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

Synthesis of Potential Anticancer Compounds. XIV. Methanesulfonic Acid Ester Analogs of Nitrogen Mustards[1,2]

ROBERT C. ELDERFIELD, RAJ N. PRASAD[3] AND TSUNG-KAI LIAO

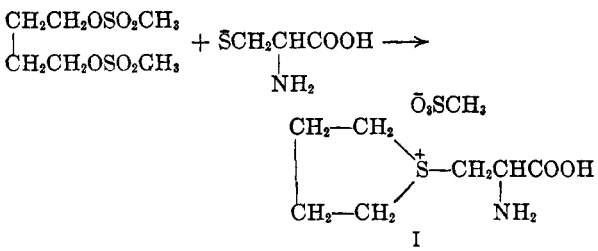
Received July 10, 1961

Derivatives were prepared by condensation of *p*-[*N*,*N*-bis(2-methanesulfonoxyethyl)amino]benzaldehyde with barbituric acid, 1,3-diphenylbarbituric acid, 2-phenylcinchoninic acid hydrazide and 6-purinylhydrazine for evaluation as cancer therapeutic agents and to provide evidence as to whether an intrinsic difference in the mode of action of nitrogen mustards and analogous methanesulfonates exists. Preparation of *p*-[*N*,*N*-bis(2-methanesulfonoxyethyl)amino]benzoic acid is also described.

The so-called alkylating agents have occupied a prominent position among compounds which have been investigated in the search for chemotherapeutic agents useful in the control of neoplastic disease.[4]

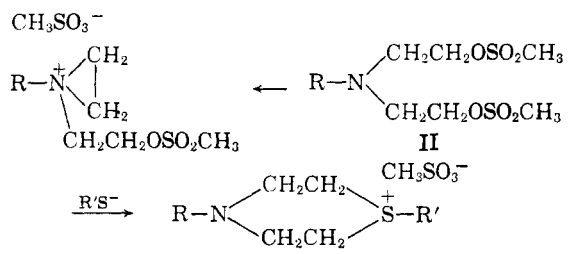
For the most part the substances carry two or more alkylating functions although a few are known which possess but a single such group. The alkylating functions encountered most frequently comprise the 2,2′-bischlorodiethylamino, the 2,2′-bischlorodiethyl sulfide, the aziridine, the methanesulfonoxy, and, less frequently, the epoxide group.

In the past the view has been expressed that all alkylating agents act substantially in the same manner against the constituents of neoplastic tissue regardless of the nature of the alkylating function.[4] There would seem to be no *a priori* chemical basis for such a generalization and recently evidence has been presented which is contradictory to such a view.[5,6] Whereas significant evidence is accumulating that the nitrogen and sulfur mustard types exert their action by alkylation of the guanine moieties of nucleic acids and to a lesser extent of the adenine moieties,[7] or of the phosphoric acid residues,[5] the methanesulfonates as exemplified by Myleran (1,4-bismethanesulfonoxybutane) apparently attack thiol groups, *e.g.* that of cysteine.[6,8] Further, maximum antitumor activity apparently occurs when the spatial arrangement in the bismethanesulfonates permits the facile formation of cyclic 5- or 6-membered sulfonium compounds.[9] Thus the action of Myleran on cysteine has been pictured as involving initial formation of the sulfonium ion (I) from which the observed products of the reaction arise by logical further transformations.[10] Similar sulfonium salt formation involving



I

a six-membered ring can easily be visualized as proceeding from the methanesulfonic ester analogs of the nitrogen mustards (II), *e.g.* It is also conceivable that compounds of the type of II could also cyclize to an ethylenimmonium ion which would then presumably function in a manner analogous to that which has been postulated for the nitrogen mustards. In the latter instance attack on nucleic acids presumably would occur in the manner suggested for attack by the nitrogen mustards—alkylation of NH rather than of SH functions. A bifunctional mode of attack also comes within the realm of speculation.



II

(1) W. R. Vaughan and M. S. Habib, *J. Org. Chem.*, **27**, 324 (1962).

(2) This work was supported by Research Grant CY-2961 from the National Cancer Institute to the University of Michigan.

(3) On leave of absence from B. N. College, Patna University,

(4) For a review of this general field see *Comparative Clinical and Biological Effects of Alkylating Agents*, Annals N. Y. Acad. Sciences, **68**, 657 (1958).

(5) P. Alexander and K. A. Stacey, *Annals N. Y. Acad. Sciences*, **68**, 1225 (1958); F. Bergel, *Annals N. Y. Acad. Sciences*, **68**, 1238 (1958).

(6) J. J. Roberts and G. P. Warwick, *Nature*, **183**, 1509 (1959); **184**, 1288 (1959), *inter. alia*.

(7) G. M. Timmis, P. D. Lawley, C. L. Leese, J. H. Lister, and G. Hems, *Proc. 4th. Int. Congr. Biochem., Vienna*, **15**, 30 (1958); I. G. Walker and W. J. Watson, *Can. J. Biochem.*, **39**, 377 (1961), *inter. alia*.

(8) W. E. Parham and J. M. Wilbur, Jr., *J. Org. Chem.*, **26**, 1659 (1961); L. A. Elson, *Biochem. Pharmacol.*, **1**, 39 (1958).

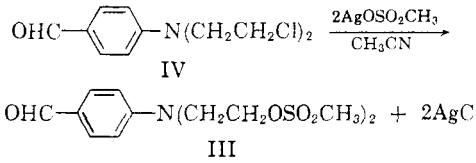
(9) J. J. Roberts and G. P. Warwick, *Annual Reports British Empire Cancer Campaign*, 40 (1960).

(10) W. E. Parham and J. M. Wilbur, Jr., *J. Am. Chem. Soc.*, **81**, 6071 (1959).

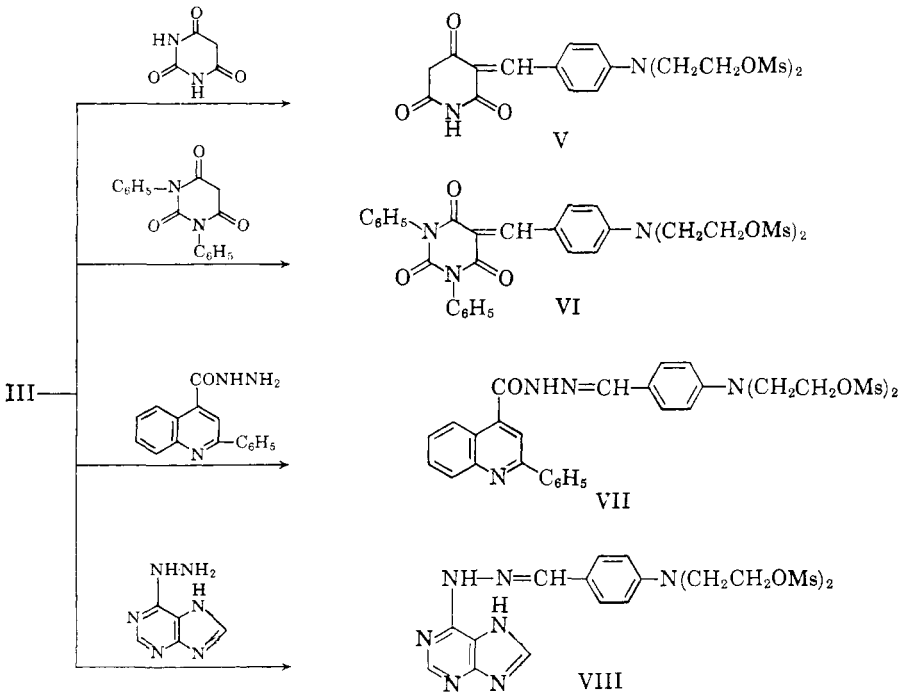
As expressed in terms of the so-called carrier hypothesis originally suggested by Ing[11] a physiologically active substance can be considered as comprising a pharmacodynamic group and a carrier part. In the compounds under discussion, the alkylating function is the pharmacodynamic group. There has been relatively little study of the comparative carcinolytic action of pairs of compounds with constant carrier moiety and varying alkylating function. Among these studies may be included a few nitrogen mustard analogs of compounds of the general Myleran type.

It therefore appeared of interest to prepare a series of bismethanesulfonates analogous to a comparable series of nitrogen mustards with the carrier portion of the molecules common in each series. If real differences in the modes of action of the two types of compounds do indeed exist, these should be apparent in the results of animal screens against experimental tumors as well as in the reactions of the compounds against selected substrates *in vitro*. Encouragement for such an approach was provided by the observation that, whereas *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzaldehyde is inactive against the Dunning rat leukemia, the corresponding bismethanesulfonoxyethyl derivative displays considerable activity against the same tumor.[12]

p-[*N*,*N*-Bis(2-methanesulfonoxyethyl)amino]-benzaldehyde (III) was prepared from IV and silver methanesulfonate by application under strictly specified conditions of a procedure described by Sprague and Engelhardt for the preparation of *N* - cycloalkylbis(2 - methanesulfonoxyethyl)-amines.[13] The benzaldehyde mesylate (III) proved



to be unexpectedly stable under a variety of conditions which enabled the preparation of a number of condensation products analogous to selected nitrogen mustards previously described.



Condensation of 6-hydrazinopurine with IV gave benzaldehyde-*p*-[bis(2-chloroethyl)amino]-6-purinylhydrazone as the hydrochloride (X) which was exceedingly difficult to purity. Reaction of the latter with silver methanesulfonate did not yield VIII presumably because of complex formation of the silver with the purine nucleus. However, VIII was readily prepared by condensation of III with 6-hydrazinopurine. Since X has displayed activity against the Dunning rat leukemia,[12] the results of similar tests of VIII are of considerable interest.

Methanesulfonic acid esters analogous to *p*-[*N*,*N*-bis(2-chloroethyl)aminobenzoic acid and its ethyl ester have also been prepared.

Results of tests of these compounds against experimental animal tumors will be reported elsewhere.

(11) H. R. Ing, *Trans. Faraday Soc.*, 39, 372 (1943).

(12) Private communication from Dr. Ralph L. Jones, Jr., Jackson Memorial Hospital, University of Miami, Miami, Fla.

(13) J. M. Sprague and E. L. Engelhardt, U. S. Patent 2,671,105 (Mar. 2, 1954); *Chem. Abstr.*, 49, 1776 (1955).

EXPERIMENTAL[14,15]

p-[N,N-Bis(2-methanesulfonoxyethyl)amino]benzaldehyde (III).[16] A mixture of 12.3 g. of IV,[17] 30.9 g. of silver methanesulfonate and 275 ml. of practical grade acetonitrile was refluxed with vigorous stirring and protection from light for 67 hr. During the reaction the mixture turned deep purple and silver chloride gradually separated. After filtering from silver chloride, the solvent was removed under reduced pressure. The residue was extracted with three 150 ml. portions of boiling absolute ethanol. The filtered extracts were concentrated to 200 ml. and on cooling 14.4 g. (79%) of material, m.p. 118–122°, separated. Further recrystallization from absolute ethanol gave pinkish crystals, m.p. 122.5–123.5°.

Anal. Calcd. for $C_{13}H_{19}NO_7S_2$: C, 42.72; H, 5.26; N, 3.83; S, 17.53. Found: C, 42.62; H, 5.27; N, 3.53; S, 17.55.

p-[N,N-Bis(2-methanesulfonoxyethyl)amino]benzoic acid. A mixture of 13.1 g.[18] of *p*-[*N,N*-bis(2-chloroethyl)amino]-benzoic acid, 20.3 g. of silver methanesulfonate and 200 ml. of acetonitrile was refluxed with vigorous stirring for 24 hr. On working up as in the above instance 10.7 g. (76%) of white leaflets, m.p. 170.5–171.5° after recrystallization from absolute alcohol, was obtained. The acid is somewhat unstable toward heat. If the reflux period was 3 days, no product was obtained.

Anal. Calcd. for $C_{13}H_{19}NO_8S_2$: C, 40.94; H, 4.99; N, 3.67; S, 16.79. Found: C, 41.10; H, 5.00; N, 3.55; S, 16.70.

Ethyl p-[N,N-bis(2-methanesulfonoxyethyl)amino]benzoate. From 27.35 g. of ethyl *p*-[*N,N*-bis(2-chloroethyl)amino]-benzoate[18] and 61.5 g. of silver methanesulfonate in 450 ml. of acetonitrile, 38 g. (98%) of the ester, m.p. 117.5–118.5° was obtained after refluxing for 48 hr. It formed white needles from 95% ethanol.

Anal. Calcd. for $C_{15}H_{23}NO_8S_2$: C, 44.01; H, 5.62; N, 3.42; S, 15.65. Found: C, 44.11; H, 5.69; N, 3.51; S, 15.55.

Benzaldehyde-p-[bis(2-chloroethyl)amino]-6-purinylhydrazone hydrochloride (X). To a stirred solution of 3.0 g. of 6-hydrazinopurine[19] in 8 ml. of concentrated hydrochloric acid and 100 ml. of water was added a solution of 5.0 g. of IV in 30 ml. of *N,N*-dimethylformamide. Stirring was continued at room temperature for 3 hr. with dropwise addition of sufficient dimethylformamide to keep the IV in solution. The yellow precipitate (3.8 g.) was collected and washed successively with cold dilute hydrochloric acid, water, methanol and ether. Purification of the material was difficult because of a strong tendency to form a colloidal suspension. A solution of it in the minimum amount of warm dimethylformamide was filtered into a stirred boiling mixture of 200 ml. of benzene and 50 ml. of methanol. After cooling, 100 ml. of ether was added. The supernatant liquid, which contained colloidal material, was decanted and the main product which settled out was collected with methanol-ether. This procedure was repeated twice to give analytically pure material which decomposed above 300°.

Anal. Calcd. for $C_{16}H_{17}Cl_2N_7 \cdot HCl$: C, 46.32; H, 4.34; N, 23.64; Cl, 25.69. Found: C, 46.29; H, 4.52; N, 23.58; Cl, 25.55.

The compound was also prepared by addition of a boiling solution of 2.5 g. of IV to a hot solution of 1.5 g. of 6-hydrazinopurine in a mixture of 80 ml. of water and 10 ml. of concentrated hydrochloric acid. An immediate precipitate separated. After boiling for 20 min. the mixture was allowed to cool with stirring during one hour. The product was purified as above.

Anal. Found: C, 46.50; H, 4.40; N, 23.47; Cl, 25.62.

p-[Bis(2-methanesulfonoxyethyl)amino]benzaldehyde-6-purinylhydrazone hydrochloride. (VIII). To a solution of 1.5 g. of 6-hydrazinopurine in 10 ml. of water and 5 ml. of concentrated hydrochloric acid was added a solution of 3.7 g. of III in 40 ml. of dimethylformamide at room temperature. On stirring for 30 min. 4.8 g (96%) of dark yellow material which melted above 160° to a viscous liquid separated. It was purified by filtering its solution in 150 ml. of warm dimethylformamide into a boiling mixture of 250 ml. of benzene and 100 ml. of absolute ethanol. After cooling, on addition of ether, an oil separated. On trituration with ether, the oil crystallized as bright yellow material, m.p. 173–176° when placed on a hot stage at 155°. Two further purifications by pouring a hot solution of the substance in 100 ml. of hot dimethylformamide into one liter of boiling acetone followed, after cooling, by addition of 800 ml. of dry ether, gave analytically pure material, m.p. 175–177° (dec.).

Anal. Calcd. for $C_{18}H_{23}N_2O_6S_2 \cdot HCl$: C, 40.48; H, 4.49; N, 18.36; S, 11.99; Cl, 6.63. Found: C, 40.11; H, 4.51; N, 17.70; S, 11.87; Cl, 6.39.

5-p-[Bis(2-methanesulfonoxyethyl)amino]benzylidene-barbituric acid. (V). To a solution of 2.24 g. of barbituric acid and 6.38 g. of III in 20 ml. of dimethylformamide was added 2 ml. of concentrated hydrochloric acid followed by 30 ml. of absolute ethanol. After stirring for one hour at room temperature the orange solid (7.7 g., 97%) was collected and recrystallized by addition of its solution in 125 ml. of hot dimethylformamide to 600 ml. of boiling methanol. The product (6.2 g.), m.p. 230° (dec.), was collected from the warm solution.

Anal. Calcd. for $C_{17}H_2N_3O_9S_2$: C, 42.94; H, 4.42; S, 13.47. Found: C, 43.06; H, 4.48; S, 13.43.

5-{p-[Bis(2-methanesulfonoxyethyl)amino]benzylidene}-1,3-diphenylbarbituric acid (VI). A solution of 2.8 g. of 1,3-diphenylbarbituric acid and 3.65 g. of III in a mixture of 70 ml. of dimethylformamide, 110 ml. of ethanol and 3 ml. of concentrated hydrochloric acid was stirred at room temperature for 22 hr. On chilling, 6.0 g. of VI which formed orange yellow needles, m.p. 163–164° after recrystallization from acetone-benzene-ligroin, separated.

Anal. Calcd. for $C_{29}H_{29}N_3O_9S_2$: C, 55.50; H, 4.62; N, 6.70; S, 10.20. Found: C, 55.71; H, 4.83; N, 6.65; S, 10.10.

2-Phenylcinchoninic acid p-[bis(2-methanesulfonoxyethyl)-amino]benzylidene hydrazide hydrochloride (VII). A solution of 2.63 g. of cinchophen hydrazide and 3.65 g. of III in 60 ml. of dimethylformamide, 10 ml. of absolute ethanol, and 4.0 ml. of concentrated hydrochloric acid was stirred at room temperature for 1.5 hr. After addition of 30 ml. of chloroform and 50 ml. of ether, stirring was continued for an additional 5.5 hr. and the red solid which was very sparingly soluble in most solvents was collected. After recrystallization first from dimethylformamide–acetone–ether and finally from boiling nitromethane-ether (1:4) the hydrochloride melted at 159–160° (dec.).

Anal. Calcd. for $C_{29}H_{30}N_4O_7S_2 \cdot HCl$: C, 53.82; H, 4.79; N, 8.66; S, 9.74. Found: C, 54.14; H, 4.73; N, 8.40; S, 9.48.

Acknowledgment. We acknowledge the valuable assistance of James L. Hudson in the preparation of several intermediates.

ANN ARBOR, MICH.

(14) All melting points are uncorrected for stem exposure.

(15) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(16) This was originally prepared in poor yield in somewhat impure state in these laboratories by Dr. J. H. Ross.

(17) R. C. Elderfield, I. S. Covey, J. B. Geiduschek, W. B. Meyer, A. B. Ross, and J. H. Ross, *J. Org. Chem.*, **23**, 1749 (1958).

(18) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, 1974 (1949).

(19) J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, 79, 2185 (1957).