which was isolated from the reaction mixture as the diflavianate yielding 370 mg. of L-histidine diflavianate, m.p. 245–247°. From the diflavianate was obtained 59.6 mg. of L-histidine, m.p. 294°. Ashley and Harington report 295°. For a 2% aqueous solution of a non-radioactive sample prepared by the same method $[\alpha]^{20}D$ −35.5°. Ashley and Harington found $[\alpha]^{20}D$ −36.0°.

Histamine-2-C^{14}-imidazole.—Histidine decarboxylase was prepared by incubating 50 mg. of acetone powder of the *Lactobacilli* with 5 ml. of McIlvaine buffer at *p*H 4.8 for 6 hours. After removing the cells by centrifugation, the enzyme preparation was added to 18.4 mg. of radioactive L-histidine in a Warburg flask and incubated at 30° for 65 minutes at which time carbon dioxide evolution was complete. The solution was transferred to a small separatory funnel, made strongly alkaline, and extracted four times with *n*-amyl alcohol. At this point 95% of the radioactivity was in the alcohol fraction. After one additional extraction the alcohol fractions were dried and evaporated to dryness *in vacuo*. The residue was dissolved in 3 ml. of water, a hot solution of 60 mg. of picric acid in 4 ml. of water added, the mixture heated to boiling and filtered; 44.3 mg. of histamine dipicrate, m.p. 238–242°, was obtained, a 65% yield from L-histidine. Pyman[10] reported m.p. 238–242°. Additional isotopic histamine dipicrate was crystallized from the mother liquor after the addition of carrier.

The activity using an internal counter was 9.3 × 10^6 c.p.m. per mg. of histamine base.

Anal. (for a non-radioactive sample synthesized in the same manner) Calcd. for $C_5H_9N_3(C_6H_3O_7N_3)_2$: C, 35.8; H, 2.66; N, 22.2. Found[11]: C, 35.7; H, 2.79; N, 22.3.

A paper chromatogram of the histamine (as the dihydrochloride) in butanol–ammonia showed a single sharp radioactive peak at R_F 0.80; under identical conditions the radioactive L-histidine produced a single sharp peak at R_F 0.15. Thus the histamine is free of demonstrable contamination by histidine. Chromatograms of the histamine in other solvents showed single peaks suggesting absence of significant amounts of other radioactive impurities.

Before use in animal experiments the radioactive histamine dipicrate was recrystallized from water, a sample dissolved in 0.15 *N* hydrochloric acid, the picric acid extracted with ether, and the solution of histamine dihydrochloride neutralized with sodium bicarbonate just before use. In a test for pharmacological activity[12] a very dilute solution of the radioactive histamine dihydrochloride produced the same contraction of guinea pig uterus as did the same amount of commercial histamine dihydrochloride.

(10) F. L. Pyman, *J. Chem. Soc.*, **49**, 668 (1911).

(11) Analysis by Micro-Tech Laboratories.

(12) Kindly performed by Dr. Georges Ungar of this Institute.

RHEUMATIC FEVER RESEARCH INSTITUTE
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CHICAGO, ILLINOIS RECEIVED AUGUST 22, 1951

Synthesis of *dl*-Adrenalin-β-C^{14} and *dl*-Adrenochrome-β-C^{14}

BY RICHARD W. SCHAYER[1]

The syntheses of radioactive adrenalin and adrenochrome were accomplished by known procedures modified for small-scale use and for conserving isotopic materials.

Experimental

Chloroacetic Acid-carboxyl-C^{14}.—Barium carbonate-C^{14} (3.0 millicuries)[2] was diluted to 4.92 g. and converted by the Grignard reaction to 1.74 g. (85% yield) of carboxyl-labeled sodium acetate.[3] Chloroacetic acid was synthesized by the method of Ostwald.[4] After recrystallization from ligroin, 2.01 g., m.p. 58°, a yield of 56% from sodium acetate after allowing for 400 mg. carrier, was obtained.

Chloroacetylcatechol.—Chloroacetic acid, 1.95 g., was heated on a steam-bath with 1.95 g. of catechol and 2.0 ml. of freshly distilled phosphorus oxychloride in an atmosphere of sulfur dioxide.[5] When the reaction was complete (about 45 minutes) the mixture was dissolved in 30 ml. of hot water, filtered and the residue washed. Crude chloroacetylcatechol, 1.50 g., m.p. 169–170°, was obtained. After recrystallization from hot water containing traces of hydrochloric acid and sodium bisulfite, 1.13 g. (29% yield from chloroacetic acid) was obtained having the reported melting point of 173°.

***dl*-Adrenalone Hydrochloride** (4-Methylaminoacetylcatechol Hydrochloride).—Chloroacetyl catechol, 1.00 g., was mixed with 5.0 ml. of 25% methylamine and allowed to stand at room temperature for 20 hours with frequent shaking.[6] Alcohol, 9 ml., was added and after standing 90 minutes in the cold, the brown precipitate was filtered, washed with 50% alcohol, absolute alcohol and finally ether. The crude adrenalone was dissolved in a minimum of dilute hydrochloric acid, diluted to about 20 ml. with water, and reprecipitated by addition of ammonia producing 0.52 g. of adrenalone (54% yield). Adrenalone, 0.52 g., was dissolved in a minimum of 3 *N* hydrochloric acid, filtered, absolute alcohol and finally ether added. Adrenalone hydrochloride, 0.50 g., crystallized, an 81% yield from adrenalone.

***dl*-Adrenalin-β-C^{14}** (Methylaminomethyl-(3,4-dihydroxyphenyl)-carbinol).—Adrenalone hydrochloride, 0.24 g., was dissolved in 10 ml. of water, 0.20 g. of catalyst (5% palladium-on-aluminum oxide) added and the mixture hydrogenated at ordinary pressure and temperature for two hours.[7] After filtering off the catalyst and adding excess ammonia 150 mg. of *dl*-adrenalin-β-C^{14} (74% yield from adrenalone hydrochloride) was obtained. The over-all yield from barium carbonate to adrenalin was 4.4%.

Anal. (for a non-radioactive sample synthesized by the same method) Calcd. for $C_9H_{13}O_3N$: C, 59.00; H, 7.27; N, 7.65. Found[8]: C, 59.11; H, 7.36; N, 7.52.

The activity measured with an internal counter was 2.72 × 10^5 c.p.m. per mg. The compound had the same effect on the blood pressure of a dog as did commercial synthetic epinephrine. A paper chromatogram of the adrenalin in butanol–acetic acid produced a single peak at R_F 0.45.

***dl*-Adrenochrome-β-C^{14}.**[9]—*dl*-Adrenalin-β-C^{14}, 40 mg., plus non-isotopic adrenalin, 60 mg., were dissolved in 3.0 ml. of absolute methanol containing 0.06 ml. of 99% formic acid. After warming to 35°, 0.7 g. of silver oxide was added, the mixture shaken and maintained at 35° for exactly one minute, filtered through a rapid filter and washed with 1 ml. of methanol. Crystals started forming immediately. After storing at −15° for 30 minutes the adrenochrome was filtered and washed successively with 1:1 methanol–ether, 1:3 methanol–ether, and ether. A first crop of 22 mg. red-brown crystals was obtained. By careful addition of ether to the mother liquor an additional 18 mg. of adrenochrome crystallized giving a total yield of 40%.

Anal. (for a non-radioactive sample synthesized by the same method, after correction for 2.12% ash) Calcd. for $C_9H_9O_3N$: C, 60.3; H, 5.06; N, 7.82. Found[8]: C, 59.2; H, 5.30; N, 7.69.

The activity measured with an internal counter was 1.06 × 10^5 c.p.m. per mg. Biological tests indicated that there was no observable contamination by adrenalin.

CONTRIBUTION FROM THE
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(2) Supplied by the Monsanto Chemical Company, on allocation from the United States Atomic Energy Commission.

(3) W. Sakami, W. E. Evans and S. Gurin, THIS JOURNAL, **69**, 1110 (1947).

(4) R. Ostwald, *J. Biol. Chem.*, **173**, 207 (1948).

(5) H. Hoberman, THIS JOURNAL, **57**, 1382 (1935).

(6) H. D. Dakin, *Proc. Roy. Soc.* (*London*), **B76**, 491 (1905).

(7) S. Sergievskaya and N. Fedotova, *Org. Chem. Ind., U. S. S. R.*, **2**, 96 (1936).

(8) Analysis by Micro-Tech Laboratories.

(9) C. L. MacCarthy, *Chim. et Ind.*, **55**, 435 (1946).