The Preparation and Antibacterial Activity of Some Sulfanilamidodiazines Bearing the N-Alkylalkanesulfonamido Group

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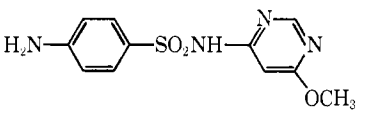
Several *N*-alkylalkanesulfonamido analogs of sulfamonomethoxine (**1**) were prepared and tested against *Streptococcus pyogenes* C203 infections in mice. The most active compound, *N*-methyl-*N*-(6-sulfanilamido-4-pyrimidinyl)butanesulfonamide (**16**), had only one-tenth the potency of **1** but was more potent than sulfisoxazole. Nearly as potent as **16** was the isomeric *N*-methyl-*N*-(2-sulfanilamido-5-pyrimidinyl)butanesulfonamide (**21**) although another isomer, *N*-methyl-*N*-(6-sulfanilamido-2-pyrazinyl)butanesulfonamide (**22**) was virtually inactive. Blood levels in mice were of short duration and generally reached a maximum in 1 hr. Essentially no N^4 conjugation was observed. Most of these new compounds were 60–90% bound to dog plasma protein and had relatively low solubility in pH 6 acetate buffer.

Certain sulfanilamidodiazines bearing one or more OCH_3 on the heterocyclic ring have demonstrated potent and useful antibacterial action [*e.g.*, sulfamonomethoxine (**1**),[1,2] sulfametin,[3] sulfamethoxypyridazine,[4] sulfadimethoxine,[5] and sulforthodimethoxine],[6]



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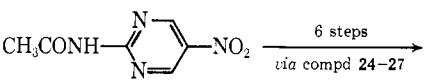
Investigations[7,8] in these laboratories, unrelated to chemotherapy, have shown interesting chemical and pharmacological relationships between the phenolic OH and the alkanesulfonamido function, RSO_2NH. The corresponding analogy between a OCH_3 and the *N*-methylalkanesulfonamido group prompted us to prepare and evaluate several sulfanilamidodiazines bearing the *N*-alkylalkanesulfonamido function on the heterocyclic moiety. One unsubstituted alkanesulfonamido derivative is included for comparison.

Chemistry.—The synthetic sequence shown in Scheme I was utilized for the preparation of some *N*-(6-sulfanilamido-4-pyrimidinyl)alkanesulfonamides, analogs of sulfamonomethoxine.

Nucleophilic displacement of a single Cl in **2** with the K salts of alkanesulfonamides (Table I) produced the corresponding *N*-(6-chloro-4-pyrimidinyl)alkanesulfonamides (**3**–**11**) (Table II). Displacement of Cl on **3**–**10** (R = alkyl) with sodium sulfanilamide provided the sulfanilamidopyrimidines (**12**–**19**) (Table III).

Preparation of the butanesulfonamido sulfa (**20**) from **11** (R = H, R′ = n-C_4H_9) was less direct. The acidic proton in **11** reacts with sodium sulfanilamide to produce a negatively charged group in the 4-position; the partial positive charge on C-6 (bearing the Cl) is diminished, rendering the Cl less reactive toward nucleophilic displacement.[9] The butanesulfonamido sulfa was obtained by fusion of **11** and N^4-acetylsulfanilamide at 200° with Cu–bronze catalyst,[10] as described in the Experimental Section.

Because **16**, bearing the *N*-methylbutanesulfonamido group, was found to be the most potent member of this series in the test system employed, we decided to test the effectiveness of the n-$C_4H_9SO_2N(CH_3)$ function in other diazine sulfas. Two additional compounds (**21**, **22**) were prepared, the CH_3O analogs[3,11] of which are effective antibacterial agents. *N*-Methyl-*N*-(2-sulfanilamido-5-pyrimidinyl)butanesulfonamide (**21**) was obtained from **23** by a sequence detailed in the Experimental Section.



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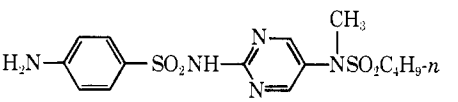

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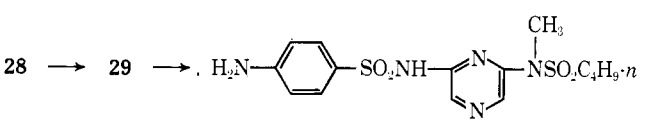
N-Methyl-*N*-(2-sulfanilamido-6-pyrazinyl)butanesulfonamide (**22**) was obtained from 2,6-dichloropyrazine (**28**) as in the 4,6-disubstituted pyrimidine series.



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Biological Results.—The *in vivo* therapeutic properties of these sulfanilamide derivatives were evaluated by the mouse protection test method of Schnitzer, *et al.*,[12]

(1) 4-Sulfanilamido-6-methoxypyrimidine; R. Clarkson and A. R. Martin, *Nature*, **192**, 523 (1961).

(2) R. G. Shepherd, W. E. Taft, and H. M. Krazinski, *J. Org. Chem.*, **26**, 2764 (1961).

(3) 2-Sulfanilamido-5-methoxypyrimidine; T. Knott, A. Kutzsche, and A. M. Walter, *Arzneim.-Forsch.*, **11**, 684 (1961).

(4) 3-Sulfanilamido-6-methoxypyridazine; H. W. Marson, M. M. Rogers, and W. E. Taft, *J. Amer. Chem. Soc.*, **80**, 980 (1958).

(5) 4-Sulfanilamido-2,6-dimethoxypyrimidine; J. Rieder, *Arzneim.-Forsch.*, **13**, 81 (1963).

(6) 4-Sulfanilamido-5,6-dimethoxypyrimidine; S. T. Madsen, *Amer. J. Med. Sci.*, **247**, 217 (1964).

(7) A. A. Larsen, and P. M. Lish, *Nature*, **203**, 1283 (1964).

(8) R. H. Uloth, J. R. Kirk, W. A. Gould, and A. A. Larsen, *J. Med. Chem.*, **9**, 88 (1966); A. A. Larsen, *et al.*, *ibid.*, **10**, 462 (1967).

(9) See J. F. Bunnett and J. J. Randall, *J. Amer. Chem. Soc.*, **80**, 6020 (1958), for a discussion of the intermediate complex in aromatic nucleophilic displacements.

(10) Swiss Patent 210,429 (1940) to Societe pour l'Industrie Chimique, a Bale, *Chem. Absr.*, **35**, 5514 (1941).

(11) M. Ghione, C. Bertazzoli, A. Buogo, T. Chieli, and V. Zavaglio, *Chemotherapia*, **6**, 344 (1963).

(12) R. J. Schnitzer, W. F. DeLorenzo, E. Grunberg, and R. Russomanno, *Proc. Soc. Exp. Biol. Med.*, **99**, 421 (1958).

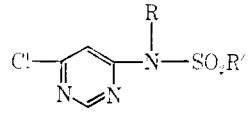
TABLE I
ALKANESULFONAMIDES

$$R\text{-}\overset{H}{\underset{|}{N}}\text{-}SO_2R'$$

R	R'	Mp or bp (mm), °C	Yield, %	$n^{25}D$	Formula	Analyses
CH_3	CH_3	100–110 (0.50)[a]	73		$C_2H_7NO_2S$	
CH_3	C_2H_5	102–105 (0.15)	90	1.4540	$C_3H_9NO_2S$	C, H, N
CH_3	C_3H_7-*i*	92–94 (0.15)[b]	88	1.4548	$C_4H_{11}NO_2S$	C, H, N
CH_3	C_4H_9-*n*	100–105 (0.10)	92	1.4523	$C_4H_{11}NO_2S$	C, H, N
CH_3	C_4H_9-*n*	115–118 (0.3)	90	1.4548	$C_5H_{13}NO_2S$	C, H, N[b]
CH_3	C_5H_{11}-*n*	40.0–41.0	31		$C_6H_{15}NO_2S$	C, H, N
C_2H_5	C_4H_9-*n*	115–120 (0.30)[c]	89	1.4516	$C_6H_{15}NO_2S$	C, H, N
C_4H_9-*n*	CH_3	115–120 (0.30)[d]	96	1.4505	$C_5H_{13}NO_2S$	C, H, S
H	C_4H_9-*n*	47.5–48.5[e]	75		$C_4H_{11}NO_2S$	

[a] B. Helferich and H. Grunert, *Ber.*, **73B**, 1131 (1940), reported 118° (0.3 mm). [b] H, calcd: 8.66; found: 8.06. [c] J. von Braun and K. Weissbach, *Ber.*, **63B**, 2836 (1930), reported 120–122° (0.1 mm). [d] Y. Ueda, H. Yano and T. Momose, *Chem. Pharm. Bull.* (Tokyo), **12**, 5 (1964); *Chem. Abstr.*, **60**, 11873*e* (1964), reported 128° (5.5 mm). [e] L. Field and F. A. Grunwald, *J. Amer. Chem. Soc.*, **75**, 934 (1953), reported 47.5–49°.

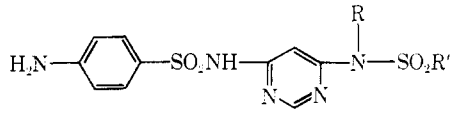
TABLE II
N-(6-CHLORO-4-PYRIMIDINYL)ALKANESULFONAMIDES



No.	R	R'	Mp or bp (mm), °C	Yield, %	Recrystn solvent[a]	Formula[b]
3	CH_3	CH_3	85.5–87.5	73	A	$C_6H_8ClN_3O_2S$
4	CH_3	C_2H_5	68.5–69.5	55	B	$C_7H_{10}ClN_3O_2S$
5	CH_3	C_3H_7-*i*	109.5–111.5	48	B, C	$C_8H_{12}ClN_3O_2S$
6	CH_3	C_3H_7-*n*	63.0–64.5	61	B, D	$C_8H_{12}ClN_3O_2S$
7	CH_3	C_4H_9-*n*	61.5–63.5	66[c]	C, E	$C_9H_{14}ClN_3O_2S$
8	CH_3	C_5H_{11}-*n*	160–166 (0.02)	67		$C_{42}H_{70}ClN_3O_2S$
9	C_2H_5	C_4H_9-*n*	45.0–46.0	43	D	$C_{10}H_{16}ClN_3O_2S$
10	C_4H_9-*n*	CH_3	58.5–59.5	51[c]	B, E	$C_9H_{14}ClN_3O_2S$
11	H	C_4H_9-*n*	141.5–142.5	60[c]	F	$C_8H_{12}ClN_3O_2S$

[a] A, EtOAc; B, (*i*-Pr)$_2$O; C, Me$_2$CO; D, Skelly F; E, cyclohexane; F, *i*-PrOH. [b] All compounds analyzed satisfactorily for C, H, N. [c] Yield after one recrystallization.

TABLE III
N-(6-SULFANILAMIDO-4-PYRIMIDINYL)ALKANESULFONAMIDES



No.	R	R'	Mp, °C	Yield, %	Recrysn Solvent[a]	Formula[b]
12	CH_3	CH_3	210.0–211.5	28	A	$C_{12}H_{15}N_5O_4S_2$
13	CH_3	C_2H_5	177.5–178.5	52	B	$C_{13}H_{17}N_5O_4S_2$
14	CH_3	C_3H_7-*i*	228.0–229.0	23	A	$C_{14}H_{19}N_5O_4S_2$
15	CH_3	C_3H_7-*n*	205.5–206.0	54	A	$C_{14}H_{19}N_5O_4S_2$
16	CH_3	C_4H_9-*n*	162.5–163.0	50	C–D	$C_{15}H_{21}N_5O_4S_2$
17	CH_3	C_5H_{11}-*n*	170.5–171.5	54	A	$C_{18}H_{27}N_5O_4S_2$
18	C_2H_5	C_4H_9-*n*	198.0–199.0	60	A–E	$C_{16}H_{23}N_5O_4S_2$
19	C_4H_9-*n*	CH_3	209.5–211.5	63	A	$C_{15}H_{21}N_5O_4S_2$
20	H	C_4H_9-*n*	244.5–245.5	31	A–E	$C_{14}H_{19}N_5O_4S_2$

[a] A, MeCN; B, EtCOMe; C, abs EtOH; D, (*i*-Pr)$_2$O; E, MeOH. [b] See Table II, footnote *b*.

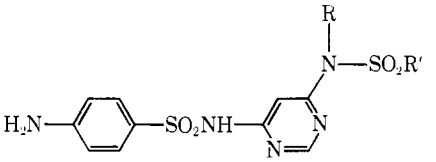
as modified by Harrison and Weikel.[13] The mice were infected by intraperitoneal injection of a standardized inoculum of *Streptococcus pyogenes* C203. Starting 2 hr after infection, the treated animals (10/dose level) received two oral doses of compound on the first and second days of infection, followed by single daily doses for the next 5 days. The mean CD_{50} (curative dose) was calculated[14] for each compound after 14 days and compared to values obtained with 1 and sulfisoxazole (3,4-dimethyl-5-sulfanilamidoisoxazole) as reference standards.

Highest antibacterial activity against streptococcal infection was obtained with the *N*-methylbutanesulfonamido derivative (**16**). Therapeutic activity increased as the substituent (R') on the aliphatic

(13) E. F. Harrison and J. H. Weikel, Jr., "Antibacterial Agents and Chemotherapy", American Society for Microbiology, Ann Arbor, Mich., 1963, pp 546–549.

(14) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96** 99 (1949).

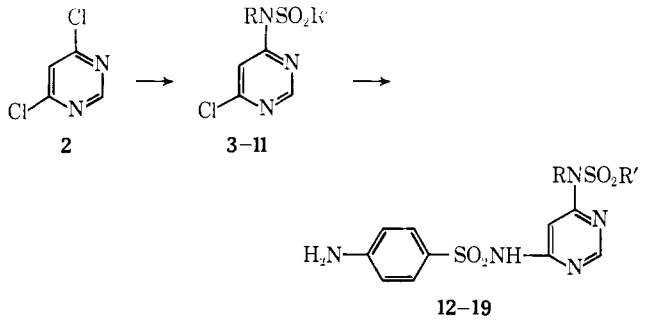
TABLE IV
PROPERTIES OF THE N-(6-SULFANILAMIDO-4-PYRIMIDINYL)ALKANESULFONAMIDES



No.	R	R′	Solubility, mg/ml[a]	Max blood level (mice), mcg/ml[b]	Partition coeff[c]	% protein binding[d]	CD_{50}, mg/kg
12	CH_3	CH_3	0.345	19	0.01	45	>400
13	CH_3	C_2H_5	0.220	85	0.24	61	>400
14	CH_3	C_3H_7-*i*	0.067	34[e]	1.04	63	>400
15	CH_3	C_3H_7-*n*	0.060	87	0.51	70	280
16	CH_3	C_4H_9-*n*	0.130	94	3.10	86	68
17	CH_3	C_7H_{15}-*n*	0.003	30	54.50	94	>400
18	C_2H_5	C_4H_9-*n*	0.014	78	8.70	89	>400
19	C_4H_9-*n*	CH_3	0.030	54	1.00	84	>400
20	H	C_4H_9-*n*	0.864	15	0.06	46	>200
1	Sulfamonomethoxine		0.460	91	0.19	32	6
	Sulfisoxazole		1.640	66	0.048	58	94

[a] Determined in 0.1 *N* NaOAc buffer (pH 6.0) on samples shaken at 37° for 18 hr. [b] After a single, oral dose of 100 mg/kg of sulfa drug; max levels were attained in 1 hr, unless otherwise noted. [c] $CHCl_3$–0.1 *N* sodium phosphate buffer (pH 7.4). [d] Binding to dog plasma protein from initial plasma concentration of 100 mcg/ml as determined by ultrafiltration at 25°. [e] Max was recorded at 3 hr.

SCHEME I



sulfonyl group was raised from Me to *n*-Bu; however, there was loss of activity when R′ was *n*-heptyl.

Replacing the *N*-Me with H (**20**) or alkyls larger than Me (**18** and **19**) resulted in diminished activity (Table IV). Branching of the alkanesulfonamido chain also decreased activity as shown by a comparison of **14** (*N*-methyl-2-propanesulfonamido) with **15** (*N*-methyl-*n*-propanesulfonamido). None of the compounds (**12**–**20**) tested had therapeutic activity equaling that obtained with sulfamonomethoxine. Compound **16** had about one-tenth the potency of **1** against experimental streptococcal infections, and was slightly more potent than sulfisoxazole in this test system.

The 2,5-disubstituted pyrimidine sulfa (**21**) corresponding to **16** had a CD_{50} of 80 mg/kg against *S. pyogenes* C203 compared with 68 mg/kg for **16**, while the pyrazine isomer (**22**) was essentially inactive against streptococcal infection (CD_{50} >400 mg/kg). Other properties of these two compounds are listed with their respective preparations.

The two most active sulfanilamides, **16** and **21**, were also tested *in vitro* against *Escherichia coli* and *Staphylococcus aureus* (Rose Strain) for inhibition of bacterial growth[15] and their effect on O_2 utilization.[16] In each case, marginal activity was noted against the former Gram-negative strain while moderate activity was obtained with the latter Gram-positive culture.

Moderately high mouse blood levels from a single, oral 100-mg dose were obtained with most of the new sulfanilamides in Table IV. Compounds **13**, **15**, and **16** gave blood levels comparable to that of sulfamonomethoxine. Levels generally reached a maximum at 1 hr and dropped to one-half the maximum in 3–5 hr. Little, if any, sulfanilamide derivative was detected in the blood after 24 hr. None of these compounds showed evidence of N^4 conjugation in total blood sulfanilamide determinations.

Most of the compounds of Table IV were 60–90% bound[17,18] to dog plasma protein, the lowest value being obtained with sulfamonomethoxine. All had rather low solubility in pH 6 acetate buffer and only **20** was more soluble than **1**. The solubility of **16** was high with respect to values for the neighboring homologs. Partition coefficients for the new compounds were generally higher than for sulfamonomethoxine at the pH of blood plasma (7.4).

pK_a values for the homologous series (**12**–**19**) showed no significant variation, values of 6.95 ±0.04 being obtained. All titrations were made in 50% aqueous Me_2CO due to the low solubility of many of the compounds in H_2O, EtOH, or even 30% Me_2CO. Values for reference standards obtained in 50% Me_2CO tended to run higher than values obtained in water[19] by about 1.3 pK units. Thus, by rough extrapolation, the 50% aqueous Me_2CO values for this series correspond to 5.6–5.7 in H_2O. The pK_a of sulfamonomethoxine was 7.17 in the 50% Me_2CO system.

Approximate oral TD_{50} values for **12**, **14**–**17**, **21**, and **22** were greater than 2000 mg/kg in mice. Compound **13** caused CNS depression accompanied by muscle weakness, ptosis, and salivation at 250 mg/kg. All of

(15) D. Grove and W. Randall, "Assay Methods of Antibiotics—A Laboratory Manual" Medical Encyclopedia, Inc., New York, N. Y., 1955, p 190.

(16) L. Neipp, W. Sackmann, and J. Tripod, *Antibiot. Chemother.*, **9**, 19 (1961).

(17) G. Zbinden in "Drug Design," Advances in Chemistry Series, No. 45, American Chemical Society, Washington, D. C., 1964, pp 33–34.

(18) A. Albert, "Selective Toxicity," 4th ed, Methuen and Co., Ltd, London, 1968, pp 82–85.

(19) P. H. Bell and R. O. Roblin, *J. Amer. Chem. Soc.*, **64**, 2905 (1942).

the sulfanilamides (**12–22**) were essentially inactive on the cardiovascular system of the anesthetized dog when administered at 10 mg/kg intravenously.

Conclusion

Substitution of the *N*-alkylalkanesulfonamido group for OCH_3 in the sulfanilamidodiazines studied generally results in lower activity. However, in one series of sulfanilamides comparing several such sulfonamido substituents, the *N*-methylbutanesulfonamido derivative best approximates the active methoxyl analog in terms of potency against *S. pyogenes* C203.

It is interesting to note that although activity varies considerably in the series of *N*-alkylalkanesulfonamido analogs of sulfamonomethoxine, the pK_a values for the various members are identical; thus, any correlative relationship between acidity[19] and activity is precluded. Although the influence of lipophilic character on activity is apparent from a measure of partition coefficients, a better relationship might be obtained with the π values[20] normally used for correlative purposes.

Experimental Section

Melting points were taken in open capillary tubes according to U. S. Pharmacopea XVI—Class I on a Thomas–Hoover apparatus and are corrected; boiling points are uncorrected. All compounds had ir spectra in agreement with their assigned structures. pK_a determinations were made on a Metrohm Herisau automatic titrimeter using a combined glass electrode in 50% (v/v) aqueous MeOAc and titrating with standardized 0.1 *N* aqueous KOH. Solubilities, blood levels, partition coefficients and protein binding values were obtained by Bracon–Marshall[21] analysis of the appropriate solutions (Table IV). Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

***N*-Alkylalkanesulfonamides** were prepared by the procedure of Baxter, *et al.*,[22] for *N*-methylmethanesulfonamide. All were purified by vacuum distillation (Table I).

***N*-(6-Chloro-4-pyrimidinyl)alkanesulfonamides (3–11, Table II). General Procedure.**—The K salts of the alkanesulfonamides, prepared by addition of 4.9 g (0.074 mol) of 85% KOH to 0.074 mol of alkanesulfonamide (Table I) in 50 ml of MeOH and distillation of the MeOH *in vacuo*, were suitable for reaction without further purification. A suspension of the K salt in 20 ml of DMSO was added in one portion to a rapidly stirred solution of 11 g (0.074 mol) of 4,6-dichloropyrimidine[23] in 25 ml of DMSO. Cooling was utilized to keep the temperature below 70°. After 30 min, the mixture was quenched in ice–water and the separated material was isolated and purified.

Two equivalents of potassium butanesulfonamide were used for the preparation of **11**, due to the acidic nature of the product.

***N*-Alkyl-*N*-(6-sulfanilamido-4-pyrimidinyl)alkanesulfonamides (12–19, Table III). General Procedure.**—A mixture of 1 equiv of the appropriate *N*-alkyl-*N*-(6-chloro-4-pyrimidinyl)alkanesulfonamide (Table II), 2 equiv of sodium sulfanilamide, and sufficient DMSO to equal three times the combined weight of the starting materials was heated at 90° for 6 hr. The resulting suspension was diluted with ice–water, chilled overnight, and filtered to remove sulfanilamide. Acidification of the filtrate with glacial AcOH produced a precipitate which was collected, air-dried, and recrystallized.

***N*⁴-Acetyl-*N*¹-(6-butanesulfonamido-4-pyrimidinyl)sulfanilamide.**—A mixture of 12.5 g (0.05 mol) of *N*-(6-chloro-4-pyrimidinyl)butanesulfonamide (**11**), 10.7 g (0.05 mol) of *N*⁴-acetylsulfanilamide, 22.5 g (0.16 mol) of anhydrous K_2CO_3, and 2.5 g of Cu–bronze catalyst (Anaconda Alloy No. 452, shavings) was heated[20] in an oil bath at 200° with occasional stirring for 1 hr, after which the melt was cooled and stirred with 100 ml of H_2O. The insoluble matter was filtered and the filtrate was acidified to pH 2 (5 *N* HCl). The yellow precipitate was collected, dried, and recrystallized twice (MeOH–Me_2CO and then absolute EtOH) to give 6.1 g (26%) of yellow powder (mp 244.5–246.5°). *Anal.* ($C_{18}H_{22}N_4O_5S_2$) C, H, N.

***N*¹-(6-Butanesulfonamido-4-pyrimidinyl)sulfanilamide (20).** A solution of 5.41 g (0.015 mol) of the *N*⁴-Ac compound, 5 g (0.125 mol) of NaOH, and 60 ml of H_2O was refluxed for 3 hr, after which the mixture was cooled and acidified to pH 5 (glacial AcOH). The crude precipitate was collected, dried, and purified (Table III).

***N*-(2-Acetamido-5-pyrimidinyl)butanesulfonamide (24).** 2-Acetamido-5-nitropyrimidine (**23**)[24] (2.7 g or 0.015 mol) was reduced catalytically (3 atm, 0.3 g of 20% Pd–C, 25°) in 150 ml of absolute MeOH. After filtration and concentration, the white solid residue was stirred at 25° in 35 ml of C_5H_5N as 2.4 g (0.015 mol) of butanesulfonyl chloride was added over 1 min. The solution was warmed 5 min at 50–55°, then stirred 1 hr at 30°. Removal of the C_5H_5N *in vacuo* left a dark, syrupy residue which was taken up in 150 ml of H_2O and acidified (pH 2) with 1 *N* HCl. The tan precipitate was collected, dried, and recrystallized (95% EtOH) to give 2.6 g (64%) of colorless needles (mp 184–185.5°). *Anal.* ($C_{10}H_{16}N_4O_3S$) C, H, N.

***N*-(2-Acetamido-5-pyrimidinyl)-*N*-methylbutanesulfonamide (25).** A stirred suspension of 2.4 g (0.0088 mol) of the diamide (**24**) in 10 ml of DMF was treated with 0.4 g (0.0089 mol) of NaH (55% in mineral oil). MeI (1.42 g, 0.01 mol) was added after 30 min and stirring was continued at 50° for 30 min. Dilution of the cooled reaction mixture with ice–water produced 2.4 g of white solid. Recrystallization (*i*-PrOH–MeCN and then EtOAc) gave 1.3 g (52%) of colorless needles, mp 154–155.5°. *Anal.* ($C_{11}H_{18}N_4O_3S$) C, H, N.

***N*-(2-Amino-5-pyrimidinyl)-*N*-methylbutanesulfonamide (26).** A suspension of 2.1 g (0.00384 mol) of the Ac compound **25** in 10 ml of 1 *N* NaOH and 1 ml of *i*-PrOH was heated at 90–95° for 30 min. The mixture was chilled and filtered to give 0.9 g of white flakes. One recrystallization (*i*-PrOH) gave 0.5 g (53%) of white waxy solid (mp 121–122°). *Anal.* ($C_9H_{16}N_4O_2S$) C, H, N.

***N*-Methyl-*N*-(2-*p*-nitrobenzenesulfonamido-5-pyrimidinyl)butanesulfonamide (27).** The aminopyrimidine (**26**) (2.44 g, 0.01 mol) in 25 ml of C_5H_5N was stirred 18 hr at 30° with 2.21 g (0.01 mol) of *p*-nitrobenzenesulfonyl chloride. The C_5H_5N was distilled *in vacuo* and the residue was taken up in dilute NH_4OH and filtered hot. Acidification of the filtrate (pH 2) with 12 *N* HCl afforded 1.5 g of tan solid (mp 182–185°). Recrystallization (MeCN and then absolute EtOH) yielded 0.85 g (20%) of off-white crystals (mp 194–195°). *Anal.* ($C_{15}H_{19}N_5O_6S_2$) C, H, N.

***N*-Methyl-*N*-(2-sulfanilamido-5-pyrimidinyl)butanesulfonamide (21).** A suspension of 12.15 g (0.0284 mol) of the NO_2 compound (**27**) in 1.2 l. of absolute MeOH was reduced catalytically (1 g of 84% PtO_2, 1 atm, 30°). The catalyst was filtered (Celite) and the filtrates were evaporated *in vacuo*. The white amorphous residue was recrystallized (*i*-PrOH and then C_6H_6–MeCN) to provide 7.3 g (65%) of off-white powder: mp 168.5–170.5°; pK_a, 7.35; solubility, 0.014 mg/ml; blood level, 41 mcg/ml; partition coefficient, 2.2; %, protein binding, 51; CD_{50}, 80 mg/kg. *Anal.* ($C_{14}H_{23}N_5O_4S_2$) C, H, N.

***N*-Methyl-*N*-(6-chloro-2-pyrazinyl)butanesulfonamide (29)** was prepared from 2,6-dichloropyrazine (**28**) (Aldrich Chemical Co.) and the K salt of *N*-methylbutanesulfonamide as described for the corresponding 4,6-disubstituted pyrimidines. The crude product was distilled at 120–130° (0.03 mm) to give a pale yellow liquid (65%): $n^{25}D$ 1.5365. *Anal.* ($C_9H_{14}ClN_3O_2S$) C, H, N.

***N*-Methyl-*N*-(6-sulfanilamido-2-pyrazinyl)butanesulfonamide (22)** was prepared according to the general procedure for the corresponding 4,6-disubstituted pyrimidine derivatives. The crude material was recrystallized twice (95% EtOH) to give beige needles: 20%; mp 203–204°; pK_a, 7.05; solubility, 0.001 mg/ml; blood level, 19 mcg/ml; partition coefficient, 2.2; %,

(20) T. Fujita and C. Hansch, *J. Med. Chem.*, **10**, 991 (1967).

(21) A. C. Bratton, E. K. Marshall, Jr., H. Bablitz, and A. R. Hendrickson, *J. Biol. Chem.*, **128**, 537 (1939).

(22) J. N. Baxter, J. Cymerman-Craig, and J. Willis, *J. Chem. Soc.*, 669 (1955).

(23) R. Holl, *ibid.*, 2214 (1951).

(24) R. C. Robbins, P. S. Winnick, and J. P. English, *J. Amer. Chem. Soc.*, **64**, 567 (1942).

protein binding, 54; CD_{50} >400 mg/kg. *Anal.* ($C_{15}H_{21}N_3O_4S_2$) C, H, N.

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Potential Antiradiation Agents. III.[1] N-Substituted Aminoethanethiosulfuric Acids

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A series of N-monoalkyl-substituted 2-aminoethanethiosulfuric acids was prepared for testing as potential antiradiation agents. The compounds were synthesized by the direct alkylation of the sodium salt of 2-aminoethanethiosulfuric acid with primary alkyl bromides, by the reaction of the appropriate *N*-alkylaminoethyl halide hydrohalides with sodium thiosulfate, or by the ring opening of 1-substituted aziridines with ammonium thiosulfate. Excellent radioprotective activity (>70% survival) was obtained with those 2-aminoethanethiosulfuric acids which were N-substituted by methyl, *n*-octyl, 2-octyl, *n*-nonyl, 2-nonyl, 3-nonyl, *n*-decyl, 2-decyl, 3-decyl, 3,7-bimethyloctyl, 4-phenylbutyl, and 5-phenylpentyl groups.

In an earlier paper[2] we described the synthesis and radioprotective properties of a series of aminoalkanethiosulfuric acids possessing a primary amino group. It was shown that optimal activity was obtained when the NH_2 and SSO_3H functions were separated by two CH_2 groups. The high antiradiation activity shown by many *N*-alkylaminoethanethiols[3] suggested that 2-aminoethanethiosulfuric acids which were N-substituted also might be useful as potential antiradiation drugs.

In this paper we report on the antiradiation properties of a series of N-monoalkyl-substituted 2-aminoethanethiosulfuric acids, the synthesis of many of which was described by us previously.[4]

Chemistry

The previously unreported *N*-alkylaminoethanethiosulfuric acids (Table I) were prepared by two general methods. Method A involved the direct alkylation of 2-aminoethanethiosulfuric acid as the Na salt with a primary alkyl bromide in EtOH–H_2O. The dialkyl-

$$RBr + H_2NCH_2CH_2SSO_3^- \longrightarrow RNHCH_2CH_2SSO_3H + Br^-$$

ated by-product was separated from the desired monoalkylated 2-aminoethanethiosulfuric acid by repeated recrystallizations.

Method B utilized the reaction of sodium thiosulfate with an *N*-alkylaminoethyl halide hydrohalide in H_2O or EtOH–H_2O. The *N*-alkylaminoethanol precursors

$$R\overset{+}{N}H_2CH_2CH_2X\ X^- + SSO_3^{2-} \longrightarrow RNHCH_2CH_2SSO_3H + 2X^-$$

were prepared either by the direct alkylation of 2-aminoethanol by the method of Wright, *et al.*,[5] or by the reaction of a carboxylic acid with 2-aminoethanol to yield an *N*-(2-hydroxylethyl)amide which was reduced with LAH in THF. The resultant N-substituted aminoethanols were converted into the amino halide form by treatment with $SOCl_2$ or 48% HBr.

Results and Discussion

Compounds **1–18** constitute a homologous series of aminoethanethiosulfuric acids N-substituted with unbranched alkyl groups. The first five members were the most water soluble and the least toxic. However, any appreciable radioprotective activity was limited to those compounds substituted with Me (**1**) or Et (**2**), while slight activity was shown by the Pr compound (**3**). Increased toxicity and absence of activity marked compounds **4–6**, but activity was restored to the series with the heptyl-substituted compound (**7**) and rose steadily, reaching a peak effect with **10**. Compound **10** not only conferred a high degree of protection to the mice, but did so at a considerably smaller dose (5 mg/kg) than that required by most radioprotective thiosulfuric acids. In contrast to 2-mercaptoethylamine (MEA), whose duration of maximum radioprotective activity extends to 15 min and then diminishes rapidly thereafter,[6] the duration of activity of **10** extends close to 1 hr. Compound **10**, while effective when given parenterally and moderately protective when given subcutaneously, is ineffective when given orally. Other agents in this class, which protected after parenteral injection, also failed to protect when administered by intubation. Attempts to induce absorption included acidification of the intestinal contents of the mouse and the use of ethylenediaminetetraacetic acid which promotes the absorption of a wide variety of poorly

(1) Part II: D. L. Klayman, M. M. Grenan, and D. P. Jacobus, *J. Med. Chem.*, **12**, 723 (1969).

(2) Part I: D. L. Klayman, M. M. Grenan, and D. P. Jacobus, *ibid.*, **12**, 510 (1969).

(3) Annual Report, FY 1964, Walter Reed Army Medical Center, Walter Reed Army Institute of Research, Division of Medicinal Chemistry, Washington, D. C. Available through the Defense Documentation Center, Cameron Station, Alexandria, Va. 22315, as Report AD 601934.

(4) D. L. Klayman and W. F. Gilmore, *J. Med. Chem.*, **7**, 823 (1964).

(5) J. B. Wright, E. H. Lincoln, R. V. Heinzelmann, and J. H. Hunter, *J. Amer. Chem. Soc.*, **72**, 3536 (1950).

(6) Z. M. Bacq, "Chemical Protection Against Ionizing Radiation," Charles C. Thomas, Springfield, Ill., 1965, pp 126–129.