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-4pyrimidinyl)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 N⁴ 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



Investigations^{7,8} in these laboratories, unrelated to chemotherapy, have shown interesting chemical and pharmacological relationships between the phenolic OH and the alkanesulfonamido function, RSO₂NH. The corresponding analogy between a OCH_3 and the Nmethylalkanesulfonamido 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-C₄H₉) was less direct. The acidic proton in 11 reacts with sodium sulfanilamide to produce a negatively charged group in the 4-position;

(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).

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.



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



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

(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.

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

^{(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).

⁽¹⁰⁾ Swiss Patent 210,429 (1940) to Societe pour l'Industrie Chimique, a Bale, Chem. Abstr., 35, 5514 (1941).
 (11) M. Ghione, C. Bertazzoli, A. Buogo, T. Chieli, and V. Zavaglio,

Chemotherapia, 6, 344 (1963).

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TABLE I Alkanesulfonamides

$R = \tilde{N} = SO_2 R'$								
R	R '	Mp or bp (mm), °C	Xield,	11 ²⁰ D	Formula	Analy ses		
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	$C_3H_5NO_2S$	С, Н, Х		
CH_3	$C_3H_{7}-i$	92-94 (0.15)	88	1.4548	$C_4H_{11}NO_2S$	С, Н, N		
CH_3	C_3H_{7} -n	100-105 (0.10)	92	1.4523	$C_4H_{11}NO_2S$	С, Н, N		
CH_3	C_4H_{p-H}	115-118 (0.3)	99	1.4548	$C_5H_{13}NO_2S$	C, H, H^{5}		
CH_3	$C_7H_{15}n$	40.0-41.0	31		$C_8H_{19}NO_28$	C, H, N		
C_2H_5	C_4H_9-n	415-120 (0.30) ^r	89	1.4516	$C_6H_{15}NO_2S$	C, H, N		
C_4H_{5} -n	CH_3	$115(120,(0,30))^d$	96	1.4505	$C_5H_{13}NO_2S$	C, H, S		
H	C_4H_9-n	$47.5{-}48.5'$	7.5		$C_4H_{11}NO_2S$			

^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**, 11873e (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)alkanesulfonamide

$C! \xrightarrow{R} V \xrightarrow{R} V \xrightarrow{N} SO_2 R'$						
X.		13.5	Mp or bp	Yield,	Recrystn	T 1
NO.	R	11 () 1 1	(mm), A	-	solvent	Formula
3	CH_3	CH_0	85.5-87.5	73	A	$C_6H_8ClN_3O_2S$
4	CH_3	C_2H_5	68.5~69.5	55	В	$C_7H_{10}ClN_3O_2S$
5	CH_3	C_3H_{7}	109.5 - 111.5	45	B C	$C_8H_{12}CIN_3O_2S$
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	661	C-E	$C_9H_{14}ClN_3O_2S$
8	CH_3	C_7H_{15} - n	160-166 (0.02)	67		$C_{12}H_{20}ClN_3O_2S$
9	C_2H_5	$C_4H_{2}-n$	45.0 - 46.0	43	D	$C_{t0}H_{16}CIN_3O_2S$
10	C_4H_9-n	CH_3	58.5 - 59.5	51°	B-E	C ₉ H ₁₄ ClN ₃ O ₉ S
11	H	C_4H_{2} - n	141.5 - 142.5	60%	F	$C_8H_{12}ClN_3O_2S$

^a A, EtOAc; B, $(i-Pr)_2O$; C, Me₂CO; D, Skelly F; E, cyclohexane; F, *i*-PrOH. ^b All compounds analyzed satisfactorily for C. II, $N_1 = 1$ Yield after one recrystallization.

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
No. R R' Mp, \circ C Yield, 'i Recrystn Solvent'' For 12 CH ₃ CH ₅ 210.0-211.5 28 A C ₁₂ H ₄ 13 CH ₃ C ₂ H ₅ 177.5-178.5 52 B C ₁₃ H ₄ 14 CH CCH 2020.0 232 A C ₁₃ H ₄	
No. R R' Mp. °C C_1 Solvent" For 12 CH ₃ CH ₃ 210.0-211.5 28 A $C_{12}H_4$ 13 CH ₃ C ₂ H ₄ 177.5-178.5 52 B $C_{13}H_1$ 14 CH CCH 232.0.230.0 233 A CCH	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	nula ⁵
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$N_5O_4S_2$
	$N_5O_4S_2$
-14 = 0.03 = 0.30777 = 220.0 = 220.0 = 20 = A = 0.4411	$N_5O_4S_2$
1.5 CH ₃ C ₃ H ₇ -n 205.5-206.0 54 A C ₁₄ H ₁	$N_5O_4S_2$
16 CH ₃ C ₄ H ₋ <i>n</i> 162.5-163.0 50 C-D C ₁₅ H ₂	$N_5O_4S_2$
17 CH ₃ C ₁ H ₁₃ - <i>u</i> 170.5-171.5 54 A C ₁₈ H ₂	$N_5O_4S_2$
18 C_2H_5 C_4H_{-n} $198.0-199.0$ 60 $A-E$ $C_{16}H_2$	$N_5O_4S_2$
$19 C_4H_{5^{-H}} CH_3 209.5-211.5 63 A C_{15}H_2$	$N_5O_4S_2$
20 H C_4H_3 - <i>n</i> 244.5-245.5 31 A E $C_{14}H_3$	$N_5O_4S_2$

^a A. MeCN; B. EtCOMe: C. abs EtOH; D. $(i-Pr)_2O$; 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 CD₅₀ (curative dose) was calculated¹⁴ 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). The rapeutic activity increased as the substituent (\mathbf{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

				R 				
				Ņ́—SO₂R′				
H_2N SO_2NH N								
No.	R	R'	Solubility, mg/ml ^a	Max blood level (mice), mcg/ml ^b	$\operatorname{Partition}_{\operatorname{coeff}^c}$	% protein binding ^d	CD₅0, 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 <i>°</i>	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	Н	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 CHCl₃-0.1 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.



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 ($CD_{50} > 400 \text{ mg/kg}$). 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 growth¹⁵ and their effect on O₂ 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 Me₂CO due to the low solubility of many of the compounds in H₂O, EtOH, or even 30% Me₂CO. Values for reference standards obtained in 50% Me₂CO tended to run higher than values obtained in water¹⁹ by about 1.3 pK units. Thus, by rough extrapolation, the 50% aqueous Me₂CO values for this series correspond to 5.6-5.7 in H₂O. The pK_a of sulfamonomethoxine was 7.17 in the 50% Me₂CO 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

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⁽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 OCH₃ 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 acidity¹⁹ 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 values²⁰ 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 Bratton-Marshall²¹ 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 $\pm 0.4\%$ 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-dichloropyrimidine²³ 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^{4} -(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 athydrous K₂CO₃, and 2.5 g of Cu-bronze catalyst. Anaconda Alloy No. 452, shavings) was heated¹⁶ in an oil bath at 200° with occasional stirring for 1 hr, after which the melt was cooled and stirred with 100 ml of H₂O. The insoluble matter was filtered and the filtrate was acidified to pH 2 (5 N HCl). The yellow precipitate was collected, dried, and ecrystallized twice. MeOH: Me₂CO and then absolute EtOH) to give 6.1 g $(26^{6})_{i}$ of yellow powder (mp 244,5-(246.5°)). Anet. (C₁₈H₂₁N₃O₂S₂) C, H, N.

 X^{1} -(6-Butanesulfonamido-4-pyrimidinyl)sulfanilamide (20). A solution of 6.44 g (0.015 mol) of the X^{3} -Ac compound, 5 g (0.125 mol) of NaOH, and 60 ml of H₂O 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°G 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.H.N 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.H.N *in racmo* left a dark, syrupy residue which was taken up in 150 ml of H₂O 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. H₂N₀S) 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 mf of DMF was treated with 0.4 g (0.0089 mol) of NaH (53 $^{\circ}_{\ell}$ in mineral of). 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. (C₁₁H₁,N₄O)S (C, H, N.

N-(2-Amino-5-pyrimidinyl)-N-methylbutanesulfonamide (26). \sim A suspension of 1.1 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'*i*) of white waxy solid mp 421-122°). Anal. (C₈H₁₆N₄O₂S) C, H, N.

N-Methyl-*N*-(2-*p*-nitrobenzenesulfonamido-5-pyrimidinyl)butanesulfonamide (27)... The animopyrimidine (26) (2.44 g, 0.01 mol) in 25 ml of C_1H_2N was stirred 18 hr at 30° with 2.21 g (0.01 mol) of *p*-nitrobenzenesulfonyl chloride. The C_8H_8N was distilled *in vacuo* and the residue was taken up in dilute NH4OH and filtered hot. Acidification of the filtrate (pH 2) with 12 *N* HCl afforded 1.5 g of tau solid (mp 182-185°). Recrystallization (MeCN and thet; absolute EtOH) yielded 0.85 g (20°7) of offwhite crystals mp 194/195°). *Anal.* ($C_{15}H_{19}N_5O_2S_2$) C, H₁ N.

N-Methyl-*N*-(2-sulfanilamido-5-pyrimidinyl)butanesulfonamide (21). -A suspension of 12.15 g (0.0284 mol) of the NO₂ compound (27) in 1.24, of absolute MeOH was reduced catalytically (1 g of 84% PrO₂, 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 C₈H₆ MeCN) to provide 7.3 g -65% of off-white powder: mp 168.5-170.5°; pK_{ay}, 7.35; solubility, 0.014 mg ml; blood level, 41 mcg ml; partition coefficient, 2.2; % protein binding, 51; CD₅₀, 80 mg kg. Anal. (C₁₅H₂(N₅O₈S₂, 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%: $w^{25}p$ 1.5365. Anal. (C₉H₁₄ClN₈O₉S) 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: 29%, mp 203-204%; $pK_{\rm a}$, 7.05; solubility, 0.004 mg ml: blood level, 19 mcg ml: partition coefficient, 2.2; %

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protein binding, 54; CD₅₀ >400 mg/kg. Anal. $(C_{15}H_{21}N_5O_4S_2)$ 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-amino-ethanethiosulfuric 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 azirdines 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, n-decyl, 2-decyl, 3-decyl, 3,7-bimethyloctyl, 4-phenylbutyl, and 5-phenylpentyl groups.

In an earlier paper² 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 NH₂ and SSO₃H functions were separated by two CH₂ groups. The high antiradiation activity shown by many N-alkylaminoethanethiols³ suggested that 2aminoethanethiosulfuric 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-H₂O. 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-H₂O. The N-alkylaminoethanol precursors

 $RNH_2CH_2CH_2X X^- + SSO_3^2 \longrightarrow$

 $RNHCH_2CH_2SSO_3H + 2X^-$

were prepared either by the direct alkylation of 2aminoethanol 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 SOCl₂ 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

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