JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

(Registered in U. S. Patent Office) (Copyright, 1952, by the American Chemical Society)

VOLUME 74

FEBRUARY 5, 1952

Number 3

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

Chemotherapeutic Dyes. I. 5-Aralkylamino-9-alkylaminobenzo[a]phenoxazines¹

By Moses L. Crossley, Paul F. Dreisbach, Corris M. Hofmann and Robert P. Parker

A series of 5-amino and 5-aralkylamino-9-(mono- and di-)-alkylaminobenzo[a]phenoxazonium salts has been prepared. Their differential tissue staining action and tumor growth-retardation activity have been determined. In addition, many of the compounds of this series exhibited strong antituberculous activity when administered orally to infected mice. About 20 of the members of this series possessed activity equivalent to or greater than that of streptomycin administered subcutaneously at optimum dosage with maximum activity being found for 5-benzylamino-9-di-*n*-butylaminobenzo[a]phenoxazine.

Investigations have shown² that the basic dyes, Nile Blue A (I) and Nile Blue 2B (II), which contain the benzo[a]phenoxazine nucleus, exert a selective tumor-staining and growth-retarding action when administered orally to mice bearing transplanted tumors. Several 5-benzylamino-9-diethylaminobenzo[a]phenoxazines containing halogen substituents in the benzyl ring have been prepared recently³ for such investigations.

Because dyes with maximum tumor-staining and growth-retarding actions are of potential importance as diagnostic and therapeutic agents in the study of cancer, the preparation of a series of chemically pure benzo[a]phenoxazines was undertaken in order to study the effects of structural modification upon these actions and the toxicity of the compounds to the host. This work was greatly stimulated and further expanded following the discovery by Dr. H. J. White and co-workers at the Stamford Laboratories of the American Cyanamid Co. that certain of these dyes prolong significantly the survival time of mice infected with a lethal dose of a boyine strain of tuberculosis.⁴

The present paper describes the preparation and properties of 5-aralkylamino-9-(mono- and di-)alkylaminobenzo [a]phenoxazonium salts illustrated by the formula

(1) Presented before the Division of Medicinal Chemistry at the American Chemical Society Meeting in Cleveland, Ohio, April 8th to 12th, 1951.

(2) M. R. Lewis, H. A. Sloviter and P. P. Goland, Anat. Record, 95, 89 (1946); M. R. Lewis, P. P. Goland and H. A. Sloviter, *ibid.*, 96, 201 (1946); M. R. Lewis and P. P. Goland, *ibid.*, 99, 369 (1947); J. F. Riley, Cancer Research, 8, 183 (1948).

(3) H. A. Sloviter, THIS JOURNAL, 71, 3360 (1949).

(4) 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.



where R is a benzyl or ring-substituted benzyl group and X designates the anion of a salt.

The term benzo [a] phenoxazonium has been applied to the cation⁵ of such compounds, indicating an ion where the charge is centered on the ring oxygen giving the nucleus a definite ortho-quinonoid structure. The term benzo [a] phenoxazonium is used in this paper to designate the cation, it being understood that this is not to indicate any particular one of the possible resonance structures. For convenience, a para-quinonoid linkage involving the 9-nitrogen atom is used in designating the salts while the free bases are conveniently written as 5-imino derivatives of benzo [a] phenoxazine.



These compounds were prepared generally by the classical method (Method A) used for the preparation of I and II which involves the reaction of a 5-dialkylamino-2-nitrosophenol hydrochloride with

(5) A. M. Patterson and L. T. Capell, "The Ring Index," Reinhold Publishing Corp., New York, N. Y., 1940, p. 354. 1-naphthylamine or one of its N-benzyl substitution products.⁶



A ratio of 1.5 moles of the nitrosophenol to one mole of the naphthylamine was generally used, the excess nitrosophenol acting as an oxidizing agent during the condensation. From this reaction, the salts of the bases are obtained directly. The bases were not isolated.

When not available, the necessary 3-dialkylaminophenols were prepared from 3-aminophenol by alkylation with alkyl halides, notwithstanding the recommendation of a less simple method for such compounds.⁷

Formation of the required 5-dialkylamino-2-nitrosophenols was effected by the usual methods involving treatment of the 3-dialkylaminophenols with sodium nitrite in acid solution.

For the preparation of the 1-(N-benzyl)-naphthylamines, a method involving reduction of the corresponding benzal derivative,⁸ was used with satisfactory results.

As an alternative to Method A and to obtain a derivative with an unsubstituted amino group in the 9-position, 4-amino- and 4-benzylamino-1,2-naphthoquinones were treated with the dihydro-chlorides of 2,5-diaminophenol and 2-amino-5-di-alkylaminophenols.



The 2-amino-5-dialkylaminophenols needed for this procedure were prepared by stannous chloride reduction of the corresponding nitroso intermediate. Using method B, satisfactory yields of II and

(6) (a) German Patents 45,268 [Frdl., 2, 173 (1887-1890)]; 60,922-[Frdl., 3, 379 (1890-1894)]; (b) R. Möhlau and K. Uhlmann, Ann., 289, 115 (1896); (c) R. Möhlau, Ber., 25, 1060 (1892).

(7) B. R. Brobranskii and I. M. Eker, J. Applied Chem. (U. S. S. R.),
14, 524 (1941); C. A., 36, 3159 (1942).

(8) L. Zechmeister and J. Truka, Ber., 63, 2883 (1930).

IV were obtained, identical in properties with the products obtained by Method A.

For purposes of comparison, 5,9-diaminobenzo-[a]phenoxazonium chloride (V) was prepared using the method of Kehrmann.⁹

The benzo[a]phenoxazonium salts, for the most part, do not show satisfactory melting or decomposition points. However, spectrophotometric measurements in the visual range of the salt and base forms in ethanol solution were made and used as a measure of purity. The wave length of maximum absorption of the base form in ethanol solution was determined on a solution of the base prepared by the addition of an alkaline reagent to the blue ethanol solution of the salt.

The shift in the wave length of maximum absorption with structural change was generally as expected, and substitution of various groups in the benzyl ring usually caused only minor changes in the maxima. Increase in the size of the group on either the 5- or 9-amino substituent generally caused the maximum to shift to a higher wave length. Replacement of alkyl groups by hydrogen or a decrease in the size of the group on the 9-amino substituent shifted the maximum toward the violet region. The bases, which generally formed red or brown solutions, showed maximum absorption at a decidedly lower wave length.

The 1-(N-benzyl)-naphthylamines used in these preparations are listed in Table I. The substituted 5,9-diaminobenzo[a]phenoxazonium chlorides are listed in Table II. Unless otherwise designated, Method A was used in the preparations.

Pharmacological Activity

Tissue Staining and Tumor Growth-retarding Action.-We are indebted to Dr. M. R. Lewis of the Wistar Institute of Biology and Anatomy for investigations of the tumor growth-retarding and staining actions of these compounds. Details of the investigations involving the oral administration to mice of a number of the described derivatives have been published elsewhere,¹⁰ and the results on the remaining compounds will be published later. As regards tumor staining activity, the results show that the compound with two unsubstituted amino groups (V) is devoid of staining action. The compound with one unsubstituted amino group in the 9-position (III) shows a slight tumorstaining effect. However, the 5-benzylamino-9dialkylaminobenzo[a]phenoxazines containing alkyl groups greater than methyl generally possess strong selective tumor-staining activity. The presence of a 2-chloro or a 4-carboxy substituent on the benzyl ring tends to weaken the effect.

Compounds containing amino, monoethylamino or diethylamino in the 9-position produce significant tumor growth-retardation. The presence of groups in the benzyl ring of 5-benzylamino-9-diethylaminobenzo[a]phenoxazine appears not to diminish this action. Of all the compounds tested, those 5-benzylamino derivatives containing 9monoethylamino and 9-amino groups appear to

(9) (a) F. Kehrmann, *ibid.*, **38**, 3605 (1905); (b) F. Kehrmann, E. Grillet and P. Borgeaud, *Helv. Chim. Acta*, **9**, 866 (1926).

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

		1-(N-BE)	NZYL)-NAPHTHY	LAMINES					
Substituent on the benzyl ring	M.p. intermediate anil., °C.	М.р., °С.	Empirical formula	Carbon Calcd, Found		Analyses, % Hydrogen Calcd. Found		Nitrogen Calcd. Found	
No substituent	73-75	71-72°	$C_{17}H_{15}N$	87.5	87.9	6.48	6.31	6.01	6.29
4-Methyl	75.5-77	66-67	$C_{18}H_{17}N$	87.4	87.8	6.93	7.12	5.67	5.74
4-Methoxy	97-100	70-71 ^b	C ₁₈ H ₁₇ NO	82.1	82.0	6.51	6.60	5.32	5.43
4-Chloro	101.5-103.5	75.5-76.5°	$C_{17}H_{14}CIN^d$	76.3	76.0	5.27	5.36	5.23	5.31
4-Carboxy	226 - 227	221 - 222.5	C18H15NO2	78.0	77.8	5.45	5.50	5.05	4.87
2-Chloro	74-75	110-112	C ₁₇ H ₁₄ ClN ^e	76.3	76.3	5.27	5.19	5.23	5.37
2,3-Dimethoxy	77-78	119-120	$C_{19}H_{19}NO_2$	77.8	77.7	6.53	6.46	4.78	4.75
3,4-Methylenedioxy	109.5-111	1	C ₁₈ H ₁₃ NO ₂ ⁹	78.5^{h}	78.3 ^h	4.76^{h}	4.92^{h}	5.09^{h}	4.99 [*]

TABLE I 1-(N-BENZYL)-NAPHTHYLAMINES

⁶ 67° reported by Zechmeister and Truka.⁸ ^b 80°, reported by Zechmeister and Truka.⁸ ^c 76-76.5° reported by Sloviter.³ ^d Calcd.: Cl, 13.2; found: Cl, 13.2. ^e Calcd.: Cl, 13.2; found: Cl, 13.2. [/] Amine isolated as an oil; m.p. 65-67° reported by R. C. Clapp, private communication. ^e Empirical formula of intermediate anil. ^h Calcd. and found values for intermediate anil.

TABLE II

Substituted 5,9-Diaminobenzo[a]phenoxazonium Chlorides $\ R_{N}$

						CI								
			At	S .		Analys			lyses, %	yses, %				
		Yield,	max,	, mμ	Empirical	Car	bon	Hydr	ogen .	Nitr	ogen .	Chlo	rine	Anti-T b
R, R'	R"	%	Base	Sait	Iormula	Caled.	Found	Caled.	Found	Caled.	Found	Calcd.	Found	activit y
н, н	н	a .	486	601										-
н, н	Benzyi	210	488	615	C22H18ClN2O-11/2H2O	66.6	66.3	5.10	5.27	10.1	9.90	8.55	8.11	- ?
H, Ethyl	н	64	502	616	C18H16CIN8O+H2O	62.9	63.2	5.28	6.07	12.2	12.7	10.3	9.98	-
H, Ethyl	Benzyl	86	507	628	C15H22CIN2O+H2O	69.2	69.2	5.58	5.48	9.68	10.0	8.17	8,30	+
H, Ethyl	4-Chlorobenzyl	81	508	625	C25H21Cl2N2O+1/2H2O	65.4	65.3	4.83	4.97	9.15	9.20	15.4	15.2	- ?
Dimethyl	Benzyl	74	512	635	C24H22ClN3O·H2O	69.2	69.1	5.58	5.30	9.68	9.87	8.17	8.30	+
Dimethyl	4-Methylbenzyl	56	512	638	CmH24CIN2O+2H2O	67.0	67.2	6.06	6.30	9.02	9.04	7.61	7.65	+ +
Diethy!	H	76 ^e	513	628	C20H20CIN2O	67.9	67.4	5.69	5.64	11.9	11.2	10.0	9.78	-
Diethyl	Benzyl	79 ^d ,•	516	642	C27H26C1N3O·H2O	70.2	70.8	6.19	5.75	9.10	9.39	7.68	7.90	++
Diethyl	4-Methylbenzyl	79	518	644	C28H28CIN;O	73.4	73.3	6.16	6.30	9.18	9.28	7.74	7.78	++
Diethyl	4-Methoxy-													
•	benzvl	85	518	642	C28H24CIN2O2	71.0	70.7	5.95	6.20	8.87	8.86	7.48	7.42	++
Diethyl	4-Carboxy-													• •
•	benzvl	53	518	644	C28H26ClNzOz+H2O	66.5	66.4	5.58	5.74	8.31	8.33	7.01	6.98	- ?
Diethyl	4-Chlorobenzyl	74	520	644	CrrHasClaNaO+HaO	65.3	65.4	5.48	5.48	8.47	8.09	14.3	14.1	++
Diethyl	2-Chlorobenzyl	88	519	646	C+7H16CleN2O-11/9H10	64.2	64.3	5.58	5.49	8.31	8.26	14.0	13.5	+
Diethy!	2.3-Dimethoxy-													•
,	benzvl	72	517	643	C++H++ClN+O++1/+H+O	67.9	67.8	6.09	6.05	8.19	8.45	6.91	7.07	- ?
Diethvl	3.4-Methylene-		•••	•										•
	dioxybenzyl	49	518	643	CraHraClN2O2+2H2O	64.2	64.4	5.77	5.89	8.02	8.20	6.77	7.38	++
D-n-propyl	н	56	516	630	Ct2H24ClN1O	69.2	68.9	6.34	6.25	11.0	11.0	9.29	9.22	
Di-m-propyl	Benzvl	641	518	646	CnHaClNsO-HO	71.1	71.3	6.58	6.87	8.58	8.59	7.24	7.64	+++
Di-n-propyl	4-Methylbenzyl	62	518	645	CatHaoClNaO	74.1	74.0	6.64	6.62	8.65	8.87	7.30	7.35	÷÷÷
Di-n-propyl	4-Chlorobenzyl	55	518	646	CmHmCl+NrO	68.8	68.7	5.77	5.97	8.30	8.22	14.0	13.7	+++
Di-z-butyl	Benzvl	61	519	647	CuH24ClNsO.H.O	71.9	71.9	7.01	6.84	8.11	8.23	6.85	6.93	++++
Di-n-butyl	4-Methylbenzyl	63	520	647	CreHatCIN ₂ O	74.8	74.7	7.06	7.18	8.17	8.03	6.90	7.05	÷÷÷.
Di-n-hutyl	4-Chlorobenzyl	53	520	650	CuHuCleN.O.H.O	67.4	67 5	6 39	6 90	7 61	7 62	12.8	12.6	<u>+++</u> +
Di-n-butyl	3.4-Methylene.													••••
2	dioxybenzyl	52	517	646	CasHan Oag	67.4	67.2	6 01	5 91	9 82	9 60			+++
D-n-amvi	Benzyl	38	518	646	CnHaClNiO.1/iHiO	73.8	73.9	7.32	7.38	7.82	8.09	6.60	6.72	<u>+++</u>
Di-n-amvl	4-Methylbenzyl	85	518	647	CuHaN404	71.8	71.7	7 09	7.29	9.85	9 89			÷÷ ′
Di-mamyl	4-Chlorobenzyl	57	517	646	CuHrrCleNtO	70 4	70 7	6 63	6 61	7 47	7 45	12.6	12 4	<u>+</u> ++
Di-n-hexvl	Benzyl	79 ^h	519	647	CarHarClN10.H10	73.2	73 1	7 73	7 61	7 32	7.52	6.18	6.17	<u>+</u> +
Di-s-hervl	4-Chlorobenzyl	234	520	650	CuH41CleN2O.H.O	69 1	68 6	7 12	7 15	6 91	7 15	11 7	11 8	÷ ÷
Ethyl.	I Childrebenbyi		010	000			00.0	•••=		0.01				• •
#-nronvl	Benzyl	81	521	651	CuHaCIN:0.11/4H+0	69 3	69.8	6 44	6 26	8 67	8 41	7 31	6 85	+ + +
Ethyl	Belleyt	~							0.20	0.01	0.11		0.00	
#-propyl	4-Chiorobenzvi	54	517	648	C#H#Cl+N20-11/+H+O	64.7	65.3	5.82	5.56	8.09	7.92	13.7	13.3	+++
Ethvi.	- Quiorobouryi	~-		010				0.04	0.00	0.00				, , (
n-hexvl	Benzvl	47 [.]	519	646	CnHHCIN:0	74.4	74.3	6.85	6.94	8,40	8.34	7.09	6.82	++
											~			

^a Reference 8a. ^b Prepared by Method B. ^c Nile Blue A chloride. ^d Nile Blue 2B. ^e 61% by Method B. ^f 62% by Method B. ^e Nitrate. ^h Prepared by T. Rees.

possess the most significant retarding action. Increase in the size of the alkylamino radical in the 9-position beyond ethyl lowers the activity.

In addition to the tumor-staining and growthretarding actions, a number of these derivatives also show a marked differential fat-staining effect when administered orally either to normal mice or to tumor-bearing mice. In the latter case, the tumor is stained blue and the fatty tissue is stained a red color. This effect was observed only for those members of the series that contained an alkyl radical of three or more carbon atoms on the 9-amino group. It appears, therefore, that the tumor growth-retarding action is diminished by increasing the size of the alkyl radical while the fat staining action is increased.

Antituberculous Action.—Nile Blue A Sulfate, New Methylene Blue GG and a mercuriated Nile Blue have been subjected to tests in experimental studies of tuberculosis¹¹ with no conclusive effects being noted.

It has been found now that by oral administration to mice previously infècted with a bovine strain of tubercle bacillus, certain of the newly prepared benzo[a]phenoxazines exert a powerful antituberculous effect as evidenced by a significantly increased median survival time of treated mice over that of infected, untreated mice.

In Table II, the ratings of 1+ to 4+ indicate simply a graded activity that is related to relative dosage required for equivalent prolongation of mouse survival time. A minus designation denotes no appreciable activity by the test method. A minus designation qualified by a question mark indicates that at the administered dosage, no activity was observed and that even if activity were observed at a higher dosage, the compound could not be rated more than a minimum activity of 1+.

Streptomycin administered at optimum dosage subcutaneously in this test receives on the basis of this grading a 2+ rating indicating that twenty of these derivatives show an activity approximately equivalent to or greater than that of streptomycin.

Activity determinations on these compounds were carried out in the Stamford Laboratories of the American Cyanamid Co. by Dr. H. J. White and co-workers, to whom we acknowledge our indebtedness for information on these tests. The testing procedure has been described¹² and the details of the results obtained will be published elsewhere.⁴

Compounds containing an unsubstituted amino group in the 5-position show no antituberculous activity. The presence of one and preferably two alkyl radicals on the 9-amino nitrogen seems necessary for activity since the unsubstituted 9-amino derivatives were inactive (III and V). One of the 9-monoethylamino derivatives possessed weak activity but the 9-dialkylamino derivatives containing alkyl radicals from ethyl through *n*-amyl generally showed a strong action. Maximum effect is reached in the 9-di-n-propylamino and 9-di-nbutylamino series. The presence of a substituent in the benzyl ring is unnecessary for high activity, although there are several instances where activity is retained or increased upon substitution in the 4position of methyl, methoxy or chloro. The 3,4-methylenedioxy derivatives also showed moderate activity. Substitution of chlorine in the 2position or of methoxy in the 2,4-positions is undesirable. The presence of an acidic group lowers the antituberculous effect.

No suggestion is offered here as to the mode of

(11) H. J. Corper, J. Infectious Diseases, **11**, 373 (1912); L. M. Dewitt, *ibid.*, **13**, 378 (1913); A. C. Hollande and G. Cremioux, Compt. rend. soc. biol., **98**, 1379 (1928); **99**, 542 (1928); A. C. Hollande and G. Hollande, *ibid.*, **102**, ⁷ 46 (1929); H. G. Wells and E. R. Long, "Chemistry of Tuberculosis," Williams and Wilkins, Baltimore, Md., 1932, p. 437.

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

action of this type of compound, but it is noted that in considering the tuberculostatic effect of phenothiazine derivatives *in vitro*, it has been postulated that the ring nitrogen should be free (unsubstituted) to take part in oxidation-reduction systems, since compounds with substituents on this nitrogen were ineffective.¹³

Experimental

1-(N-Benzyl)-naphthylamines.—These compounds were prepared from the corresponding benzal derivatives by the method of Zechmeister and Truka,⁸ using a somewhat larger excess of magnesium than recommended.

3-Dimethylaminophenol.—To 504 g. (4 moles) of dimethyl sulfate was added with stirring, 218 g. (2 moles) of 3-aminophenol in small portions over a period of 1.5 hours. At the start, the temperature increased spontaneously to about 65° and after the addition was complete, the mixture was heated on the steam-bath with stirring for two hours, then cooled and drowned in approximately one liter of iced water. The mixture was made alkaline by the addition of sodium carbonate and then extracted with ether.

Extraction of the product from the ether solution with dilute hydrochloric acid and liberation of the product by making alkaline with sodium carbonate afforded a partial separation of colored impurities. The product was finally taken up in ether, dried over Drierite, and the ether was removed, leaving an amber-colored residue.

separation of colored impurities. The product was finally taken up in ether, dried over Drierite, and the ether was removed, leaving an amber-colored residue. Distillation of this crude product at reduced pressure gave 129 g. (47%) of slightly yellow viscous liquid, b.p. $155-157^{\circ}$ (21 mm.) (152° (17 mm.) on another similar preparation), n^{26} D 1.5895. Various boiling ranges are given in the literature for this product. Brobranskii and Eker⁷ list a boiling point of 152° at 12 mm. This product quickly acquires a deep red-brown color

This product quickly acquires a deep red-brown color upon exposure to the atmosphere. To confirm the identity of this phenol, a crystalline nitroso derivative as the hydrochloride was prepared. This decomposed gradually from 176° , in agreement with the figure recorded in the literature.⁶

Anal. Calcd. for $C_8H_{10}N_2O_2$ ·HCl·H₂O: C, 43.5; H, 5.94; N, 12.7; Cl, 16.1. Found: C, 43.2; H, 5.70; N, 12.1; Cl, 15.8.

Treatment of the hydrochloride with sodium carbonate solution liberated the 5-dimethylamino-2-nitrosophenol base in the form of brown crystals, m.p. 167-169°, which agrees with that reported in the literature.⁶

Anal. Calcd. for $C_8H_{10}N_2O_2$: C, 57.8; H, 6.07; N, 16.9. Found: C, 57.7; H, 5.93; N, 16.8.

5-Dialkylamino-2-nitrosophenol Hydrochlorides.—The dimethyl and diethyl derivatives were prepared by described methods.⁶

For the preparation of the di-*n*-propyl and di-*n*-butyl derivatives, 3-aminophenol was alkylated with *n*-propyl and *n*-butyl bromides according to the procedure which follows:

To a solution of 1 mole of 3-aminophenol in 300 ml. of ethanol was added 1 mole of the alkyl bromide and the resulting mixture was heated with stirring at steam-bath temperature until there was no longer any appreciable reflux of the alkyl halide. The whole was then drowned in one liter of water, made alkaline by the addition of sodium carbonate and the separated 3-monoalkylaminophenol in ethanol solution was again treated in the manner described above with another mole of the alkyl halide. The resulting 3-dialkylaminophenol liberated from its hydrobromide was taken up in ether or benzene solution, separated and purified by distillation.

takin up in cluct of benchmarks and the second sec

⁽¹³⁾ D. F. Houston, E. B. Kester and F. DeEds, THIS JOURNAL, 71, 3816 (1949); B. L. Freedlander, *Proc. Soc. Exptl. Biol. Med.*, 67, 106 (1944).

throughout this addition which required about 45 minutes. After stirring an additional hour, the product which separated was removed by filtration and washed with dilute hydrochloric acid giving 48 g. (87%) of yellow crystalline hydrochloride. This was recrystallized from 190 ml. of ethanol containing a few ml. of hydrochloric acid giving 5-di-*n*-propylamino-2-nitrosophenol hydrochloride as bright yellow crystals, m.p. 151-152° (60% based on the 3-amino-phenol used as starting material).

Anal. Calcd. for $C_{12}H_{18}N_2O_2$ ·HCl·H₂O: C, 52.1; H, 7.63; N, 10.1; Cl, 12.8. Found: C, 52.2; H, 8.02; N, 10.2; Cl, 12.9.

The distilled **3-di**-*n*-butylaminophenol (50% yield), b.p. 173-176° (6 mm.), $n^{31}D$ 1.5270, was nitrosated in alcoholic hydrochloric acid. To a warm solution of 22.1 g. (0.1 mole) of 3-di-*n*-butylaminophenol in 65 ml. of ethanol was added 30 ml. of concentrated hydrochloric acid. After cooling this solution to 10°, a paste prepared from 7.6 g. (0.11 mole) of sodium nitrite and water was then added portionwise with stirring over three hours, the temperature being maintained below 10° during this time. After stirring for another one-half hour the mixture was warmed to 40° and then filtered to remove sodium chloride. The nitroso derivative was not isolated, but the reaction solution was used directly in the condensation with the naphthylamine derivative.

Alkylation of 3-aminophenol with *n*-amyl bromide and *n*-hexyl bromide to the corresponding 3-dialkylaminophenols was carried out as follows:

A solution of one mole of 3-aminophenol and one mole of the alkyl bromide was heated to the reflux temperature in 150 ml. of ethanol with stirring for about four hours; an additional mole of the alkyl bromide was then added along with a mole of sodium carbonate in 200 ml. of water. The whole mixture was again heated to steam-bath temperature with efficient stirring for about 15 hours. The dark oily product was then separated from the aqueous layer and after washing with water, was purified by vacuum distillation, giving an extremely viscous pale yellow liquid.

The 3-di-n-amylaminophenol (a partially purified product was obtained in 89% yield, observed boiling range, 170 to 189° at 5 mm., n^{26} D 1.5253) was nitrosated under the conditions described for the nitrosation of 3-di-n-butylaminophenol, the nitroso derivative also being used without isolation.

The 3-di-n-hexylaminophenol (73% yield), b.p. 190-195° (3 mm.), was also nitrosated in alcoholic hydrochloric acid. A paste of 69 g. (1.0 mole) of sodium nitrite in water was added gradually to 277 g. (1.0 mole) of 3-di-n-hexylaminophenol dissolved in 500 ml. of ethanol containing 250 ml. of concentrated hydrochloric acid at about 10°. After stirring for a total of one hour, the precipitated product was removed by filtration (59%). After purification by recrystallization several times from ethanol, the purified 5-di-n-hexylamino-2-nitrosophenol hydrochloride was obtained as an orange crystalline solid which decomposed at 139-139.5° after darkening at 133°.

Anal. Calcd. for C₁₈H₃₀N₂O₂·HCl·H₂O: C, 59.9; H, 9.22; N, 7.76; Cl, 9.83. Found: C, 59.9; H, 8.96; N, 7.64; Cl, 9.77.

The preparation of 3-ethylaminophenol was effected by alkylation of 3-aminophenol with one mole of ethyl bromide under conditions similar to those described above. The 3-ethylaminophenol was obtained in 70% yield as a pale yellow liquid, b.p. $137-143^{\circ}$ (4 to 5 mm.).¹⁴ This was alkylated with *n*-propyl bromide under similar conditions giving an 87% yield of 3-ethyl-*n*-propylaminophenol as a viscous straw-colored product, b.p. $138-140^{\circ}$ (3 mm.). This was nitrosated by the method employed for the nitrosation of the diethyl derivative, giving 5-ethyl-*n*-propylamino-2-nitrosophenol hydrochloride as yellow crystalline material which decomposed at about 156° after darkening from 150°.

Anal. Calcd. for $C_{11}H_{16}N_2O_2 \cdot HCl^{-1}/_2H_2O$: C, 52.1; H, 7.15; N, 11.0; Cl, 14.0. Found: C, 52.1; H, 7.10; N, 10.7; Cl, 14.1.

By a similar alkylation of 3-ethylaminophenol with *n*-hexyl bromide, **3-ethyl**-*n*-hexylaminophenol was obtained as a viscous straw-colored liquid (52%), b.p. $139-141^{\circ}$ (1

(14) R. Gnehm and T. Scheutz, J. prakt. Chem., [2] 63, 423 (1901), give 176° at 12 mm, as the b.p. for this compound.

mm.), n^{29} D 1.5403. This was nitrosated using the method described above for 3-di-*n*-butylaminophenol, the nitrosation product being used in the condensation without isolation.

Anal. Calcd. for $C_{14}H_{23}NO$: C, 76.0; H, 10.5; N, 6.33. Found: C, 75.8; H, 10.2; N, 6.24.

Nitrosation of 3-ethylaminophenol according to the procedure used for the diethylamino compound gave a 54% yield of the nitrosamine, N-nitroso-3-ethylaminophenol, which separated directly from the reaction mixture as a brown water-insoluble solid. Recrystallization from aqueous methanol gave the purified nitrosamine as brown crystal-line material, m.p. 115-115.5°.

Anal. Calcd. for $C_8H_{10}N_2O_2$: C, 57.8; H, 6.07; N, 16.9. Found: C, 57.9; H, 6.11; N, 17.1.

The nitrosamine derivative was rearranged using hydrogen chloride in alcoholic ether solution by the method of Fischer and Hepp¹⁵ to **5-ethylamino-2-nitrosophenol hydrochloride**. Recrystallization of this hydrochloride from an alcohole ether solution gave the purified product as brownish glassy crystals decomposing at 175–180° (44% based on nitrosamine derivative).¹⁶

Anal. Calcd. for $C_8H_{10}N_2O_2$ ·HCl: C, 47.4; H, 5.47; N, 13.8. Found: C, 47.5; H, 5.60; N, 13.8.

2-Amino-5-di-*n*-propylaminophenol Dihydrochloride.—To a solution of 135 g. (0.6 mole) of stannous chloride dihydrate in 150 ml. of concd. hydrochloric acid was added portionwise with stirring over a one-hour period, 77.6 g. (0.3 mole) of 5-di-*n*-propylamino-2-nitrosophenol hydrochloride. During the reduction, the temperature was maintained below 20° by cooling in a bath. After the reduction was complete, the solution was saturated with hydrogen chloride and the stannic chloride addition compound of the product in the form of a white crystalline precipitate was removed by filtration. After dissolving the white product in 1250 ml. of water, hydrogen sulfide was passed into the solution until the tin was precipitated completely as the sulfide which was removed by filtration. Concentration of the tin-free solution at reduced pressure gave a moist white solid which was purified by precipitation from solution in 300 ml. of ethanol by the addition of 250 ml. of ether. After desiccation over solid caustic soda, 40 g. (55%) of product was obtained, dec. at 245°.

Anal. Calcd. for $C_{12}H_{20}N_2O$ ·2HCl·¹/₂H₂O: C, 49.7; H, 7.99; N, 9.65; Cl, 24.4. Found: C, 49.7; H, 7.60; N, 9.68; Cl, 24.4.

Benzo[a]phenoxazines. 5-Benzylamino-9-(mono- or di-)alkylaminobenzo[a]phenoxazonium Salts.--These were made by either of two methods which are illustrated by the detailed procedures given below for the preparation of 5benzylamino - 9 - di - n - propylaminobenzo[a] phenoxazonium chloride. Method A was used in most cases. The chloride salts were purified by recrystallization from acetic acid or ethanol to which had been added a trace of hydrochloric acid. In several instances, failure to obtain the chlorides as crystalline products prompted conversion to the nitrates by treatment of the reaction mixture with an excess of nitric acid. The nitrate salts were also purified by recrystallization from ethanol. The salts were generally obtained as glistening green crystalline solids, insoluble in ether, sparingly soluble in water, and forming intensely blue-colored solutions in ethanol. Ethanol solutions of the 9-monoalkylamino derivatives appeared blue by transmitted light and red by reflected light. Occasionally, samples of the salts were obtained which appeared as brown or copper colored crystals. However, spectrophotometric examination showed no difference from the same compound exhibiting a different color in the solid state. The bases were formed by treatment of a slurry of the salt in ethanol or water with aqueous ammonia, but they were not isolated in the solid form for characterization because they tended to decompose.

5-Benzylamino-9-di-n-propylaminobenzo[a]phenoxazonium Chloride. Method A.—To a suspension of 117 g. (0.5 mole) of 1-(N-benzyl)-naphthylamine in 600 ml. of ethanol was added 194 g. (0.75 mole) of 5-di-n-propylamino-2-nitrosophenol hydrochloride and 7 ml. of concd. hydrochloric acid. The whole was heated to the reflux tempera-

(16) This substituted phenol is mentioned in German Patent 127,425 [Frdl., 6, 499 (1900-1902)].

⁽¹⁵⁾ O. Fischer and E. Hepp, Ber., 19, 2991 (1886).

ture with stirring for three hours during which time the color of the mixture changed through brown and green to an intense blue. After cooling, the precipitated chloride was separated by filtration and washed with ethanol, giving 160 g. (64%) of glistening green crystalline material. This was recrystallized from acetic acid in which the product is sparingly soluble.

A sample of the free base was prepared by treatment of a slurry of the chloride in ethanol with a slight excess of aqueous ammonium hydroxide solution. The base was obtained as a red-brown solid showing a reddish color in ethanol solution. However, since the solid base tended to decompose on storage, this derivative and other similar ones were retained as the chlorides.

Method B.—To 90 ml. of ethanol was added 7 g. (0.025 mole) of 2-amino-5-di-*n*-propylaminophenol dihydrochloride and 6.5 g. (0.025 mole) of 4-benzylamino-1,2-naphthoquinone.¹⁷ The mixture was heated to the reflux temperature with stirring for five hours, during which time it acquired an intense blue color. The glistening green product which precipitated on cooling was removed by filtration, giving 7.3 g. (62%) of the chloride. This product was identical with that prepared above under Method A.

9-Amino-5-benzylaminobenzo[a]phenoxazonium Chloride. —The procedure is based on the method of Kehrmann⁹ for the preparation of the corresponding 5,9-diamino derivative.

To a solution of 30 g. (0.114 mole) of 4-benzylamino-1,2naphthoquinone¹⁷ in 500 ml. of ethanol was added 30 g. (excess) of 2,5-diaminophenol dihydrochloride.¹⁸ After stirring at the reflux temperature for a 3-hour period, the mixture was allowed to cool and the precipitated green product was removed by filtration and washed with ethanol and ether.

(17) L. F. Fieser and M. Fieser, THIS JOURNAL, 57, 494 (1935).

After extraction of the crude green product with a total of 800 ml. of acetic acid, there remained a black insoluble residue which was discarded. Upon cooling the extract, 25 g. (57%) of product was obtained in the form of dark olive-green crystalline material which was recrystallized from butanol. This material was insoluble in ether but moderately soluble in ethanol, forming an intensely colored solution which appeared blood-red by reflected light and blue by transmitted light through a thin layer. Addition of a trace of ammonia solution releases the base from the chloride, forming a red-brown solution. The analytical data for this compound are recorded in Table II.

5-Amino-9-(mono or di)-alkylaminobenzo[a]phenoxazonium Salts.—These compounds were prepared by described methods⁶ using aqueous acetic acid as the vehicle for the condensation of the nitrosophenols with the 1naphthylamine derivatives. Purification was effected by recrystallization from acetic acid in which these derivatives are only sparingly soluble.

Spectrophotometric Determinations.—These were carried out on ethanol solutions of the dyes in 1-cm. glass cells using a General Electric type of automatic recording spectrophotometer.

Acknowledgments.—We are grateful to Dr. M. E. Hultquist and Dr. R. J. Turner for their coöperation on this project and we wish to thank Mr. H. L. Komarowski for his assistance in the preparation of some of these compounds. We are indebted to Mr. O. E. Sundberg and associates for the microanalyses and to Mrs. R. Kaselis and Miss M. R. O'Rourke for the spectrophotometric determinations.

BOUND BROOK, N. J.

RECEIVED JULY 26, 1951

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

Chemotherapeutic Dyes. II. 5-Arylamino-9-dialkylaminobenzo [a]phenoxazines¹

By Moses L. Crossley, Richard J. Turner, Corris M. Hofmann, Paul F. Dreisbach and Robert P. Parker

Because of the tumor growth-retarding and antituberculous actions of various 5-aralkylamino-9-dialkylaminobenzo[a]phenoxazines, a series of related 5-arylamino-9-(mono- and di)-alkylaminobenzo[a]phenoxazines was prepared for study. The preferred method of preparation involved amination of 9-dialkylaminobenzo[a]phenoxazines with aromatic amines although some members of the series were synthesized by reaction of 5-(N-mono- and N,N-di-)-alkylamino-2-nitrosophenols with 1-(N-aryl)-naphthylamines. In this series of compounds, the alkyl radicals on the 9-amino group were varied from methyl through n-hexyl. Maximum antituberculous activity resulted when two propyl radicals were present on the 9-amino group. Twenty-six of the compounds of this series when administered orally to mice possessed activity equivalent to or greater than that of streptomycin administered subcutaneously at optimum dosage.

In a previous communication,² a series of 5-benzylamino-9-dialkylaminobenzo[a]phenoxazonium salts has been described (I, where R is benzyl and R' is lower alkyl) which, administered orally, retard the growth of transplanted tumor tissue in mice and greatly increase the survival time of mice previously infected with a bovine strain of tubercle bacillus.



⁽¹⁾ Presented before the Division of Medicinal Chemistry at the American Chemical Society Meeting in Cleveland, Ohio, April 8th to 12th, 1951.

The high activities exhibited by certain of the dyes of type I led us to investigate further the effect of structural modifications upon the antituberculous and tumor growth-retarding activities. The present paper describes a series of benzo[a]phenoxazines containing aryl- and substituted arylamino groups in the 5-position (I, R is aryl and R' is alkyl). A few compounds are reported which are 9-monoalkylamino derivatives (*i.e.*, I, one R' is H).

The preparation of compounds of type I in which R is aryl and R' is alkyl has been accomplished preferably by two methods, A and B outlined below, which are described in the literature.⁸

(3) Method A: (a) R. Meldola, Ber., 12, 2065 (1879); (b) R. Hirsch and F. Kalckhoff, *ibid.*, 23, 2992 (1890); (c) C. C. Schlarb, Chem. Zeit., 15, 1281 (1891); (d) German Patent 56,722, Frdl., 3, 374 (1890-1894); and others. Method B: (e) German Patent 45,268, Frdl., 2, 173 (1887-1890); (f) R. Möhlau and K. Uhlmann, Ann., 289, 115 (1896).

⁽¹⁸⁾ F. Kehrmann and G. Betsch, Ber., 30, 2098 (1897).

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