tetrahydro-1-methyl-2-phenyl-3*H*-thieno[3,4-*c*]pyrazolone (**7a**): mp 136–137°; ir 5.95 μ; nmr δ 3.03 (s, 3, NCH_3), 3.95 (m, 4, CH_2SCH_2). Partition chromatog of the mother liquors provided two additional compds. Eluted first from the column was 1.42 g (12%) of colorless cryst, mp 109–111°. Recrystn from hexane gave colorless cryst of **2,3a,4,6-tetrahydro-3a-methyl-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one** (**8a**): mp 115–116°; ir 5.83 μ; nmr δ 1.67 (s, 3, CCH_3), 2.72 and 3.02 (dd, J = 10.6 Hz, 2, CH_2 AB), 3.66 and 3.75 (dd, J = 13.5 Hz, 2, CH_2 AB). Further elution gave 0.3 g (3%) of **9** as colorless cryst, mp 86–87°.

C. **With $PhCH_2Br$.**—A mixt of 2.2 g (0.01 mole) of **5**, 6 g of K_2CO_3, 50 ml of Me_2CO, and 1.5 ml (0.013 mole) of $PhCH_2Br$ was stirred at room temp for 24 hr and filtered. The filtrate was concd to an oily solid which was chromatog on basic alumina. Eluted with C_6H_6–hexane (1:1) was 0.8 g (26%) of colorless oil. Evaporative distn at 150° (0.1 mm) gave **3a-benzyl-2,3a,4,6-tetrahydro-2-phenyl-3*H*-thieno[3,4-*c*]pyrazole-3-one** (**8b**) as a viscous colorless liq, ir 5.83 μ.

Elution of the column with C_6H_6–MeOH (49:1) gave a yellow oil. Trituration with Et_2O provided 1.18 g (38%) of colorless cryst, mp 134–135°. Recrystn from EtOH gave colorless crystals, mp 134–135°, ir 5.92 μ, of **1-benzyl-1,2,4,6-tetrahydro-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one** (**7b**).

D. **With Allyl Bromide.**—A mixt of 7.9 g (0.036 mole) of **5**, 22 g of K_2CO_3, 180 ml of Me_2CO, and 5.4 ml of allyl bromide was stirred at room temp for 24 hr and was filtered. The filtrate was concd to an oily solid. Trituration with Et_2O provided 2.8 g (30%) of colorless cryst, mp 132–133°. Recrystn from C_6H_6 gave colorless cryst, mp 135–136°, ir 6.00 μ, of **1-allyl-1,2,4,6-tetrahydro-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one** (**7c**).

The mother liquors were subjected to partition chromatog. Eluted from the column was 4.1 g (44%) of pale yellow liq. Evaporative dist at 170° (20 mm) gave **3a-allyl-2,3a,4,6-tetrahydro-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one** (**8c**), as a viscous colorless oil, ir 5.83 μ.

5,6-Dihydro-3-methoxy-2-phenyl-2*H*-thieno[3,2-*c*]pyrazole (**11**).—A stirred soln of 1.09 g (5.0 mmoles) of 5,6-dihydro-2-phenyl-2*H*-thieno[3,2-*c*]pyrazol-3-ol (**6**), 0.28 g (5.0 mmoles) of NaOMe, and 25 ml of anhyd DMF was heated to 80°, and 0.93 g (5.0 mmoles) of MeOTs was added. The soln was heated at 120° for 2 hr, cooled, dil with H_2O, and extd with Et_2O. The Et_2O soln was dried ($MgSO_4$) and concd to a yellow oil which was chromatog on alumina. Eluted with *c*-C_6H_{12}–EtOAc (9:1) was 0.32 g (27%) of pale yellow crystals, mp 62–65°. Recrystn from *c*-C_6H_{12} gave colorless crystals; mp 68–69°; ir, no C=O below 6.2 μ; nmr δ 2.93 (t, J = 7.5 Hz, 2, CH_2), 3.65 (t, J = 7.4 Hz, 2, CH_2), 3.90 (s, 3, OCH_3).

2-Methyl-4-methylthio-1-phenyl-3-vinyl-3-pyrazolin-5-one (**12**).—A mixt of 1.09 g (5.0 mmoles) of **6**, 2.8 g of K_2CO_3, 1 ml of MeI, and 25 ml of Me_2CO was stirred at room temp for 20 hr and was filtered. The filtrate was concd to a brown oil, which was chromatog on basic alumina. Eluted with C_6H_6–MeOH (99:1) was 50 mg (4%) of colorless solid, mp 87–88°. Recrystn from *c*-C_6H_{12} gave colorless cryst; mp 89–90°; ir 6.03 μ; nmr ($CDCl_3$) δ 2.40 (s, 3, SCH_3), 3.08 (s, 3, NCH_3), 5.71–6.97 (3, vinyl ABC system), and 7.44 (m, 5, phenyl-H).

2,6-Dihydro-3-methoxy-2-phenyl-4*H*-thieno[3,4-*c*]pyrazole 5,5-Dioxide (**16**).—To a stirred soln of 2.32 g (0.01 mole) of **9** and 100 ml of CH_2Cl_2 was added 3.60 g (0.021 mole) of *m*-chloroperoxybenzoic acid. After 1 hr at room temp, the soln was washed with $NaHCO_3$, dried (K_2CO_3), and concd to a solid. Recrystn from C_6H_6–hexane provided 2.0 g (76%) of tan crystals, mp 148–149°, finally 149–150°.

1,2,4,6-Tetrahydro-1-methyl-2-phenyl-3*H*-thieno[3,4-*c*]pyrazole 5,5-dioxide (**14a**) was prepd from 0.93 g (4.0 mmoles) of **7a** by the above method. Recrystn of the crude solid from MeCN gave 0.50 g (47%) of yellow cryst, mp 159–160°. Recrystn from EtOH gave colorless cryst, mp 159–160°.

2,3a,4,6-Tetrahydro-3a-methyl-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one 5,5-dioxide (**15**) was prepd from 0.46 g (2.0 mmoles) of **8a** by the above method. Recrystn of the crude solid from C_6H_6 gave 0.30 g (57%) of colorless cryst, mp 198–199°.

1-Benzyl-1,2,4,6-tetrahydro-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one 5,5-dioxide (**14b**) was prepd from 0.31 g (1.0 mmole) of **7b** by the above method. Recrystn of the crude solid from C_6H_6–hexane gave 0.20 g (59%) of colorless cryst, mp 137–139°, finally 139–140°.

2,6-Dihydro-3-methoxy-2-phenyl-3*H*-thieno[3,4-*c*]pyrazole 5-Oxide (**18**).—To a cold, stirred soln of 2.32 g (0.01 mole) of **9** and 50 ml of CH_2Cl_2 was added dropwise during 1 hr a soln of 1.72 g (0.01 mole) of *m*-chloroperoxybenzoic acid in CH_2Cl_2. The soln was washed with $NaHCO_3$, dried (K_2CO_3), and concd to a solid. Recrystn from C_6H_6–hexane gave 1.80 g (73%) of tan cryst, mp 120–121°. Recrystn gave colorless cryst, mp 122–123°.

1,2,4,6-Tetrahydro-1-methyl-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one 5-oxide (**17**) was prepd from 0.93 g (4.0 mmoles) of **7a** by the above method. Crystn of the crude product from EtOAc gave 0.50 g (51%) of yellow crystals, mp 129–130°, finally pale yellow cryst, mp 136–137°.

cis- (**19**) **and** *trans*-**2,3a,4,6-tetrahydro-3a-methyl-2-phenyl-3*H*-thieno[3,4-*c*]pyrazol-3-one 5-oxide** (**20**) were prepd from 1.09 g (4.7 mmoles) of **8a** by the above method. The crude product, 1.12 g of colorless cryst, mp 125–126°, was subjected to preparative tlc (Analtech, Inc. silica gel GF, 1000-μ plates) with a C_6H_6–EtOH (9:1) solvent. Eluted farthest from the origin was 0.41 g of colorless solid. Recrystn from C_6H_6–hexane gave 0.34 g (30%) of **19** as colorless cryst, mp 142–143°.

A second fraction consisted of 0.35 g of colorless solid. Recrystn from C_6H_6 provided 0.31 g (27%) of **20** as colorless cryst, mp 164–165°.

Synthesis and Biological Study of a Series of S-Substituted α-Mercaptohippuramides and Nitriles[1]

VINOD P. SHAH, GRACE LAM, AND ROGER KETCHAM*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122

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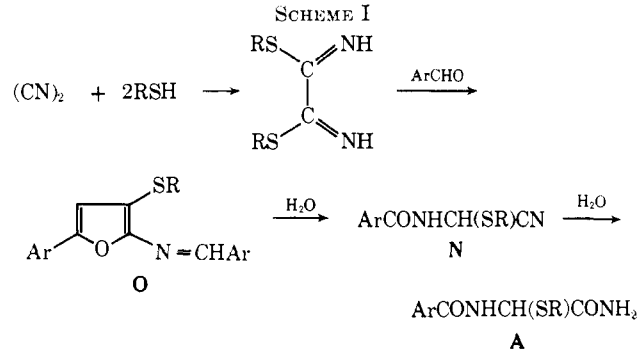
S,S′-Disubstituted dithiooxaldiimidates react with aromatic aldehydes to form 5-arylidene-4-substituted-mercapto-2-phenyloxazoles (**O**).[2] As part of the structural proof it was shown that one of these (**1-O**) could be hydrolyzed to α-methylmercaptohippuronitrile (**1-N**) and the hippuramide **1-A**. This amide was evaluated for its somnifacient properties because of its structural relationship to trimeglamide and thalidomide.[3] We are now reporting the synthesis and biological evaluation of a series of these amides and nitriles having modifications in both the aryl group and the S substituent. The oxazoles were also included in the studies since their facile chemical hydrolysis should make them easily degraded in biological systems as well. The reactions are outlined in Scheme I; the data for the synthesis of new compounds are in Table I.

SCHEME I



(1) Supported in part by a grant (NIMH 08787) from the U. S. Public Health Service.

(2) A. R. Martin and R. Ketcham, *J. Org. Chem.*, **31**, 3612 (1966).

(3) A. R. Martin, F. H. Meyers, and R. Ketcham, *J. Pharm. Sci.*, **56**, 753 (1967).

TABLE I

OXAZOLES (O), NITRILES (N), AND AMIDES (A)

Compd	Ar	R	Mp, °C	Yield, %	Solvent[a]	Formula[b]
4-O	C_6H_5	p-$CH_3OC_6H_4CH_2$	110–111	65	Abs EtOH	$C_{24}H_{20}N_2O_2S$
5-O	p-$NO_2C_6H_4$	CH_3	235–237	60	Abs EtOH	$C_{17}H_{12}N_4O_5S$
7-O	p-$NO_2C_6H_4$	$C_6H_5CH_2$	198–200	60	Abs EtOH	$C_{23}H_{16}N_4O_5S$
3-N	C_6H_5	$C_6H_5CH_2$	105–106	45	EtOH	$C_{16}H_{14}N_2OS$
5-N	p-$NO_2C_6H_4$	CH_3	150–151	50	EtOH	$C_{10}H_9N_3O_3S$
6-N	p-$NO_2C_6H_4$	C_6H_5	157–158	75	EtOH	$C_{15}H_{11}N_3O_3S$
8-N	p-$CH_3OC_6H_4$	CH_3	143–144	50	EtOH	$C_{11}H_{12}N_2O_2S$
2-A	C_6H_5	C_6H_5	191–192	42	EtOH	$C_{15}H_{14}N_2O_2S$
3-A	C_6H_5	$C_6H_5CH_2$	154–155	58	EtOH	$C_{16}H_{16}N_2O_2S$
4-A	C_6H_5	p-$CH_3OC_6H_4CH_2$	184–185	52	Me_2CO	$C_{17}H_{17}N_2O_3S$
5-A	p-$NO_2C_6H_4$	CH_3	190–192	63	EtOH	$C_{10}H_{11}N_3O_4S$
6-A	p-$NO_2C_6H_4$	C_6H_5	204–206	36	Me_2CO	$C_{15}H_{13}N_3O_4S$
7-A	p-$NO_2C_6H_4$	$C_6H_5CH_2$	168–171	30	A–H[c]	$C_{16}H_{15}N_3O_4S$
8-A	p-$CH_3OC_6H_4$	CH_3	188–190	50	Me_2CO	$C_{11}H_{14}N_2O_3S$

[a] Crystn solvent. [b] All compds were analyzed for C, H, N, and S. [c] Acetone–hexane, 1:1.

The only new thioimidate is the S,S'-di-p-methoxybenzyl dithiooxaldiimidate which was prepared by addition of p-methoxybenzylmercaptan to $(CN)_2$.

In view of the observations of Hodnett and Willie[4] on the antitumor activity of Schiff bases we screened some of the oxazoles **O** for their antitumor properties.

The highest dose used in the acute toxicity studies was 1 g/kg. All compounds produced decreased locomotor activity, a sedative effect, and physiologic sleep, from which the animals were easily aroused by tactile and auditory stimuli. Sleep was produced in about 20–30 min after injection and lasted from 4 to 8 hr. The nitriles were the most toxic compounds whereas the oxazoles did not produce death at 1 g/kg. With the amides and nitriles death resulted from respiratory failure, and they also showed increased spontaneous motor activity such as stretching motions. Compound **7-A** demonstrated the highest somnifacient effect. The data are given in Table II.

TABLE II

ACUTE TOXICITY AND SLEEPING TIME

	Sleeping time in min, ave ± S.E.	
Compd	Dose, 250 mg/kg	Dose, 50 mg/kg
1-O[a]	277.1 ± 38.0	176.5 ± 32.8
1-N[b]		124.0 ± 20.7
1-A[a]	99.3 ± 8.2	146.6 ± 45.5
2-O[a]	149.1 ± 24.9	145.7 ± 34.9
2-A[a]	250.7 ± 44.9	106.6 ± 10.2
3-O[a,c]	142.6 ± 39.7	147.0 ± 31.4
3-A[a]	147.7 ± 23.4	100.5 ± 23.7
4-A[a,d]		121.1 ± 10.0
5-O[a]	172.9 ± 46.5	129.2 ± 18.4
5-N[e]	149.9 ± 34.2[f]	
5-A[a]	115.9 ± 9.2	211.1 ± 27.6
6-O[a]	184.6 ± 25.6	177.5 ± 24.7
6-A[a]	106.3 ± 9.6[f]	79.4 ± 7.7
7-O[a,g]	279.1 ± 86.8	151.4 ± 27.5
7-A[a]	189.0 ± 27.8	169.1 ± 17.3
8-O[a,h]	175.3 ± 12.4	146.2 ± 22.8
8-A[a]	116.6 ± 15.5	124.0 ± 19.1

[a] Acute toxicity (AT) no deaths at 1 g/kg. [b] AT—3/10 at 1 g/kg. [c] AT for 3-N: 3/10 at 250 mg/kg, 9/10 at 1 g/kg. [d] AT for 4-O: 0/10 at 1 g/kg. [e] AT 0/10 at 100 mg/kg; 10/10 at 500 mg/kg. [f] At 10 mg/kg. [g] AT for 7-N: 0/10 at 1 g/kg. [h] AT for 8-N: 0/10 at 500 mg/kg; 10/10 at 1 g/kg.

All the compounds show potentiation of barbiturate sleeping time (Table II). In the same series, the oxazole has a higher effect than either the corresponding nitrile or amide. Sleeping time varied from 79.4 ± 7.7 min to 279.1 ± 96.8 min. The controls had a sleeping time of 44 ± 5.4 min, with pentobarbital and 61.8 ± 8.4 min with vehicle followed by pentobarbital.

The antitumor activity of the oxazoles against sarcoma S180 in mice was found to be low (Table III). The first member of the series (**1-O**) was the most effective, inhibiting the cancer growth by 38%. Substituents reduced the activity.

TABLE III

EFFECT OF OXAZOLES IN MICE CANCER GROWTH

Compd	Cell count per mouse[a,b] T (treated)	% inhibition $100 \times \frac{C - T}{C}$
1-O	228,499,000	37.76
2-O	297,040,000	19.1
3-O	352,162,000	4.08
5-O[c]	345,993,000	5.76
8-O	412,158,000	

[a] Average of 10. [b] Control (C) 367,135,000. [c] Drug poorly absorbed, particles seen when the cavity was opened.

Experimental Section[5]

Pharmacology.—The acute toxicities of the oxazoles, amides, and nitriles were studied in Swiss Webster male mice, weighing 18–22 g. Ten mice were used at each dose level, and were observed for 24 hr. The compd under study was injected ip as a suspension prepd by trituration with 5 drops of Tween 80 and making the required vol by adding a soln of 5% acacia and 20% w/v of propylene glycol with trituration. The vehicle, containing Tween 80, acacia, and propylene glycol, was used as a control.

Potentiation of pentobarbital sleeping time by these compds was studied in albino male mice (18–22 g) as a measure of their somnifacient activity. Ten mice were used at each dose level for each compd. The mice were injected ip with sublethal doses of the compd (suspension prepd as described above). This was followed in 15 min by an ip injection of an anesthetic dose (65 mg/kg) of pentobarbital Na. The sleeping time was taken as the interval between administration of the pentobarbital Na and when the animal regained and maintained its righting reflex for 1 min.

(4) E. M. Hodnett and W. Willie, *Proc. Okla. Acad. Sci.*, **46**, 107 (1966).

(5) Melting points were measured using a Thomas-Hoover Capillary melting point apparatus and are corrected. Elemental analyses were carried out by the microanalytical laboratory of the University of California at Berkeley, Berkeley, Calif.

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

GEORGE M. SIEGER, WILLIAM C. BARRINGER, AND JAMES E. KRUEGER

Pharmaceutical Product and Process Development Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York 10965

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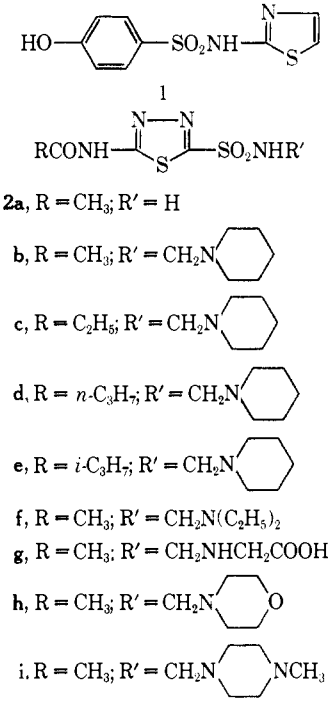
An earlier report[1] described the preparation of a series of nuclear-substituted Mannich bases of the phenolic sulfonamide **1**. This paper describes the results of an investigation of Mannich-type derivatives of the carbonic anhydrase inhibitor sulfonamides **2a** (acetazolamide) and **3a** (methazolamide).[2] 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).

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(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).