line solid, mp 136–138° (ligroin) (65.6% yield). *Anal.* ($C_{16}H_{12}O_4$) C, H.

3-Epoxypropoxyxanthone (16) was obtained as a white crystalline product, mp 162–163° (ligroin) (71% yield). *Anal.* ($C_{16}H_{12}O_4$) C, H.

2-(3-Isopropylamino-2-hydroxypropoxy)xanthone (14). In a stainless vessel containing 5 g of 2-epoxypropoxyxanthone (**13**) (0.0186 mole) and 50 ml of BzH, a slight excess of isopropylamine (1.2 g, 0.02 mole) was added, and the mixture was kept, under stirring, at 50° for 12 hr. After removing of the solvent, the residue was crystallized from ligroin, giving 4 g (54% yield) of white product, mp 113–115°. *Anal.* ($C_{19}H_{21}NO_4$) C, H, N.

The hydrochloride salt (15) was obtained as a white solid, mp 222–224° (from MeOH–Et_2O). *Anal.* ($C_{19}H_{22}ClNO_4$) C, H, Cl, N.

3-(3-Isopropylamino-2-hydroxypropoxy)xanthone (17). In a similar manner, starting from 5 g (0.0186 mole) of compd **16**, 3.2 g (54% yield) of white solid, mp 143–144° (ligroin), was obtained. *Anal.* ($C_{19}H_{21}NO_4$) C, H, N.

The hydrochloride salt (18) was obtained as a white solid, mp 220–222° (MeOH–Et_2O). *Anal.* ($C_{19}H_{22}ClNO_4$) C, H, Cl, N.

References

(1) P. Da Re, P. Valenti, A. Borraccini, and G. P. Primofiore, *J. Med. Chem.*, **15**, 198 (1972).
(2) J. D. Fitzgerald, *Clin. Pharmacol. Ther.*, **10**, 292 (1969).
(3) S. R. Kottegoda, *Brit. J. Pharmacol.*, **8**, 83 (1953).
(4) R. C. Garry and J. S. Gillespie, *J. Physiol.*, **128**, 557 (1955).
(5) J. W. Blank, W. A. M. Duncan, and R. G. Shanks, *Brit. J. Pharmacol.*, **25**, 577 (1965).
(6) M. S. K. Ghouri and T. J. Haley, *J. Pharm. Sci.*, **58**, 511 (1969).

Preparation of *N*-(2-Chloroethyl)-*N*-(2-hydroxyethyl)arylamines. Possible Intermediates to Potential Carcinolytic Agents Bearing Dissimilar Reactive Functions

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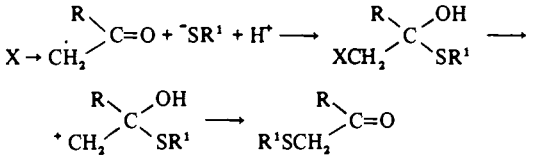
Although *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)arylamines are potentially useful intermediates in the preparation of bifunctional alkylating agents containing dissimilar reactive groupings, few syntheses of these compounds have been reported. Ross and his coworkers[1] prepared *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)anthranilic acid *via* the internal ester formed from the corresponding *N,N*-bis(2-hydroxyethyl) derivative. Yaguzhinskii and Chinaeva[2] applied a potentially more general procedure, the reaction of ethylene oxide with an *N*-(2-chloroethyl)arylamine, to the synthesis of *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)aniline (I).

Since *N,N*-bis(2-chloroethyl)arylamines bearing a wide range of carrying structures are known,[3] partial hydrolysis to the corresponding *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)-arylamines was investigated. Davis and Ross[4] have shown that, near neutrality, the rate constant ($k_1 = 13.8 \times 10^{-4}$ sec^{-1}) for the formation from 3-[4-bis(2-chloroethyl)aminophenoxy]propionic acid of the *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl) derivative was less than the value ($k_2 = 20.8 \times 10^{-4}$ sec^{-1}) for the hydrolysis of the latter. A similar relationship between k_1 and k_2 was obtained for a range of derivatives studied by Yaguzkinskii and Chinaeva.[2] Hence, the partial hydrolysis products do not accumulate under neutral conditions.

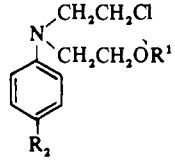
In contrast, when the hydrolysis of the three *N,N*-bis(2-chloroethyl)arylamines, aniline mustard [*N,N*-bis(2-chloroethyl)aniline],[5] *p*-hydroxyaniline mustard [*N,N*-bis(2-chloroethyl)-4-hydroxyaniline],[6] and chlorambucil {4-[4-bis(2-chloroethyl)aminophenyl]butyric acid},[1] was conducted in unbuffered aqueous acetonitrile, accumulation of the corresponding partial hydrolysis products (I–III) occurred, with little further hydrolysis to the *N,N*-bis(2-hydroxyethyl) derivatives. Clearly, the protonation of the basic nitrogen atom in the products of partial hydrolysis prevented further hydrolysis by the SN1 mechanism. Eventually, protonation of the more weakly basic *N,N*-bis(2-chloroethyl)arylamines proceeds as the hydrogen ion concentration rises, causing virtual cessation of the hydrolysis. In aqueous dioxan, partial hydrolysis is less complete when this terminal stage is approached, presumably owing to the lower dielectric constant of the medium. Since the above procedure exploits a feature inherent in the bis(2-chloroethyl)amino function, namely the lower pK_b of its derivatives compared with corresponding *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)-substituted analogs, it is potentially of general applicability.

The use of such an intermediate for the preparation of agents of mixed functionality is exemplified by the conversion of *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)aniline (I) into the halogenoacetyl derivatives (IV–VI). It has been suggested that the cross-linking of DNA to protein could be a significant factor contributing to the cytotoxic properties of bifunctional alkylating agents.[7] The halogenoacetyl group should confer reactivity toward thiol functions under physiological conditions according to the following mechanism.[8]

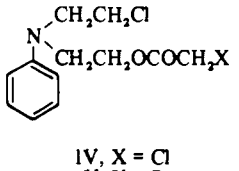


Hence the derivatives (IV–VI) might form cross-links between DNA and the cysteine residues of a protein molecule.

However, screening data revealed that the two compounds examined, IV and VI, had no curative effect on rats bearing the transplanted Walker 256 carcinoma at single intraperitoneal doses of up to 125 mg/kg for the chloro derivative (IV) (LD_{50} 595 mg/kg) and 25 mg for the iodo derivative (VI) (LD_{50} 25 mg/kg). The higher toxicity of the iodo derivative (VI) is consistent with the greater reactivity toward cellular thiol functions which would be conferred by the superior inductive effect (see above mechanism) of the iodo grouping. In contrast, the corresponding bis-nitrogen mustard, *N,N*-bis(2-chloroethyl)aniline gave 90% inhibition of tumor growth at a dose (ED_{90}) of 13.6 mg/kg.



I, $R^1 = R^2 = H$
II, $R^1 = H$; $R^2 = OH$
III, $R^1 = H$; $R^2 = CH_2CH_2CH_2CO_2H$



IV, X = Cl
V, X = Br
VI, X = I

The LD_{50} was 146 mg/kg, giving a therapeutic index (LD_{50}/ED_{90}) of 10.8.[9] Presumably, therefore, the reaction of the halogenoacetyl moiety is preponderantly with thiol groups not in proximity to DNA. Ross pointed out that compounds, such as halogeno ketones, unsaturated lactones, and vinyl sulfones, which react with thiol groups

under physiological conditions, also proved ineffective as inhibitors of the Walker carcinoma in rats.[10] Since the iodoacetyl grouping reacts more readily than does the nitrogen mustard grouping with thiol groups, the ineffectiveness against the Walker tumor of the iodoacetyl derivative (VI) and of *N,N'*-bis(2-iodoacetyl)-*o*-phenylenediamine[11] may be related to an extensive reaction with extracellular or extranuclear thiol groupings, thus preventing their reaction at the required site of action, assuming this to be DNA and its associated macromolecules.

Experimental Section†

N-(**2-Chloroethyl**)-*N*-(**2-hydroxyethyl**)**aniline (I).** A solution of *N,N*-bis(2-chloroethyl)aniline[5] (5 g) in CH_3CN–H_2O (3:2 v/v) (50 ml) was refluxed for 3 hr, then concd under reduced pressure. The concentrate was partitioned between HCl (1 *N*, 50 ml) and Et_2O (50 ml). The organic phase contained starting material (3 g). The aqueous phase was treated with Et_2O (50 ml) and then with satd aqueous $NaHCO_3$ (*ca.* 50 ml) to neutrality. The dried ($MgSO_4$) organic phase was concd and applied to a column of silicic acid (25 cm × 3 cm^2) which was eluted with the same solvent (10-ml fractions). Concn of fractions 10–19, which contained the reqd product (R_f 0.6 on tlc in Et_2O; starting material, R_f 0.8), gave a colorless oil (1.13 g, 25%: 62% based on unrecovered starting material) which turned blue on exposure to light. *Anal.* ($C_{10}H_{14}ClNO$) C, H, Cl, N.

N-(**2-Chloroethyl**)-*N*-(**2-hydroxyethyl**)-**4-hydroxyaniline (II).** A soln of *N,N*-bis(2-chloroethyl)-4-hydroxyaniline (from the hydrochloride,[6] 10 g) in CH_3CN–H_2O (1:1 v/v) (100 ml) was refluxed for 2 hr, and the product isolated as for I (column 25 cm × 8 cm^2, product in fractions 21–56, R_f 0.45 in Et_2O; starting material, R_f 0.7). On crystn from PhH, the product formed pale pink needles. (3.35 g, 42%), mp 83–85°. *Anal.* ($C_{10}H_{14}ClNO_2$) C, H, Cl, N.

4-[4-*N***-(2-Chloroethyl)-***N***-(2-hydroxyethyl)aminophenyl]butyric Acid (III).** A soln of 4-[4-bis(2-chloroethyl)aminophenyl]butyric acid[1] (5 g) in CH_3CN–H_2O (1:1 v/v) (100 ml) was refluxed for 2 hr. After concn and extn with Et_2O from a soln in 1 *N* HCl, the pH was adjusted to *ca.* 1.0 with 1 *N* NaOH. Further extn with Et_2O (50 ml) removed some product (III) together with all remaining starting material (which otherwise chromatographed in partial admixture with the product). The aqueous phase was further basified to pH 3.0 at which value the CO_2H group is still largely un-ionized and then extd with Et_2O (50 ml). The concd organic phase was chromatographed as for I (column 35 cm × 4 cm^2, product in fractions 8–35, R_f 0.35 in Et_2O; starting material, R_f 0.6). On crystn from *i*-Pr_2O (40 ml) at −15°, the product formed hemispherical clusters of colorless needles (0.82 g, 17%), mp 56–58°. *Anal.* ($C_{14}H_{20}ClNO_3$) C, H, Cl, N.

N-(**2-Chloroacetoxyethyl**)-*N*-(**2-chloroethyl**)**aniline (IV).** To a stirred soln of *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)aniline (2 g) in 2,6-lutidine (5 ml) at −10° was added dropwise chloroacetyl chloride (1.13 g). After 0.5 hr, the soln was stirred at room temperature for a further 2 hr, then poured into ice-cold dil H_2SO_4, and then extd with $CHCl_3$. The organic phase was washed (10% aqueous Na_2CO_3), dried ($MgSO_4$), and then concd. A soln of the concentrate in PhH was applied to a column of silicic acid (18 cm × 9 cm^2) which was eluted with the same solvent (5-ml fractions). On concn, the fractions containing the component of R_f 0.5 (tlc in PhH) gave a colorless oil (1.65 g, 60%), $n^{23.5}$D 1.5578°. *Anal.* ($C_{12}H_{15}Cl_2NO_2$) C, H, Cl, N.

N-(**2-Bromoacetoxyethyl**)-*N*-(**2-chloroethyl**)**aniline (V).** The title compound, prepared as for IV, using bromoacetyl chloride, was obtained as a colorless oil (2.08 g, 65%) of the same R_f in PhH, $n^{22.9}$D 1.5734°. *Anal.* $C_{12}H_{15}BrClNO_2$) C, H, Br, Cl, N.

N-(**2-Chloroethyl**)-*N*-(**2-iodoacetoxyethyl**)**aniline (VI).** To a stirred soln of the bromo derivative (V, 3.2 g) in Me_2CO (10 ml) was added a soln of NaI (2.3 g) in this solvent. NaBr separated during 0.5 hr. The filtered soln was concd, and a soln of the concentrate in PhH was chromatographed as for IV. On concn, the fractions containing the component of R_f 0.7 in PhH gave a slightly colored oil (2.13 g, 58%), $n^{21.8}$D 1.5875°. *Anal.* ($C_{12}H_{15}ClINO_2$) C, H, Cl, N; I: calcd, 34.5; found, 34.0.

†Melting points, which were corrected, were determined with a Kofler hot-stage apparatus. Merck Kieselgel G was used for column chromatography and for thin-layer chromatography (tlc) on coated microscope slides.

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References

(1) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).
(2) L. S. Yaguzhinskii and A. D. Chinaeva, *J. Gen. Chem. USSR*, **36**, 685 (1966).
(3) W. C. J. Ross, "Biological Alkylating Agents," Butterworths, London, 1962.
(4) W. Davis and W. C. J. Ross, *Annu. Rep. Brit. Empire Cancer Campgn.*, **37**, 39 (1959).
(5) W. C. J. Ross, *J. Chem. Soc.*, 183 (1949).
(6) M. H. Benn, A. M. Creighton, L. N. Owen, and G. R. White, *ibid.*, 2365 (1961).
(7) R. J. Rutman, W. J. Steele, and C. C. Price, *Cancer Res.*, **21**, 1124 (1961).
(8) M. Dixon, *Biochem. Soc. Symp.*, **2**, 39 (1948).
(9) M. Artico and W. C. J. Ross, *Biochem. Pharmacol.*, **17**, 893 (1968).
(10) W. C. J. Ross, *Ann. N. Y. Acad. Sci.*, 68, 669 (1958).
(11) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, 1972 (1949).

ω-(*N,N*-Diethylamino)-*n*-alkyl 3,4,5-Trimethoxybenzoates as Local Anesthetics†

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A series of ω-(*N,N*-diethylamino)-*n*-alkyl 3,4,5-trimethoxybenzoates (TMB) were prepared by the esterification of the appropriate ω-(*N,N*-diethylamino)-*n*-alkanol with 3,4,5-trimethoxybenzoyl chloride (I) according to the general synthetic method described by Sastry and Lasslo.[2] These compounds have the structural characteristics of a typical local anesthetic:[3] a lipophilic part and a hydrophilic part connected by an intermediate chain. Therefore, they were tested for their local anesthetic activities.

Experimental Section‡

Synthetic Methods. Each TMB was prepd as a white cryst HCl salt and purified by recrystn from C_6H_6 unless otherwise stated.

2-(*N,N*-Diethylamino)ethyl 3,4,5-trimethoxybenzoate·HCl (II) was prepd from 10.6 g (0.05 mole) of I and 6.6 g (0.05 mole) of 2-(*N,N*-diethylamino)ethanol, yield 12.4 g (72%), mp 153–154.5° (lit.[4] 152–155°).

3-(*N,N*-Diethylamino)propyl 3,4,5-trimethoxybenzoate·HCl (III) was prepd from 10.6 g (0.05 mole) of I and 7.0 g (0.05 mole) of 3-(*N,N*-diethylamino)-1-propanol, yield 14.1 g (80%), mp 169.5–171° (lit.[5] 172°).

†A part of this investigation[1] was presented orally at the Meetings of the American Society for Pharmacology and Experimental Therapeutics in Pittsburgh, Pa., Aug 1969. This investigation was supported by a Graduate Traineeship to one of the authors (C.P.R.), Grant No. GZ-604 from the National Science Foundation; U. S. Public Health Service Training Grant No. GM 00058 from the National Institute of General Medical Sciences and U. S. Public Health Service Research Grant No. NS-04699 from the National Institute of Neurological Diseases and Stroke.

‡Microanalysis was reported by International Chemical and Nuclear Corp., City of Industry, Calif. Analytical results obtained were within ±0.4% of the theoretical values. All melting points were uncorrected.