The anticancer activity in five oxazoles, **1-, 2-, 3-, 5-,** and **8-O** was screened by the method of Apple and Greenberg[6] using sarcoma S180 in white albino mice weighing 20–25 g. The animals were injected ip with 2×10^6 cells of exponentially growing ascites cancers. After 24 hr they were injected ip with 666 mg/kg of oxazole in suspension. Normal saline was used in control animals. Ten mice were used for each test. After 7 days, the animals were sacrificed by cervical dislocation, the body was slit open, and tumor cells were washed into a 50-ml cylinder with a spray jet of isotonic saline. Subsequently, dilutions were made to 10^4–10^5 cells/ml for counting. The number of tumor cells were counted using a Coulter Counter (a particle impedance transducer, Coulter Electronics, U.S.A.) The cell count of the treated animals (T) is compared with that of the control animals (C). The effect of the drug, in preventing cancer cell growth is calcd and expressed as per cent inhibition. The data are given in Table III.

***S,S'*-Dialkyl or -diaryl dithiooxaldiimidates** were prepd as previously described.[2] *S,S'*-Di-*p*-methoxybenzyl dithiooxaldiimidate was prepd from *p*-methoxybenzyl mercaptan and $(CN)_2$ in 55% yield, mp 169–170°. *Anal.* ($C_{18}H_{20}N_2O_2S_2$), C, H, N, S.

Oxazoles were prepd by modifications of the previously described procedure.[2] The prepn of 5-benzylindenamino-4-*p*-methoxybenzylmercapto-2-phenyloxazole (**4-O**) illustrates the procedure.

S,S'-Di-*p*-methoxybenzyl dithiooxaldiimidate (7.2 g, 0.02 mole) and 5.3 g (0.05 mole) of PhCHO were heated at 100° for 1.5 hr and 50 ml of abs EtOH was added to the reaction mixt. On cooling yellowish orange cryst sepd, mp 105–108°. Crystn from EtOH afforded 5.2 g (65%) of the oxazole, mp 110–111°.

Hydrolysis of Oxazoles to Nitriles and Amides.—The oxazoles were subjected to mild hydrolysis to give the corresponding nitrile. The procedure is illustrated by the prepn of α-benzoylamino-α-benzylmercaptoacetonitrile (**3-N**). To 3.7 g (0.01 mole) of 5-benzylmercapto-2-phenyloxazole (**3-O**) in 150 ml of Me_2CO was added 100 ml of 5% HCl. The mixt was allowed to stand for 5 min at 25° and was neutralized with Na_2CO_3, dild to 400 ml with H_2O, satd with NaCl, and extd with Et_2O. The Et_2O ext was dried (Na_2SO_4) and evapd to a residue which when crystd from PhH gave 1.2 g (45%) of the nitrile **3-N**, mp 100–105°; further crystn from EtOH gave the anal. sample, mp 105–106°. The hydrolysis of oxazoles to amides is illustrated by the prepn of α-benzoylamino-α-benzylmercaptoacetamide (**3-A**).

To a clear soln of 3 g (0.008 mole) of 5-benzylideneamino-4-benzylmercapto-2-phenyloxazole (**3-O**) in 50 ml of Me_2CO was added 50 ml of 5% HCl. The reaction mixt was heated under reflux for 2 hr, cooled, and neutralized with $NaHCO_3$. Removal of Me_2CO under reduced pressure afforded the crude amide, mp 144–151°. Crystn from abs EtOH yielded 1.4 g (58%) of amide **3-A**, mp 154.5–155°.

Acknowledgments.—We are indebted to Mr. Richard Simmons for technical assistance.

(6) M. Apple and D. M. Greenberg, *Cancer Chemother. Rep.*, **51**, 455 (1967).

Mannich Derivatives of Medicinals. 2. Derivatives of Some Carbonic Anhydrase Inhibitors

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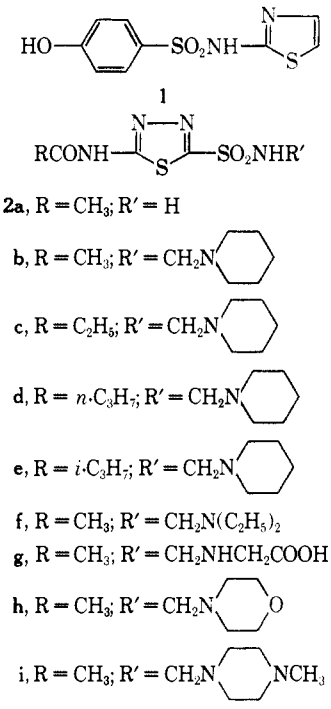
An earlier report[1] described the preparation of a series of nuclear-substituted Mannich bases of the phenolic sulfonamide **1**. This paper describes the results of an investigation of Mannich-type derivatives of the carbonic anhydrase inhibitor sulfonamides **2a** (acetazolamide) and **3a** (methazolamide).[2] The present objective was to obtain derivatives having increased solubility which would also rapidly regenerate the parent compounds under physiological conditions. It was expected that for these materials, derivatization would occur at the amide or sulfonamide group, giving a hydrolytically labile $>NCH_2N<$ link,[3] rather than at the aromatic nucleus.



Chemical Studies.—The Mannich-type derivatives and related compounds summarized in Table I were prepared by condensation of the sulfonamides with CH_2O and the corresponding amines.[4] The composition and purity of the products were confirmed by elemental analyses. Uv spectra (Table II) show that the heterocyclic chromophores of the parent compounds are present and unchanged in the derivatives. In the **3** series this is sufficient to show that the newly introduced aminomethyl group is linked to the sulfonamide N. Furthermore, the similarity of preparation and properties suggests analogous structures for the derivatives of **2a**, which is confirmed by nmr and ir data. The nmr spectrum[5] of **2a** contains broad peaks centered at 790 cps (δ 13.17, 1 H) and 514 cps (8.57, 2 H) arising from the N-protons of the carboxamide and sulfonamide groups, respectively. The spectrum of **2b**·HCl contains 3 one-proton peaks at 740 cps (δ 12.38, broad), 607 cps (10.12, broad), and 502 cps (8.37, sharp).

(1) G. M. Sieger, W. C. Barringer, F. M. Callahan, N. Gruenfeld, and J. F. Weidenheimer, *J. Pharm. Sci.*, **50**, 869 (1961).

(2) (a) R. O. Roblin, Jr., and J. W. Clapp, *J. Amer. Chem. Soc.*, **72**, 4890 (1950). (b) W. W. Miller, A. M. Dessert, and R. O. Roblin, Jr., *ibid.*, **72**, 4893 (1950). (c) J. W. Clapp and R. O. Roblin, U. S. Patent 2,554,816 (May 29, 1951). (d) R. W. Young, K. H. Wood, and J. R. Vaughan, Jr., U. S. Patent 2,783,241 (Feb 26, 1957).

(3) (a) C. M. Suter, "Organic Chemistry of Sulfur," Wiley, New York, N. Y., 1945, p 585. (b) C. Maselli, *Gazz. Chim. Ital.*, **30**, 33 (1900). (c) G. M. Sieger and W. C. Barringer, U. S. Patent 3,213,092 (1965).

(4) Attempts to prepare derivatives of **2a** using $EtNH_2$, diethanolamine, and iminodiacetic acid and a derivative of **3a** using Et_2NH were unsuccessful.

(5) The nmr spectra were obtained with a Varian A-60 spectrometer on 10–15% solutions in deuterated DMSO (Me_4Si).

TABLE I

PHYSICAL DATA OF NEW COMPOUNDS

No.	Formula	Analyses	% yield	Mp, °C[a]
2b	$C_{10}H_{17}N_5S_2O_3$	C, H, N, S	82	169–171
2b·HCl	$C_{10}H_{18}ClN_5S_2O_3$	C, H, N, Cl; S[b]	45	150–154
2c	$C_{11}H_{19}N_5S_2O_3$	C, H, N, S	57	155–157
2c·HCl	$C_{11}H_{20}ClN_5S_2O_3$	C, H, Cl	65	141–143
2d	$C_{12}H_{21}N_5S_2O_3$	C, H, N, S	76	151–154
2e	$C_{12}H_{21}N_5S_2O_3$	C, H, N, S	62	153–156
2f	$C_9H_{17}N_5S_2O_3$	C; H[c]	97	
2g	$C_7H_{11}N_5S_2O_5$	C, H	82	
2h	$C_9H_{15}N_5S_2O_4$	C, H	65	
2h·HCl	$C_9H_{16}ClN_5S_2O_4$	C, H, Cl		
2i	$C_{10}H_{18}N_6S_2O_3$	C, H, S	40	
3b	$C_{11}H_{19}N_5S_2O_3$	C, H, N, S	50	138–140
3c	$C_8H_{15}N_5S_2O_3$	C, S; H, N[d]	81	
4a	$C_6H_8N_4S_2O_4$	C, H	63	
4b	$C_{12}H_{19}N_5S_2O_4$	H, N, S; C[e]		200–210, 212–222[g]
5	$C_9H_{12}N_8S_4O_6$	C, H; N, S[f]	71	

[a] Uncorr capillary mp, all decomp. [b] S: calcd, 18.02; found, 18.47. [c] H: calcd, 5.58; found, 5.15. [d] H: calcd, 5.15; found, 5.60; N: calcd, 23.87; found, 23.35. [e] C: calcd, 39.87; found, 39.42. [f] N: calcd, 24.55; found, 24.08; S: calcd, 28.10; found, 27.43. [g] Polymorphs, from *i*-PrOH–EtOH–hexane.

TABLE II

ULTRAVIOLET ABSORPTION MAXIMA

	MeOH		0.1 *N* NaOH	
No.	λ, mμ	ε	λ, mμ	ε
2a	263	10,600	292	13,600
2b	264	10,000	292	13,500
5	263	21,600	292	27,000
3a	292	12,600	290	13,200
3b	290	12,500	288	14,000

This eliminates the alternative structure in which the aminomethyl moiety is attached to the carboxamide N and thereby confirms the structure shown; the bands are assigned to the carboxamide, ammonium, and sulfonamide protons, respectively.

The nmr spectrum of **2b** itself contains a single, two-proton band at 565 cps (δ 9.42) for both N-hydrogens, indicative of rapid exchange. An explanation of this observation is provided by analysis of the ir spectrum of **2b** in which a typical ammonium ion band is seen at about 4.0 μ and the sulfonamide SO_2 bands, found at 7.6 and 8.5 μ for **2a**, are shifted to 7.9 and 8.3 μ, reminiscent of a sulfonate ion. Similar bands are seen in the ir spectra of the other aminomethyl derivatives of **2a** and **3a**. It is, therefore, probable that these derivatives are internally ionized and have the partial structure $SO_2N^-CH_2NH^+$. The ir spectra of the hydrochlorides of these compounds contain normal sulfonamide bands as well as an ammonium ion band (at about 3.2 μ), which supports this interpretation. An attempt to relate the internal ionization to the ionization constants of the sulfonamide and ammonium protons of **2b** failed because the compound was hydrolyzed too rapidly by aq acid to permit the determination of pK_a values.[6]

The aq solubilities of **2b** and its hydrochloride (Table III) are only slightly greater than the values predicted for **2a** at the same pH levels from its pK_a values. However, the propylene glycol solubilities of the derivatives are sufficient for pharmaceutical utility. In addition, ethanolamine salts of the aminomethyl compounds, formed *in situ*, are soluble to about 10% in H_2O.

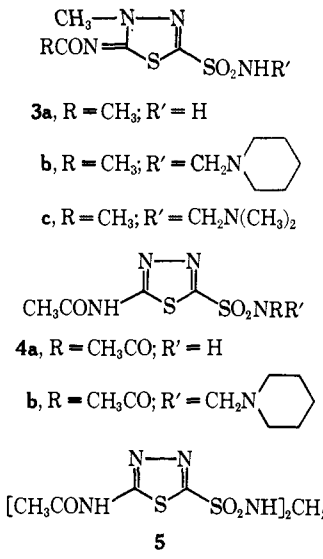


TABLE III

SOLUBILITIES

No.	H_2O, % w/v	Propylene glycol, % w/v
2a	0.07	1.1
3a	0.07	0.8
5	0.03	9.8
2b	1.01	4.4
2b·HCl	0.16	
2c	1.07	8.8
2c·HCl	0.14	
2d	0.33	7.8
2e	0.25	9.5
3b	1.25	8.2

Pharmacological Studies.—Data obtained from a detailed study of the diuretic activity of the compounds are presented in Tables IV and V.[7]

TABLE IV

ORAL DIURETIC ACTIVITY IN RATS

No.	Dose, mg/rat	Collection period, hr	Urine volume[a]	Cl^- excreted[a]
2a	10	0–5	290	−22
2b	10	0–5	328	−6
		0–24	49	−16
2c	10	0–5	364	−5
		0–24	50	−30
2d	10	0–5	366	52
		0–24	93	33
2e	10	0–5	450	32
		0–24	97	35
3a	5	0–5	227	−39
		0–24	47	−33
3b	10	0–5	533	31
		0–24	100	−36
5	10	0–5	433	52
		0–24	78	3

[a] Per cent increase over that produced by 2% starch soln.

(6) Compounds **2a** and **2b** were acetylated, giving **4a** and **4b**, whose structure is indicated by the sharply increased acidity of **4a** (pK_a = 2.6, 8.4) compared to **2a** (pK_a = 7.2, 8.8).

(7) The methodology involved in the diuretic studies has been described by J. R. Cummings, J. D. Haynes, L. M. Lipchuck, and M. A. Ronsberg, *J. Pharmacol. Exp. Ther.*, **128**, 414 (1960); J. R. Cummings and E. H. Stokey, *Arzneim.-Forsch.*, **13**, 661 (1963).

TABLE V
ORAL DIURETIC ACTIVITY IN DOGS

No.	Dose, mg/kg	Collection period, hr	Urine[a] volume	Ions excreted[a] Na^+	K^+	Cl^-
2a	10	0–6	246	16.1	8.9	6.2
		0–24	346	26.1	21.0	11.4
2b	10	0–6	251	8.2	3.7	2.1
		0–24	308	8.9	6.4	3.0
2c	10	0–6	303	11.0	4.2	2.8
		0–24	364	12.2	7.6	7.2
2e	10	0–6	382	13.4	8.3	7.7
		0–24	422	13.8	12.4	8.6
3b	10	0–6	270	8.1	3.6	2.5
		0–24	330	9.2	6.1	4.2
5	5	0–6	338	9.3	4.7	4.9
		0–24	408	11.5	9.7	8.3

[a] Per cent increase over that produced by starch.

In rat studies the derivatives produced 5-hr and 24-hr urine volumes comparable to those of the parent sulfonamides, and several were more efficient as stimulants of Cl^- excretion. In dogs, the urinary electrolyte patterns produced by the aminomethyl derivatives resembled those of the parents, *i.e.*, the new compounds appear to be carbonic anhydrase inhibitors (Table V). In addition, the urine flow in all cases was comparable with that produced by **2a**. Quantitatively, the excretion of Na^+, K^+, and Cl^- stimulated by the test compounds in dogs was somewhat lower than that produced by **2a**. The similarity in the activities of all of the derivatives and the parent compounds in rats and dogs supports the hypothesis that the derivatives are readily hydrolyzed *in vivo* to the parent sulfonamides, as does their instability during the attempted pK_a measurements.

Experimental Section

5-Acetamido-*N*-piperidinomethyl-1,3,4-thiadiazole-2-sulfonamide (2b).—To a soln of 5.5 g (0.025 mole) of 5-acetamido-1,3,4-thiadiazole-2-sulfonamide (**2a**) and 5 ml (4.31 g, 0.05 mole) of piperidine in 50 ml of MeOH was added 5 ml (0.067 mole) of 37% CH_2O. The reaction mixt was kept at room temp overnight, and the product was collected by filtration and dried *in vacuo* at 60°. Compounds **2c–i**, **3b–c**, and **4b** were also made by this procedure; when the product did not crystallize spontaneously, the soln was concd as needed. For **2g** the initial pH was adjusted to 7.5.[8]

***N*,*N*′-Methylenebis(5-acetamido-1,3,4-thiadiazole-2-sulfonamide) (5).**—A suspension of **2a** (5.5 g, 0.025 mole) in 50 ml of MeOH was heated on a steam bath. After 2 min 37% CH_2O (5.0 ml, 0.067 mole) was added, and the mixt was heated for 30 min, allowed to cool for 1 hr, and filtered. The product was washed with MeOH and dried *in vacuo*. The yield was 4.0 g (72%).

2c·HCl.—Dry HCl was bubbled for 5 min through a soln of 1.8 g of **2c** in 10 ml of Et_2O and 30 ml of Me_2CO. After 30 min the product was collected and dried *in vacuo*. The yield was 1.3 g (65%).

5-Acetamido-*N*-acetyl-1,3,4-thiadiazole-2-sulfonamide (4a).—A mixt of 5 g of **2a**, 25 g of anhyd NaOAc, and 35 ml of Ac_2O was heated on a steam bath for 1 hr and poured into 300 ml of ice water. The resulting mixt was kept at 4° overnight and filtered. The solid was resuspended in H_2O, filtered, washed (H_2O), and dried to give 4.1 g of crude product. Recrystn from EtOH gave 3.75 g (63%) of **4a**.

Solubility Studies.—To determine the H_2O solubilities the solute content of a measured vol of filtered, satd soln was ascertained by evapn and weighing. To determine the propylene glycol solubilities, a nearly satd soln was prepd at 60–70° from a weighed sample of compound and kept at room temp overnight. The solid material which sepd on standing was filtered, dried, and weighed.

(8) See ref 3c for specific procedures for several of these compounds.

Acknowledgment.—The authors are indebted to Mr. L. Brancone and his staff for microanalytical data, to Mr. W. Fulmor and his staff for infrared and nmr spectra and for valuable discussions about their interpretation, and to Dr. W. Gray, Dr. J. Cummings, and their coworkers of the Experimental Therapeutics Research Section for the biological and pharmacological studies. The authors especially wish to thank Messrs. M. A. Stead and D. F. Deyo for their competent technical assistance.

Synthetic Biologically Active Polymers. 8. Antibacterial Activity of Some Sulfonamide–Dimethylolurea Copolymers

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Chemistry.—Previous publications in this series have described the synthesis, characterization, and certain biological activities of a number of polymers and copolymers.[2] Papers 3–7 deal with formaldehyde copolymers of sulfonamide drugs.[2c–f] This publication will describe the antibacterial activity of a second type of sulfonamide copolymer, namely, sulfonamide–dimethylolurea copolymers. These polymers were prepared so that the biological effect of employing a comonomer other than formaldehyde with the sulfonamides might be observed. The sulfonamide–dimethylolurea condensates were prepared and characterized as reported previously.[2g]

Biological Activity.—Table I displays information concerning the antibacterial activity of certain sulfonamides and sulfone vinylogs and their respective dimethylolurea copolymers. As can be seen, the antibacterial activities of the sulfonamides (M) and the copolymers (P) do not really differ appreciably. Thus, in general, while all three logical occurrences which might be expected to be observed relative to the antibacterial activity of the sulfonamides upon incorporation into the copolymer (antibacterial activity: (1) stays the same, (2) increases, (3) decreases) can be observed, the differences are very small. However, this is relatively interesting because even though the sulfonamide content of the copolymers (P) is smaller than that in equivalent test dosages of the sulfonamide monomers (M), the antibacterial activity did not drop markedly in the copolymers (P). This same phenomenon was observed in the case of antibacterial activity in the sulfonamide drugs relative to the CH_2O copolymers of the same

(1) Taken in part from the thesis submitted by Mr. John R. Dombroski in partial fulfillment of the requirements for the Master of Science degree.

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