Some N-Sulfinylhydrazine Analogs of Nitrogen Mustard[1]

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N′-Methyl-N′-chloroalkyl-N-sulfinylhydrazines and N′,N′-bis(chloroalkyl- and -chloroallyl)-N-sulfinylhydrazines have been prepared by treatment of suitably substituted hydroxy- or chlorohydrazines with thionyl chloride. N′,N′-Bis(2-chloroethyl)-N-sulfinylhydrazine is the most active of these compounds in antitumor screening.

In view of the anticancer activity of compounds related to 2-chloroethyl-N-sulfinylamine,[2] we are interested in preparing other sulfinylamines which might also be considered as nitrogen mustards. The well-known greater anticancer activity of bifunctional alkylating agents over monofunctional alkylating agents led us to prepare bifunctional derivatives closely related to 2-chloroethyl-N-sulfinylamine. In order to prepare compounds of greater stability, we have taken advantage of the greater stability of N-sulfinylhydrazines relative to N-sulfinylamines. With these ideas in mind, and in order to evaluate the relative importance of bi- *vs.* monofunctionality, we have synthesized two groups of haloalkyl-N-sulfinylhydrazines, one bifunctional and the other monofunctional, but with a methyl group replacing the second of the "bis" groups of the bifunctional molecules.

Some of these compounds were synthesized by the reaction of thionyl chloride with suitable hydroxyalkylhydrazines.

$$[HO(CH_2)_n]_2NNH_2 + SOCl_2 \xrightarrow{\text{pyridine}} [Cl(CH_2)_n]_2NNSO$$

The hydroxyalkylhydrazines were prepared by reaction of the appropriately substituted oxiranes with hydrazine. N-Methyl-N-substituted hydrazines were prepared similarly from an oxirane and methylhydrazine. This route was used for the synthesis of N′,N′-bis(2-chloroethyl)-N-sulfinylhydrazine, N′,N′-bis(2-chlorophenethyl)-N-sulfinylhydrazine, N′-methyl-N′-(2-chloroethyl)-N-sulfinylhydrazine, and N′-methyl-N′-(2-chlorobutyl)-N-sulfinylhydrazine.

Since the activity of nitrogen mustard analogs is related to both the basicity of the nitrogen and the chemical reactivity of the halogen, we have sought to modify activity by preparing some compounds such as N′,N′-bis(2-chloroallyl)-N-sulfinylhydrazine in which the halogen is expected to be less reactive by virtue of its incorporation as part of a vinyl-type halide. Our method of synthesis of these compounds took advantage of the difference in reactivity of the halogens in dihalopropenes, in which one of the halogens is allylic and reactive and the other halogen is vinylic and unreactive. Thus, reaction of 2,3-dichloropropene with hydrazine in the presence of sodium hydroxide gave N′,N′-bis(2-chloroallyl)hydrazine in 66% yield.

$$CH_2{=}CClCH_2Cl + NH_2NH_2 \xrightarrow{\text{NaOH}} (CH_2{=}CClCH_2)_2NNH_2$$

It was also possible to prepare the *cis* and *trans* isomers of N′,N′-bis(3-chloroallyl)hydrazine by starting with the respective isomers of 1,3-dichloropropene and proceeding through the above reaction. The substituted hydrazines prepared in this way were readily converted to the bis(haloallyl)-N-sulfinylhydrazines by reaction with thionyl chloride in pyridine.

Acetylhydrazine was also alkylated, using 2,3-dichloropropene and 2,3-dibromopropene. The resulting N′,N′-bis(2-haloallyl)-N-acetylhydrazines were hydrolyzed to give bis(2-haloallyl)hydrazine hydrochlorides. Hydrolysis of N′,N′-bis(2-chloroallyl)-N-sulfinylhydrazine (whose preparation involved alkylation of free hydrazine) gave a hydrazine hydrochloride identical with that obtained by alkylation of acetylhydrazine. Screening of these hydrazine hydrochlorides provides the opportunity to compare their antitumor activity with that of the corresponding N-sulfinyl derivatives.

Biological Evaluation.[3]—The biological data on these compounds are summarized in Tables I and II.

TABLE I

ACTIVITY OF R(CH$_3$)NNSO AGAINST WALKER CARCINOSARCOMA 256

No.	R	T/C, %	Dose, mg/kg
1	$ClCH_2CH_2$	112	10
2	$CH_3CHClCH_2$	91	10
3	$CH_3CH_2CHClCH_2$	103	50

TABLE II

ACTIVITY OF R$_2$NNSO AGAINST WALKER CARCINOSARCOMA 256

No.	R	T/C, %	Dose, mg/kg
4	$ClCH_2CH_2$	0	1.62
		4	0.81
5	$C_6H_5CHClCH_2$	*a*	
6	$CH_2{=}CClCH_2$	117	50.0
7	*cis*-ClCH=CHCH$_2$	112	3.0
8	*trans*-ClCH=CHCH$_2$	82	3.0

[a] This compound had an ED_{50}[4,5] of 21 μg/ml in cell culture cytotoxicity tests. N′,N′-Bis(2-chloroethyl)-N-sulfinylhydrazine had an ED_{50} of 6.6 μg/ml when tested by the same procedure.

The hydrochlorides of N,N-bis(2-chloroallyl)hydrazine and N,Nbis(2-bromoallyl)hydrazine each had an ED_{50}[4,5] of 100 μg/ml in cell culture cytotoxicity tests.

N′-Methyl-N′-(2-chloroethyl)-N-sulfinylhydrazine has appreciably less activity than the previously

(1) This work was supported by Research Grant CA-06586 from the National Cancer Institute, National Institutes of Health, to the University of Kentucky Research Foundation.

(2) W. T. Smith, Jr., and W. Y. Chen, *J. Med. Chem.*, **8**, 748 (1965).

(3) The evaluations were done through the facilities of the Cancer Chemotherapy National Service Center.

(4) The concentration required to inhibit the growth of KB cells in culture to 50% of controls.

(5) H. Eagle and G. F. Foley, *Cancer Res.*, **18**, 1017 (1958).

Table III
RR'NNH$_2$

No.	R	R'	Bp (mm), °C	Yield, %	Formula	Analyses
9	Me	$HOCH_2CH_2$	59–62 (0.2)	44	$C_3H_{10}N_2O$	C, H
10	Me	$MeCH(OH)CH_2$	54–62 (0.2)	72	$C_4H_{12}N_2O$	H; C[a]
11	CH_2=$CClCH_2$	CH_2=$CClCH_2$	58–71 (0.1)	66	$C_6H_{10}N_2Cl_2$	C, H, N
12	ClCH=$CHCH_2$ (*cis*)	ClCH=$CHCH_2$ (*cis*)	57–77 (0.1)	12	$C_6H_{10}Cl_2N_2$	H; C[b]
13	ClCH=$CHCH_2$ (*trans*)	ClCH=$CHCH_2$ (*trans*)	45–72 (0.1)	16	$C_6H_{10}Cl_2N_2$	C,[c] H[d]

[a] C: calcd, 46.10; found, 45.63. [b] C: calcd, 39.79; found, 39.02. [c] C: calcd, 39.79; found, 40.37. [d] H: calcd, 5.56; found, 6.32.

reported 2-chloroethyl-N-sulfinylamine.[2] It would be expected that the presence of a phenyl group on C-2, as in N',N'-bis(2-chlorophenethyl)-N-sulfinylhydrazine, might be expected to make the halogen more reactive. While this compound does show some biological activity, its relatively poor activity as compared to the related compound without the phenyl group indicates the possibility of a steric requirement in the alkylation reaction. N',N'-Bis(2-chloroethyl)-N-sulfinylhydrazine is by far the most active compound described here. In addition to its favorable anticancer activity, it has a LD_{50}[6] in mice greater than 100 mg/kg. It is difficult to make precise comparison of the activity of $(ClCH_2CH_2)_2NNSO$ with the activity of $(ClCH_2CH_2)_2$-NNH_2,[7,8] but it does appear that introduction of the N-sulfinyl group has appreciably decreased toxicity.

Experimental Section[9]

N,N-Bis(2-hydroxyethyl)hydrazine.—A stirred mixture of 95% NH_2NH_2 (34 g, 1 mole) and ethylene oxide (100 g, 2.25 moles) was kept at 0° for 16 hr, followed by 8 hr at room temperature. Distillation gave 50 g (42%), bp 120–140° (0.5 mm). A similar procedure was used for preparation of **9**–11 listed in Table III.

N,N-Bis(chloroallyl)hydrazines.—Compounds **12**–14 in Table III were prepared from the appropriate dichloropropene (0.5 mole), 95% NH_2NH_2 (0.25 mole), and NaOH (0.5 mole). The mixture was shaken for 9 hr, with intermittent cooling during the first hour, then cooled and filtered. The aqueous layer was removed and the organic layer was distilled twice.

N',N'-Bis(2-chloroethyl)-N-sulfinylhydrazine.—A mixture of N,N-bis(2-hydroxyethyl)hydrazine (54 g, 0.45 mole) and pyridine (145 g, 1.8 moles) was stirred at 0° while a solution of $SOCl_2$ (162 g, 1.35 moles) in 100 ml of $CHCl_3$ was added dropwise over a period of 2 hr. The reaction mixture was extracted with five 200-ml portions of Skellysolve A, the extracts were combined, and the black residue was discarded. Distillation gave 10 g (11%), bp 107–112° (0.5 mm). A similar procedure was used for the preparation of compounds 1–4 and **6**–8.

N',N'-Bis(2-chlorophenethyl)-N-sulfinylhydrazine.—A mixture of styrene oxide (60 g, 0.5 mole) and 95% NH_2NH_2 (17 g, 0.25 mole) was stirred at room temperature for 24 hr. The resulting crude N',N'-bis(2-hydroxyphenethyl)hydrazine was treated as in the above procedure with pyridine (79 g, 1.0 mole) and $SOCl_2$ (90 g, 0.75 mole). After the solvent was removed from the Skellysolve A extract, the residual viscous oil was chromatographed on an alumina column, using Skellysolve A as eluent. The first 300-ml portion of eluent was discarded, the second 300-ml portion contained sulfur, and the third 300-ml portion gave 2 g (2%) of the desired product (Table IV).

Table IV
RR'NNSO

No.	Bp (mm), °C	Yield, %	Formula	Analyses
1	93–98 (0.2)	7	$C_3H_7ClN_2OS$	N; C,[a] H[a]
2	72–75 (0.1)	6	$C_4H_9ClN_2OS$	N; C,[b] H[b]
3	70–94 (0.2)	23	$C_5H_{11}ClN_2OS$	N, S
4	107–112 (0.5)	11	$C_4H_8Cl_2N_2OS$	N,[c] S[c]
5	mp 94–96	2	$C_{16}H_{16}Cl_2N_2OS$	C, H, N
6	78–88 (0.1)	24	$C_6H_8Cl_2N_2OS$	N, S
7	80–95 (0.1)	36	$C_6H_8Cl_2N_2OS$	N, S
8	75–91 (0.1)	16	$C_6H_8Cl_2N_2OS$	N, S

[a] C: calcd, 23.31; found, 24.30. H: calcd, 4.51; found, 5.21. [b] C: calcd, 28.49; found, 29.49. H: calcd, 5.38; found, 6.06. [c] N: calcd, 13.79; found, 14.39. S: calcd, 15.79; found, 16.62. Hydrolysis of this product in concentrated HCl gave N,N-bis(2-chloroethyl)hydrazine hydrochloride, mp 133–135° [R. Preussman, *Angew. Chem.*, **70**, 743 (1958), reports 133–135°].

N',N'-Bis(2-chloroallyl)-N-acetylhydrazine.—A mixture of 2,3-dichloropropene (22.2 g, 0.2 mole), acetylhydrazine (7.4 g, 0.1 mole), NaOH (8 g, 0.2 mole), and 50 ml of absolute EtOH was shaken with intermittent cooling for 1 hr, followed by additional shaking for 8 hr. The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the residue was extracted with Skellysolve A. Recrystallization (Skellysolve A) gave product, mp 58–60°.

Anal. Calcd for $C_8H_{12}Cl_2N_2O$: C, 43.03; H, 5.42; N, 12.55. Found: C, 43.48; H, 5.60; N, 12.05.

Hydrolysis in concentrated HCl gave **N,N-bis(2-chloroallyl)-hydrazine hydrochloride,** mp 159–160°. *Anal.* ($C_6H_{11}Cl_3N_2$) C, H, N.

N',N'-Bis(2-bromoallyl)-N-acetylhydrazine.—The above procedure, using 2,3-dibromopropene (25 g, 0.13 mole), acetylhydrazine (4.7 g, 0.06 mole), NaOH (5 g, 0.13 mole), and 60 ml of absolute EtOH gave 5 g (12%), mp 80–81°. *Anal.* ($C_8H_{12}Br_2N_2O$) C, H, N.

Hydrolysis in concentrated HCl gave **bis(2-bromoallyl)-hydrazine hydrochloride,** mp 162–163°. *Anal.* ($C_6H_{11}Br_2ClN_2$) C, H, N.

(6) C. S. Weil, *Biometrics*, **8**, 249 (1952).

(7) R. Preussman, *Arzneimittel-Forsch.*, **12**, 260 (1962).

(8) M. Ishidate, Y. Sakurai, and Y. Kuwada, *Chem. Pharm. Bull.* (Tokyo), **7**, 391 (1959).

(9) Melting points were taken on a Fisher-Johns melting point block and are corrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.