After drying for several hours at 100°, this analyzed as the monohydrate. The analytical data for this compound are recorded in Table II D.

Method B.—To a solution of 219 g. (1.0 mole) of 1-(N-phenyl)-naphthylamine in 2000 ml. of ethanol was added 388 g. (1.5 moles) of 5-di-*n*-propylamino-2-nitrosophenol hydrochloride[2] and several drops of concd. hydrochloric acid. The mixture was heated to the reflux temperature with stirring for seven hours and then cooled to room temperature. After diluting with an equal volume of ethanol, the solution was treated with an excess of concd. aqueous ammonium hydroxide which caused precipitation of the base as a slightly tacky material which congealed to a dark green solid after standing for a short time. This was suspended in just sufficient ethanol to completely cover the material and a small excess of concd. nitric acid was added slowly with agitation, causing the formation of the solid nitrate. After removal by filtration, the product was recrystallized from ethanol, giving 260 g. (57%) of glistening green nitrate. This was identical with the product obtained above by Method A.

9-(*n*-Butyl-*n*-propylamino)-5-(N-methyl-N-4-methylphenylamino)-benzo[a]phenoxazonium Nitrate.—A mixture of 22.2 g. (0.05 mole) of 9-(*n*-butyl-*n*-propylamino)-benzo[a]-phenoxazonium nitrate, 18.2 g. (0.15 mole) of N-methyl-4-toluidine and 125 ml. of ethanol was warmed gently to give a clear solution. A fine stream of air was bubbled through the solution for 18 hours during which time a green crystalline product separated. Filtration gave 6.3 g. (25%) of green crystals which were recrystallized from 250 ml. of ethanol. The wave length of maximum absorption of the nitrate in ethanol solution was 664 mμ. A solution of the base in ethanol showed maximum absorption at 534 mμ.

Anal. Calcd. for $C_{31}H_{34}N_4O_4 \cdot H_2O$: C, 68.4; H, 6.66; N, 10.3. Found: C, 68.1; H, 6.35; N, 10.5.

9-Di-*n*-propylamino-5-(N-ethyl-N-phenylamino)-benzo[a]-phenoxazonium Nitrate.—A mixture of 19.7 g. (0.05 mole) of 9-di-*n*-propylaminobenzo[a]phenoxazonium nitrate, 18.2 g. (0.15 mole) of N-ethylaniline and 125 ml. of ethanol was warmed gently until the reactants dissolved. A fine stream of air was bubbled through the solution for four hours and the solution was then allowed to stand in an open beaker at room temperature for several days. The color of the solution gradually changed from reddish-blue to a greenish-blue. Addition of water caused precipitation of an oil which upon stirring repeatedly with diethyl ether congealed to a dark solid, 14.8 g. (58%). Addition of ammonium hydroxide to an ethanol solution of the crude nitrate caused precipitation of a brown base which was reconverted to the purified nitrate by rubbing up with dilute nitric acid. The absorption maxima of the salt and the base in ethanol solution were found to be at 659 and 531 mμ, respectively. This product was given an antituberculous rating of 2+.

Anal. Calcd. for $C_{30}H_{32}N_4O_4$: C, 70.3; H, 6.29; N, 10.9. Found: C, 70.4; H, 6.17; N, 10.8.

Spectrophotometric Determinations.—These were carried out in ethanol solution as described in paper I of this series.[2]

Acknowledgments.—We are grateful to Dr. M. E. Hultquist and Dr. J. J. Denton for their interest and coöperation on this project and we acknowledge the assistance of Mr. H. L. Komarowski in preparing some of these compounds. We are indebted to Mr. O. E. Sundberg and associates for the microanalyses and to Mr. F. C. Dexter and Miss M. R. O'Rourke for the spectrophotometric determinations.

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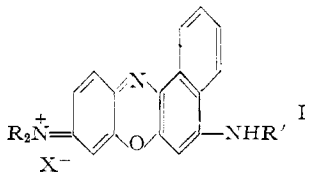
[CONTRIBUTION FROM THE CHEMICAL RESEARCH DEPARTMENT OF THE CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Chemotherapeutic Dyes. III. 5-Heterocyclicamino-9-dialkylaminobenzo[a]phenoxazines[1]

BY MOSES L. CROSSLEY, CORRIS M. HOFMANN AND PAUL F. DREISBACH

In order to study the effect on tumor growth-retardation and antituberculous action caused by introduction of a heterocyclicamino group in the benzo[a]phenoxazines, a series of 9-dialkylamino-5-heterocyclicaminobenzo[a]phenoxazines was prepared. The method of preparation involved amination of 9-dialkylaminobenzo[a]phenoxazines with various amino-heterocycles. None of the compounds of this series possessed significant activity.

In the two preceding papers in this series,[2,3] there are described the preparation and properties of a number of benzo[a]phenoxazine dyes of the general formula, I, in which R designates alkyl, R′



I

aralkyl or aryl and X the anion of a salt. Some of these compounds exhibited strong antituberculous activity in mice and showed differential staining and growth-retarding effects on transplanted tumor tissue in mice.

In view of the marked changes in activity that were noted when various arylamino and aralkylamino groups were substituted in the 5-position of the benzo[a]phenoxazine nucleus, it was considered important to investigate other structural modifications. The introduction of a heterocyclic group in many systems has been shown to modify the pharmacological activity of the parent compound, a striking example of this being in the sulfanilamide series.

Therefore, a series of 9-dialkylaminobenzo[a]-phenoxazines was prepared which contained a 5-heterocyclicamino group. These compounds were obtained by a procedure similar to that described in paper II of this series[3] for the preparation of the related 5-arylamino derivatives. The procedure involved reaction of a 9-dialkylaminobenzo[a]phenoxazonium nitrate with an excess of the aminoheterocycle in ethanol solution. The pK_a values of the aminoheterocycles used in this series fall in the range of 3 to 7, which is the range of pK_a values of the aromatic amines used successfully in the preparation of similar aryl derivatives. When more basic heterocyclic amines such as piperidine were

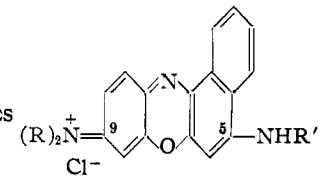
(1) Presented before the Division of Medicinal Chemistry at the American Chemical Society Meeting in Cleveland, Ohio, April 8th to 11th, 1951.

(2) M. L. Crossley, P. F. Dreisbach, C. M. Hofmann and R. P. Parker, THIS JOURNAL, **74**, 573 (1952).

(3) M. L. Crossley, R. J. Turner, C. M. Hofmann, P. F. Dreisbach and R. P. Parker, *ibid.*, **74**, 578 (1952).

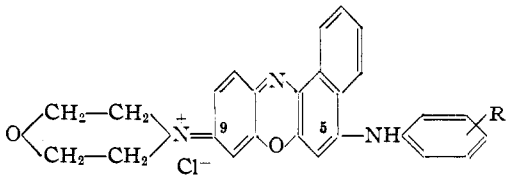
TABLE I

A. SUBSTITUTED 5,9-DIAMINOBENZO[a]PHENOXAZONIUM CHLORIDES



R	R'	Yield, %	Abs. max., mμ Base	Salt	Empirical formula	Analyses, % Carbon Calcd.	Found	Hydrogen Calcd.	Found	Nitrogen Calcd.	Found	Chlorine Calcd.	Found	Anti-Tb activity
Ethyl	2-Pyridyl	25	542	674	$C_{26}H_{23}ClN_4O$	69.6	69.5	5.38	5.43	13.0	13.2	8.23	8.07	–
Ethyl	2-Pyrimidyl	25	550	668	$C_{24}H_{21}N_5O$[a]	72.9	72.7	5.35	5.50	17.7	17.7			±
Ethyl	2-Pyrazinyl	50	558	675	$C_{24}H_{22}ClN_5O$	66.7	67.0	5.13	5.08	16.2	16.2	8.21	8.26	+
Ethyl	2-Thiazolyl	45	576	684	$C_{23}H_{21}ClN_4OS\cdot H_2O$[b]	60.7	61.2	5.10	5.08	12.3	12.3	7.79	7.98	– ?
n-Propyl	2-Pyridyl	80	543	678	$C_{27}H_{27}N_5O_4$[c]	66.8	67.0	5.61	5.84	14.4	14.3			+
n-Propyl	2-Pyrazinyl	32	561	678	$C_{26}H_{26}N_6O_4$[c]	64.2	64.3	5.39	5.46	17.3	17.3			– ?
n-Butyl	2-Pyridyl	63	544	678	$C_{29}H_{31}N_5O_4$[c]	67.8	67.8	6.09	6.15	13.6	13.4			+

B. SUBSTITUTED 5,9-DIAMINOBENZO[a]PHENOXAZONIUM CHLORIDES



H	60	495	648	$C_{26}H_{22}ClN_5O_2\cdot{}^1/_2H_2O$	68.9	68.6	5.12	4.92	9.28	9.35	7.83	7.28	–
4-CH_3	53	502	648	$C_{27}H_{24}ClN_5O_2\cdot 2H_2O$	65.7	65.7	5.71	5.75	8.51	8.71	7.18	7.28	– ?

[a] Base. [b] Calcd. S, 7.05. Found: S, 6.75. [c] Nitrate.

employed in the reaction, the corresponding 9-dialkylaminobenzo[a]phenoxazone-5 was obtained. This is in accord with observations noted previously[2,3] that strong bases lead to the formation of hydroxyl ion which reacts with the postulated carbonium ion centered in the 5-position.

Morpholine, when employed in reaction with 9-dimethylaminobenzo[a]phenoxazine, gave an anomalous product, the analysis of which indicated the desired compound as a chloride hydrochloride hemihydrate. Its absorption maxima in the visual range differed from those of other substituted 5-amino derivatives, that of the base form being at a decidedly lower wave length. In view of these facts, it cannot be stated definitely that the morpholine derivative was the product indicated.

Two related heterocyclic derivatives were prepared wherein the nitrogen in the 9-position itself formed part of a ring substituent. For this preparation, N-(4-nitrosophenyl)-morpholine was condensed with 2-naphthol according to the procedure described in paper II of this series[3] for the preparation of the 9-dialkylaminobenzo[a]phenoxazines. The 9-(4-morpholinyl)-benzo[a]phenoxazine was aminated with aniline and 4-toluidine under the conditions generally used giving the corresponding 5-phenylamino- and 5-(4-methylphenylamino)-derivatives.

To note the effect of substitution of oxygen for nitrogen in the 9-position of these benzo[a]phenoxazines, the 5-amino and 5-phenylamino derivatives of 9-hydroxy-benzo[a]phenoxazine were prepared, the latter by treatment of 4-nitroso-1,3-resorcinol with 1-(N-phenyl)-naphthylamine which reaction is similar to Method B in paper II of this series.[3]

The benzo[a]phenoxazine derivatives are listed in Table I.

Some members of the present series of compounds were isolated and purified as nitrate salts and tested pharmacologically as such, while in other cases, the nitrates were converted through the base form to the chloride salts for purification and testing purposes. The absorption maxima were determined in the visual range for the salt and base forms in ethanol solution and used for estimation of purity. The bases were generally not isolated.

Pharmacological Activity

Tumor Staining and Growth-retarding Action.—Details of the testing results on a number of these compounds have been reported by Dr. M. R. Lewis and associates,[4] to whom we are indebted for information on the activity of these compounds when administered orally to mice bearing transplanted tumors. Those members of the series possessing a 9-diethylamino group showed a definite growth-retarding effect on the tumor transplants, the most active compound in this respect being the 9-diethylamino-5-(2-pyridylamino)-benzo[a]phenoxazine derivative. The 9-di-*n*-butylamino homolog stained the tumor tissue but showed no appreciable growth retarding effect. None of the other derivatives listed in the table showed any significant staining or growth-retarding action.

Antituberculous Action.—The rating system used for antituberculous activity here is the same as that described in papers I and II in this series.[2,3] Although a slight activity was shown by three of the products listed in the table, no significant antituberculous activity was found in any of the 5-heterocyclicamino derivatives. We are indebted to Dr. H. J. White and co-workers at the Stamford Laboratories of the American Cyanamid Co. for the antituberculous testing results on these compounds. The testing procedure has been de-

(4) M. R. Lewis, P. P. Goland and H. A. Sloviter, *Cancer Research*, **9**, 736 (1949).

scribed[5] and the details of the testing results will be published elsewhere.[6]

Experimental

9-Dialkylamino-5-heterocyclicaminobenzo[a]phenoxazines.—The procedure used for the preparation of these compounds was similar to that described under Method A in paper II in this series.[3] One equivalent of the 9-dialkylaminobenzo[a]phenoxazonium nitrate[7] was treated with three equivalents of the aminoheterocycle in ethanol solution to give the 5-heterocyclicamino derivative in the form of the nitrate salt. In some instances the products were isolated in the base form and occasionally the base was converted to the hydrochloride and this salt was analyzed. The procedure described below for the 5-(2-pyridylamino)-9-di-*n*-propylamino derivative is typical of these preparations.

9-Di-*n*-propylamino-5-(2-pyridylamino)-benzo[a]phenoxazonium Nitrate.—To a solution of 47 g. (0.12 mole) of 9-di-*n*-propylaminobenzo[a]phenoxazonium nitrate in 250 ml. of ethanol was added 34 g. (0.36 mole) of 2-aminopyridine. After warming gently for a few minutes, the solution was allowed to stand at room temperature in an open beaker for several days, during which time the product precipitated as a green crystalline solid with a metallic luster. Filtration gave 44 g. (80%) of crude product which was recrystallized from two liters of ethanol to give 33 g. of green solid. The analytical data for this compound are recorded in Table I.

N-(4-Nitrosophenyl)-morpholine.—This was prepared by nitrosation of N-phenylmorpholine according to Marckwald[8] in 57% yield. The crude hydrochloride was used in the condensation described in the following preparation. The base melted at 99–100°.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.5; H, 6.30; N, 14.6. Found: C, 62.7; H, 6.36; N, 14.5.

9-(4-Morpholinyl)-benzo[a]phenoxazonium Nitrate.—A solution of 79 g. (0.55 mole) of 2-naphthol, 42.2 g. (0.31 mole) of anhydrous zinc chloride and 550 ml. of ethanol was stirred and warmed to the reflux temperature for 30 minutes during which time 122.5 g. (0.55 mole) of N-(4-nitrosophenyl)-morpholine hydrochloride was added portionwise. After refluxing one hour more, the precipitated black solid zinc chloride double salt was removed by filtration of the hot mixture, giving 172.2 g. (74%) of product.

For conversion to the nitrate, the zinc chloride double salt was dissolved in 9750 ml. of hot water. Filtration removed a small amount of tacky insoluble material. After adding 430 ml. of concd. nitric acid to the cooled filtrate, the nitrate separated as a dark brown solid which was removed by filtration, giving 85.6 g. of product.

The crude nitrate was recrystallized from one liter of ethanol, giving 76.2 g. of purified 9-(4-morpholinyl)-benzo[a]phenoxazonium nitrate as a dark brown solid.

Anal. Calcd. for $C_{20}H_{17}N_3O_5$: C, 63.3; H, 4.52; N, 11.1. Found: C, 63.4; H, 4.52; N, 10.9.

9-(4-Morpholinyl)-5-phenylaminobenzo[a]phenoxazonium Chloride.—A mixture of 19 g. (0.05 mole) of 9-(4-morpholinyl)-benzo[a]phenoxazonium nitrate, 14 g. (0.15 mole) of aniline and 125 ml. of ethanol was warmed slightly for five minutes. After standing for several days at room temperature in an open beaker, the precipitated product was removed by filtration and converted to the base by stirring in 100 ml. of ethanol and adding 10 ml. of concd. ammonium hydroxide solution. The base precipitated as a red-brown solid. This was removed by filtration and converted to the chloride by slurrying in 50 ml. of ethanol and adding 3 ml. of concd. hydrochloric acid. The precipitated chloride was removed by filtration and recrystallized from 800 ml. of ethanol acidified with gaseous hydrogen chloride. The purified chloride was obtained as a reddish brown solid. The analytical data for this and the following preparation are found in Table I.

5-(4-Methylphenylamino)-9-(4-morpholinyl)-benzo[a]-phenoxazonium Chloride.—Amination of 19 g. (0.05 mole) of 9-(4-morpholinyl)-benzo[a]phenoxazonium nitrate with 16.1 g. (0.15 mole) of *p*-toluidine by the procedure described above for the 5-phenylamino derivative gave 12.1 g. (53%) of purified chloride.

9-Dimethylamino-5-(4-morpholinyl)-benzo[a]phenoxazonium Chloride.[9]—A solution of 33.8 g. (0.1 mole) of 9-dimethylaminobenzo[a]phenoxazonium nitrate (Meldola Blue Nitrate), 26 g. (0.3 mole) of morpholine and 250 ml. of ethanol was warmed slightly for ten minutes and then allowed to stand at room temperature for three days. A small amount of brown solid separated. This solid nitrate was converted to the base by stirring it in 100 ml. of water and adding 20 ml. of concd. ammonium hydroxide solution. The mixture was heated to 85° and the crude brown base was removed by filtration. It was then converted to the chloride by dissolving it in 100 ml. of hot ethanol and treating the solution with 20 ml. of concd. hydrochloric acid. After cooling, the chloride salt which had precipitated was recrystallized from 150 ml. of ethanol containing a few drops of hydrochloric acid, giving 5.5 g. (14%) of a Paris green solid which was soluble in ethanol, giving a greenish blue solution.

The maximum absorption of an ethanol solution of this salt was found to be at 667 mμ. The absorption maximum of an ethanol solution of the base obtained by the addition of a few drops of ammonium hydroxide solution to a solution of the salt was found to be at 420 mμ.

Anal. Calcd. for $C_{22}H_{22}ClN_3O_2 \cdot HCl \cdot {}^1/_2H_2O$: C, 59.9; H, 5.48; N, 9.52; Cl, 16.1. Found: C, 59.8; H, 5.91; N, 9.86; Cl, 15.8.

5-Aminobenzo[a]phenoxazone-9.[9]—This compound was prepared in 37% yield by the method of Kehrmann and de Gottrau.[10]

5-Phenylaminobenzo[a]phenoxazone-9.[9]—A solution of 44 g. (0.3 mole) of 4-nitroso-1,3-resorcinol[11] dissolved in 800 ml. of ethanol was added to a solution of 44 g. (0.2 mole) of 1-(N-phenyl)-naphthylamine dissolved in 35 ml. of concd. hydrochloric acid. The mixture was stirred for 20 hours at room temperature and then filtered. The precipitate was extracted with a total of 500 ml. of hot pyridine in two portions and the combined pyridine extracts were treated with 500 ml. of water. After cooling, the precipitated product was removed by filtration, giving 32 g. (47%) of dark brown solid.

Anal. Calcd. for $C_{22}H_{14}N_2O_2$: C, 78.1; H, 4.17; N, 8.28. Found: C, 78.2; H, 4.32; N, 8.36.

Spectrophotometric Determinations.—These were carried out in ethanol solution as described in paper I of this series.[2]

Acknowledgments.—We are grateful to Dr. R. P. Parker, Dr. M. E. Hultquist and Dr. J. J. Denton for their interest and coöperation on this project and we appreciate the aid of Dr. R. J. Turner in the preparation of some of the compounds. We also acknowledge the assistance of Mr. H. L. Komarowski on this project. We are indebted to Mr. O. E. Sundberg and associates for the microanalyses and to Mr. F. C. Dexter and Miss M. R. O'Rourke for the spectrophotometric determinations.

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(5) M. J. Baker, M. E. Schlosser and H. J. White, *Ann. N. Y. Acad. Sci.*, **52**, 678 (1949).

(6) H. J. White, M. E. Schlosser and M. J. DiCenza, to be published. Paper presented before the Society of American Bacteriologists, Detroit, Mich., April, 1951.

(7) Preparation described in ref. 3.

(8) German Patent 119,785; *Frdl.*, **6**, 1158 (1900-1902).

(9) Prepared by R. J. Turner.

(10) F. Kehrmann and H. de Gottrau, *Ber.*, **38**, 2574 (1905).

(11) F. Heurich, *ibid.*, **35**, 4191 (1902).