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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fluoren-1-one from indene.[11] The treatment of 3-(3′-indenyl)propyl bromide[11] with potassium cyanide-C^{14} gave 4-(3′-indenyl)butyronitrile-1-C^{14}. Cyclization of the nitrile and hydrolysis to the labeled tetrahydrofluorenone was accomplished by an adaptation of the method of Howell and Taylor[11] to the semimicro scale. A variety of catalysts, solvents, and reaction times were investigated in attempts to effect the dehydrogenation of the ketone to 1-fluorenol. In each case the ketone and catalyst were heated at the reflux temperature of the solvent for periods of 10 minutes to 24 hours. No detectable 1-fluorenol was formed with the use of palladium black in *m*-xylene or naphthalene, with 10% palladium-on-charcoal in *p*-cymene or naphthalene, or with chloranil in *m*-xylene. With fluorene as solvent, 10% palladium-on-charcoal gave a maximum yield of 30% 1-fluorenol. The optimum conditions, however, were found using palladium black as catalyst, fluorene as solvent, and a reaction time of 4–5 hours. Crude yields of 65% of 1-fluorenol-1-C^{14} were realized under these conditions. Nitration of 1-fluorenol-1-C^{14} gave 2-nitro-1-fluorenol-1-C^{14}.[8] Reduction by zinc dust, calcium chloride, and ethanol[12] converted the nitrofluorenol to 2-amino-1-fluorenol-1-C^{14}. Acetylation was carried out in the usual manner.[13] The overall radioactive yield of *N*-(1-hydroxy-2-fluorenyl-1-C^{14})acetamide was 5.1%, and the final product had a specific radioactivity of 0.66 mC./mM.

This synthetic route to the formation of 2-amino-1-fluorenol and its acetamide, in addition to being the only feasible method at present for incorporation of radioactive carbon in the fluorene nucleus, has been used successfully for large scale preparations of the unlabeled compounds. The overall yield is comparable to that which can be obtained by the method using fluoranthene as starting material,[7,8] but the reaction sequence is shorter by one step.

During the large scale preparation of 1,2,3,4-tetrahydrofluoren-1-one it was found that more extensive purification of the intermediate 4-(3′-indenyl)butyronitrile resulted in crystallization of this material which had previously been reported as an impure liquid.[11] This compound has now been more fully characterized.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point block and are uncorrected as are boiling points. Radioactivity measurements were made by the micro method previously described.[14]

4-(3′-Indenyl)butyronitrile-C^{14}. A solution of 1.28 g. (5.4 mmoles) of 3-(3′-indenyl)propyl bromide[11] in 0.9 ml. of absolute ethanol was placed in a 10 ml. pear-shaped flask. Potassium cyanide-C^{14} (0.163 g., 2.51 mmoles)[15] and sodium cyanide (0.170 g., 3.47 mmoles) were added, followed by 0.4 ml. of water. The reaction flask was fitted with a reflux condenser carrying at the top a glass tube which led into a 10% potassium hydroxide solution. This trapping arrangement was necessary to prevent the escape of small quantities of hydrogen cyanide-C^{14} during the reaction. The solution was then heated under reflux for 9.5 hr. The mixture was allowed to stand at room temperature overnight and was then treated with 2 ml. of 10% potassium hydroxide solution. Without delay the solution was extracted with 3-ml. portions of ether until the extract was colorless, about 5 portions being required. The ether extracts were combined in a 50-ml. dropping funnel which contained some anhydrous sodium sulfate supported on glass wool, and the solution was percolated through a column (1 × 14 cm.) of acid-washed, activated alumina using a total volume of 40–50 ml. of ether. With the small quantities of compound used in the radioactive run purification by column chromatography was more practical than the vacuum distillation recommended by Howell and Taylor.[11] The solvent was evaporated by means of an infrared lamp. Vapors of ether and hydrogen cyanide-C^{14} were trapped by an aspirating funnel placed over the flask containing the eluate. 4-(3′-Indenyl)butyronitrile-C^{14} was obtained as 0.99 g. of light yellow oil. This oil probably contained some unreacted bromide, but it was of sufficient purity for the subsequent reaction. Additional purification of nonradioactive preparations by vacuum distillation gave a yellow oil, b.p. 134–136° (0.5 mm.), 89% yield. Three crystallizations from benzene–petroleum ether (b.p. 30–60°) (3:1), followed by one crystallization from ethanol, gave colorless plates, m.p. 118–119°. The infrared spectrum showed the characteristic nitrile absorption band at 2250 $cm.^{-1}$

Anal. Calcd. for $C_{13}H_{12}N$: C, 85.2; H, 7.15; N, 7.64. Found: C, 85.0; H, 7.10; N, 7.89.

1,2,3,4-Tetrahydrofluoren-1-one-C^{14}. The nitrile (0.99 g.) was placed in a 10 ml. pear-shaped flask having a small side arm. Anhydrous ether (5 ml.) and powdered zinc chloride (0.5 g., 3.7 mmoles) were added, and a reflux condenser carrying a glass tube leading into water was attached. Dry hydrogen chloride was passed into the reaction mixture for 2 hr. by means of a tube which extended through the side arm of the reaction flask. At 0.5 hr. intervals the flow of hydrogen chloride was interrupted, and the precipitated solids were stirred manually to insure complete reaction. The ether lost by entrainment was replaced periodically. At the end of the reaction time, the solid 1,2,3,4-tetrahydrofluoren-1-imine-1-C^{14} hydrochloride was allowed to settle, and the overlying ether was drawn off. The product was washed three times by adding fresh ether, passing hydrogen chloride through the mixture and removing the ether. These washings were necessary for removal of impurities. Water (5 ml.) was then added, and air was slowly passed through the gradually warmed mixture to entrain the ether and thus prevent bumping. When the ether had been removed, the mixture was heated under reflux for 15 min. After thorough cooling, the crude product was collected on a sintered glass funnel and dried *in vacuo*. The purification of the product was accomplished by treating it with hot petroleum ether C (Skelly). When all the light-colored product had dissolved, the solution was separated from the residual brown tar.

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(15) Supplied by Volk Radiochemical Company, Chicago 40, Ill. This material had been assayed by the manufacturer to contain 3 mC. However, the final specific radioactivity of our product which was checked against a standard sample of benzoic acid-C^{14} obtained from the National Bureau of Standards indicated that the activity of the cyanide-C^{14} was actually 32% greater.

Evaporation of the solvent by a stream of air gave 0.462 g. (2.53 mmoles) of slightly yellow, crystalline 1,2,3,4-tetrahydrofluoren-1-one-1-C^{14}, m.p. 92–98°, representing a 46.5% yield for the two-step sequence. This product was sufficiently pure for the succeeding steps of the synthesis. Further crystallizations of unlabeled preparations from petroleum ether C gave white plates, m.p. 103–105°.

1-Fluorenol-1-C^{14}. 1,2,3,4-Tetrahydrofluoren-1-one-1-C^{14} (0.462 g., 2.53 mmoles) was mixed with 5 g. of sublimed fluorene and 0.241 g. of purified palladium black catalyst[16] in a 35-ml. round-bottom flask fitted with a reflux condenser. The mixture was heated under reflux by means of a Wood's metal bath for 4.5 hr. The cooled solid solution was dissolved in 40 ml. of ether and filtered through a layer of Celite into a 60-ml. centrifuge tube. The ether solution was extracted 3 times with 8-ml. portions of a 15% potassium hydroxide solution. The alkaline extract was warmed to remove any dissolved ether and was then filtered through a layer of Celite. The filtrate was acidified with concentrated hydrochloric acid and cooled. The precipitate was collected on a sintered glass funnel and dried to give 0.294 g. (1.61 mmoles) of crude 1-fluorenol-1-C^{14}, m.p. 115–119°, 64.5% yield. For the subsequent reaction further purification was not necessary. When desired, this could be accomplished by recrystallization from a large volume of hot water giving material melting at 120–121° with a resulting loss of 13% of 1-fluorenol. In large scale runs of this reaction, the materials were mixed in a ratio of 2.5 g. of palladium black catalyst, 5.0 g. of ketone and 25 g. of fluorene. With heating at the reflux temperature for 4 hr., the yield of crude 1-fluorenol was 64%. Following solution of the reaction mixture in ether, the catalyst may be recovered by filtration and reused for this reaction without purification.

2-Nitrofluorenol-1-C^{14}. 1-Fluorenol-1-C^{14} (0.294 g., 1.61 mmoles) was dissolved in 6.4 ml. of glacial acetic acid in a 25-ml. Erlenmeyer flask. The solution was stirred by a magnetic stirrer and cooled in an ice bath while a solution of 0.145 ml. (2.27 mmoles) of concentrated nitric acid in 0.145 ml. of water was added dropwise from a pipet. The pipet was washed with 1 ml. of glacial acetic acid and the wash liquid added to the reaction mixture. The flask was stoppered and the solution stirred at room temperature for 3.5 hr. The mixture was then cooled in ice. The yellow precipitate was collected on a sintered glass funnel, washed with 15 ml. of water and dried *in vacuo*. Addition of the wash water to the mother liquor caused precipitation of a second crop which was collected, washed with water, and dried. The first crop was purified by chromatography on a 1 × 18 cm. column of acid-washed, activated alumina using benzene as eluent and collecting only the leading yellow band. Crop 2 was purified in a like manner on the same column. Evaporation of the combined eluates by means of an air stream and infrared lamp gave 0.182 g. (0.80 mmole) of 2-nitro-1-fluorenol-1-C^{14}, m.p. 162–165°, 50% yield.

2-Amino-1-fluorenol-1-C^{14}. A solution of 2-nitro-1-fluorenol-1-C^{14} (0.182 g., 0.80 mmole) in 29 ml. of hot ethanol was prepared in a 50-ml. flask equipped with a reflux condenser and magnetic stirrer. Zinc dust (1.46 g.) was added and the mixture was stirred and heated under reflux while a solution of 0.4 g. of calcium chloride in 4.4 ml. of water was added dropwise. Stirring and heating were continued for 2.5 hr. at the end of which time the hot slurry was filtered through a layer of Celite into 1.6 ml. of concentrated hydrochloric acid. The zinc dust was washed with 5 ml. of ethanol. The combined filtrate and wash liquid were evaporated by means of an air stream and infrared lamp until there remained only a slurry of precipitated salts in a minimum volume of water. The salts were collected on a sintered glass funnel, washed with ether, and dried. The material was dissolved in 8 ml. of water, and the solution was filtered through a bed of Celite into 12 ml. of a 15% sodium acetate solution. The resulting suspension was cooled, and the precipitate was collected without delay. After washing with water, the product was dried *in vacuo* over calcium chloride to yield 0.0958 g. (0.49 mmole) of 2-amino-1-fluorenol-1-C^{14}, 60.5% yield.

N-(1-hydroxy-2-fluorenyl-1-C^{14})acetamide. 2-Amino-1-fluorenol-1-C^{14} (0.0958 g., 0.49 mmole) was dissolved in 22.5 ml. of hot water containing 0.09 ml. of concentrated hydrochloric acid. The solution was filtered through a coarse sintered glass funnel and cooled to room temperature. Freshly distilled acetic anhydride (0.07 ml., 0.74 mmole) was added all at once with magnetic stirring. A solution of 0.09 g. (0.11 mmole) of sodium acetate in 4.9 ml. of water was added, and the reaction mixture was stirred and cooled in an ice bath for 15 min. The gray precipitate was collected and dried. An ethyl acetate solution of the product was percolated through a 0.6 × 15 cm. column of acid-washed, activated alumina. The eluate was evaporated to dryness *in vacuo* and the residue was recrystallized by being dissolved in 3 ml. hot ethanol which was then added to 15 ml. of water. A second pass through an alumina column using the same eluent followed by recrystallization gave pure, white *N*-(1-hydroxy-2-fluorenyl-1-C^{14})acetamide, m.p. 210–212°. The yield was 0.0726 g. (0.30 mmole, 62.6%) and the specific radioactivity was 0.66 mC./mM.

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Convenient Syntheses of 3-Indolesuccinic and 3-Indolepropionic Acids

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In a recent publication[1] Noland and Hammer have shown that the dibasic acid obtained by the alkaline hydrolysis of maleyldiindole[2] is 3-indolesuccinic acid. The preparation of this acid from indole and diethyl diazosuccinate has been reported previously by Jackson and Manske.[3]

The facile preparation, in 95% yield, of 3-indolealdehyde[4] prompted us to use this compound as starting material for new and convenient syntheses of 3-indolesuccinic and 3-indolepropionic acids.

Compound I, prepared by a modification of a previously described procedure,[5] when treated with potassium cyanide in refluxing ethanol[6] gave rise to 3-indolesuccinonitrile (III). The dinitrile was readily hydrolyzed with aqueous base to give an al-

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