, using methanol as the reaction solvent, or by including 0.25 mmole of potassium iodide per mmole of bromoacetate ester in the reaction mixture. A higher reaction temperature (150° C.) or a twofold increase or decrease in ethanol volume greatly reduced the yield of 6a,12a-dehydrorotenone.

Preparation of Rotenone-6a-C^{14} from 6a,12a-Dehydrorotenone-6a-C^{14}. 6a,12a-Dehydrorotenone-6a-C^{14} (765 mg., 2.36 mc. per mmole) was dissolved in 16 ml. of anhydrous dioxane and warmed to 60° to 65° C. Six milliliters of sodium boron hydride (80 mg.) in anhydrous ethanol were added slowly. The reaction mixture was kept at 60° to 65° C. for 30 minutes and, subsequently, at 25° C. for 18 hours. After decomposition of excess sodium boron hydride with acetone (5 ml.), all solvents were evaporated off under reduced pressure. The residue was dissolved in 15 ml. of chloroform and 15 ml. of water. After separation of the chloroform layer, an additional 15 ml. of chloroform were used to rinse the reaction flask and again extract the water phase. The combined chloroform phase was washed with water and dried with anhydrous potassium carbonate, and the solvent was evaporated. The dry residue was dissolved in 64 ml. of anhydrous benzene and 40 ml. of anhydrous acetone, after which aluminum isopropoxide (6.4 grams) was added.

Following 10 hours of refluxing, the solution was cooled to 5° C. and 20 ml. of concentrated hydrochloric acid were added. The benzene layer was washed with water and then dried with anhydrous potassium carbonate. After the benzene was removed by evaporation, 10 ml. of anhydrous methanol were added to the black residue. Light yellow crystals appeared and these were recrystallized from absolute ethanol to yield deoxy-12,12a-dehydrorotenone-6a-C^{14} (m.p. 160–61° C., 2.36 mc. per mmole, 15.6% yield from 6a,12a-dehydrorotenone-6a-C^{14}). The identity of the radioactive material with deoxy-12,12*a*-dehydrorotenone was confirmed by mixed melting point and infrared spectral comparisons. The methanol in the filtrate was completely removed by evaporation and 1.0 ml. of carbon tetrachloride was added. Crystals obtained on cooling the carbon tetrachloride were recrystallized from absolute ethanol to yield rotenone-6a-C^{14} (m.p. 162–63° C., 2.36 mc. per mmole, 7.5% yield from 6a,12a-dehydrorotenone-6a-C^{14}). The identity of the radioactive material with natural rotenone was confirmed by mixed melting point, and by infrared spectral and cochromatographic comparisons. Further, rotenone-6a-C^{14} was identical in potency with natural rotenone as an inhibitor of glutamic dehydrogenase.

This method for conversion of 6a,12a-dehydrorotenone to the 12-hydroxy compound with sodium boron hydride and then to rotenone by the Oppenauer oxidation procedure was identical to that of Miyano and Matsui (*15*). With nonlabeled 6a,12a-dehydrorotenone, yields of natural rotenone were routinely 17 to 25% and no deoxy-12,12a-dehydrorotenone was ever obtained. Three radiosyntheses (2.0, 2.37, and 3.0 mc. per mmole) gave less than half the rotenone-6a-C^{14} yields anticipated from the nonlabeled runs made in the same way, and with the identical reagents and at the same scale of operation. In each radioactive run, deoxy-12,12a-dehydrorotenone-6a-C^{14} was obtained as a by-product. The specific activities of the rotenone-6a-C^{14} and deoxy-12,12a-dehydrorotenone-6a-C^{14} were the same as that of

the 6a,12a-dehydrorotenone-6a-C^{14} used in the reaction. Therefore, the reduction and oxidation steps were not preferential for the labeled or nonlabeled compounds within the same reaction mixture. Deoxy-12,12a-dehydrorotenone-6a-C^{14} might result from incomplete oxidation by aluminum isopropoxide of the 12-hydroxy compound 6a-C^{14}, and its conversion on addition of hydrochloric acid to decompose the aluminum isopropoxide. An alternative possibility for the lower rotenone-6a-C^{14} yield and formation of the by-product, deoxy - 12,12a - dehydrorotenone - 6a-C^{14}, might be a radiation-induced autoxidation during the sodium boron hydride reduction of 6a,12a-dehydrorotenone-6a-C^{14}, but no information is available on the specific intermediates formed.

Oxidation of 12-Hydroxy Compound to Rotenone by Active Manganese Dioxide or by CrO_3-Pyridine Complex. Two other oxidation methods were compared with the Oppenauer procedure for conversion of the 12-hydroxy compound to rotenone.

Active manganese dioxide was used by Crombie *et al.* (*4*) to oxidize the 12-hydroxy compound (obtained from natural rotenone reacting with lithium aluminum hydride) to rotenone in 45% yield; however, they noted that it is difficult to prevent some overoxidation to 6a,12a-dehydrorotenone. In the present study, active manganese dioxide [prepared according to Attenburrow *et al.* (*1*)] was used to oxidize the 12-hydroxy compound prepared as described above from 6a,12a-dehydrorotenone with sodium boron hydride. The active manganese dioxide, used under reaction conditions apparently identical to those reported by Crombie *et al.*, yielded no natural rotenone or 6a,12a-dehydrorotenone. When one fourth to one half the amount of active manganese dioxide specified by Crombie was used, yields of 7 to 12% rotenone and 4 to 6% 6a,12a-dehydrorotenone were obtained. Twofold increase or decrease in the acetone volume for the reaction had little effect on the yield. Thus, active manganese dioxide of the type used was less efficient than the Oppenauer procedure for conversion of the 12-hydroxy compound from 6a,12a-dehydrorotenone to rotenone.

The 12-hydroxy compound prepared from 6a,12a-dehydrorotenone (788 mg.) was dissolved in 10 ml. of anhydrous pyridine and cooled to −50° C. Chromium trioxide (2.4 grams) in 40 ml. of anhydrous pyridine was then added and the mixture left for 2 hours at 25° C. After dilution with water, extraction with chloroform, and evaporation of the chloroform, the residual material was recrystallized from anhydrous methanol, giving a product with the melting point of the methanol solvate of natural rotenone (124–25° C.), and, subsequently, from absolute ethanol to yield rotenone (m.p. 163° C., 23% yield from 6a,12a-dehydrorotenone). Identical reaction conditions except for use of half the amount of chromium trioxide gave 21% rotenone.

The optimal temperatures for the first and second phases of the reaction were further investigated. With the temperature of the second phase held constant at 25° C., temperatures of −10° to −15° C. in the first phase yielded 15% rotenone, while 0° C. gave no rotenone. When the first phase temperature was held constant at −50° C., the yields varied with the temperature during the second phase. For example, 34% rotenone was obtained after 24 hours at −10° C., 40% after 48 hours at −10° C., 25% after 12 hours at −10° C. followed by 2 hours at 25° C., and only 8% after 24 hours at 25° C. [This oxidation procedure using the CrO_3-pyridine complex was adapted from one reported for terpenoid oxidations (*5*).] Thus, the yields for conversion of 6a,12a-dehydrorotenone to rotenone varied considerably with temperature, although under optimal conditions they were higher than with the Oppenauer procedure. In an analogous oxidation reaction, a related chemical, the hydroxy compound formed on borohydride reduction of 6*H*-rotoxen-12-one, was oxidized with chromium trioxide in acetone at room temperature to form 6a,12a-dihydro-6*H*-rotoxen-12-one in good yield (*2*). While the CrO_3-pyridine complex and chromium trioxide in acetone were not studied with 6a,12a-dehydrorotenone-6a-C^{14}, they might be worthy of consideration for radiosyntheses of rotenone-6a-C^{14} in future work.

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