[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE]

Synthesis of 2-Methyl-1,4-naphthoquinone-4-C^{14}[1]

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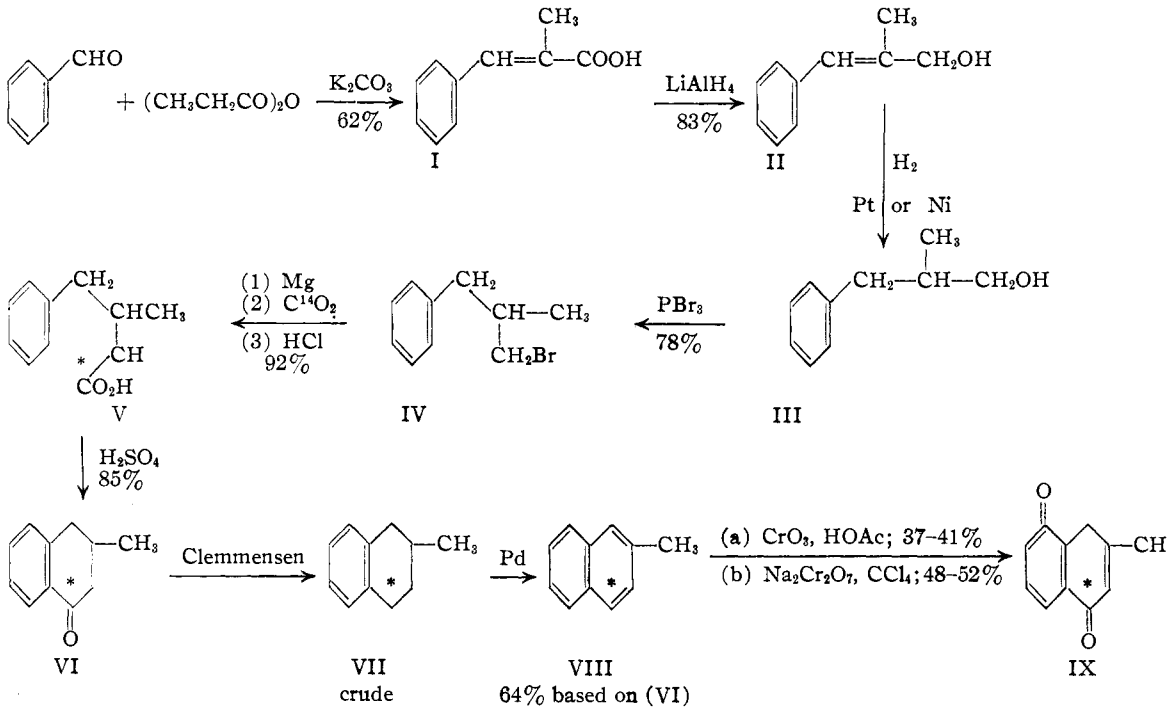
A new synthesis of the vitamin K substitute, 2-methyl-1,4-naphthoquinone, has been developed and used to incorporate carbon-14 into the 4-position of this compound. The reaction sequence has been used successfully to prepare the quinone in an over-all yield of 25% based on the isotopic carbon dioxide using 5 mM. of barium carbonate-C^{14} of activities of 1 μc., 100 μc. and 2 mc.

The availability of an isotopically labeled sample of the vitamin K substitute, 2-methyl-1,4-naphthoquinone, should make possible a number of biological studies with this substance that hitherto have been impossible. Collins[4] has reported an interesting synthesis of this quinone, incorporating carbon-14 into position 8 in the aromatic ring. This report presents a new preparation of the quinone permitting inclusion of carbon-14 in position 4 of the quinoid ring. We have preferred to label in this position because of the report of Shemiakin, *et al.*,[5] of the vitamin K activity of phthalic acid and its diethyl ester and its isolation from the urine of men and dogs after administration of 2-methyl-1,4-naphthoquinone.

over-all yield of 65% from 2-methylcinnamic acid (I) according to the reaction sequence (I) → (II) → (III) → (IV).

Reduction of (I) was carried out successfully at ice-bath temperature; at higher temperatures, a mixture of the allylic and saturated alcohol was obtained. A purer product (III) was always obtained by isolation of (II) before reduction at 3 atmospheres pressure with hydrogen and platinum or Raney nickel. Since dehydrogenation of (VI) with palladium produced a mixture of phenols and hydrocarbon which could not be readily purified, the ketonic oxygen was removed by the Clemmensen reduction to give (VII). Oxidation of (VIII) to (IX) by a modification of Fieser's method[6] was



2-Benzylpropyl bromide (IV) was prepared in an not as satisfactory as a modification of the method of Hyman and Peter,[7] the yields being 37–41% and 48–52%, respectively. This over-all procedure has been used with no appreciable variation in results with 5 mM. of barium carbonate-C^{14} of activities of 1 μc., 100 μc. and 2 mc., the over-all yield being 25% based on the carbon dioxide used. Biological studies will be reported elsewhere.

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(2) (a) This paper is based in part on a thesis submitted by Liang Li to the Graduate School of St. Louis University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry, November, 1949. (b) Fellow, American Bureau of Medical Aid to China, 1947.

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(4) C. J. Collins, THIS JOURNAL, **73**, 1038 (1951).

(5) M. M. Shemiakin, L. A. Schukina and J. B. Shvezov, *Nature*, **151**, 585 (1943); **154**, 513 (1944).

(6) L. F. Fieser, *J. Biol. Chem.*, **133**, 391 (1940).

(7) J. Hyman and C. F. Peter, U. S. Patent 2,402,226 (June 1946).

Experimental[8]

2-Methylcinnamic Acid (I).—This material was prepared in 62% yield from benzaldehyde and propionic anhydride by a modified Perkin reaction,[9] m.p. 82°.[10]

2-Methylcinnamyl Alcohol (II).—A solution of 24.3 g. of (I) in 300 ml. of absolute ether was added dropwise with continuous stirring during the course of two hours to a solution of 5.8 g. of $LiAlH_4$ in 300 ml. of absolute ether maintained at 0–5°. After stirring 24 hours, the temperature was permitted to rise to 20° during the course of an hour. The solution was then cooled below 5° and hydrolyzed with 60 ml. of water and 300 ml. of 10% H_2SO_4. After washing the ether layer successively with water, 20% Na_2CO_3 and water, there was obtained from the dried ether extract 20 g. of (II), b.p. 96–97° at 1.5 mm., representing an 83% yield. Three grams of unchanged acid was recovered. Crystallization of the alcohol from cold petroleum ether yielded colorless, elongated plates, melting at 24.5°.

Anal. Calcd. for $C_{10}H_{12}O$: C, 81.04; H, 8.16; quantitative microhydrogenation, 1 mole of H_2. Found: C, 81.76; H, 8.19; quantitative microhydrogenation, 1.06 moles of H_2.

A 3,5-dinitrobenzoate was prepared from 50 mg. of (II) which crystallized from petroleum ether (30–60°) as colorless leaflets, m.p. 125.5–126°.

Anal. Calcd. for $C_{17}H_{14}O_6N_2$: C, 59.65; H, 4.12. Found: C, 59.48; H, 3.78.

2-Benzylpropanol-1 (III).—Ten grams of (II) was reduced in the usual manner with Raney nickel or platinum and hydrogen to give (III), b.p. 77.5–78° at 0.5 mm. or 126–126.5° at 15 mm.[11]

2-Benzylpropyl Bromide (IV).—Phosphorus tribromide (19 g.) was slowly added dropwise to 10 g. of (III) with stirring at 0°. The reaction mixture was allowed to stand 18 hours in an ice-bath until the temperature gradually rose to room temperature. It was then heated to 90–100° for 2–3 hours, cooled, and poured onto crushed ice. The ether extract was washed with water and dried over anhydrous calcium chloride. After removal of the ether, 11 g. of (IV), b.p. 77.5–78° at 0.5 mm., was obtained in a yield of 78%.[12]

3-Benzylbutyric-1-C^{14} Acid (V).—The apparatus used for preparation of this material was similar to that suggested by Calvin.[13] The Grignard reagent was prepared in an atmosphere of nitrogen in the usual manner from 1.5 g. (7.5 mM.) of (IV) and 182 mg. of acid-washed magnesium turnings. The CO_2 for carbonation was obtained from 987 mg. of barium carbonate-C^{14} containing 99 μc. of carbon-14[14] by acidification with 50 ml. of 80% H_2SO_4 and was flushed through a U-shaped column of anhydrous calcium chloride and Drierite into the gas manifold where it was distilled twice from traps cooled with liquid nitrogen. The flask containing the Grignard reagent was attached to the gas manifold, cooled with liquid nitrogen, the nitrogen atmosphere in the flask removed, and evacuated to a pressure of 5 μ. The CO_2 was slowly distilled into the Grignard reagent over a period of not less than 30 minutes, the liquid nitrogen was replaced with an ice–salt-bath at −15° and the reaction mixture was stirred with a magnetic stirrer for 30 minutes and then brought to atmospheric pressure with nitrogen gas. After ten hours, the reaction mixture was hydrolyzed with dilute HCl, the ether layer removed, and extracted with 10% NaOH. The alkaline extract was acidified, extracted with ether and dried over anhydrous $MgSO_4$. After evaporation of the ether, 818 mg. of colorless oil was obtained (91.5%). A non-radioactive preparation was distilled at 127° at 0.1 mm. Levene and Marker[12] reported b.p. 160° at 1 mm.

3-Methyltetralone-1-C^{14} (VI).—The 818 mg. of (V) was stirred at ice-bath temperature and 5.5 ml. of H_2SO_4 (sp. gr. 1.84) was added dropwise from a buret during the course of 40 minutes. After standing 24 hours at room temperature, it was poured onto crushed ice and the oily product taken up in ether. After washing the ether extract with water, 5% $NaHCO_3$ and water, the ether was removed leaving 625 mg. (85%) of a slightly yellow oil. An oxime was prepared from 37 mg. of this product and found to melt at 123°.[15]

2-Methylnaphthalene-4-C^{14} (VIII).—The tetralone (588 mg.) was reduced by Clemmensen reduction using zinc amalgam, 6 ml. of 38% HCl, 0.3 ml. of glacial acetic acid and 6 ml. of toluene in the usual manner for a period of 26 hours. From the reaction mixture, 455 mg. of a golden yellow oil was obtained which was immediately dehydrogenated according to the procedure of Linstead.[16] The oil was heated at 250° for 3 hours over a palladium–charcoal catalyst in an 18 × 160 mm. Pyrex tube fitted with an air condenser. After extraction of the catalyst with ether and removal of the ether, 420 mg. of solid was obtained. Microsublimation of this product produced 330 mg. of colorless crystals (m.p. 34.5°) of (VIII) in a yield of 64% based on the tetralone. This product did not depress the melting point of authentic 2-methylnaphthalene. The picrate of a sample of non-radioactive 2-methylnaphthalene also prepared by this procedure, m.p. 116°, did not depress the melting point of an authentic sample upon admixture.

2-Methyl-1,4-naphthoquinone-4-C^{14} (IX).—In a 50-ml. flask provided with a small magnetic stirrer and reflux condenser, 1.65 g. of $Na_2Cr_2O_7 \cdot 2H_2O$ was dissolved in 0.8 ml. of water. To this was added the 330 mg. of (VIII) dissolved in 5.5 g. of CCl_4. The contents of the flask were stirred at 50–55° while 1.7 ml. of 80% H_2SO_4 was added dropwise. The temperature of the bath was gradually raised to 80° in the course of 10 minutes, held there for 5 minutes, and then the flask was chilled with ice-water.

The CCl_4 layer was removed and the aqueous layer was extracted with CCl_4 and ether; the combined CCl_4–ether layer was extracted several times with 5-ml. portions of a freshly prepared alkaline hydrosulfite solution (2% sodium hydrosulfite in 2% NaOH) until no color remained in the extract. The unoxygenated fraction remaining in the CCl_4 was reoxidized with 1 g. of $Na_2Cr_2O_7 \cdot 2H_2O$ in 0.5 ml. of water and 1.0 ml. of 80% H_2SO_4. The hydroquinone from both oxidations (225 mg.) was oxidized with 450 mg. of of Ag_2O in the presence of a small amount of anhydrous $MgSO_4$. The crude quinone, 212 mg., m.p. 98–100°, was sublimed *in vacuo* to give 185 mg. melting at 104.5–105° which did not depress the melting point of authentic non-radioactive 2-methyl-1,4-naphthoquinone. Recrystallization from 50% methanol raised the melting point to 106.5°. The yield was 50%. Using this method of oxidation of Hyman and Peter,[7] the over-all yield of (IX) based on the $C^{14}O_2$ used was 25%. Adaptation of Fieser's method[6] of oxidation of the hydrocarbon consistently gave yields of only 37–41%.

Radioactivity Assay.—A sample of the product was oxidized by means of a modified Van Slyke–Folch wet oxidation procedure.[17]

Anal. 6.739 mg. of (IX) gave 80.49 mg. of $BaCO_3$. Calcd. for $C_{11}H_8O_2$: C, 76.73. Found: C, 76.78.

The radioactivity of this sample of $BaC^{14}O_3$ was compared with that of a Bureau of Standards Carbon-14 Standard using a thin window Geiger tube (window thickness, 1.7 mg./cm.^{2}). *Anal.* 19.1 microcuries of carbon 14 per millimole of (IX).

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(8) All melting points taken on the Fisher–Johns apparatus.

(9) J. R. Johnson, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 251.

(10) P. Raikow, *Ber.*, **20**, 3397 (1887), reported m.p. 81–82°.

(11) J. V. Braun, A. Grabowski and G. Kirschbaum, *ibid.*, **46**, 1278 (1913), reported b.p. 128–129° at 16 mm.

(12) P. A. Levene and R. E. Marker, *J. Biol. Chem.*, **110**, 303 (1935), reported b.p. 85° at 1 mm.

(13) M. Calvin, C. Heidelberger, J. Reid, B. Tolbert and P. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 142.

(14) The radioactive $BaCO_3$ was obtained on allocation from the Atomic Energy Commission, Isotopes Division, Oak Ridge, Tennessee.

(15) W. E. Bachmann and W. S. Struve, THIS JOURNAL, **62**, 1618 (1940), reported m.p. 122.5–123.5° for this oxime.

(16) R. P. Linstead and S. L. S. Thomas, *J. Chem. Soc.*, 1127 (1940).

(17) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).