

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, DEPARTMENT OF SURGERY OF BETH ISRAEL HOSPITAL AND HARVARD MEDICAL SCHOOL]

Synthesis of Naphthalene and Quinoline Derivatives Related to Acetylcholine¹

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Hydrolytic intracellular enzymes have been demonstrated histochemically by the use of synthetic substrates which yield naphthols after enzymatic hydrolysis. Incubation of tissue sections in a solution of the substrate at appropriate pH and in the presence of an appropriate diazonium salt resulted in the production by coupling of an insoluble azo dye at the site of enzymatic activity. In this way methods for alkaline phosphatase,² acid phosphatase,³ esterase,⁴ sulfatase⁵ and β -glucuronidase⁵ were developed. In order to develop a method for the histochemical demonstration of acetylcholinesterase (nerve enzyme) it was necessary to synthesize a substrate sufficiently similar in structure to acetylcholine (I) to be hydrolyzed specifically by this enzyme. Furthermore, it was necessary that the substrate be colorless and slightly soluble in water, possess a slow rate of hydrolysis at neutral pH, and produce an insoluble azo dye with good pigment quality, by coupling with an appropriate diazonium salt after enzymatic hydrolysis.

Quinoline derivatives were prepared which contained the carbon-nitrogen skeleton of acetylcholine and an acetoxy group on carbon beta to a quaternary ammonium ion. 3-Acetoxy-1-methylquinolinium chloride (II) proved to be unsatisfactory because 3-hydroxy-1-methylquinolinium chloride would not couple with tetrazotized diorthoanisidine. 8-Acetoxy-1-methylquinolinium chloride (III) was readily hydrolyzed by fresh brain tissue,⁶ but the rate of spontaneous hydrolysis at pH above 6.8 was so rapid that a satisfactory histochemical method could not be evolved.⁶ A suitable substrate for acetylcholinesterase was found in 2-acetoxy-3-dimethylaminonaphthalene methiodide (X). Its rate of hydrolysis at neutrality was slow compared to the rapid hydrolysis in the presence of fresh brain tissue.⁶ The hydrolysis product, 2-hydroxy-3-dimethylaminonaphthalene methiodide (VII) coupled with tetrazotized diorthoanisidine⁷ to

form an insoluble purplish-blue pigment. The specificity for acetylcholinesterase was tested by preparation of the propionoxy derivative (XII). This was found to be hydrolyzed less readily by brain tissue⁶ than the acetoxy analog.

2-Acetoxy-3-dimethylaminonaphthalene methiodide (X) was obtained from 2-hydroxy-3-aminonaphthalene (VI), prepared by a modification of the method of Fries.⁸ He reported initially that 2-hydroxy-3-isocyanatonaphthalene (IV) was isolated in the Curtius rearrangement of the azide of 2-hydroxy-3-naphthoic acid. However, 2-hydroxy-3-isocyanatonaphthalene (IV) would be expected to couple with diazonium salts. Since this reaction failed to occur, it was considered probable that internal cyclization had occurred to form β,β -naphthoxazolone (V). An analogous reaction has been reported in the production of benzoxazolone by a Curtius rearrangement of salicylic acid azide.⁹ Fries, Walter and Schilling¹⁰ later corrected their original statement and mentioned the naphthoxazolone without giving any details. The naphthoxazolone could be hydrolyzed to 2-hydroxy-3-aminonaphthalene (VI), in yields better than reported⁸ by prolonged heating with alkali. N-Methylation (VII) with methyl iodide and anhydrous sodium acetate proceeded in good yield. In the presence of sodium carbonate, the hydroxyl group was methylated (XIII) as well. This is probably due to the increased acidity conferred by quaternary nitrogen on the ortho hydroxyl group. For the same reason, difficulties were experienced in the acetylation of 2-hydroxy-3-dimethylaminonaphthalene methiodide (VII). After conversion to the sodium salt (VIII), it could be made to react with acetic anhydride to give 2-acetoxy-3-dimethylaminonaphthalene (IX), although the elements of methyl iodide were lost. Similarly, sodium acetate in the presence of acetyl chloride and glacial acetic acid demethylated 2-hydroxy-3-dimethylaminonaphthalene methiodide (VII) to 2-hydroxy-3-dimethylaminonaphthalene hydrochloride (XIV). Remethylation of the nitrogen in IX without deacetylation was difficult owing to the instability of the acetoxy group neighboring a quaternary nitrogen in the product formed (X). When the reaction was conducted in boiling anhydrous methanol, deacetylation occurred. 2-Acetoxy-3-dimethylaminonaphthalene methio-

5% zinc chloride and 20% aluminum sulfate, under the name of Naphthanil Diazo Blue B. It was provided through the courtesy of Dr. E. R. Laughlin, du Pont de Nemours and Company, Chicago, Illinois.

(8) Fries, *Ber.*, **56**, 2845 (1925).

(9) Stoermer, *ibid.*, **42**, 3133 (1909).

(10) Fries, Walter and Schilling, *Ann.*, **516**, 248 (1935), footnote p.

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(2) (a) Menten, Junge and Green, *J. Biol. Chem.*, **138**, 471 (1944); (b) Manheimer and Seligman, *J. Nat. Cancer Inst.*, **9**, 181 (1948).

(3) Seligman and Manheimer, *ibid.*, **9**, 427 (1948).

(4) Nachlas and Seligman, *ibid.*, **9**, 415 (1948).

(5) Seligman and Nachlas, to be published.

(6) Seligman, unpublished observations.

(7) Available commercially as a powder (20%) stabilized with

Anal. Calcd. for $C_{14}H_{18}NOI$: C, 48.98; H, 5.29. Found: C, 48.94; H, 5.41.

2-Hydroxy-3-dimethylaminonaphthalene Methiodide (VII).—This compound was prepared in moderate yield by heating hydroxyaminonaphthalene in a sealed tube at 100° for eighteen hours with methyl iodide and a small amount of methanol. It was obtained in nearly quantitative yield by dissolving 1 g. of hydroxyaminonaphthalene in 30 cc. of methanol and boiling under reflux for twenty-four hours with 1.1 g. of anhydrous sodium acetate and 2.5 cc. of methyl iodide. The solution was then concentrated, whereupon the desired compound crystallized. It was recrystallized from absolute alcohol and obtained in colorless prisms, m. p. 200° (dec.), 1.95 g. (94%). The compound was soluble in water and coupled with tetrazotized di-*o*-anisidine to give an insoluble, purplish-blue precipitate. The picrate crystallized from alcohol in yellow prisms, m. p. 260° (dec.).

Anal. Calcd. for $C_{12}H_{16}ON \cdot C_6H_5O_7N_3$: C, 52.90; H, 4.43. Found: C, 53.25; H, 4.14.

2-Acetoxy-3-dimethylaminonaphthalene (IX).—2-Hydroxy-3-dimethylaminonaphthalene methiodide (5 g.) was dissolved in 15 cc. of water, one equivalent (7.5 cc.) of 2 *N* sodium hydroxide solution was added and the water was removed under reduced pressure at 100° . The solid which crystallized was the sodium salt of hydroxydimethylaminonaphthalene methiodide. That ethyl iodide was not lost was shown by its solubility in water after neutralization. The sodium salt was then boiled under reflux for fifteen minutes with 12.5 cc. of acetic anhydride. The solvent was removed under reduced pressure at 100° and the residue treated with hot benzene to extract the 2-acetoxy-3-dimethylaminonaphthalene (IX) from the residual sodium salts. On removal of the benzene, the oily residue crystallized and could be recrystallized from 85% alcohol in colorless prisms, m. p. 92° , 2.4 g. (69%). The compound was soluble in dilute acid from which it was precipitated by base. The picrate crystallized from alcohol in yellow needles, m. p. 179° (dec.).

Anal. Calcd. for $C_{14}H_{18}O_2N \cdot C_6H_5O_7N_3$: C, 52.36; H, 3.95. Found: C, 51.96; H, 3.51.

Other methods for the acetylation of hydroxydimethylaminonaphthalene methiodide were tried without success. These included heating the hydroxy-compound in glacial acetic acid with acetyl chloride with or without pyridine, heating in acetic anhydride with pyridine or concentrated sulfuric acid, and heating the sodium salt with acetyl chloride. Acetoxydimethylaminonaphthalene (IX), when refluxed in methanol with methyl iodide for eight hours, is hydrolyzed and gives hydroxydimethylaminonaphthalene methiodide (VII) in almost quantitative yield.

2-Hydroxy-3-dimethylaminonaphthalene Hydrochloride (XIV).—This compound was obtained in an attempt to acetylate hydroxydimethylaminonaphthalene methiodide (VII). One gram of the latter compound was dissolved in 10 cc. of glacial acetic acid and boiled under reflux for one hour with one equivalent (0.35 g.) of acetyl chloride and 0.35 g. of anhydrous sodium acetate. The precipitated sodium chloride was removed by filtration and the acetic acid evaporated under reduced pressure at 100° . The residue was recrystallized from chloroform m. p. 214° (with decomposition). The salt is soluble in water, is precipitated by bicarbonate and redissolved by sodium hydroxide solution.

Anal. Calcd. for $C_{12}H_{14}ONCl$: C, 64.42; H, 6.30. Found: C, 64.40; H, 6.41.

2-Acetoxy-3-dimethylaminonaphthalene Methiodide (X).—Acetoxydimethylaminonaphthalene (IX) (4 g.) was dissolved in 10 cc. of benzene and allowed to stand at room temperature with 6.8 cc. of methyl iodide for four to five days. Colorless rhombic prisms appeared and were collected by filtration; 5.1 g. (78.5%). An almost quantitative yield could be obtained if a reaction period of ten to twelve days was allowed. All attempts to speed up the reaction failed; methylation in a sealed tube at 100° or in boiling benzene or ethyl acetate were unsuccessful. The compound was recrystallized from chloroform by the addition of ethyl acetate, m. p. 154° (dec.).

Anal. Calcd. for $C_{15}H_{18}O_2NI$: C, 48.52; H, 4.88. Found: C, 48.38; H, 5.05.

The picrate crystallized from alcohol in orange prisms, m. p. 184° .

Acetoxydimethylaminonaphthalene methiodide hydrolyzed slowly to hydroxydimethylaminonaphthalene methiodide in cold water and hydrolyzed rapidly in hot water or in bicarbonate solution. Acetoxydimethylaminonaphthalene could not be made to react with ethyl iodide, butyl iodide or benzyl iodide in benzene solution either at room temperature or in boiling benzene.

2-Propionoxy-3-dimethylaminonaphthalene (XI).—Hydroxydimethylaminonaphthalene methiodide (2 g.) was converted into the sodium salt as described in the preparation of IX. The sodium salt was then boiled under reflux for thirty minutes with 10 cc. of propionic anhydride. The solvent was evaporated under reduced pressure at 100° , the residue was treated with benzene, sodium salts were removed by filtration and the filtrate was evaporated to dryness. The residual oil crystallized and was recrystallized from 80% alcohol, m. p. 72° , 1.2 g. (81%). The picrate crystallized from alcohol in long, yellow needles, m. p. 200° (dec.).

Anal. Calcd. $C_{15}H_{17}O_2N \cdot C_6H_5O_7N_3$: C, 53.39; H, 4.17. Found: C, 53.58; H, 4.04.

2-Propionoxy-3-dimethylaminonaphthalene Methiodide (XII).—One gram of propionoxydimethylaminonaphthalene (XI) was dissolved in 5 cc. of benzene to which 1 cc. of methyl iodide was added. After standing at room temperature for two days, long prismatic needles separated. They were recrystallized from a mixture of benzene and alcohol, m. p. 176° (dec.). A nearly quantitative yield was obtained after standing four to five days.

Anal. Calcd. for $C_{16}H_{20}O_2NI$: C, 49.89; H, 5.20. Found: C, 50.20; H, 5.35.

Hydroxydimethylaminonaphthalene methiodide was obtained from this compound by hydrolysis which proceeded slowly in cold water and rapidly in hot water or in bicarbonate solution.

Summary

The synthesis of 3-acetoxy-1-methylquinolinium chloride, 8-acetoxy-1-methylquinolinium chloride, 2-propionoxy-3-dimethylaminonaphthalene methiodide and 2-acetoxy-3-dimethylamino-naphthalene methiodide is described. The last named compound appears to possess very favorable properties on which to base a method for the histochemical demonstration of acetylcholinesterase.

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