Synthesis of Propranolol Mustard as a Possible Lung-Specific Antitumor Agent

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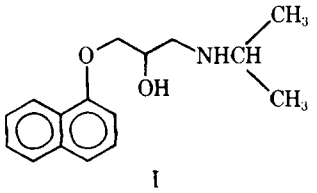
Abstract □ A nitrogen mustard analog of propranolol was synthesized as a potential lung-specific antitumor agent. Since *dl*-propranolol concentrates in lung tissue and β-blocking activity resides only with the *l*-enantiomer, the *d*-modification could serve as a lung-directed carrier for a cytotoxic group. Reaction of 1-(1-naphthyloxy)-3-[bis(2-hydroxyethyl)amino]-2-propanol with thionyl chloride resulted in replacement of all three hydroxyl groups with chlorine. The necessary chlorination selectivity was achieved with *p*-toluenesulfonyl chloride in dimethylformamide solution to provide propranolol mustard, 1-(1-naphthyloxy)-3-[bis(2-chloroethyl)amino]-2-propanol. Both the trichloro compound and propranolol mustard showed reproducible activity against P-388 leukemia. Neither compound was active against the B16 tumor or Lewis lung carcinoma.

Keyphrases □ Propranolol—nitrogen mustard analog, synthesis, potential lung-specific antitumor activity □ Antitumor agents, potential—lung specific, propranolol mustard, synthesis □ Alkylating agents—propranolol mustard, synthesis, potential lung-specific antitumor activity

Alkylating agents, especially the nitrogen mustards, continue to be an important class of drugs in the management of clinical cancer. Although early analogs involved the attachment of the mustard group to carriers seemingly randomly selected (1), cancer chemotherapists of the last decade have attempted to design new mustard derivatives based on exploitable differences between cell types and with the best attainable cytotoxic specificity toward neoplastic tissue (2).

A carrier moiety that has potential for selectively directing a mustard group to lung tissue was suggested recently (3) as having possible utility in the treatment of pulmonary carcinoma. It was reported (4) that high levels of *dl*-propranolol (I) concentrate in the lungs. Although the *l*-enantiomer produces a β-adrenergic blockade, the antipode is inactive as a β-blocker. Therefore, *d*-propranolol might serve effectively as a carrier for lung-directed alkylating agents that would be devoid of undesired effects on the peripheral autonomic nervous system. For example, replacement of the isopropylamino substituent of I with a bis(2-chloroethyl)amino group would result in mustard V shown in Scheme I.

As a continuation of the development of drugs for the treatment of lung cancer (5), the synthesis of a propranolol mustard (V) was initiated. Although the preliminary plan called for working with racemic synthetic intermediates leading to racemic V, the synthetic sequence (Scheme I) provided the option of resolution of optical isomers if biological test data of the racemic mixtures were encouraging.



I

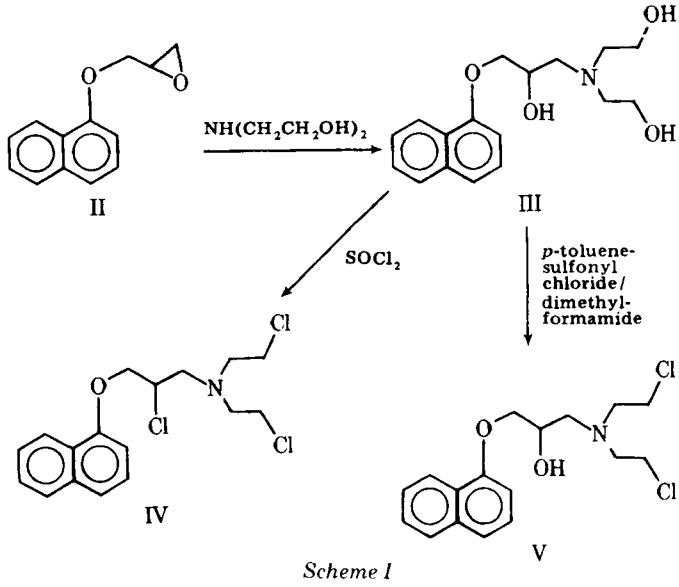
RESULTS AND DISCUSSION

Chemistry—Condensation of (1-naphthyloxy)methyloxirane (II) with diethanolamine (Scheme I) gave a triol (III), which was isolated in excellent yield as the hydrochloride salt. Conversion of III to V necessitated a regioselective chlorination of the two primary hydroxyl groups to generate the mustard functionality. Chlorination of III with thionyl chloride, using a procedure (6) that was utilized successfully in the preparation of a similar nitrogen mustard, nonspecifically gave the trichloro compound IV as the only isolated product.

The ^{13}C-NMR spectrum[1] of IV showed chemical shifts at 42.0 and 57.2 ppm due to the carbon atoms bearing the chlorine substituents and the carbon atoms bonded to the nitrogen atom of the mustard group, respectively. These assignments compare well with those reported (7) for the bis(2-chloroethyl)amino group of cyclophosphamide. The remaining aliphatic carbon atoms gave resonance signals at 69.2 (OCH_2), 58.6 (ClCH), and 57.5 (CH_2N) ppm. These assignments were verified by observing the chemical shift multiplicities in a spectrum obtained in the partially decoupled mode. The shifts recorded for the carbon atoms of the aromatic nucleus were in line with those reported (8) for I.

The necessary regiospecific chlorination of III was obtained using *p*-toluenesulfonyl chloride in dimethylformamide solution to provide the targeted compound, propranolol mustard (V). The poor water solubility of the hydrochloride salt of V allowed it to be separated easily in a 44% yield from other reaction products. Although used to advantage in the present work, the formation of alkyl chlorides first was reported (9) as a side reaction occurring with the tosylation of primary alcohols with *p*-toluenesulfonyl chloride in dimethylformamide solution.

The mass spectra of mustards IV and V showed molecular ions at *m/e* 359 and 341, respectively, and both gave their base peaks at *m/e* 154 due to the $[CH_2{=}N(CH_2CH_2Cl)_2]^+$ ion, which resulted from cleavage of the



Scheme I

[1] Determined in deuterochloroform solution (0.3 *M*) by R. J. Highet, National Heart, Lung, and Blood Institute, National Institutes of Health, with a JEOL FX-60 spectrometer.

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Table I—Antitumor Evaluations

Compound	Dose[b], mg/kg/day	Percentage Increase of Lifespan[a] P-388 Leukemia Trial 1	P-388 Leukemia Trial 2	B16 Melanoma Trial 1	B16 Melanoma Trial 2	Lewis Lung Carcinoma (Subcutaneous) Trial 1	Lewis Lung Carcinoma (Subcutaneous) Trial 2	Lewis Lung Carcinoma (Intravenous) Trial 1	Lewis Lung Carcinoma (Intravenous) Trial 2
IV	40	33	39	2	30	−3	T[c]	13	−2
	20	34	41	22	24	6	−4	5	−2
	10	19	27	12	15	10	−8	−3	0
	5	27	20	2	11	1	−3	−2	−10
	2.5			3	13	−4	3	−3	
	1.25			9		3	−8	−8	−9
V	40	T	T	T	T	T	T	T	T
	20	−9	−9	T	T	T	T	T	T
	10	49	44	12	6	T	T	−8	12
	5	46	40	17	17	3	3	−4	−5
	2.5	40	31	19	15	1	0	−6	−2
	1.25	30	22	13	11	4	−6	5	−11

[a] The percentage increase (decrease) of the median lifespan of test animals beyond the median survival time of untreated control animals was used to evaluate antitumor activity. Activity is defined as a percentage increase of lifespan of ≥25%. [b] Drugs were suspended in 0.9% saline with hydroxypropylcellulose and administered daily for 9 days after tumor implantation. [c] The T indicates that deaths occurred due to drug-related toxicity.

carbon–carbon bond located α,β to the nitrogen atom. The ^{1}H-NMR spectra of IV and V were consistent with the structures assigned.

Antitumor Evaluation—Propranolol mustard (V) as well as the trichloro Compound IV was evaluated extensively *in vivo* for antitumor activity using three transplantable murine tumors: P-388 leukemia (PS), B16 melanoma (B16), and Lewis lung carcinoma (LL). The last tumor was employed in one system (10) where Lewis lung carcinoma tumor cells were given intravenously and in another system (11) where the Lewis lung carcinoma tumor inoculum was implanted subcutaneously. The P-388 and B16 test systems were used according to established protocols (12). In each of the four test systems, test compounds were administered intraperitoneally daily for 9 days beginning 24 hr after tumor implantation. Dose–response data for IV and V against each test system are recorded in Table I; duplicate determinations were carried out for each system.

Both IV and V prolonged the lifespan of mice bearing P-388 leukemia beyond that of untreated control animals. The percentage increase of lifespan achieved in leukemic mice by IV was between 34 and 41% at a dosage of 20 mg/kg/day. Propranolol mustard (V) gave a somewhat more potent antileukemic effect than IV since just 10 mg/kg/day was sufficient to produce a similar range increase (44–49%). That the bis(2-chloroethyl)amino group is necessary for activity was supported by the lack of activity observed with triol III against P-388 leukemia over a dose range of 400–12.5 mg/kg/day.

Neither IV nor V gave significant activity indications against B16 melanoma, a test system model for solid tumors (Table I). Table I also indicates that neither IV nor V was active in the lung tumor models. The Lewis lung tumor, both in the subcutaneous and intravenous implantation procedures, was completely unaffected by mustards IV and V.

The absence of activity shown by propranolol mustard in the Lewis lung system could be attributed to the net increase in the partition coefficient (log P) resulting from replacement of the $NHCH(CH_3)_2$ group in propranolol with the more lipophilic $N(CH_2CH_2Cl)_2$ group. However, the lack of activity also could be ascribed to an inappropriate utilization of propranolol as a carrier moiety. If the aliphatic portion of I is the essential element in the molecule to effect preferential concentration in lung tissue, then a rational design of a lung-specific antitumor agent would suggest attachment of the cytotoxic mustard group at a point distant from the side chain. Future work also might consider the use of a nitrosourea substituent as the cytotoxic functionality since members of the nitrosourea series have greater incidence of activity against the Lewis lung tumor than do the nitrogen mustards (13).

EXPERIMENTAL[2]

(1-Naphthyloxy)methyloxirane (II)—A literature method (14) was used to convert 110 g (0.76 mole) of 1-naphthol into II. It was obtained as a pale-yellow oil in a 21% yield after distillation, bp 141–144° (0.07 mm) [lit. (15) bp 140° (2 mm)]. The integrated NMR spectrum (60 Hz) was consistent with the assigned structure.

1-(1-Naphthyloxy)-3-[bis(2-hydroxyethyl)amino]-2-propanol (III)—A solution of 16.0 g (0.08 mole) of II and 10.1 g (0.096 mole) of diethanolamine in 200 ml of 1-propanol was refluxed for 2 hr. The reaction solution was concentrated under vacuum at 40°, and the residue was treated with excess methanolic hydrogen chloride. Four volumes of ether were added, and the mixture was allowed to stand overnight at 4°, giving 24.8 g (91% yield) of III as the crystalline hydrochloride salt, mp 140–142°. The melting point was raised to 142–142.5° after recrystallization from methanol–ether (1:2).

Anal.—Calc. for $C_{17}H_{23}NO_4$·HCl: C, 59.73; H, 7.08; Cl, 10.37; N, 4.10. Found: C, 59.71; H, 6.88; Cl, 10.13; N, 4.13.

The free base was extracted with chloroform from an aqueous solution of III·HCl, which was made basic with dilute sodium hydroxide solution. Evaporation of the dried extracts gave the liberated base (III) as a pale-yellow syrup. This syrup could not be induced to crystallize but was homogeneous by TLC; IR: 3360, 1627, 1596, 1579, 1458, 1397, 1262, 1100, 1066, and 1033 cm^{-1}; NMR (60 MHz): δ 2.68 (t, J = 5.5 Hz, 4H, NCH_2CH_2) and 3.52 (t, J = 5.5 Hz, 4H, CH_2OH).

The picrate of III was formed from ethanol and gave a melting point of 161–162° after recrystallization from ethanol [lit. (16) mp 161–163°].

1-(1-Naphthyloxy)-2-chloro-3-[bis(2-chloroethyl)amino]propane (IV)—To a solution of purified (17) thionyl chloride (16.3 g, 0.137 mole) in 60 ml of dry (sodium) benzene, which was stirred and cooled to 2°, was added dropwise a solution of II (10.45 g, 0.0342 mole) in 60 ml of dry benzene at a rate such that the solution temperature did not exceed 5°. After the addition was complete, the reaction solution was refluxed and stirred for 2.5 hr and then evaporated under reduced pressure at room temperature. Thionyl chloride traces were removed by codistillation with two 100-ml portions of dry benzene.

The residue was partitioned between 100 ml of benzene and 100 ml of 2 N aqueous NaOH solution. The layers were separated, and the aqueous layer was saturated with sodium chloride and extracted twice with benzene. The combined benzene solutions were washed twice with water, dried with anhydrous sodium sulfate, treated with activated charcoal, and evaporated to give 9.5 g of an oil. The oil was distilled in a short-path apparatus (150–170°, 0.2 mm) in 0.5–1.7-g portions to give a combined distillate of 7.0 g as a pale-yellow oil. The oil was extracted with hot petroleum ether.

Storage of the extract overnight at ~−15° gave 4.72 g (38%) of white crystals (IV), mp 45–46°; IR: 1583, 1510, 1460, 1404, 1267, 1240, and 1103 cm^{-1}; NMR (100 MHz): δ 2.96 (t, J = 6 Hz, 4H, NCH_2CH_2), 3.08–3.26 (m, 2H, $CHCH_2N$), 3.48 (t, J = 6 Hz, 4H, CH_2Cl), 4.23–4.56 (m, 3H, $ArOCH_2CH$), and 6.78–8.30 (m, 7H, aromatic); mass spectrum: m/e 359 (M^+, 4.4%), 323 (M − HCl, 1.2), 310 (M − CH_2Cl, 8.8), 216 (8.2), 202 (M − $CH_2OC_{10}H_7$, 8.2), 183 (13), 154 [$CH_2{=}N(CH_2CH_2Cl)_2$, 100], 144 (20), 127 (9.8), and 115 (32).

Anal.—Calc. for $C_{17}H_{20}Cl_3NO$: C, 56.61; H, 5.59; Cl, 29.49; N, 3.88. Found: C, 56.71; H, 5.62; Cl, 29.44; N, 4.02.

The picrate of IV was formed from chloroform and gave a melting point of 123–124° after recrystallization from ethanol.

[2] PMR spectra were determined with a Varian T-60 or HA100 spectrometer in deuterochloroform solution. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane, which was used as an internal standard. IR spectra were recorded in chloroform solution with a Perkin-Elmer model 621 spectrophotometer. Mass spectra were obtained by direct probe insertion with a DuPont 21-492 spectrometer operated with a 75-ev ionizing voltage. Melting points were measured on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation, National Institute of Arthritis, Metabolism, and Digestive Disease, National Institutes of Health.

Anal.—Calc. for $C_{17}H_{20}Cl_3NO \cdot C_6H_3N_3O_7$: C, 46.84; H, 3.93; Cl, 18.03; N, 9.50. Found: C, 46.87; H, 4.09; Cl, 17.83; N, 9.45.

1-(1-Naphthyloxy)-3-[bis(2-chloroethyl)amino] -2- propanol (V)—*p*-Toluenesulfonyl chloride (6.25 g, 0.0328 mole) was added to a solution of III (5.0 g, 0.0164 mole) in 50 ml of dry (molecular sieves) dimethylformamide. The reaction mixture was stirred and heated for 2 hr at 55–65°. The solvent was removed under reduced pressure at 40° with a rotary evaporator, and the residual syrup was partitioned between 250 ml of benzene and 100 ml of 1 *N* aqueous NaOH solution. The benzene layer was separated, washed with water, and dried over anhydrous sodium sulfate.

Under vigorous stirring, 250 ml of 2 *N* HCl was added dropwise over 30 min to the benzene solution. The stirring was continued for an additional hour after the addition was completed. The white crystals that formed were removed by filtration and washed with 2 *N* HCl, benzene, and ether to give 2.71 g (44% yield) of V as the hydrochloride salt, mp 149–153°. Analytically pure hydrochloride was obtained by chromatographing the free base and by converting the purified base back to the hydrochloride. Accordingly, the base was liberated by benzene extraction from 1 *N* NaOH.

After evaporation of the dried extracts, the residue was chromatographed over silica[3]. Elution of the column with chloroform gave V as a noncrystallizable oil, which was treated with ethanol saturated with hydrogen chloride. The resulting crystalline hydrochloride was recrystallized from the same solvent, mp 155.5–157°; IR (mineral oil mull): 3260 (br), 2580 (br), 1580, 1400, 1391, 1270, 1239, 1126, and 1100 cm^{-1}.

Anal.—Calc. for $C_{17}H_{21}Cl_2NO_2 \cdot HCl$: C, 53.91; H, 5.86; Cl (total), 28.08; Cl (ionic), 9.36; N, 3.70. Found: C, 53.80; H, 5.83; Cl (total), 27.98; Cl (ionic), 9.50; N, 3.70.

The free base appeared as one spot when analyzed by TLC (silica, chloroform); NMR (100 MHz): δ 2.78 (d, J = 7 Hz, 2H, $CHCH_2N$), 2.95 (t, J = 6 Hz, 4H, NCH_2CH_2), 3.55 (t, J = 6 Hz, 4H, CH_2Cl), 4.02–4.32 (m, 3H, $ArOCH_2CH$), and 6.76–8.26 (m, 7H, aromatic); mass spectrum: *m/e* 341 (M^+, 2.3%), 305 (M − HCl, 2.1), 292 (M − CH_2Cl, 1.7), 256 (M − CH_2Cl − HCl, 3.3), 198 (1.1), 186 (2.3), 183 (2.9), 154 [$CH_2{=}N(CH_2CH_2Cl)_2$, 100], 144 (13), and 115 (17).

[3] Bio-Sil A, Bio-Rad Laboratories, Richmond, CA 94804.

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Centrally Active *N*-Substituted Analogs of 3,4-Methylenedioxyphenylisopropylamine (3,4-Methylenedioxyamphetamine)

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Received April 29, 1979, from the **Institute of Pharmacology, University of Bonn, 53 Bonn, West Germany*, and ‡*1483 Shulgin Road, Lafayette, CA 94549.* Accepted for publication September 20, 1979.

Abstract □ The known central nervous system activity of 3,4-methylenedioxyphenylisopropylamine and its *N*-methyl homolog prompted the synthesis of a series of analogs with substituents on the nitrogen atom. Most of these analogs (R = alkyl, alkenyl, hydroxy, alkoxy, and alkoxyalkyl) were prepared by the reductive alkylation of 3,4-methylenedioxyphenylacetone with the appropriate amine and sodium cyanoborohydride. Hindered isomers were synthesized indirectly. Measurements of their pharmacological activity in several animal assays and in human subjects indicated that the central activity decreased with the increasing bulk of the *N*-substituent.

Keyphrases □ Amines, phenylalkyl, substituted—*N*-substituted analogs of psychotomimetic agents □ Psychotomimetic agents—*N*-substituted analogs of known centrally active agents □ 3,4-Methylenedioxyphenylisopropylamine—synthesis of centrally active *N*-substituted analogs

The structural nucleus of amphetamine has served as the basis of numerous compounds of pharmacological interest. Amphetamine itself is a sympathomimetic stimulant at nominal dosages and leads to a psychotomimetic syndrome only at high dose levels (1). Nuclear alkyl substitution maintains the stimulant properties of the parent compound (2, 3), but the substitution of methoxyl groups leads to compounds that are primarily psychotomimetic

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