The present method simplifies the existing procedures by utilizing the readily available 2,7-diaminofluorene dihydrochloride as the starting material.[4] 2-Amino-7-hydroxyfluorene, m.p. 265–268°, was obtained in yields ranging from 24–37%.

The quantitative estimation of the compound in solution or in biological materials may be based on the measurement of the red dye which is formed when 2-amino-7-hydroxyfluorene is diazotized and coupled with sodium 2-naphthol-3,6-disulfonate (R-salt). The dye absorbs maximally at a wave length of 530 mμ (Fig. 1). The molar extinction coefficient at 530 mμ is 34,300. The spectrophotometric measurement of the dye under the conditions described in the experimental part permits the detection of as little as 5 micrograms of 2-amino-7-hydroxyfluorene. Beer's law is followed with quantities of 2-amino-7-hydroxyfluorene ranging from 5–80 micrograms. The great sensitivity of of the method makes it particularly suited for the estimation of the compound in metabolic studies.

Experimental

2-Acetylaminofluorene-7-diazonium Chloride.—Thirteen grams of 2,7-diaminofluorene dihydrochloride[4] (0.049 mole) was dissolved in 300 ml. of distilled water with slight warming and filtered. After the solution had attained room temperature 5.6 ml. of freshly distilled acetic anhydride (0.060 mole) was added dropwise with vigorous stirring. After the addition of the acetic anhydride had been completed, stirring was continued for one hour. The 2-amino-7-acetylaminofluorene hydrochloride which had precipitated was filtered with suction and washed with 400 ml. of distilled water. The washed product was dried in air for 12 hours and used without further purification in the next step of the synthesis. All of the material was mixed in small succeeding portions in a Waring blendor with 500 ml. of 0.18 *M* hydrochloric acid and the mixture transferred to a 1-l. beaker. To the rapidly stirred suspension there was added 1.95 g. of sodium nitrite (0.028 mole) in 30 ml. of distilled water. After the suspension had been stirred for 2 hours it was warmed to 60° and filtered with suction. The residue on the filter was washed with 145 ml. of distilled water. The filtrate was made 80% saturated with 200 g. of sodium chloride. After standing at 5° for 12 hours the fine, reddish-brown precipitate was filtered with suction and washed with cold 50% saturated sodium chloride solution. The use of hard filter paper (Whatman No. 52) facilitates the quantitative collection of the compound. The material was recrystallized by dissolving it in methanol (90 ml./g.) and adding sufficient ether (100 ml./g.) to cause incipient precipitation. After standing at 5° for 1 hour the precipitate was filtered with suction and dried over calcium chloride. Additional material was obtained from the mother liquor after standing at 5° for 12 hours. After it had been collected with suction the product was added to the material which had been obtained previously. The compound weighed 3 g. and melted at 143–145°. Ray and Peters[5] report a melting point of 146° for this compound.

2-Amino-7-hydroxyfluorene.—A solution of 1.7 g. of 2-acetylaminofluorene-7-diazonium chloride (0.0062 mole) in 150 ml. of distilled water was added dropwise to 300 ml. of boiling 3 *M* hydrochloric acid in the course of 1.5 hours. Nitrogen was passed through the solution during the hydrolysis. The hot solution was filtered and the filtrate cooled in an ice-bath. The solution was neutralized with concentrated ammonium hydroxide. The very fine gray precipitate was collected with suction on hard filter paper (Whatman No. 52) and dried *in vacuo* over calcium chloride. There was obtained 0.65 g. of material which melted at 258–263° (with decomposition). The compound was recrystallized from 30 ml. of refluxing absolute ethanol. After standing in the refrigerator for 12 hours the gray, crystalline powder was collected and washed with cold ethanol and ether; m.p. 265–268° (with decomposition).

(4) S. Schulman, *J. Org. Chem.*, **14**, 382 (1949).

(5) F. E. Ray and J. H. Peters, *Brit. J. of Cancer*, in press.

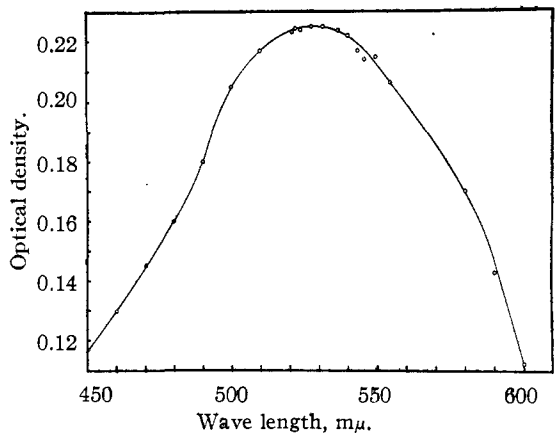

Fig. 1.

Anal. Calcd. for $C_{18}H_{11}ON$: N, 7.11. Found: N, 7.19.

The compound was soluble in acetone, amyl acetate and glacial acetic acid. It darkened quickly on standing in contact with air.

Measurement of the Absorption Spectrum of the Sodium Salt of 7-Hydroxyfluorenyl-2-azo-2′-naphthol-3′,6′-disulfonic Acid.—To 0.0678 mg. of 2-amino-7-hydroxyfluorene in 2 ml. of 4.5 *M* glacial acetic acid and 0.3 ml. of concentrated hydrochloric acid there was added 1 ml. of 0.029 *M* sodium nitrite. The mixture was shaken and, after 1 minute, it was added to 10 ml. of 0.031 *M* sodium 2-naphthol-3,6-disulfonate in 5 *M* aqueous ammonium hydroxide. The solution was cooled to room temperature, 5 ml. of acetone was added and the absorption spectrum of the red dye determined with a Beckman model DU spectrophotometer (Fig. 1).

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O-*p*-Toluenesulfonyl-L-tyrosine, Its N-Acetyl and N-Benzoyl Derivatives

BY ERNEST L. JACKSON

O-*p*-Toluenesulfonyl-L-tyrosine was reported by Fischer[1] as having m.p. 218° (cor., dec.) and $[\alpha]^{20}D$ −4.6° in *N* hydrochloric acid (*c* 6.5). Fischer expressed doubt of the optical purity of the compound, which he prepared by the reaction of hydriodic acid and phosphonium iodide with O,N-di-*p*-toluenesulfonyl-L-tyrosine.

Hydrolysis of the pure methyl ester of O-*p*-toluenesulfonyl-N-acetyl-L-tyrosine with a mixture of hydrochloric and acetic acids yields O-*p*-toluenesulfonyl-L-tyrosine showing m.p. 213–214° (uncor., dec.) and $[\alpha]^{20}D$ +9.0° in *N* hydrochloric acid (*c* 0.42) or +9.5° (*c* 3.16). The rapid separation of crystals from a 6.5% solution of the compound in *N* hydrochloric acid at 20° prevented the determination of the rotation at this concentration. The methyl ester of O-*p*-toluenesulfonyl-N-acetyl-L-tyrosine was prepared by the reaction of *p*-toluenesulfonyl chloride in alkaline acetone solution with the methyl ester of N-acetyl-L-tyrosine, which resulted from acetylation of the known methyl ester of L-tyrosine. Acetylation and benzoylation of O-*p*-toluenesulfonyl-L-tyrosine

(1) E. Fischer, *Ber.*, **48**, 100 (1915).

yielded, respectively, the crystalline N-acetyl and N-benzoyl derivatives.

Experimental

N-Acetyl-L-tyrosine Methyl Ester.—Fifteen grams of the methyl ester[2] of L-tyrosine was acetylated by Fischer's[3] method with mechanical stirring and the use of 7.6 g. of acetyl chloride, 800 cc. of absolute chloroform, 9 g. of anhydrous sodium carbonate and 60 cc. of water. At the end of the reaction the suspended solid was collected on a filter, washed with chloroform and extracted with 400 cc. of hot ethyl acetate in several portions. The ethyl acetate solution upon concentration deposited in two crops 16.6 g. or 91% of nearly pure product. The chloroform solution yielded a small amount of product. The compound was purified by recrystallization from ethyl acetate as colorless, rod-shaped prisms which, after being dried overnight in an evacuated desiccator over calcium chloride, were usually in a solvated state and melted at 118–120°. Sometimes the crystals were almost solvent-free and melted near 135°. Drying at 100° *in vacuo* yielded solvent-free crystals; m.p. 136–137° (uncor.); $[\alpha]^{20}D$ +29.7° in methanol (*c* 0.41).

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.91. Found: C, 60.56; H, 6.38; N, 5.88.

O-*p*-Toluenesulfonyl-N-acetyl-L-tyrosine Methyl Ester.—A solution of 8.8 g. of methyl ester of N-acetyl-L-tyrosine and 7.4 g. of *p*-toluenesulfonyl chloride in 185 cc. of acetone, after being mixed with 37 cc. of *N* sodium hydroxide solution, was refluxed for one hour and then concentrated at 25° *in vacuo* to 50 cc. The sirupy product was separated from the aqueous solution, which was extracted thoroughly with chloroform. The sirup was combined with the chloroform extract; the solution was extracted with 10% sodium carbonate solution, washed with water and dried over sodium sulfate. After removal of the solvent *in vacuo*, the thick sirup was crystallized as colorless needles from ethyl acetate–petroleum ether (b.p. 30–75°); yield 10.4 g. or 72%; m.p. 90–91° (cor.); $[\alpha]^{20}D$ +15.5° in methanol (*c* 0.8). Samples for analysis and rotation were dried in an evacuated desiccator over calcium chloride.

Anal. Calcd. for $C_{19}H_{21}NO_6S$: C, 58.30; H, 5.41; N, 3.58; S, 8.19. Found: C, 58.36; H, 5.38; N, 3.59; S, 8.11.

O-*p*-Toluenesulfonyl-L-tyrosine.—A solution of 5.7 g. of pure methyl ester of O-*p*-toluenesulfonyl-N-acetyl-L-tyrosine in a mixture of 100 cc. of glacial acetic acid and 100 cc. of 38% hydrochloric acid was refluxed for two hours. The solution was cooled, mixed with 850 cc. of water and neutralized to litmus paper with ammonium hydroxide. The precipitated crystals were collected on a filter and washed with water; air-dried; yield 4.7 g. Recrystallized from water as colorless needles and dried at 79° *in vacuo*, it melted at 213–214° (uncor., dec.); $[\alpha]^{20}D$ +9.0° in *N* hydrochloric acid (*c* 0.42) or +9.5° (*c*, 3.16).

Anal. Calcd. for $C_{16}H_{17}NO_5S$: C, 57.30; H, 5.11; N, 4.18; S, 9.56. Found: C, 57.02; H, 5.13; N, 4.14; S, 9.49.

O-*p*-Toluenesulfonyl-N-acetyl-L-tyrosine.—The acetylation of 2.3 g. of O-*p*-toluenesulfonyl-L-tyrosine was carried out according to Fischer,[3] using 0.7 g. of acetyl chloride, 100 cc. of absolute chloroform, 1.2 g. of anhydrous sodium carbonate and 8 cc. of water. The solvent was removed at 25–30° *in vacuo*. A filtered solution of the residual oil in 20 cc. of water was made slightly acid (litmus paper) by addition of hydrochloric acid which precipitated 0.8 g. of starting compound. The addition of more acid to the filtrate precipitated a sirup. The decanted aqueous layer was extracted with two 10-cc. portions of ethyl acetate which, after being combined with an ethyl acetate solution of the sirup, deposited at 25° 0.1 g. of starting compound. After removal of the solvent at 25° the sirup was stirred with 2% hydrochloric acid to yield 1.2 g. of crystals. The compound crystallized, slowly the first time, as rosettes of colorless short blades from its solution in ethyl acetate–petroleum ether; m.p. 134–135° (uncor.); $[\alpha]^{20}D$ +29.4° in methanol (*c* 0.83).

Anal. Calcd. for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.07; N, 3.71; S, 8.50. Found: C, 57.38; H, 5.00; N, 3.79; S, 8.31.

(2) E. Fischer and W. Schrauth, *Ann.*, **354**, 34 (1907).

(3) E. Fischer, *Ber.*, **37**, 2495 (1904).

O-*p*-Toluenesulfonyl-N-benzoyl-L-tyrosine.—O-*p*-Toluenesulfonyl-L-tyrosine (1.5 g.) was benzoylated by the method of Fischer,[4] using 1.9 g. of benzoyl chloride, 3 g. of sodium bicarbonate and 40 cc. of water. After the crystalline product had been extracted thoroughly with petroleum ether, it was recrystallized as colorless, hexagonal plates from acetone–petroleum ether; yield 0.9 g.; m.p. 194–195° (uncor.); $[\alpha]^{20}D$ −1.3° (*c* 2.61) in water containing 1.1 molecular equivalents of sodium hydroxide.

Anal. Calcd. for $C_{23}H_{21}NO_6S$: C, 62.85; H, 4.82; N, 3.19; S, 7.30. Found: C, 63.02; H, 5.10; N, 3.12; S, 7.39.

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(4) E. Fischer, *ibid.*, **32**, 2454 (1899).

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Some N-(β-Substituted Ethyl)-N,N-dibenzylamines

BY IRVING ALLAN KAYE AND HERMAN HORN

The importance of the dibenzylamino residue as a contributor to the activity of Dibenamine[1] [N-(2-chloroethyl)-dibenzylamine] hydrochloride, a compound commercially available as a potent and specific adrenergic blocking agent,[2] suggested the preparation of several N,N-dibenzyl-N′-aralkyl-N′-heterocyclic-ethylenediamines for pharmacological screening tests. The products, structurally related to several histamine antagonists on the market,[3] were prepared by alkylating an N-aralkyl-N-heterocyclicamine with 2-dibenzylaminoethyl chloride hydrochloride in the presence of lithium amide. The benzohydryl ether of 2-dibenzylaminoethanol (an analog of Benadryl[4]),

$$R{-}NH{-}CH_2R' + LiNH_2 + (C_6H_5CH_2)_2NCH_2CH_2Cl\cdot HCl \longrightarrow R{-}N(CH_2R'){-}CH_2CH_2N(CH_2C_6H_5)_2$$

R = 2-pyridyl, 2-pyrimidyl, 2-thiazolyl, or 2-lepidyl

R′ = phenyl, *p*-methoxyphenyl or *p*-chlorophenyl

also synthesized, was obtained by heating the benzohydryl ether of ethylene chlorohydrin with dibenzylamine.

$$(C_6H_5)_2CHOCH_2CH_2Cl + 2(C_6H_5CH_2)_2NH \longrightarrow (C_6H_5)_2CHO{-}CH_2CH_2N(CH_2C_6H_5)_2 + (C_6H_5CH_2)_2NH\cdot HCl$$

Three of the products, N,N-dibenzyl-N′-(benzyl and *p*-chlorobenzyl)-N′-(2-pyridyl)-ethylenediamines and N,N-dibenzyl-N′-benzyl-N′-(2-thiazolyl)-ethylenediamine, tested against histamine using the isolated guinea pig ileum strip, showed less than 0.1% of the activity of Pyribenzamine.[5a,6] None of these compounds showed any evidence of

(1) Trademark of Smith, Kline and French Laboratories.

(2) W. S. Gump and E. J. Nikawitz, THIS JOURNAL, **72**, 1309 (1950); J. F. Kerwin, T. F. Herdegen, R. Y. Heisler and G. E. Ullyot, *ibid.*, **72**, 940 (1950).

(3) B. Idson, *Chem. Revs.*, **47**, 377 (1950).

(4) Trademark of Parke Davis & Co.

(5) The authors wish to thank (a) Dr. Harold Blumberg and Mr. Eric Meyer of Endo Products, Inc., and (b) Dr. C. Chester Stock of The Sloan-Kettering Institute for Cancer Research for this information.

(6) "Pyribenzamine" is the trademark of Ciba Pharmaceutical Products, Inc., for N,N-dimethyl-N′-benzyl-N′-(2-pyridyl)-ethylenediamine.