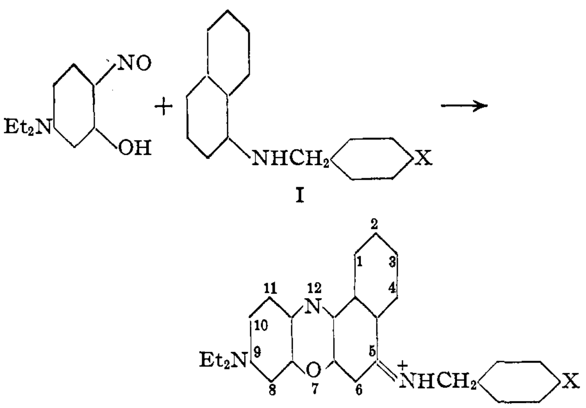
[CONTRIBUTION FROM THE HARRISON DEPARTMENT OF SURGICAL RESEARCH, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA, AND THE DEPARTMENT OF NEUROSURGERY, HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA]

Halogenated Benzophenoxazine Dyes[1,2]

BY HENRY A. SLOVITER

The discovery[3] that certain oxazine dyes stain and inhibit the growth of cancer cells when administered to living animals stimulated the investigation of related compounds. Since these oxazine dyes showed some selective affinity for tumor tissue, the present work was undertaken to prepare derivatives which might ultimately be used as "carriers" of radioactivity when radioactive isotopes were incorporated in the molecule. The halogens were selected as the intended radioactive isotopes because of their availability and because their emission characteristics are suitable for the intended biological studies.

Since one of these dyes, 5-benzylamino-9-diethylaminobenzo[a]phenoxazine[4] (II, X = H) was found to have optimal physiologic activity, its halogen derivatives (II, X = Cl, Br, I) were prepared by the reaction

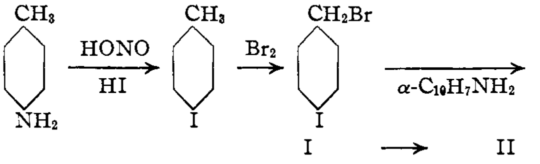


II

The N-(p-halobenzyl)-α-naphthylamines (I) have not been described previously. They were prepared by treating the proper p-halobenzyl halide with a large excess of α-naphthylamine in a manner similar to that described[5] for the preparation of the N-(nitrobenzyl)-α-naphthylamines.

The preparation of p-iodobenzyl bromide[6,7] by the bromination of p-iodotoluene without a solvent was found to give the product in poor yield and accompanied by considerable tarry matter. Good yields of pure p-iodobenzyl bromide were obtained by the modification of a procedure[8] used for the study of the photochemical bromination of p-iodotoluene in carbon tetrachloride.

In a complete synthesis of II where X is radioactive iodine, the sequence of reactions is



This synthesis has been successfully carried out and has yielded a product whose radioactivity is of the order of 0.5 millicurie per gram. The details of this preparation will be described in a subsequent report.

Since this synthesis involves carrying the radioactive iodine through four steps, an attempt was made to develop a shorter method of preparation. Accordingly, the preparation of I (X = Iodine) was attempted, without success, by replacement with iodine of the amino group of N-(p-aminobenzyl)-α-naphthylamine. The hydrochloride of the latter compound was prepared as described previously[5] by reduction of N-(p-nitrobenzyl)-α-naphthylamine. The product of this reduction on treatment with nitrous acid and iodide did not yield the desired N-(p-iodobenzyl)-α-naphthylamine.

An attempt was also made to prepare a benzo[a]phenoxazine dye containing a halogen atom in the ring structure. The reaction of 5-bromo-1-naphthylamine with 2-nitroso-5-diethylaminophenol which might have been expected to yield 1-bromo-5-amino-9-diethylaminobenzo[a]phenoxazine resulted in a substance which was apparently not a benzophenoxazine compound.

N-Allyl-α-naphthylamine was prepared with the intent of adding two atoms of iodine to obtain N-(2,3-diiodopropyl)-α-naphthylamine, from which the corresponding benzophenoxazine dye containing two atoms of iodine per molecule could be prepared. Thus far, N-(2,3-diiodopropyl)-α-naphthylamine has not been successfully isolated. However, 5-allyl-9-diethylaminobenzo[a]phenoxazine has been prepared.

Experimental

p-Iodobenzyl Bromide.—Sixteen grams (5.4 ml.) of bromine in 50 ml. of pure carbon tetrachloride was added to 19 g. of freshly distilled p-iodotoluene (m. p. 35.0–35.3°) in 50 ml. of carbon tetrachloride. The flask was attached to an efficient reflux condenser and then illumi-

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[1] Presented at the Meeting-in-Miniature of the Philadelphia Section, American Chemical Society, January 20, 1949.

[2] Aided in part by a grant to Doctor Margaret Lewis from the National Cancer Institute.

[3] Lewis, Sloviter and Goland, *Anatomical Record*, **95**, 89 (1946).

[4] The nomenclature is that of the Ring Index. This dye is known commercially as Nile blue 2B; number 914 in the Colour Index, Society of Dyers and Colourists, Great Britain, 1924.

[5] Darier and Mannassewitch, *Bull. soc. chim.*, [3] **27**, 1055 (1902).

[6] Wheeler and Clapp, *Am. Chem. J.*, **40**, 460 (1908).

[7] Jackson and Mabery, *ibid.*, **2**, 250 (1880).

[8] Sampey, Fawcett and Morehead, THIS JOURNAL, **62**, 1839 (1940).

TABLE I

N-SUBSTITUTED-α-NAPHTHYLAMINES

N-Substituent	Halide used	Formula	Yield, %	M. p., °C.	B. p., 4 mm., °C.	Halogen, % Calcd.	Halogen, % Found
p-Chlorobenzyl	Chloride	$C_{17}H_{14}NCl$	56	76.0–76.5	184–188	13.26	13.39[b]
p-Bromobenzyl	Bromide	$C_{17}H_{14}NBr$	62	88.5–89.0	190–195	25.62	25.69[b]
p-Iodobenzyl	Bromide	$C_{17}H_{14}NI$	70	84.5–85.0	200–205	35.36	35.73[c]
Allyl[a]	Chloride	$C_{13}H_{13}N$	95		110–120	[d]	[d]

[a] Distills as colorless liquid, rapidly becomes light amber. [b] Parr bomb method. [c] Chromic–sulfuric acid digestion, gravimetric. [d] Calcd.: iodine no., 139; N, 7.65. Found: iodine no. (two minutes contact with Wijs soln.), 144; N, 7.74.

nated by two 200-watt clear Mazda lamps placed almost in contact with the flask. Heat was applied to maintain the mixture at gentle reflux whereupon copious evolution of hydrogen bromide took place. After two hours the evolution of hydrogen bromide had almost ceased, and no vapors of bromine were visible in the reaction flask. The heat and light were continued for thirty minutes more. The flask was cooled, and 5 g. of potassium iodide in 50 ml. of water was added. Sodium thiosulfate solution (approximately 0.1 *N*) was added in slight excess (about 80 ml.). The carbon tetrachloride layer was separated and washed with water. After removal of the carbon tetrachloride by distillation, the residue solidified rapidly on cooling to a clean white crystalline mass. Recrystallization from ethanol yielded 15 g. (60% yield) of clean white crystals, m. p. 78.5–79.5°. The *p*-bromobenzyl bromide was similarly prepared.

N-(*p*-Halobenzyl)-α-naphthylamines.—To a solution of 14.3 g. (0.1 mole) of α-naphthylamine in 50 ml. of ethanol was added a solution of 0.03 mole of *p*-halobenzyl halide in 50 ml. of ethanol. The solution was refluxed for two hours and the alcohol was removed by distillation. About 100 ml. of hot water was added to the viscous residue and, after swirling the mixture, 1:1 hydrochloric acid was added dropwise until the aqueous phase became clear. The supernatant liquid was removed by siphon. This procedure was repeated two times, and the residue was then washed with hot water. This procedure served to remove most of the excess α-naphthylamine. The residue was then cooled in an ice-bath, whereupon it hardened sufficiently so that remaining water could be drained off. It was then washed with about 10 ml. of cold ethanol to remove water adhering to the flask and residue. The residue was then recrystallized from ethanol. The product tended to separate as an oil but very slow cooling and seeding gave clean, almost white crystalline products. The amines could be distilled under reduced pressure prior to recrystallization. The yields thus obtained were somewhat lower and the products were white, but the melting points were the same in both cases.

The yields, properties and analyses of these amines are listed in Table I. The melting points shown are corrected values.

N-Allyl-α-naphthylamine.—This was prepared in the same manner as the *p*-halobenzyl-α-naphthylamines using 25 g. of α-naphthylamine and 5 ml. of allyl chloride. After removal of the excess α-naphthylamine, the crude product was dissolved in ether and washed with water. After removal of the ether, the product was distilled under reduced pressure.

5-(N-Substituted Amino)-9-diethylaminobenzo[a] phenoxazine Chloride.—A mixture of 0.02 mole of N-substituted-α-naphthylamine, 5 ml. of concentrated hydrochloric acid and 30 ml. of ethanol was warmed gently until a clear solution was obtained. To this was added a solution of 5.8 g. (0.03 mole) of 2-nitroso-5-diethylaminophenol[9] in 15 ml. of ethanol, and the mixture was boiled under reflux for three hours. After standing overnight, the mixture was cooled in ice, and the crystalline dye was filtered, washed with a small quantity of cold ethanol and finally with ether. The products were pure as shown by analysis (Table II) and adsorption chromatograms on paper. The dyes could be recrystallized in small batches from ethanol containing a trace of hydrochloric acid.

TABLE II

5-R-9-DIETHYLAMINOBENZO[a]PHENOXAZINE CHLORIDES

R	Formula	Yield, %		Analyses, % Calcd.	Analyses, % Found
p-Chlorobenzylamino	$C_{27}H_{25}ON_3Cl_2$	75	Cl	14.84	14.77[a]
p-Bromobenzylamino	$C_{27}H_{25}ON_3ClBr$	80	Br	15.30	15.08[b]
p-Iodobenzylamino	$C_{27}H_{25}ON_3ClI$	88	I	22.29	22.43[c]
Allylamino	$C_{23}H_{24}ON_3Cl$	40			

[a] Parr bomb method. [b] Robertson method followed by weighing of mixed silver halides and after conversion of silver bromide to silver chloride. [c] Chromic–sulfuric acid digestion, gravimetric.

Attempts to prepare the sulfates of these dyes always yielded a resinous product which could not be crystallized.

Reaction of N-(*p*-Aminobenzyl)-α-naphthylamine with Nitrous Acid and Iodide.—A suspension of 5 g. of N-(*p*-aminobenzyl)-α-naphthylamine dihydrochloride[5] in a mixture of 40 ml. of water and 5 ml. of 1:1 hydrochloric acid was kept below 5°, and a solution of 0.75 g. of sodium nitrite in 10 ml. of water was added dropwise until a slight excess was present (starch–iodide paper). A clear solution resulted. To this was added slowly a solution of 1.75 g. of potassium iodide in 10 ml. of water. A dark brown precipitate formed slowly which floated to the surface. The mixture was kept below 5° for two hours and allowed to warm to room temperature overnight. The mixture was filtered and washed with water. The very dark brown powdery product was not appreciably soluble in water, dilute hydrochloric acid, alcohol or acetone.

Attempted Preparation of 1-Bromo-5-amino-9-diethylaminobenzo[a]phenoxazine Chloride.—To a boiling solution of 2 g. of 5-bromo-1-naphthylamine[10] in a mixture of 15 ml. of glacial acetic acid, 10 ml. of water and 3 ml. of 1:1 hydrochloric acid was added 2.2 g. of 2-nitroso-5-diethylaminophenol in small portions. The mixture was kept near boiling for one hour during which time it became almost black in color. After standing overnight, it was filtered, washed with water until the washings were only faintly colored and allowed to dry. This almost black, granular product gave a blue solution in glacial acetic acid and a cherry red solution in xylene. It was insoluble in water and dilute hydrochloric acid.

Acknowledgment.—The author is indebted to Mr. Louis Goldberg for the preparation of several batches of 2-nitroso-5-diethylaminophenol and for assistance with the analyses, and to Dr. Milton H. Paul and Mr. Bernard Shapiro for assistance in the preparation of adsorption chromatograms.

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(9) Mohlau, *Ber.*, **25**, 1060 (1892), reports m. p. 84°; we consistently obtained m. p. 89–90°.

(10) Ullmann and Consonno, *ibid.*, **35**, 2804 (1902).

Summary

1. The 5-(*p*-halobenzylamino)-9-diethylaminobenzo[a]phenoxazine chlorides and corresponding 5-allylamino compound have been prepared. The intermediates, N-(*p*-halobenzyl)-α-naphthylamines and N-(allyl)-α-naphthylamine, have been prepared.

2. The attempted preparation of N-(*p*-iodobenzyl)-α-naphthylamine by treatment of N-(*p*-aminobenzyl)-α-naphthylamine with nitrous acid and iodide was unsuccessful.

3. The reaction of 5-bromo-1-naphthylamine with 2-nitroso-5-diethylaminophenol does not yield a benzophenoxazine dye.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE OHIO STATE UNIVERSITY]

The Reduction of Terminal Epoxides[1]

BY MELVIN S. NEWMAN, GERALD UNDERWOOD AND MARY RENOLL

Although the reduction of the epoxide function has been studied,[2] little systematic work has been done on the production of primary alcohols from terminal epoxides. The formation of *n*-propyl, *n*-butyl[3,4] and β-phenylethyl alcohols by catalytic reduction of the corresponding epoxide by hydrogen has been mentioned in the patent literature.

In this paper we report the successful reduction of 1,2-epoxydecane and of styrene oxide to the corresponding primary alcohols by catalytic methods. Reductions involving sodium amalgam and lithium aluminum hydride[5] gave mainly secondary alcohols and hence were not studied in detail.

TABLE I

REDUCTION OF 1,2-EPOXYDECANE AND STYRENE OXIDE

	Reaction conditions	*T*, °C.	Pr (p. s. i.)	$n^{20}\text{D}$[a]	Analyses,[b] % Epoxide	Sec. alc.	Pri. alc.
		1,2-Epoxydecane					
1	Raney nickel[c]	20	37	1.4292	+[d]		
2	Raney nickel[c] (large amount)[e]	20	15	1.4355			+[d]
3	Platinic oxide (Adams)	20	37	1.4289	+[d]		
4	Raney nickel[c]	150	900	1.4362	5	10	83[g]
5	Raney nickel[f]	150	900	1.4360	<5	<10	90
6	Raney nickel[c] + 0.01 g. NaOH	150	900	1.4353	5	10	85
7	Raney nickel[c] + 0.1 g. NaOH	150	900	1.4347	5	85	7.5[g]
8	Raney nickel[f] + 0.1 g. NaOH	150	900	1.4343	<5	95	<5
9	Raney nickel[c] + 0.1 g. H_3PO_4[h]	150	900	1.4359	<5	50	50[g]
10	Raney nickel[f] + 0.1 g. H_3PO_4[h]	150	1100[i]	1.4354	<5	50	50
11	Raney nickel[f]	25	1000	1.4298	+[d]		
12	Ni-on-kieselguhr (UOP)	200	1450	1.4358	5	10	85
13	Sodium amalgam				60	35	5
14	Lithium aluminum hydride				10	90	
Standards	1,2-Epoxydecane			1.4289			
	2-Decanol			1.4340			
	1-Decanol			1.4373			
		Styrene Oxide					
15	Raney nickel[c] and [f]	25	700	1.5309	0	0	100[g]
16	Raney nickel[c] and [f] + 0.1 g. NaOH	25	700	1.5317	0	0	100[g]
17	Raney nickel[c] + 0.1 g. H_3PO_4	25	700	1.5299	41[j]	0	75[h]
18	Raney nickel[f] + 0.1 g. H_3PO_4	25	700	1.5295	40	0	60[h]
19	Raney nickel[c]	150	700	1.4770[k]			

[a] The indices of refraction are those of the crude distillate obtained directly from the hydrogenation experiments. [b] The analytical results reported were carried out by Dr. J. J. Shipman of the B. F. Goodrich Co. using an infrared spectrophotometer. Calibration curves for pure 1-decanol, 2-decanol and 1,2-epoxydecane were obtained and synthetic

Our results are summarized in Table I. Of particular interest is the effect of small amounts of acidic or basic substances on the course of the reduction of 1,2-epoxydecane but *not* of styrene oxide. In the absence of such additives reduction

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[1] The work herein reported was carried out during 1946 and 1947 on research project 162 of the Ohio State University Research Foundation and was sponsored by the B. F. Goodrich Company of Akron, Ohio.

[2] Grignard, "Trâité de chime organique," Vol. VI, Masson et Cie, Paris, 1940, p. 286.

[3] Usines de Melle and H. M. E. Guinot, British Patent 496,264 (1938).

[4] I. G., German Patent 573,535 (1933).

[5] Nystrom and Brown, THIS JOURNAL, **69**, 1197 (1947); Finholt, Bond and Schlesinger, *ibid.*, **69**, 1199 (1947).