N^4-(2-Bromoethyl)-N^4-ethylsulfanilamide, a Monofunctional Nitrogen Mustard with Antitumor Action[1]

P. HEBBORN AND D. J. TRIGGLE

Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo 14, New York

Received January 14, 1965

A very large number of 2-halogenoethylamines have been tested for their antitumor action.[2,3] However, effective action is apparently confined to the bifunctional derivatives ("nitrogen mustards") with the monofunctional analogs exhibiting toxicity but no significant antitumor action.[2,4,5] However, the suggestion has recently been made[5] that bifunctionality of alkylating potential may not be a necessary prerequisite for antitumor action in compounds that act at a specific cellular site or receptor.

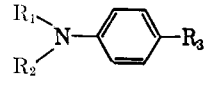
Hawkins, *et al.*,[6] reported that antitumor action in an extensive series of alkylating sulfonamides, $(BrCH_2CH_2)_2NC_6H_4SO_2NHR$-4, was observed only when R = H, NH_2, or CH_2COOH. This strict structure–activity relationship (with other evidence[6]) led to the suggestion that alkylation of a specific cellular site (possibly a folate utilizing enzyme) was responsible for the antitumor action of these compounds and that monofunctional analogs, *i.e.*, N^4-(2-bromoethyl)-N^4-ethylsulfanilamide, might also prove to be effective antitumor agents.[5]

Some alkylating and nonalkylating sulfonamides are compared with N-(2-bromoethyl)-N-ethylaniline in Table I. On subacute administration, the monofunctional compounds were more toxic than the bifunctional analog **4**, while the nonalkylating analog **5** was nontoxic at a relatively high dose (400 mg./kg./day). None of the monofunctional compounds produced leucopenia at the LD_{50} dose level, which contrasts with the marked leucopenia produced by the bifunctional compound. However, it was possible to distinguish between the monofunctional nitrogen mustards by comparing their antitumor activities. Compound **1** produced moderate growth inhibition of the Murphy–Sturm lymphosarcoma only at a dose which approximated the LD_{50} value; the Walker carcinosarcoma was not affected. Compound **2** was inactive against both tumors. Compound **3** had marked activity against the Murphy–Sturm tumor at nonlethal dose levels, and complete and permanent regression of established tumors was observed. The Walker tumor was less sensitive than the Murphy–Sturm tumor, but a significant growth inhibition was observed with **3** at the maximum tolerated dose (25 mg./kg./day). The bifunctional alkylating sulfonamide **4** produced complete regression of both tumors. The nonalkylating analog **5** was devoid of activity at the highest dose level tested.

Compound **3** is thus a monofunctional nitrogen mustard which, in contrast with other monofunctional nitrogen mustards, *e.g.*, **1**, shows marked antitumor activity. Moreover, **3** is more potent than the bifunctional analog **4** in producing complete regression of the Murphy–Sturm tumor. Confirmation of the prediction that a monofunctional alkylating sulfonamide may have antitumor activity lends support to the suggestion that a specific macromolecular cell constituent is alkylated. The lack of activity of **2** may be due to a number of factors, including an alteration of the basicity of N-4, which may adversely affect the adsorption of the compound at a specific receptor. Further studies of the mechanism of action of these compounds are in progress.

TABLE I

TOXICITY AND ANTITUMOR ACTIVITIES



No.	R_1	R_2	R_3	Acute LD_{50}, mg./kg. mouse	Acute LD_{50}, mg./kg. rat	Leucopenia at LD_{50}, %	Therapeutic evaluation[a] LD_{50}, rat,[b] mg./kg.	Therapeutic evaluation[a] Dose,[b] mg./kg.	Therapeutic evaluation[a] Murphy–Sturm T/C[c]	Therapeutic evaluation[a] Walker 256 T/C[c]
1	CH_2CH_3	CH_2CH_2Br	H	44	35	0	11	10	0.3	0.95
								5	0.5	1.05
2	CH_2CH_3	$COCH_2Cl$	SO_2NH_2	150	120	0	18	15	1.1	1.0
3	CH_2CH_3	CH_2CH_2Br	SO_2NH_2	290	70	0	30	25	0.0[d]	0.4
								15	0.0[d]	0.9
								10	0.3	
4	CH_2CH_2Br	CH_2CH_2Br	SO_2NH_2	215	285	90	110	50	0.0[d]	0.3[d]
								25	0.2	0.6
5	CH_2CH_3	CH_2CH_3	SO_2NH_2	>400	>400	...	>400	400	0.9	0.9

[a] Compounds administered i.p. in oil. [b] Administered daily from fifth day after tumor implantation to the ninth day. [c] Ratio of mean volume of tumors of treated and control animals, estimated on day 12. [d] Complete and permanent regression of tumor.

Experimental[7]

N^4-(2-Bromoethyl)-N^4-ethylsulfanilamide.—N-Ethyl-N-(2-hydroxyethyl)aniline (82 g., 0.5 mole) in $CHCl_3$ (200 ml.) was added dropwise to a solution of PBr_3 (150 g., 0.55 mole) in

(1) This investigation was supported by Public Health Service Grant No. CA 06645-02 from the National Cancer Institute.

(2) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co. Ltd., London, 1962.

(3) R. P. Bratzel, *Cancer Chemotherapy Rept.*, **26**, 1 (1963).

(4) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).

(5) D. J. Triggle, *J. Theoret. Biol.*, **7**, 241 (1964).

(6) R. Hawkins, L. N. Owen, and J. F. Danielli, *ibid.*, **5**, 236 (1963).

(7) Melting points are recorded on a Thomas-Kofler micro hot stage and are corrected. Analyses are by Galbraith Laboratories, Knoxville, Tenn., and Dr. A. Bernhardt, Mülheim, Germany.

$CHCl_3$ (200 ml.) at 0°. The solution was stirred at room temperature for 60 min., refluxed for 4 hr., and then filtered. The filtrate was neutralized ($NaHCO_3$ solution), dried, and distilled to give N-(2-bromoethyl)-N-ethylaniline, b.p. 110–112° (0.1 mm.), in 45% yield.

Anal. Calcd. for $C_{10}H_{14}BrN$: Br, 35.08. Found: Br, 34.84.

N^4-(2-Bromoethyl)-N^4-ethylsulfanilamide was prepared, in 17% yield, from N-(2-bromoethyl)-N-ethylaniline according to the method of Benn, *et al.*[8] It had m.p. 130–131° (benzene).

Anal. Calcd. for $C_{10}H_{15}BrN_2O_2S$: C, 39.1; H, 4.92, Br, 26.02; N, 9.12. Found: C, 39.26; H, 5.1; Br, 25.98; N, 8.7.

N^4-Chloroacetyl-N^4-ethylsulfanilamide.—N-Chloroacetyl-N-ethylaniline[9] (10 g., 0.05 mole) in CCl_4 (25 ml.) was treated with chlorosulfonic acid (15 ml.) at 0°. The mixture was then maintained at 100–110° for 1 hr. and poured onto ice. The precipitate of N^4-chloroacetyl-N^4-ethylsulfanilyl chloride was filtered and dissolved in acetone (30 ml.) and added to NH_4OH (25 ml., *d* 0.88) and stirred for 10 min. Dilution with water and crystallization of the precipitate from benzene gave the sulfonamide as white plates, m.p. 138° (9.8 g., 70%), which were soluble in 10% NaOH.

Anal. Calcd. for $C_{10}H_{13}ClN_2O_3S$: C, 43.4; H, 4.73; Cl, 12.82; N, 10.12. Found: C, 43.27; H, 4.69; Cl, 12.90; N, 9.61.

Toxicity Determinations.—Male Holtzman rats (180–200 g.) and male Swiss mice (22–26 g.) were used. Compounds dissolved or suspended in cottonseed oil were administered by intraperitoneal injection to groups of 3 to 6 animals per dose level. Deaths within a 21-day period were recorded and approximate LD_{50} values were estimated graphically from per cent mortality/log dose plots.

Therapeutic Evaluation.—Murphy–Sturm lymphosarcoma and Walker carcinosarcoma 256 were implanted subcutaneously in the flank region of male Holtzman rats using a trochar and cannula. Five days later, when the tumors weighed 5–10 g., the compound was injected intraperitoneally daily for 5 days to groups of 6–10 rats. Control animals received injections of cottonseed oil. Changes in volume of the tumors were estimated from daily measurements taken by caliper.[10]

Development of leucopenia was determined from leucocyte counts performed 72 hr. after a single intraperitoneal injection of an LD_{50} dose.

Acknowledgment.—The authors acknowledge the able assistance of Mr. Paul L. Stanley in the biological testing.

(8) M. H. Benn, A. M. Creighton, B. J. Johnson, L. N. Owen, and G. R. White, *J. Chem. Soc.*, 3395 (1964).

(9) E. B. Kelsey, *J. Am. Chem. Soc.*, **46**, 1694 (1924).

(10) P. Hebborn and J. F. Danielli, *Biochem. Pharmacol.*, **1**, 19 (1958).

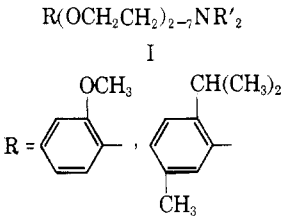
Basic Ethers of Guaiacol and Thymol with a Polyoxyethylenic Chain and Their Main Pharmacological Activities. New Antitussives

M. CARISSIMI, A. CATTANEO, R. D'AMBROSIO, V. DE PASCALE, E. GRUMELLI, E. MILLA, AND F. RAVENNA

Research Laboratories of Maggioni and Company, S.p.A., Milan, Italy

Received January 2, 1965

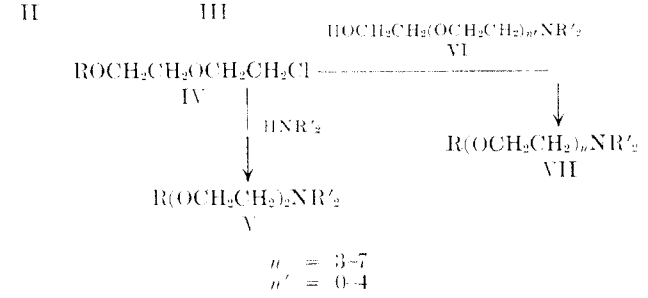
Among the compounds showing antitussive activity are found methoxypoly(ethyleneoxy)ethyl *p*-butylaminobenzoate (benzonatate) and 2-(diethylaminoethoxy)ethyl 1-phenylcyclopentyl-1-carboxylate (carbetapentane), both containing a polyoxyethylenic chain, which according to Bucher[1] seems to possess a selective affinity for the myelin surrounding the afferent pathways of stretch and tactile receptors. We synthetized two series of substances (I) by introducing such a chain ending with a basic group into two molecules, guaiacol and thymol, which are employed in the therapy of the respiratory apparatus. They were screened as antitussives.



Since the activity of many antitussive drugs such as codeine and its derivatives involves some more or less pronounced central effects (respiratory and circulatory impairments, general sedation, nausea, tolerance) as well as the inhibition of intestinal peristalsis and bronchoconstriction, and since several recently described thymol derivatives[2] showed marked sedative properties, we tried to determine the degree of such activities in our compounds in order to ascertain through a pharmacological profile as complete as possible the selectivity of their antitussive activity.

Chemistry.—The synthesis of the compounds listed in Table I was carried out according to Scheme I.

SCHEME I



2-Chloroethyl 2-aryloxyethyl ethers (IV) were prepared by condensing sodium guaiacolate or thymolate (II) with bis(2-chloroethyl) ether (III); IV reacted with a secondary amine in 1-hexanol in the presence of pyridine and gave compounds V (n = 2). For the synthesis of higher homologs (VII) the same compounds IV were condensed with a polyoxyethylenic amino alcohol (VI) in xylene in the presence of an alkali metal. Most of the amino alcohols VI used were prepared according to literature methods (see Table II) suitably improved when yields and purity of the products were unsatisfactory. Members where n' = 2, 3, and 4 were synthesized by treating 2-(2-aminoethoxy)-ethanols (VI, n' = 1) with ethylene oxide; in turn, compounds VI were prepared by condensation of 2-chloroethyl 2-acetoxyethyl ether with a secondary amine, followed by hydrolysis of the acetoxy group.

Experimental[3]

2-(2-Aminoethoxy)ethanols (Table II, 5 and 8–10).—2-Chloroethyl 2-acetoxyethyl ether[4] (300 g., 1.8 moles), 540 g. (5.4 moles) of 4-methylpiperazine or 1 equiv. wt. of the other heterocyclic

(1) K. Bucher, *Schweiz. Med. Wochschr.*, **86**, 94 (1956).

(2) A. Ashford, C. J. Sharpe, and F. F. Stephens, *Nature*, **197**, 969 (1963).

(3) Boiling and melting points are uncorrected, the work being completed before the announcement of the requirement for corrected data.

(4) F. F. Blike and J. M. Biel, *J. Am. Chem. Soc.*, **76**, 3163 (1954).