case of the bromination of *dl*-dichloride. The crude reaction product gave a 73% yield of starting dichloride, m. p. 189–190° on recrystallization from ethanol. The only other product was an oil which yielded an oily mixture when treated with zinc in acetic acid. No crystals could be isolated from these oily products.

The Stability of the α,α'-Dichlorobibenzyls.—Solutions of both dichlorobibenzyls in carbon tetrachloride containing iodine gave virtually quantitative yields of the starting dichlorides after standing in direct sunlight for ten days. Starting material was recovered in high yields when carbon tetrachloride solutions of either dichlorobibenzyl were treated with chlorine, bromine or iodine in the dark for as long as ninety-five days.

Chlorination of α,α,α'-Trichlorobibenzyl.—A solution of 0.5 g. of the trichloride in 30 ml. of carbon tetrachloride was saturated with chlorine and placed in direct sunlight for two hours. Crystallization of the product from the oily residue yielded 0.20 g. of $\alpha,\alpha,\alpha'\alpha'$-tetrachlorobibenzyl, m. p. 160°. An oily by-product with a camphor-like odor was also observed.

Summary

1. Bromine vapor has been found to bring about an isomerization of solid *dl*-α,α'-dibromobibenzyl to the *meso*-isomer in 90% yield, both in the dark and in daylight. Bromine and iodine in carbon tetrachloride effected the isomerization of *dl*-dibromobibenzyl in light with high yields. Chlorine gave a much lower yield of the *meso*-isomer and a mixture of products containing trihalides.

2. Chlorine and bromine have been found to isomerize *dl*-α,α'-dichlorobibenzyl to the *meso*-isomer in sunlight, but only very small yields of the *meso*-isomer could be isolated. Several other halogenation products were detected. Iodine did not affect the *meso*- or *dl*-dichlorobibenzyl in sunlight.

3. Displacement of one halogen by another is evidently the preferred reaction during the halogenation of the dibromobibenzyls while with the dichlorobibenzyls substitution of halogen for hydrogen predominates.

4. A mode of isomerization of *dl*-α,α'-dihalobibenzyl involving a series of displacements of one halogen atom for another until the more stable *meso*-α,α'-dihalobibenzyl is formed has been suggested.

IOWA CITY, IOWA RECEIVED DECEMBER 23, 1949

[CONTRIBUTION FROM THE RADIOACTIVITY CENTER OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY AND THE MEDICAL LABORATORIES OF THE COLLIS P. HUNTINGTON MEMORIAL HOSPITAL, MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MASSACHUSETTS]

Synthesis of C-14 Carboxyl-labeled Tyrosine and Diiodotyrosine[1]

BY ROBERT BERNER LOFTFIELD

When carboxyl-labeled tyrosine and diiodotyrosine were required for certain studies of animal metabolism, a survey of the available literature revealed no synthesis of tyrosine which could reasonably be adapted to the preparation of carboxyl-labeled tyrosine. Erlenmeyer's[2,3] classic syntheses involve the preparation of either hippuric acid or phenylalanine followed by several reactions whose comparatively poor yields are not easily improved.

Accordingly the much more direct approach, that of the Strecker synthesis, was tried. The required aldehyde, *p*-hydroxyphenylacetaldehyde, is so sensitive that it is known only as its *p*-nitrophenylhydrazone. In this investigation the corresponding methyl ether was prepared by the Darzens[4] aldehyde synthesis using conditions differing from those used by Rosenmund and Dornsaft.[5] Anisaldehyde was treated with ethyl chloroacetate in a solution of sodium in a mixture of ethanol and methanol. The mixed alcohols gave a mixed ester which stays in solution during the subsequent extractions and hydrolysis. The ester was hydrolyzed almost instantaneously by a solution of sodium hydroxide in methanol and ether to give sodium *p*-methoxyphenylglycidate hemihydrate.

Treatment of this salt with weak acids gave the expected aldehyde. However, this aldehyde was always accompanied by varying quantities of resin, even immediately after distillation. Hence, the sodium glycidate was decomposed by dissolving it in a hot solution of sodium bisulfite in water. On cooling, pure stable *p*-methoxyphenylacetaldehyde–sodium bisulfite separated in 56% over-all yield.

The Strecker synthesis and various modifications of it were investigated in order to prepare tyrosine. The only substance which could be isolated was apparently the imino-compound, bis-(α-cyano-β-*p*-methoxyphenylethyl)-amine (m. p. 112–113°). Erlenmeyer[3] had had similar difficulty with the preparation of phenylalanine.

However, excellent results were obtained with the Bucherer[6] hydantoin synthesis which, followed by hydrochloric acid hydrolysis at 140–160°, gave tyrosine in an over-all yield of about 65% based on cyanide.[7] Since the yield based on anisaldehyde is more than 35%, the scheme may find application in the preparation of ordinary synthetic *dl*-tyrosine.

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(1) This research was supported in part by U. S. Navy Contract N5 ori-76; and by an institutional grant from the American Cancer Society to the Massachusetts General Hospital. The C-14 was supplied by the Atomic Energy Commission. Publication No. 701 of the Cancer Commission of Harvard University.

(2) Erlenmeyer and Halsey, *Ann.*, **307**, 138 (1899).

(3) Erlenmeyer and Lipp, *ibid.*, **219**, 161 (1883).

(4) Darzens, *Compt. Rend.*, **139**, 1214 (1904).

(5) Rosenmund and Dornsaft, *Ber.*, **52**, 1740 (1919).

(6) Bucherer, *et al.*, *J. prakt. Chem.*, [2] **140**, 291; **141**, 5 (1934).

(7) Prepared from potassium, ammonia and $C^{14}O_2$ as described earlier; "Nucleonics," Vol. I, No. 3, p. 54 (1947).

The tyrosine thus prepared yielded diiodotyrosine when treated with iodine and potassium hydroxide according to the method of Wheeler and Jamieson.[8] The alternative method of Block and Powell[9] was not as conveniently adapted to the present small scale of operations. Dibenzoyl *dl*-tyrosine (m. p. 213–214°) was obtained by reaction of the tyrosine with benzoyl chloride and sodium hydroxide.

Experimental

***p*-Methoxyphenylacetaldehyde–Sodium Bisulfite Compound.**—Seven grams of sodium was dissolved in a mixture of 50 cc. of absolute ethanol and 50 cc. of absolute methanol. With stirring and salt–ice cooling, a solution of 36.6 g. of ethyl chloroacetate (0.3 mole) and 40.8 g. of anisaldehyde (0.3 mole) in 70 cc. of anydrous ether was added at such a rate that the temperature did not rise above 5°. This required about one hour. The temperature was then allowed to rise to 20°. The mixture was poured into 650 cc. of cold water and extracted with three 100-cc. portions of ether. The ether extract was washed with 300 cc. of water, 300 cc. of 3% sodium bicarbonate solution, and 300 cc. of water.

The washed ether extract was diluted with ether to a volume of 500 cc., chilled to 5°, and then poured into an ice cold solution of 6.9 g. of sodium in 100 cc. of methanol and 6.0 cc. of water. After standing ten minutes, the mixture was filtered with suction. The solid sodium salt was worked into a paste with 50 cc. of ether and 10 cc. of methanol and the sucked to near dryness. (This material could be recrystallized from 1:1 ethanol–water to yield pure crystalline sodium *p*-methoxyphenylglycidate hemihydrate. *Anal.* Calcd. for $C_{10}H_{10}O_{4.5}Na$: C, 53.55; H, 4.48. Found: C, 53.72; H, 4.47.) Before the sodium glycidate could dry completely to an intractible hard cake, it was worked into a paste with 40 cc. of ethanol and 30 cc. of water. This was added in small portions to a vigorously stirred solution of 70 g. of sodium bisulfite in 250 cc. of boiling water contained in a one-liter beaker. Eight cc. of acetic acid was added and the clear solution was slowly cooled to 0°. The ***p*-methylphenylacetaldehyde–sodium bisulfite compound** weighing 48.8 g. still contained about 13% sodium bisulfite; over-all yield 56%.

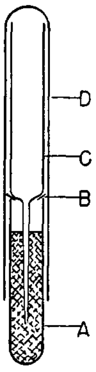

Fig. 1.

The **dinitrophenylhydrazone** was prepared by the usual method[10] and recrystallized twice from ethyl acetate; m. p. 137–138°. *Anal.* Calcd. for $C_{15}H_{14}O_5N_4$: C, 54.58; H, 4.27. Found: C, 54.45; H, 4.37.

4-(*p*-Methoxybenzyl)-hydantoin-5-C^{14}.—The HC^{14}N prepared from 196 mg. of BaC^{14}O$_3$ (1.0 mmole, 500 μcuries)[7] was collected in 1.2 mmoles of sodium methoxide and evaporated to dryness in a 12 × 200-mm. Pyrex tube. To this was added 65 mg. of potassium cyanide, 600 mg. of *p*-methoxyphenylacetaldehyde–sodium bisulfite compound, 600 mg. of powdered ammonium carbonate, and 3 cc. of 50% ethanol–water. The tube was sealed and heated for four hours at 100°. After cooling and opening the tube, the mixture was gradually warmed to 104° to decompose the ammonium carbonate and remove the ethanol; 2.0 cc. of hot water was added and the clear solution chilled two hours in ice. The tube (A, Fig. 1) was cut off squarely just above the surface of the crystal mass, a gasket of filter paper (B) was put on the rim, and supporting tube C was inserted. Tube D was slipped on and the whole assembly was now inverted and centrifuged. A, B and C were removed as a unit from D. C was rinsed with water and 2.0 cc. of water was centrifuged from C into A in order to wash the crystalline product which, when dry, weighed 330 mg. (75%). On recrystallization from alcohol we obtained colorless leaflets of ***p*-methoxybenzylhydantoin**, m. p. 176–179°. *Anal.* Calcd. for $C_{11}H_{12}O_3N_2$: H, 5.49; C, 60.00. Found: H, 5.75; C, 59.67.

C^{14}-Carboxyl-labeled *dl*-Tyrosine.—The above hydantoin (240 mg.) was heated in a sealed tube with 2.0 cc. of concentrated hydrochloric acid for two hours, beginning at 140° and ending at 160°. The tube was cooled in ice, opened, and its contents diluted to 10 cc. with water. The solution was heated to boiling, adjusted with ammonium hydroxide and acetic acid to a *p*H about 6, filtered hot through charcoal and then cooled. (Frequently no crystals appear unless the solution is filtered through charcoal even though the solution is clear and colorless.) The tyrosine, m. p. (dec.) 314–315°, weighed 175 mg. (89%). Dissolved in 2.5% aqueous NaOH and treated with benzoyl chloride, this tyrosine yielded dibenzoyl-*dl*-tyrosine m. p. 213–214°. *Anal.* Calcd. for $C_{23}H_{19}O_5N$: C, 70.98; H, 4.92. Found: C, 71.19; H, 5.17; radioassay[11]; 271 microcuries per millimole; radioactivity yield from BaC^{14}O$_3$, 52%.

Example of Attempted Strecker Synthesis.—Two hundredths of a mole of the aldehyde bisulfite compound was dissolved in 50 cc. of water and cooled to 20°. This solution was shaken with 1.50 g. of potassium cyanide and allowed to stand one hour before it was extracted with ether. The cyanohydrin was isolated by removing the ether and water in a vacuum desiccator. This was then refluxed overnight with 20 cc. of absolute ethanol in a stream of ammonia. On addition of water and cooling, 0.8 g. of what is presumed to be **bis-(α-cyano-β-*p*-methoxyphenylethyl)-amine** separated, which after two recrystallizations from ethyl alcohol melted at 112–113°. *Anal.* Calcd. for $C_{20}H_{21}O_2N_3$: C, 71.63; H, 6.31. Found: C, 71.45; H, 6.38.

C^{14}-Carboxyl-labeled *dl*-Diiodotyrosine.—Seven mg. of the above C-14 tyrosine and 127 mg. of ordinary *dl*-tyrosine were dissolved in a solution of 146 mg. of potassium hydroxide in 3.0 cc. of water in a centrifuge tube. With stirring and under nitrogen 408 mg. of iodine was added. After fifteen minutes, 0.10 cc. of acetic acid was added. After fifteen minutes of standing the precipitate was filtered by centrifugation and washed with water. The solid was largely dissolved by stirring with 0.10 cc. concd. hydrochloric acid in 4.0 cc. of water. The resulting suspension was filtered through 50 mg. of Norite into 200 mg. of sodium acetate in 1.0 cc. of water. Very shortly, light buff colored crystals of diiodotyrosine separated, which after washing and drying weighed 182 mg. (57%), m. p. (dec.) 195°; radioactivity,[10] 14.1 microcuries per millimole.

Summary

A new synthesis of *dl*-tyrosine has been developed and applied to the preparation of C^{14}-carboxyl-labeled *dl*-tyrosine and *dl*-diiodotyrosine.

BOSTON, MASS. RECEIVED NOVEMBER 9, 1949

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[8] Wheeler and Jamieson, *Am. Chem. J.*, **33**, 365 (1905).

[9] Block and Powell, THIS JOURNAL, **65**, 1430 (1943).

[10] Shriner and Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1940, p. 143.

[11] For radio-assays the amino acid was decarboxylated by the ninhydrin technique and counted as barium carbonate (Zamecnik, Frantz, Loftfield and Stephenson, *J. Biol. Chem.*, **175**, 299 (1948)). The reference standard was the BaC^{14}O$_3$ starting material.