[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TENNESSEE]

Potential Carcinostatic Derivatives of Benzo[a]- and Benzo[b]phenoxazine

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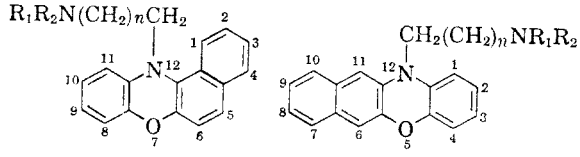
This paper reports the synthesis of "nitrogen mustard" type derivatives and dialkylaminoalkyl derivatives of benzo[a]- and benzo[b]phenoxazine.

It has been demonstrated that a number of benzo[a]phenoxazonium salts related to Nile Blue dyes possess tumor growth retarding action in mice as well as antituberculous action in mice.[3] We have synthesized for evaluation as potential cancer chemotherapeutic agents some *N*-dialkylaminoalkyl and *N*-bis(β-chloroethyl)aminoethyl derivatives of benzo[a]phenoxazine (I) and benzo[b]-phenoxazine (II). The so-called "nitrogen mustard" types ($R_1 = R_2 = ClCH_2CH_2-$, $n = 1$) are of particular interest in view of the anticancer



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activity of a number of compounds containing the β-chloroethylamino group.[4]

The methods used for synthesis of these types parallel routes reported earlier from this laboratory.[5] *n*-Butyllithium used to form the *N*-lithio derivatives of the heterocycle which was then treated with appropriate alkyl halides or alkyl tosylates. The present commercial availability of *n*-butyllithium provides a significant advantage to use of this reagent over formation of the *N*-sodio types with sodamide. Yields of *N*-alkyl types from the *N*-lithio and *N*-sodio derivatives appear comparable. For synthesis of the nitrogen mustards the *N*-lithiobenzophenoxazine was converted to the *N*-β-chloroethyl derivative with 2-chloroethyl *p*-toluenesulfonate. Subsequent reaction with diethanolamine produced the $>NCH_2CH_2N(CH_2CH_2OH)_2$ derivatives which were converted with phosphoryl chloride to the nitrogen mustard types, $>NCH_2CH_2N(CH_2CH_2Cl)_2$. Dialkylaminoalkyl derivatives of the benzophenoxazines were prepared by reaction of the appropriate dialkylaminoalkyl halide with the *N*-lithiobenzophenoxazine.

Benzo[a]phenoxazine has *been reported in* the literature,[6] but it was apparently formed in quite poor yield and was not adequately characterized. Modification of the reported method, including product chromatography over alumina, has allowed formation of benzo[a]phenoxazine in a consistent though rather poor yield of 30%. We have prepared and characterized the previously unreported *N*-acetyl and *N*-methyl derivatives of benzo[a]phenoxazine.

Tests for carcinostatic activity of the compounds reported in this paper are being obtained by the National Cancer Chemotherapy Service Center, Bethesda, Maryland. Significant results of these tests will be reported elsewhere. The authors acknowledge with thanks the financial support of the National Cancer Institute under Grant CY-4068.

EXPERIMENTAL[7]

Benzo[a]phenoxazine. A modified procedure giving significantly better results than reported in the literature[6] was as follows. An intimate mixture of 10.0 g. (0.051 mole) of freshly crystallized 1-amino-2-naphthol hydrochloride and 6.0 g. (0.055 mole) of *o*-aminophenol was heated under a nitrogen atmosphere at a temperature of 185–190° for 3 hr. The product was extracted repeatedly with a mixture of petroleum ether (b.p. 39–52°) and acetone (9:1) and the extracts passed through a 3 × 40 cm. chromatographic column containing Alcoa F-20 activated alumina. Elution was with the same solvent. The benzo[a]phenoxazine moved down the column well ahead of a band of darker unwanted material. The solvent was evaporated from the eluate to incipient precipitation and cooling precipitated 3.5 g. (30%) of yellow product, m.p. 104–107°. Recrystallization from petroleum ether (b.p. 39–52°) raised the melting point to 112° (lit.[6] m.p. 107°).

Benzo[a]phenoxazine is rapidly decomposed by exposure to light and to the atmosphere.

12-Acetylbenzo[a]phenoxazine. Benzo[a]phenoxazine was acetylated at room temperature with excess acetic anhydride containing anhydrous zinc chloride. Recrystallization from methanol gave 42% of product, m.p. 126°.

Anal. Calcd. for $C_{18}H_{13}NO_2$: C, 78.56; H, 4.76; N, 5.09. Found: C, 78.76, 78.40; H, 4.72, 4.93; N, 5.28, 5.35.

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(7) Elementary microanalyses by Weiler and Strauss, Oxford, England. Melting points were determined on a "Mel-Temp" apparatus.

TABLE I

12-DIALKYLAMINOALKYL DERIVATIVES OF BENZO[a]- AND BENZO[b]PHENOXAZINE

Compound	B.P.	Yield, %	Molecular Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
I. $n = 2$ $R_1 = R_2 = CH_3$—	208°/1 mm.	72	$C_{21}H_{22}N_2O$	79.24	79.21	6.92	6.94	8.80	8.92
					79.23		6.84		8.95
II. $n = 1$ $R_1 = R_2 = CH_3CH_2$—	250°/1 mm.	73	$C_{22}H_{24}N_2O$	79.52	79.70	7.23	7.36	8.43	8.25
					79.61		7.12		8.67
II. $n = 2$ $R_1 = R_2 = CH_3$—	242°/1 mm.	72	$C_{21}H_{22}N_2O$	79.24	79.34	6.92	6.86	8.80	8.75

12-Methylbenzo[a]phenoxazine. To a solution of 5.2 g. (0.022 mole) of benzo[a]phenoxazine in benzene was added with stirring under a nitrogen atmosphere 20 ml. of a solution of *n*-butyllithium in hexane (Foote Mineral Co. product), containing 0.026 mole of organometallic. To the resulting slurry of *N*-lithio compound was added 4.88 g. (0.026 mole) of methyl *p*-toluenesulfonate and the reaction mixture became homogeneous within a few minutes. The mixture was heated under reflux for 14 hr., after which it was treated with water. The benzene layer was washed with water, dried, and the benzene evaporated. The resulting solid was recrystallized from 95% ethanol-water to give 67% of product, m.p. 107°.

Anal. Calcd. for $C_{17}H_{13}NO$: C, 82.57; H, 5.30; N, 5.66. Found: C, 82.61, 82.29; H, 5.43, 5.22; N, 5.30, 5.58.

Benzo[b]phenoxazine. This compound was prepared from 2,3-dihydroxynaphthalene and *o*-aminophenol essentially in accordance with the procedure of Kehrmann and Neil.[8] The yield was 55% of product, m.p. 289°. Kehrmann and Neil[8] report 302°. The 12-acetyl derivative melted at 151° which corresponds to the literature[8] value. Benzo[b]-phenoxazine is more stable to light and moist air than is benzo[a]phenoxazine. With both compounds the *N*-acyl and *N*-alkyl derivatives are quite stable.

12-Chloroacetylbenzo[a]phenoxazine. A solution of 1 equivalent of benzo[a]phenoxazine and 1.3 equivalents of chloroacetyl chloride in benzene was heated under reflux for 10–12 hr. The solution was washed repeatedly with water, the benzene evaporated and the residue crystallized from benzene. There was obtained a 70% yield of product, m.p. 184°.

Anal. Calcd. for $C_{18}H_{12}ClNO_2$: C, 69.79; H, 3.88; N, 4.52. Found: C, 69.68, 69.60; H, 3.81, 4.13; N, 4.23, 4.43.

12-Chloroacetylbenzo[b]phenoxazine, m.p. 131°, was prepared in similar fashion and 60% yield.

Anal. Calcd. for $C_{18}H_{12}ClNO_2$: C, 69.79; H, 3.88; N, 4.52. Found: C, 69.84, 69.65; H, 3.85, 4.08; N, 4.65, 4.79.

The hitherto unreported *10-chloroacetylphenoxazine,* m.p. 139–140°, was prepared in 58% yield.

Anal. Calcd. for $C_{14}H_{10}ClNO_2$: C, 64.74; H, 3.85; N, 5.39. Found: C, 65.44, 65.37; H, 3.75, 4.00; N, 5.20, 5.33.

10-Diethylaminoacetylphenoxazine. A solution of 1.0 g. of 10-chloroacetylphenoxazine and 3.0 ml. of diethylamine in 20 ml. of benzene was heated under reflux for 5 hr. The precipitated white solid was filtered off and the filtrate extracted with 5% aqueous hydrochloric acid. The extract was neutralized with sodium carbonate and the precipitated oil extracted with ether. The ether was evaporated and the residual oil chromatographed over 60–100 mesh "Florisil" absorbent using 1:9 acetone-benzene. The product was obtained in greater than 40% yields upon evaporation of the eluent. It melted at 39–40°.

Anal. Calcd. for $C_{18}H_{20}N_2O_2$: C, 72.97; H, 6.76; N, 9.46. Found: C, 72.83, 72.78; H, 6.71, 6.66; N, 9.30, 9.51.

The *methiodide* derivative of the above melted at 149°.

Anal. Calcd. for $C_{19}H_{23}N_2IO_2$: C, 52.06; H, 5.25; N, 6.39. Found: C, 52.56, 52.16; H, 5.78, 5.64; N, 5.94, 6.06.

(8) F. Kehrmann and A. Neil, *Ber.*, **47**, 3102 (1914).

Attempts to convert 10-chloroacetylphenoxazine to 10-bis-(2-hydroxyethyl)aminoacetylphenoxazine apparently resulted in alcoholysis of the amide to the unstable 10-phenoxazinecarboxylic acid, since only phenoxazine could be isolated as the major reaction product. We were thus blocked in an attempt to use this route to produce nitrogen mustard derivatives of the $>NCOCH_2N(CH_2CH_2Cl)_2$ type.

10-(2-Piperidinoethyl)phenoxazine. A mixture of 3.7 g. (0.015 mole) of 10-(2-chloroethyl)phenoxazine[5a] and 6.8 g. (0.080 mole) of piperidine in 80 ml. of dry xylene was heated under reflux until precipitation of piperidine hydrochloride no longer occurred (144 hr.). The xylene solution was cooled and excess 5% aqueous hydrochloric acid was added whereupon there was formed a light violet precipitate of the hydrochloride of 10-(2-piperidinoethyl)phenoxazine. The salt was formed in 58% yield and melted at 242°.

Anal. Calcd. for $C_{19}H_{23}ClN_2O$; C, 68.99; H, 6.96; N, 8.47. Found: C, 68.95, 68.86; H, 6.82, 7.00; N, 8.83, 8.78.

12-(2-Chloroethyl)benzo[a]phenoxazine. The procedure described in our earlier work[5a] was used without significant modification. The product was recrystallized from methanol and was formed in 50% yield, m.p. 76°.

Anal. Calcd. for $C_{18}H_{14}ClNO$: C, 73.09; H, 4.77; N, 4.74. Found: C, 73.32, 73.25; H, 4.82, 4.91; N, 5.03, 4.89.

12-(2-Chloroethyl)benzo[b]phenoxazine was prepared as above. The yield was 69%, m.p. 108°.

Anal. Calcd. for $C_{18}H_{14}ClNO$: C, 73.09; H, 4.77; N, 4.74. Found: C, 72.89, 73.16; H, 4.91, 4.69; N, 4.74, 4.97.

12-{2-[Bis(2-hydroxyethyl)amino]ethyl}benzo[b]phenoxazine. (II, $n = 1$, $R_1 = R_2 = HOCH_2CH_2-$). A solution of 2.0 g. (0.0068 mole) of 12-(2-chloroethyl)benzo[b]phenoxazine in 70 ml. of diethanolamine was heated and stirred at 130–140° for 18 hr. The reaction mixture was cooled, diluted with water, and extracted several times with a mixture of benzene and chloroform. Evaporation of the extracts yielded an oil which was crystallized from acetone and dry ether to yield 2.2 g. (89%) of white product, m.p. 96°.

Anal. Calcd. for $C_{22}H_{24}N_2O_3$: C, 72.53; H, 6.59; N, 7.69. Found: C, 72.58, 72.38; H, 6.59, 6.57; N, 7.78, 7.74.

A *hydrochloride* of the above amine was crystallized from ethanol and ether and melted at 209°.

Anal. Calcd. for $C_{22}H_{25}ClN_2O_3$: C, 65.92; H, 6.24; N, 6.99. Found: C, 66.11, 65.91; H, 6.16, 6.10; N, 7.15, 7.23.

12-{2-[Bis(2-chloroethyl)amino]ethyl}benzo[b]phenoxazine. (II, $n = 1$, $R_1 = R_2 = ClCH_2CH_2$—). A solution of 5.0 g. (0.014 mole) of the hydroxyethyl compound described above in 15 ml. of phosphoryl chloride was heated on a steam bath for 1 hr., after which the excess phosphoryl chloride was removed under vacuum at the same temperature. The residue was extracted with hot chloroform, and the chloroform solution washed several times with water and the chloroform evaporated. The residue was then suspended in benzene and the suspension washed with aqueous sodium carbonate solution, after which the benzene layer was separated and the aqueous layer was extracted twice with benzene. The combined solutions were chromatographed over 60–100 mesh "Florisil" using benzene as the eluting agent. The product came from the column as the first frac-

TABLE II

PICRATE AND METHIODIDE DERIVATIVES OF 12-DIALKYLAMINOALKYLBENZO[a]- AND -BENZO[b]PHENOXAZINE

Compound	Derivative	M.P.	Recrystn. Solvent	Molecular Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
I. $n = 2$ $R_1 = R_2 = CH_3$—	Methiodide	210°	95% Ethanol and ether	$C_{22}H_{25}IN_2O$	57.39	57.49 57.24	5.43	5.48 5.57	6.09	6.07 6.45
II. $n = 1$ $R_1 = R_2 = CH_3CH_2$—	Picrate	190°	Dioxane and ethanol	$C_{28}H_{27}N_5O_8$	59.89	59.96 59.95	4.81	4.93 5.09	12.44	12.28 12.78
II. $n = 2$ $R_1 = R_2 = CH_3$—	Picrate	234°	Propylene glycol and ethanol	$C_{27}H_{25}N_5O_8$	59.23	59.16 59.01	4.57	4.54 4.39	12.80	12.78 12.50
	Methiodide	242°	95% Ethanol and ether	$C_{22}H_{25}IN_2O$	57.39	56.95 57.20	5.43	5.61 5.53	6.09	5.88 5.93

tion and evaporation of the benzene yielded a green-yellow oil.

Anal. Calcd. for $C_{22}H_{22}Cl_2N_2O$: C, 65.86; H, 5.49; N, 6.98. Found: C, 65.95, 65.73; H, 5.69, 5.53; N, 6.85, 6.56.

The above oil was converted to the *hydrochloride* in ethanol-ether solvent. The over-all yield was 62% and the salt melted at 160°.

Anal. Calcd. for $C_{22}H_{23}Cl_3N_2O$: C, 60.34; H, 5.23; N, 6.40. Found: C, 60.48, 60.42; H, 5.16, 5.32; N, 6.33, 6.80.

12-{2-[Bis(2-chloroethyl)amino]ethyl}benzo[a]phenoxazine. (I, $n = 1$, $R_1 = R_2 = ClCH_2CH_2$—]. This preparation was conducted in accord with the procedure given above. The intermediate bis(hydroxyethyl)compound was not purified, but treated directly with phosphoryl chloride. The product was a solid, m.p. 68°.

Anal. Calcd. for $C_{22}H_{22}Cl_2N_2O$: C, 65.86; H, 5.49; N, 6.98. Found: C, 65.72, 65.67; H, 5.67, 5.75; N, 6.90, 6.97.

The *hydrochloride*, m.p. 140°, was formed in 64% over-all yield from 12-(2-chloroethyl)benzo[a]phenoxazine. The carbon analysis was somewhat high on this salt, although hydrogen and nitrogen values were satisfactory.

Anal. Calcd. for $C_{22}H_{23}Cl_3N_2O$: C, 60.34; H, 5.23; N, 6.40. Found: C, 61.30, 61.53; H, 5.37, 5.24; N, 6.45, 6.20.

Preparation of 12-dialkylaminoalkyl derivatives of benzo[a]- and benzo[b]phenoxazine. A solution of 1 equivalent of the benzophenoxazine in benzene was treated with 1.1 equivalents of *n*-butyllithium dissolved in hexane. After stirring under a nitrogen atmosphere for 30 min., 1 equivalent of the appropriate dialkylaminoalkyl chloride was added to the red-yellow slurry. The mixture was stirred and heated under reflux (nitrogen atmosphere) for 16 hr., during the early part of which a clear yellow solution was formed. Excess water was added and the benzene layer was extracted with several portions of 4% aqueous hydrochloric acid solution. The combined acid extracts were made basic with sodium hydroxide solution, and the precipitated base extracted with ether. After drying, the ether was evaporated and the residual oil was distilled.

The compounds prepared by this method are listed in Table I.

Table II lists some picrate and methiodide derivatives of the bases.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anticancer Agents. XI. Synthesis and Reactions of Derivatives of 6-Methyluracil-5-sulfonic Acid[1,2]

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On reaction with chlorosulfonic acid 6-methyluracil gives the 5-sulfonyl chloride. The sulfonic acid function in 6-methyluracil-5-sulfonic acid and its derivatives is remarkably susceptible to displacement as shown by a variety of reactions. A series of sulfonic acid esters and sulfonamides derived from 6-methyluracil-5-sulfonic acid has been prepared for evaluation as anticancer agents.

It is well known that alkylating agents such as ethylenimines, methanesulfonates, and sulfur or nitrogen mustards exert carcinostatic action against a number of transplanted animal tumors. However, lack of selectivity towards tumor cells and frequent high toxicity have often limited their use as practical chemotherapeutic agents in the management of human neoplastic disease. In this laboratory attempts have been made and are continuing to design agents which may not be active themselves but which possibly can be converted to active substances *in vivo*.[3] Such a goal can, at least in principle, be achieved by incorporating an alkylating function into a so-called "carrier molecule."

(1) The work here reported was done under Research Grant CY-2961 from the National Cancer Institute to The University of Michigan.

(2) For the preceding communication in this series, see R. C. Elderfield, M. Israel, J. H. Ross and J. A. Waters, *J. Org. Chem.*, **26**, 2827 (1961).

(3) See ref. 2 and earlier papers in this series.