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N-Heteroaralkyl Substituted α-Amidinium Thiolsulfates (1)

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The syntheses of a number of *N*-substituted α-amidinium thiolsulfates, $CH_2(S_2O_3^-)$-$C(=NH_2^+)NH(CH_2)_nR$ are described, where n was varied from 1 to 3 and R represents such heteroaryl groups as 2-furyl, 2-thienyl, 3-indolyl and 2-, 3- and 4-pyridyl. The preparation of *S*-(2-imidazolinemethyl)thiolsulfuric acid, as an example of an *N,N'*-disubstituted α-amidinium thiolsulfate, is also reported.

In view of the fact that a number of α-amidinium thiolsulfates bearing aralkyl groups,

$$CH_2(S_2O_3^-)\text{-}C(=NH_2^+)NH(CH_2)_nAr$$

have shown protective activity against otherwise lethal X-radiation in mice (2), a series of analogs were synthesized where Ar represents a heterocyclic system. In designing these molecules, the heteroaromatic systems which were selected were furan, thiophene, indole and pyridine and these were attached to the amidine function by a short aliphatic chain (n < 3). The syntheses of the required α-amidinium thiolsulfates were adapted from the ones developed earlier (2,3). The base-catalyzed addition of methanol to chloroacetonitrile gave methyl chloroacetimidate, $ClCH_2C(=NH)OCH_3$, which was reacted with an amide hydrochloride, $RNH_3^+Cl^-$, to furnish the corresponding α-chloroacetamidinium chloride,

$$ClCH_2C(=NH_2^+)NHR\ Cl^-$$

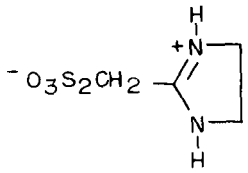
which could sometimes be isolated in crystalline form. The reaction of the α-chloroacetamidinium chlorides with sodium thiosulfate led to the formation of the highly crystalline α-amidinium thiolsulfates which are listed in Table I.

The amines chosen for incorporation into the syntheses of these α-amidinium thiolsulfates were methyl or ω-substituted ethyl- or propylamines bearing 2-furyl, 2-thienyl, 3-indolyl or 2-, 3- and 4-pyridyl groups. The syntheses involving tryptamine and homotryptamine (which attached the 3-indolyl system) were of particular interest since it had been shown that tryptamine and 5-hydroxytryptamine (serotonin) itself possessed radiation-protective properties (4). In the same vein, since tyramine itself had also afforded some protection (4a), the α-amidinium thiolsulfate derived from it was prepared and is included in Table I. The synthesis of a non-heterocyclic derivative is described at this time. It is the one in which an γ-(methylmercapto)propyl group is attached to one of the amidine nitrogen atoms, *viz.*,

$$CH_2(S_2O_3^-)C(=NH_2^+)NH(CH_2)_3SCH_3.$$

It was selected to ascertain how the introduction of the sulfide function would influence the activity since the corresponding *n*-propyl analog has shown some activity previously (2). Furthermore, it would serve as an aliphatic analog to the 2-thiophenemethyl derivative listed in Table I.

A heterocyclic system was also synthesized at this time, which involved substitution on both the nitrogen atoms of the amidinium moiety. When ethylenediamine was used as the amine in the synthetic sequence, chloroacetonitrile was converted to the imidazolinium thiolsulfates, I, in good yield.



I

All of the α-amidinium thiolsulfates described herein are currently being evaluated for radiation protection (5).

EXPERIMENTAL (6)

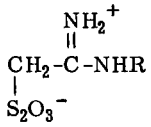
Starting Materials.

The following amines were purchased: γ-(methylmercapto)propylamine (Eastman Organic Chemicals); 2-, 3- and 4-pyridylmethylamine, β-(2-pyridyl)ethylamine, (6-methyl-2-pyridyl)methylamine, 2-furylmethylamine, tryptamine hydrochloride, homotryptamine hydrochloride and tryamine hydrochloride (Aldrich Chemical Company); 2-thienylmethylamine (Beacon Division, Lehn and Fink Products Corp., Cambridge, Massachusetts).

β-(2-Thienyl)ethylamine was prepared according to the procedure reported in the literature. Reaction of 2-thiophenecarboxaldehyde with nitromethane in the presence of sodium hydroxide furnished ω-nitro-2-vinylthiophene (7) which on reduction with lithium aluminum hydride furnished the desired amine (8). In a similar fashion, β-(2-furyl)ethylamine was prepared from freshly distilled furfural (9).

Amine hydrochlorides were prepared by either passing hydrogen chloride through an ethereal solution of the amine or by adding an ether solution saturated with hydrogen chloride to an ethanolic solution of the amine until no further precipitate was formed. Diamines were handled preferably as the dihydrochlorides. The melting point of all amine hydrochlorides checked with those in the literature.

TABLE I

α-Amidinium Thiosulfates, 

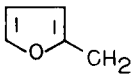
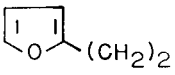
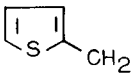
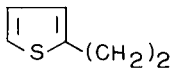
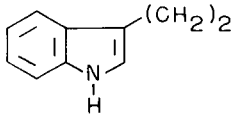
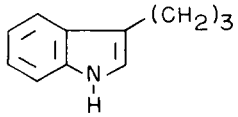
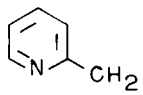
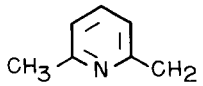
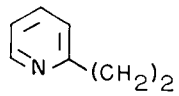
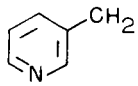
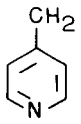
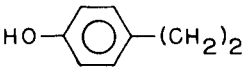
R	Yield, %	M.P., °C, dec.	Formula		Analysis C,%	H,%	N,%	S,%
	67	166-167	$C_7H_{10}N_2O_4S_2$	Calcd.	33.58	4.02	11.19	25.61
				Found	33.67	4.11	10.98	25.45
	44	123.5-124.5	$C_8H_{12}N_2O_4S_2$	Calcd.	36.35	4.57	10.59	24.25
				Found	36.40	4.41	10.45	23.50
	60	163-165	$C_7H_{10}N_2O_3S_3$	Calcd.	31.56	3.78	10.51	36.11
				Found	31.64	3.89	10.79	35.94
	41	114-115	$C_8H_{12}N_2O_3S_3$	Calcd.	34.26	4.31	9.99	34.30
				Found	33.86	4.35	9.79	33.65
	41	90-92	$C_{12}H_{15}N_3O_3S_2$	Calcd.	45.98	4.82	13.40	20.46
				Found	46.27	4.99	13.52	20.74
	71	159-160	$C_{13}H_{17}N_3O_3S_2$	Calcd.	47.68	5.23	12.83	19.58
				Found	48.02	5.61	12.65	19.61
	32	131-132	$C_8H_{11}N_3O_3S_2$	Calcd.	36.76	4.28	16.08	24.53
				Found	36.52	4.50	15.93	24.43
	10	91.5-93	$C_9H_{13}N_3O_3S_2$	Calcd.	39.26	4.75	15.26	23.28
				Found	39.29	4.79	14.81	22.89
	32	165-166	$C_9H_{13}N_3O_3S_2$	Calcd.	39.26	4.75	15.26	23.28
				Found	39.03	4.57	15.00	23.27
	37	186-189	$C_8H_{11}N_3O_3S_2$	Calcd.	36.76	4.28	16.08	24.53
				Found	36.97	4.26	15.80	23.72

TABLE I (Continued)

R	Yield, %	M.P., °C, dec.	Formula		Analysis C,%	H,%	N,%	S,%
	51	165-167	$C_8H_{11}N_3O_3S_2$	Calcd.	36.76	4.28	16.08	24.53
				Found	36.80	4.38	16.22	24.70
$(CH_2)_3SCH_3$	48	118-119	$C_6H_{14}N_2O_3S_3$	Calcd.	27.89	5.46	10.84	37.22
				Found	28.11	5.38	10.62	37.00
	71	170-171	$C_{10}H_{14}N_2O_4S_2$	Calcd.	41.36	4.85	9.99	22.08
				Found	41.36	5.15	9.79	21.73

α-Chloroacetamidinium Chlorides.

Whenever possible, the crystalline salts were isolated and were purified prior to the next step. Several examples are described to illustrate the procedure.

N-[β-(2-Thienyl)ethyl]chloroacetamidinium Chloride.

To a methanolic solution of sodium methoxide [from sodium (0.057 g., 0.0025 g. atom) in 20 ml.] was added chloroacetonitrile (1.88 g., 0.025 mole) and the mixture stirred for 1 hour at 25°. The progress of the reaction was followed by its n.m.r. spectrum, changes being monitored on internal TMS and the solvent, methanol (δCH_3 = 3.2). The CH_2 signal from $ClCH_2CN$ at 4.46 δ decreased to give rise to two new singlets from $ClCH_2C(=NH)OCH_3$, at 4.13 (CH_2) and 3.76 (CH_3) δ. After the addition of β-(2-thienyl)ethylammonium chloride, the signals due to the methyl imidate commenced to diminish and a new singlet appeared at 4.41 δ ($ClCH_2$) of the chloroacetamidinium chloride. The other protons of this salt were observed as two sets of multiplets, one between 3.0 and 3.6 δ (CH_2's) and the other between 6.8 and 7.3 δ (arene protons). After 2 hours at 25°, the reaction seemed complete and the mixture was worked up. The solution was filtered and solvents were removed *in vacuo*, at 25°. The remaining orange syrup was triturated with ether to give the salt (4.6 g., 77%) which was recrystallized from ethanol-ether, m.p. 149-150°.

Anal. Calcd. for $C_8H_{12}Cl_2N_2S$: C, 40.17; H, 5.05; N, 11.71. Found: C, 39.98; H, 5.09; N, 11.62.

N-(2-Pyridylmethyl)chloroacetamidinium Chloride Hydrochloride.

Chloroacetonitrile (5.66 g., 0.075 mole) was added to sodium methoxide (from 0.172 g. sodium, 0.0075 g. atom) in methanol (45 ml.) and the mixture stirred at 25° for 1 hour. 2-Pyridylmethylamine hydrochloride (9.75 g., 0.075 mole) was added and stirring continued for 2 hours at 25°. Salt was filtered off and the filtrate saturated with hydrogen chloride gas. Solvents were removed *in vacuo* (< 45°) and the solid (11.3 g., 56%) was crystallized from ethanol-ether, m.p. 240-245°. Its n.m.r. spectrum (deuterium oxide) showed singlets at 4.80 ($ClCH_2$) and 5.31 (NCH_2) and multiplet between 8.0 and 9.1 δ (arene). This assignment is based on the fact that in concentrated sulfuric acid (from internal TPS) the signal at 4.96 δ appeared as a doublet due to coupling with the NH proton (J = 6 c.p.s.) and the other singlet at 4.48 δ had then to be due to the $ClCH_2$ protons.

Anal. Calcd. for $C_8H_{12}Cl_3N_3$: C, 37.44; H, 4.71; N, 16.37. Found: C, 37.92; H, 4.89; N, 16.82.

Three other *N*-[ω-(2-pyridyl)alkyl]chloroacetamidinium chloride hydrochlorides were prepared in this manner and obtained in a crystalline form. Starting from (6-methyl-2-pyridyl)methylamine hydrochloride, the corresponding *N*-[(6-methyl-2-pyridyl)methyl]chloroacetamidinium chloride hydrochloride was obtained in 74% yield, m.p. 245-247°.

Anal. Calcd. for $C_9H_{14}Cl_3N_3$: C, 39.94; H, 5.21; N, 15.52. Found: C, 39.98; H, 5.33; N, 15.28.

Similarly, *N*-[β-(2-pyridyl)ethyl]chloroacetamidinium chloride hydrochloride was prepared from β-(2-pyridyl)ethylamine dihydrochloride in 54% yield, m.p. 195-196°.

Anal. Calcd. for $C_9H_{14}Cl_3N_3$: C, 39.94; H, 5.21; N, 15.52. Found: C, 40.37; H, 5.20; N, 15.17.

2-(Chloromethyl)imidazolinium Chloride.

To a stirred methanolic solution of sodium methoxide (0.172 g. sodium in 45 ml.) containing chloroacetonitrile (5.66 g.) was added, after 1 hour, ethylenediamine dihydrochloride (9.97 g., 0.075 mole) and the mixture stirred 3 hours longer at 25°. Salt was filtered off and the filtrate evaporated *in vacuo* to furnish the product (8 g., 68%), which crystallized from ethanol-ether, m.p. 193-196°, lit. m.p. 202-204° (15). Its n.m.r. spectrum (deuterium oxide) showed two singlets in the ratio of 1:2 at 4.58 ($ClCH_2$) and 4.03 δ (NCH_2).

Anal. Calcd. for $C_4H_8Cl_2N_2$: C, 30.98; H, 5.20; N, 17.42. Found: C, 30.92; H, 5.32; N, 17.65.

α-Amidinium Thiolsulfates.
General Method.

A solution of the α-chloroacetamidinium chloride (either crystalline or as a syrup) from 0.05 mole of amine hydrochloride was dissolved in water (10 to 100 ml.) and added to a solution of sodium thiosulfate pentahydrate (0.05 mole) in about 40 ml. water. The mixture was heated with intermittent swirling at 70°-100° for 1 hour. If the reactants were initially sparingly soluble in water, the reaction was carried out in hot 50% aqueous ethanol.

The work-up of this reaction mixture was varied somewhat. In those cases where two phases were obtained, the best method consisted of decanting the hot solution at the end of the reaction from any residual gum and chilling this aqueous supernatant liquid. Usually, the product crystallized out and was recrystallized best from either water, aqueous alcohol or ethanol alone. However, the dark gum may have trapped a considerable quantity of the α-amidinium thiolsulfate. The residue was extracted several times with hot water or hot aqueous ethanol to insure that all of the product was isolated.

When the hot reaction mixture was in the form of a solution, it was chilled in an ice-water bath and the product so obtained was then recrystallized from an appropriate solvent.

When the α-amidinium thiolsulfate was quite soluble in water, the reaction mixture was concentrated, *in vacuo*, prior to isolation. When the salt still refused to crystallize, all solvents were removed and the product separated from the residual inorganic salts by recrystallization from an appropriate solvent. The structure of each salt was then checked by its n.m.r. spectrum in trifluoroacetic acid.

The specific examples described below illustrate these general procedures. The other examples followed these procedures and are listed in Table I, along with their physical constants. The yields are based in each instance on starting chloroacetonitrile.

S-[*N*-β-(2-thienylethyl)carboxamidinomethane]thiolsulfuric Acid.

To a solution of *N*-[β-(2-thienyl)ethyl]chloroacetamidinium chloride (4.0 g., 0.0165 mole) in water (15 ml.) was added an aqueous solution of sodium thiosulfate pentahydrate (4.12 g., 0.0165 mole in 20 ml.). The mixture was heated at 85° for 1 hour with intermittent swirling. After allowing the mixture to settle for a few minutes, the aqueous phase was decanted from a dark residue and cooled to give the product (2.6 g.). Its n.m.r. spectrum (trifluoroacetic acid) showed two multiplets at 3.30 ($C-CH_2-C$) and 3.78 (NCH_2), a singlet at 4.25 (SCH_2) and a multiplet between 6.8 and 7.4 (arene) and a broad signal between 7.6 and 8.4 δ (NH). For further data see Table I.

S-[*N*-(6-Methyl-2-picolyl)carboxamidinomethane]thiolsulfuric Acid.

To a solution of the α-chloroacetamidinium chloride hydrochloride (10.0 g., 0.039 mole) obtained starting from 2-aminomethyl-6-picoline dihydrochloride in water (10 ml.) was added an aqueous solution of sodium thiosulfate pentahydrate (9.7 g., 0.039 mole in 15 ml.). The mixture was heated on a steam bath for 1 hour and was concentrated *in vacuo* to give an orange-colored syrup. The syrup was dissolved in the minimum of hot water and on standing bright yellow crystals were deposited (see Table I). Its n.m.r. spectrum (trifluoroacetic acid) showed singlets at 2.95 (CH_3) and 4.53 (SCH_2), a doublet at 5.40 (NCH_2, J = 6 c.p.s.) and a complex multiplet between 7.8 and 9.0 δ (arene and NH). Addition of deuterium oxide removed the CH_2-NH coupling and the doublet at 5.40 δ collapsed to a singlet.

S-[*N*-(γ-3-Indolylpropyl)carboxamidinomethane]thiolsulfuric Acid.

The gummy chloroacetamidinium chloride prepared from chloroacetonitrile and homotryptamine hydrochloride (12.57 g., 0.06 mole) was dissolved in water (10 ml.) and added to a solution of sodium thiosulfate pentahydrate (14.8 g., 0.06 mole) in water (40 ml.) and ethanol (50 ml.). After 0.75 hour at 80°, the mixture was concentrated *in vacuo* at 35° until the product just started to crystallize out. At this point, the mixture was chilled and the product isolated and purified (see Table I).

S-(2-Imidazolinemethyl)thiolsulfuric Acid (V).

To a solution of (2-chloromethyl)imidazolinium chloride (15.5 g., 0.1 mole) in water (25 ml.) was added an aqueous solution of sodium thiosulfate pentahydrate (24.8 g., 0.1 mole in 35 ml.). After 1 hour at 100°, the solution was concentrated *in vacuo* at 50° to about two-thirds of its volume, cooled and the crystals (16.5 g., 84%) filtered off. The salt was crystallized from aqueous ethanol, m.p. 160-160.5°. Its n.m.r. spectrum (trifluoroacetic acid) showed two singlets (ratio 2:1) at 4.18 (NCH_2) and 4.32 δ (SCH_2) and a broad signal at 8.3 δ (NH).

Anal. Calcd. for $C_4H_8N_2O_3S_2$: C, 24.48; H, 4.11; N, 14.28; S, 32.68. Found: C, 24.74; H, 4.10; N, 14.16; S, 32.40.

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(1) This project was sponsored by the U. S. Army Medical Research and Development Command, (Research Contract DA-49-193-MD-2047) whose generous support is gratefully acknowledged.

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(5) The biological tests are being conducted under the auspices of Drs. D. P. Jacobus and T. R. Sweeney, Walter Reed Army Institute of Research.

(6) All melting and boiling points are uncorrected. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Illinois. Nitrogen analyses were obtained in this Department by Mr. Leo Horner using a Coleman Nitrogen Analyzer, Model D-29. Infrared spectra were recorded on a Perkin-Elmer Model 337 spectrophotometer. The proton magnetic resonance spectra were obtained by means of the Varian A-60 spectrometer. Signals are recorded in p.p.m. (δ) downfield from tetramethylsilane (TMS) in organic solvents or sodium 3-(trimethylsilyl-1-propanesulfonate) (TPS) in deuterium oxide as internal standards. Assignments of protons are based upon integral information from the n.m.r. spectrum.

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