ω-(2-Mercaptoethylamino)-1-alkanesulfonic Acid Inner Salts and Related Compounds as Potential Antiradiation Agents[1]

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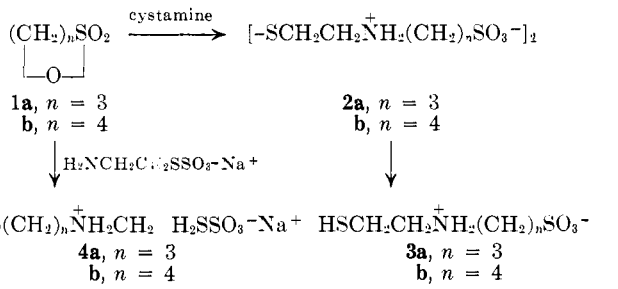
Reactions of sultones **1a** and **1b** with cystamine, sodium S-2-aminoethylthiosulfate, and thiones, such as thiosemicarbazide and pyridine-2(1H)-thione, provided a number of sulfonic acid inner salt derivatives for testing as antiradiation agents. Catalytic hydrogenolysis of the cystamine derivatives afforded ω-(2-mercaptoethylamino)-1-alkanesulfonic acids **3a** and **3b**. Sulfoalkylation products of 2-thiazolidinethione were observed to be particularly labile toward hydrolysis giving S-2-aminoethyl S'-ω-sulfoalkyl dithiocarbonates **9a** and **9b**. 3,3'-[Dithiobis(ethylenimino)]bis(1-propanesulfonic acid) (**2a**) and the derived thiol **3a** showed good radioprotective activity in contrast to the inactivity of the butane derivatives **2b** and **3b**, the Bunte salts **4a** and **4b**, and the dithiocarbonates **9a** and **9b**. None of the isothiuronium-type sulfonates showed significant activity with the possible exception of 4-(acetimidoylthio)-1-butanesulfonic acid.

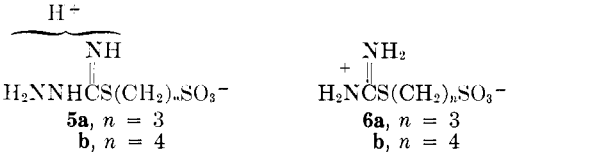
In the search for antiradiation agents 2-alkylaminoethanethiols functionally substituted on the alkyl group constitute a potentially fertile area of investigation in view of the reported[2] radioprotective properties of N-(2-mercaptoethyl)glycine. One such type is the taurine-related ω-(2-mercaptoethylamino)-1-alkanesulfonic acids, an approach to the synthesis of which was suggested by the previously described[3] facile ring openings of 4-hydroxy-1-butanesulfonic acid sultone (**1b**) with primary and secondary amines. Respective reactions of 3-hydroxy-1-propanesulfonic acid sultone (**1a**) and **1b** with 2,2'-dithiobisethylamine (cystamine) provided 3,3'-[dithiobis(ethylenimino)]bis(1-propanesulfonic acid) and 4,4'-[dithiobis(ethylenimino)]bis-(1-butanesulfonic acid) as the inner salts **2a** and **2b**[4] (see Scheme I). Conversion of these disulfides to the desired 3-(2-mercaptoethylamino)-1-propanesulfonic acid (**3a**) and 4-(2-mercaptoethylamino)-1-butanesulfonic acid (**3b**) was accomplished by hydrogenolysis in aqueous solution over 30% palladium-on-charcoal catalyst at 3.5 kg/cm². Optimal iodometric assays of **3a** and **3b** were obtained when the catalyst weight was 50% of that of the starting disulfide; hydrogenolysis was incomplete when the weight of catalyst was 30% and negligible (with **2b**, and presumably would be with **2a**) when it was only 10 and 20%.[5] Reactions of **1a** and **1b** with sodium S-2-aminoethylthiosulfate in methanol afforded sodium S-2-(3-sulfopropylamino)-ethylthiosulfate (**4a**) and sodium S-2-(4-sulfobutylamino)ethylthiosulfate (**4b**), respectively, in low yields.

Sultone ring openings with thiourea, thiosemicarbazide, thioacetamide, and a number of heterocyclic thiones provided isothiuronium sulfonates and related inner salts for evaluation as sulfonic acid analogs of radioprotective isothiuronium salts.[6] Several examples of this type of reaction have been reported, such as, reactions of thiourea with **1a** and **1b**,[7a] of substituted thioureas and related heterocyclic compounds with **1a**,[7a] and of thio-

Scheme I



acetamide with **1a**.[7b] The products of sulfoalkylations of thiosemicarbazide are designated as ω-sulfoalkyl thiocarbazinidates (**5a** and **5b**) rather than 1-substituted thiosemicarbazides because of the close similarity of their infrared spectra with those of the ω-(amidinothio)-alkanesulfonic acids **6a** and **6b**; all show strong C=N stretching bands near 1660 cm⁻¹, but only **5a** and **5b** show sharp NH stretching bands near 3330 cm⁻¹.



Sulfoalkylations of heterocyclic thiones that were slow or incomplete in refluxing ethanol, particularly with the less reactive **1b**, were effected in refluxing 1-propanol, which probably could have been used advantageously in all reactions of this type. The products, as inner salts, crystallized from the reaction mixtures, often in pure form. The reaction of 2-thiazolidinethione (**7**) with **1a** in ethanol was no exception and gave the expected 3-(2-thiazolin-2-ylthio)-1-propanesulfonic acid (**8a**), but the reaction of **7** with **1b** in ethanol gave a low yield of a water-recrystallized product, whose elemental analyses indicated partial hydration but whose infrared absorption in the carbonyl region was more consistent with a dithiocarbonate[8] than with hydrated4 -(2-thiazolin-2-ylthio)-1-butanesulfonic acid (**8b**). Repetition of the reaction

(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2028.

(2) (a) E. Felder, F. Bonati, and S. Bianchi, *Experientia*, **15**, 32 (1959); (b) E. Felder and S. Bianchi, German Patent 1,062,705 (1959).

(3) B. Helferich and V. Böllert, *Ann. Chem.*, **647**, 37 (1961).

(4) The products derived from sultones in this work are named as sulfonic acids but are actually inner sulfonate salts.

(5) *Cf.* the hydrogenolysis of 3,3-dithiobispropionamidine dihydrochloride [T. P. Johnston and A. Gallagher, *J. Org. Chem.*, **28**, 1436 (1963)].

(6) J. F. Thomson, "Radiation Protection in Mammals," Reinhold Publishing Corp., New York, N. Y., 1962, pp 69–77.

(7) (a) Böhme Fettchemie G.m.b.H., British Patent 775,026 (1957); *Chem. Abstr.*, **51**, 16534 (1957); (b) W. Ried and E. Schmidt, *Ann. Chem.*, **676**, 114 (1964).

(8) (a) R. J. Gaul and W. J. Fremuth, *J. Org. Chem.*, **25**, 869 (1960); (b) T. P. Johnston and A. Gallagher, *ibid.*, **29**, 2442 (1964).

TABLE I
SULFOALKYLATIONS OF HETEROCYCLIC THIONES

Reactants			Reaction solvent (ml)	Reaction time, hr	Yield, %	Mp,[a] °C	Product data							
								Calcd, %				Found, %		
Thione	Sultone	mmoles					Formula	C	H	N	S	C	H	N	S
2-Imidazolidinethione	1a	9.8	Ethanol (10)	18	87	247–248[b]	$C_6H_{12}N_2O_3S_2$	32.12	5.39	12.49	28.59	32.24	5.23	12.50	28.6
2-Imidazolidinethione	1b	39.1	Ethanol (50)	64	89	232–234	$C_7H_{14}N_2O_3S_2$	35.28	5.92	11.75	26.91	35.36	5.73	11.76	26.4
2-Thiazolidinethione	1a	25.2	Ethanol (25)	1.5	70	223–225	$C_6H_{11}NO_3S_3$	29.82	4.59	5.80	39.86	29.52	4.88	5.53	40.02
2(1H)-Pyridinethione	1a	18.0	Ethanol (50)	2	80	227–229	$C_8H_{11}NO_3S_2$	41.19	4.75	6.00	27.49	41.20	4.82	5.84	27.6
2(1H)-Pyridinethione	1b	30.0	Ethanol (15)	3	60	195–197	$C_9H_{13}NO_3S_2$	43.71	5.30	5.66	25.93	43.92	5.36	5.57	26.1
2-Benzimidazolidinethione	1a	26.6	Ethanol (30)	1	85	270–290 dec	$C_9H_{12}N_2O_3S_2$	44.10	4.44	10.28	23.52	43.86	4.52	10.06	23.0
2-Benzimidazolidinethione	1b	26.6	Ethanol (50)	2	33	290–300 dec	$C_{10}H_{14}N_2O_3S_2$	46.13	4.93	9.78	22.39	46.05	5.00	9.59	21.8
2-Benzothiazolidinethione	1a	29.9	Ethanol (40)	19	47[c]	190–193 dec	$C_9H_9NO_3S_3$	41.50	3.83	4.84	33.24	41.33	3.80	4.68	33.1
2-Benzothiazolidinethione	1b	29.9	1-Propanol (50)	17	25[c]	*d*	$C_{10}H_{11}NO_3S_3$	43.54	4.62		31.70	43.22	4.68		31.2
Purine-8(1H)-thione	1a	29.6	1-Propanol (50)	1.0	23[c]	~290 dec	$C_8H_8N_4O_3S_2$	35.03	3.68	20.43	23.38	34.74	3.26	19.93	23.35
Purine-8(1H)-thione	1b	29.0	1-Propanol (50)	18	26[f]	279–281 dec	$C_9H_{10}N_4O_3S_2$	37.48	4.20	19.43	22.24	37.08	4.57	18.92	22.48

[a] Determined in a Mel-Temp apparatus. [b] Lit.[7a] mp 245°. [c] Recrystallized from water. [d] Indefinite. [e] Recrystallized from water–ethanol. [f] Recrystallized from water–acetone (5:450 ml).

of **7** with **1b** in 1-propanol gave a product that was indicated by its infrared spectrum to be hydrolyzed for the most part and whose recrystallization from water afforded analytically pure S-2-aminoethyl S′-4-sulfobutyl dithiocarbonate (**9b**). Vapor phase chromatography showed that the water content of the 1-propanol used was sufficient to meet the requirements for hydrolysis of **8b** to **9b** and that the product of hydrolysis contained no water of crystallization, but the reaction of **7** with **1a** in the same solvent gave a product spectrally identical with **8a** obtained in ethanol (see Scheme II). Recrystallization of **8a** from water

SCHEME II

7

8a, $n = 3$
b, $n = 4$

9a, $n = 3$
b, $n = 4$

after a short reflux period produced S-2-aminoethyl S′-3-sulfopropyl dithiocarbonate (**9a**). Previous preparations of analogous dithiocarbonates have involved hydrolysis of appropriately substituted 2-thiazolines in hydrochloric acid or hydrobromic acid.[8b,9]

Comparisons of the ultraviolet spectra of the sulfoalkylation products of pyridine-2(1H)-thione [*i.e.*, 3-(2-pyridylthio)-1-propanesulfonic acid (**10a**) and 4-(2-pyridylthio)-1-butanesulfonic acid (**10b**)] with those reported for 2-(alkylthio)pyridines and 1-alkyl-2-(1H)-pyridinethiones[10] provide evidence for the assigned structures. The structures of 3-(purin-8-ylthio)-1-propanesulfonic acid (**11a**) and 4-(purin-8-ylthio)-1-butanesulfonic acid (**11b**) were similarly supported by ultraviolet spectral comparisons with 8-(methylthio)-purine.[11] Although structure proof of the other heterocyclic compounds prepared (see Table I) has not been pursued, the assigned structures are more adaptable to stable inner salt formation than the corresponding N-substituted isomers.

10a, $n = 3$
b, $n = 4$

11a, $n = 3$
b, $n = 4$

The compounds described above were tested at the Walter Reed Army Institute of Research, Washington, D. C., for radioprotective activity in mice exposed to lethal radiation; the tests were performed as previously described,[12] and some of the results obtained are sum-

(9) J. C. Crawhall and D. F. Elliott, *J. Chem. Soc.*, 3094 (1952).
(10) V. A. Koroblov, A. V. Voroqueva, and L. K. Fel'dman, *J. Gen. Chem. USSR*, **31**, 2922 (1961).
(11) D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 682 (1957).
(12) L. Field, A. Ferretti, R. Crenshaw, and T. Owen, *J. Med. Chem.*, **7**, 39 (1964).

Table II

Radioprotective Activities of ω-(2-Mercaptoethylamino)-1-alkanesulfonic Acid Inner Salts and Related Compounds

Compd	Drug dose, mg/kg	Vehicle of administration	% survival
2a	2500	Water	87
	2000		93
	1000		54
	500		0
2b	1000	Saline	0
	500		0
3a	4000	Water	0
	2000		60
	1500	Water	53
	750		7
3b	1000	Water	0
	500		0
4a	600	Water	0
	300		0
4b	1000	Water	0
	500		0
9a	600	MC/Tw[a]	0
	300		0
9b	250	MC/Tw	0
	125		0

[a] Compound suspended in saline solution containing 0.2% methylcellulose and 0.4% Tween 80.

marized in Table II.[13] The disparity of activity noted in comparable pairs of disulfides and thiols (**2a** *vs.* **2b** and **3a** *vs.* **3b**) may be due simply to toxicity factors; the butane derivatives were toxic at the high optimal dose levels of the propane derivatives. The disulfide **2a** appears to be more active than the corresponding thiol **3a**, but neither the Bunte salt **4a** nor the dithiocarbonate **8a**, was active. No significant activity has as yet been observed among the isothiuronium sulfonates and related heterocyclic sulfonates with the possible exception of 4-(acetimidoylthio)-1-butanesulfonic acid (33% survival at 900 mg/kg).

Experimental Section[14]

3,3'-[Dithiobis(ethylenimino)]bis(1-propanesulfonic acid) (2a).—The sultone **1a**[15] (16.6 g, 0.136 mole) and 2,2'-dithiobisethylamine[16] (9.3 g, 0.061 mole) were mixed in a small volume of ethanol. A vigorous reaction ensued, which was quickly moderated by the addition of more solvent (300 ml). The mixture was heated under reflux for 1 hr and cooled. The supernant liquid was decanted from the brown gum, which was extracted with methanol (250 ml) under reflux for 2 hr with intermittent trituration. The residual white solid was washed with methanol and ether and dried *in vacuo* over P_2O_5; yield 13.2 g (54%); σ^{KBr} (SO_3^-) in cm^{-1}: 1220–1150 (s, broad), 1040 (s).

Anal. Calcd for $C_{10}H_{24}N_2O_6S_4$: C, 30.28; H, 6.10; S, 32.35. Found: C, 30.08; H, 6.16; S, 32.0.

4,4'-[Dithiobis(ethylenimino)]bis(1-butanesulfonic acid) (2b).—A solution of 2,2'-dithiobisethylamine[16] (3.60 g, 23.6 mmoles) and **2b**[17] (6.60 g, 48.5 mmoles) in ethanol (120 ml) was heated under reflux for 1 hr and allowed to cool. The precipitate was washed with methanol and ether and dried *in vacuo* over P_2O_5 at 80°; yield of white solid, 2.94 g (29%); σ^{KBr} (SO_3^-) in cm^{-1}: 1190–1170 (s, broad), 1040 (s).

Anal. Calcd for $C_{12}H_{28}N_2O_6S_4$: C, 33.94; H, 6.65. Found: C, 33.66; H, 6.73.

Recrystallization of a similarly prepared product from water–ethanol gave a fractional hydrate that was dried as described above for 3 hr.

Anal. Calcd for $C_{12}H_{28}N_2O_6S_4 \cdot 0.25H_2O$: C, 33.58; H, 6.69; S, 29.88; H_2O, 1.05. Found: C, 33.39; H, 6.47; S, 29.9; H_2O, 0.93 (Karl Fischer method).

3-(2-Mercaptoethylamino)-1-propanesulfonic Acid (3a).—A solution of **2a** (15.0 g, 37.8 mmoles) in water (250 ml) was shaken in a Parr hydrogenator with 30% Pd-C (7.5 g) and hydrogen at 3.5 kg/cm^2. When the hydrogen uptake ceased (4–5 hr), the catalyst was removed by filtration under nitrogen and the filtrate was evaporated to dryness under reduced pressure leaving an oil. Several successive *in vacuo* evaporations after additions of methanol left a tan solid, which was triturated twice in methanol and dried *in vacuo* over P_2O_5; yield 10.3 g (69%); infrared absorption quite similar to that of **2a.**

Anal. Calcd for $C_5H_{13}NO_3S_2$: C, 30.13; H, 6.67; N, 7.03; SH, 16.6. Found: C, 29.69; H, 6.44; N, 6.74; SH, 16.1.

4-(2-Mercaptoethylamino)-1-butanesulfonic Acid (3b).—A solution of **2b** (4.84 g, 11.4 mmoles) in water (75 ml) was shaken in a Parr hydrogenator with 30% Pd-C (2.42 g) and hydrogen at 3.5 kg/cm^2. When the hydrogen uptake ceased (4–5 hr), the catalyst was removed by filtration through Celite and under nitrogen, and the filtrate was evaporated to dryness under reduced pressure (oil pump). The residue, a mixture of yellow crystals and an oil, was extracted with boiling methanol (300 ml). The methanol-insoluble solid was dissolved in water (50 ml), and the solution after treatment with Norit was evaporated to dryness under reduced pressure. The yellow-orange residue was triturated in methanol leaving **3b** as a yellow solid, which melted at 210–212° dec after being dried *in vacuo* over P_2O_5; yield, 1.66 g (34%); infrared absorption quite similar to that of **2b.**

Anal. Calcd for $C_6H_{15}NO_3S_2$: C, 33.78; H, 7.09; S, 30.06; SH, 15.5. Found: C, 33.87; H, 7.43; S, 29.8; SH, 14.9.

Sodium S-2-(3-Sulfopropylamino)ethylthiosulfate (4a) Hemihydrate.—S-2-Aminoethylthiosulfuric acid[18] (10.0 g, 63.6 mmoles) was added in portions to a solution of sodium methoxide [from sodium (1.46 g, 63.6 mg-atoms)] in dry methanol (200 ml); the mixture was stirred until solution was complete. Sultone **1a**[15] (8.65 g, 63.6 mmoles) was added, and the resulting solution was refluxed for 1.5 hr and allowed to stand overnight. Crude **4a** (11.0 g) was obtained in 4 crops with precipitation being aided by subsequent additions of ethanol. Recrystallization from methanol–ethanol gave 6.2 g (32%) of **4a** as a hemihydrate in 2 crops; σ^{KBr} (SO_3^-) in cm^{-1}: 1200 (s, broad), 1025 and 1045 (m–s, doublet), 635 (m–s).

Anal. Calcd for $C_5H_{12}NNaO_6S_3 \cdot 0.5H_2O$: C, 19.35; H, 4.22; N, 4.51; S, 30.99. Found: C, 19.66; H, 4.32; N, 4.13; S, 30.67.

Sodium S-2-(4-sulfobutylamino)ethylthiosulfate (4b) was prepared from equimolar amounts (63.6 mmoles) of S-2-aminoethylthiosulfuric acid[18] and **1b**[17] by a procedure similar to that used for **4a**; a longer reflux period might have improved the yield. The reaction solution was concentrated *in vacuo* to half-volume. The addition of ethanol (300 ml) caused the precipitation of **4b** as a white solid, which was washed with ethanol and dried *in vacuo* over P_2O_5; yield, 1.98 g (10%); σ^{KBr} (SO_3^-) in cm^{-1}: 1190 (s, broad), 1025 (m–s), 630 (m–s).

Anal. Calcd for $C_6H_{14}NNaO_6S_3$: C, 22.84; H, 4.47; S, 30.52. Found: C, 22.62; H, 4.66; S, 30.5.

3-Sulfopropyl Thiocarbazimidate (5a).—A solution of thiosemicarbazide (8.32 g, 91.3 mmoles) and **1a**[15] (11.2 g, 91.3 mmoles) in ethanol (100 ml) was refluxed overnight. The white, crystalline **5a** was collected, washed with ethanol, triturated in boiling methanol, and dried *in vacuo* over P_2O_5; yield, 13.7 g (70%); mp 223–224° dec; σ^{KBr} in cm^{-1}: 3325 (s, NH), 1665 (s, C=N), 1150–1200 (s, broad multiplet), and 1040 (s, SO_3^-).

Anal. Calcd for $C_4H_{11}N_3O_3S_2$: C, 22.52; H, 5.20; N, 19.70; S, 30.07. Found: C, 22.63; H, 4.95; N, 19.38; S, 29.5.

(13) The authors are indebted to Drs. D. P. Jacobus and T. R. Sweeney for the antiradiation test data.

(14) Melting points were determined ina Mel-Temp apparatus; no melting points are reported for those compounds (**2a**, **2b**, **3a**, **4a**, and **4b**) that decomposed over such an indefinite range as to be considered meaningless as a purity criterion. Infrared absorption spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 521 or 221-G spectrophotometer.

(15) Distillation Products Industries, Rochester, N. Y.

(16) T. P. Johnson and A. Gallagher, *J. Org. Chem.*, **26**, 3780 (1961); *ibid.*, **27**, 2452 (1962). Care should be taken to avoid an excess of sodium methoxide in freeing the base.

(17) W. E. Truce and F. D. Haerger, *J. Am. Chem. Soc.*, **76**, 5357 (1954).

(18) Kindly furnished by Dr. T. R. Sweeney, Walter Reed Army Institute of Research; also available commercially.[15]

4-Sulfobutyl Thiocarbazimidate (5b).—The reaction of thiosemicarbazide (2.00 g, 22.0 mmoles) with an equimolar amount of **1b**[17] in ethanol (10 ml) gave **5b** in low yield after a relatively short (not optimal) reflux period. Crude **5b** was purified by extraction into boiling methanol and reprecipitation by the addition of ether; yield, 1.01 g (20%) as white crystals; mp 174–178°; σ^{KBr} in cm^{-1}: 3340 (m–s, sharp, NH), 1670 (m, C=N), 1160–1190 (s, broad), and 1040 (s, SO_3^-).

Anal. Calcd for $C_5H_{13}N_3O_3S_2$: C, 26.42; H, 5.76; S, 28.21. Found: C, 26.77; H, 5.72; S, 27.9.

4-(Acetimidoylthio)-1-butanesulfonic Acid.—A solution of thioacetamide (19.3 g, 25.8 mmoles) and **1b** (35.1 g, 25.8 mmoles) in benzene (100 ml) was refluxed for 2 hr and then chilled. The white crystalline precipitate was collected in two crops and triturated in boiling acetone; yield 6.90 g, mp 193–195° dec. The analytical sample, mp 196–200° dec, was recrystallized from acetic acid.

Anal. Calcd for $C_6H_{13}NO_3S_2$: C, 34.11; H, 6.20; S, 30.35. Found: C, 34.17; H, 5.99; S, 30.2.

3-(2-Thiazolin-2-ylthio)-1-propanesulfonic Acid (8a) and S-2-Aminoethyl S'-3-Sulfopropyl Dithiocarbonate (9a).—A solution of 2-thiazolidinethione (**7**) (5.00 g, 41.8 mmoles) and the sultone **1a**[18] (5.13 g, 41.8 mmoles) in 1-propanol[19] (25 ml) was refluxed for 1.5 hr and allowed to cool to room temperature. The white crystals, which had begun to deposit during the reflux period, were collected, washed with 1-propanol, acetone, and ether, and dried *in vacuo* over P_2O_5; yield of **8a**, 8.70 g (80%); mp 219–221° (melting point of analytical sample obtained from reaction in ethanol, 223–225°); σ^{KBr} in cm^{-1}: 1580 (s, C=N), 1215 (s), 1150 (s), and 1015 (s, SO_3^-).

Anal. Calcd for $C_6H_{11}NO_3S_3$: C, 29.82; H, 4.59; N, 5.80; S, 39.86. Found: C, 29.52; H, 4.88; N, 5.53; S, 40.02.

A solution of the **8a** described above in hot water (100 ml) was refluxed for 15 min and allowed to cool to room temperature. The dithiocarbonate **9a** was deposited in 2 crops (5.40 g, 50%, from **7**) as white crystals: mp 266–267°; σ^{KBr} in cm^{-1}: 1650 (s, C=O), 880 (s, SCS), 1155 (s, broad), and 1035 (s), (SO_3^-).

Anal. Calcd for $C_6H_{13}NO_4S_4$: C, 27.78; H, 5.05; N, 5.40; S, 37.08. Found: C, 28.07; H, 5.15; N, 5.32; S, 37.2.

S-2-Aminoethyl S'-4-Sulfobutyl Dithiocarbonate (9b). A solution of 2-thiazolidinethione (**7**) (15.2 g, 0.128 mole) and sultone **1b**[17] (17.4 g, 0.128 mole) in 1-propanol (125 ml) was refluxed for 3 hr and then cooled. The white crystalline precipitate (11.3 g, mp 240–245° dec), washed with ethanol and acetone, was recrystallized from water. The yield of **9b** as white crystals, mp 254–255° dec, in two crops was 4.91 g (14%); σ^{KBr} in cm^{-1}: 1640 (s, C=O), 875 (s, SCS), 1155, 1175 (broad doublet), and 1040 (SO_3^-).

Anal. Calcd for $C_7H_{15}NO_4S_4$: C, 30.75; H, 5.53; N, 5.12; S, 35.19. Found: C, 30.76; H, 5.39; N, 4.99; S, 35.1.

General Procedure for the Sulfoalkylation of Heterocyclic Thiones. Individual preparations are summarized in Table I. A solution (or suspension) of equimolar amounts of thione and sultone **1a**[18] or **1b**[17] in the appropriate solvent (ethanol or preferably 1-propanol) was refluxed for the indicated period. (Sometimes the use of a slight excess of sultone made isolation of pure products easier.) The reaction mixture was allowed to cool to room temperature; the crystalline product, which usually precipitated during the reflux period, was collected, washed thoroughly with ethanol, acetone, and ether (in that order), and dried *in vacuo* over P_2O_5. In most instances the products so obtained were analytically pure; some, however, required recrystallization.

(19) Water content by vpc: 0.5%.

Anabolic Agents. A-Ring Oxygenated Androstane Derivatives

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The synthesis of several A-ring modified dihydrotestosterone derivatives is described in detail. A comparison of the androgenic and anabolic responses produced by these A-ring isomers revealed that the C-1 oxygenated derivatives were the most potent, having a favorable separation of anabolic from the less desirable androgenic activity.

In a recent publication,[1] we reported on the interesting biological properties of various A-ring conjugated enone androstane derivatives. The most potent orally active anabolic agent of this series was found to be 17β-hydroxy-17α-methyl-5α-androst-1-en-3-one. In addition, it was found that saturation of the double bond of compound Id[1] and retention of either the carbonyl or hydroxyl function produced compounds with superior anabolic properties. These observations prompted interest in making a biological comparison (Table III) of the compounds which had a carbonyl or hydroxyl group in all of the possible positions (C-1 to C-4) of the A ring. This paper will discuss the chemistry and biology of these modifications.

The facile conversion of the 1,2α-epoxy-5α-androstan-3-one series of compounds to 1α-hydroxy-5α-androst-2-ene derivatives by treatment with hydrazine hydrate[1] afforded a convenient pathway to the 1-oxygenated A-ring androstane derivatives (III and IV) (see Table I). When the 2-dehydro-1α-hydroxy compounds (I) were reduced under catalytic conditions with platinum oxide, the corresponding saturated analogs (III) were obtained. Subsequent oxidation using chromic acid in acetone[2] afforded the 1-keto-5α-androstane derivatives (IV). An alternate pathway to the ketones IV involved oxidation of I with chromic acid in acetone[2] followed by catalytic hydrogenation of II (see Scheme I).

The synthesis of 5α-androstan-1α-ol-17-one proved to be somewhat more lengthy than that of the other 1α-hydroxy derivatives (III). This method involved protecting the 17α-hydroxy group while performing the necessary chemical changes in the A ring of the androstane molecule. The 17-tetrahydropyranyl ether of 5α-androst-2-ene-1α,17β-diol[1] was acetylated to protect the 1α-hydroxyl group. Subsequent removal of the tetrahydropyranyl group afforded a good yield of 5α-androst-2-ene-1α,17β-diol 1-acetate. Finally, treatment with chromic acid in acetone,[2] followed by base hydrolysis gave the desired 1α-hydroxy-5α-androst-2-en-17-one analog (Ia).

For the synthesis of the 2-oxygenated steroids, the reductive removal of the bromine from the appropriate

(1) P. D. Klimstra and R. E. Counsell, *J. Med. Chem.*, **8**, 48 (1965).

(2) K. Bowden, C. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).