TABLE V

S-SUBSTITUTED SODIUM HYDROGEN PHOSPHOROTHIOATES AND DIHYDROGEN PHOSPHOROTHIOATES

No.	ml of H_2O/ mmole of Na_3PSO_3	Yield, %	Mp, °C dec	Formula[a]
2b	1.5	45		$C_{12}H_{14}N_2NaO_5PS \cdot 6H_2O$
2e	1.5	93	131–132	$C_{13}H_{17}N_2O_5PS \cdot H_2O$
2g	5.0	77		$C_{14}H_{18}N_2NaO_5PS \cdot 5H_2O$
2i	4.2	45	148–150	$C_{15}H_{21}N_2O_5PS$
2k	2.3	44	158–160	$C_{14}H_{19}N_2O_5PS \cdot 1.3H_2O$
2n	3.5	56	151–152	$C_{15}H_{21}N_2O_5PS$
2p	3.5	43	179–180	$C_{16}H_{23}N_2O_5PS$
16c[b]	1.0	59	184–186	$C_{16}H_{23}N_2O_5PS$
26c	2.0	55		$C_{14}H_{18}N_2NaO_5PS_2 \cdot 4.5H_2O$
26d	2.0	70		$C_{15}H_{20}N_2NaO_5PS_2 \cdot 5H_2O$
32d	2.0	65		$C_{12}H_{16}N_2NaO_6PS_2 \cdot 6.5H_2O$
32e	2.0	89	182–183	$C_{13}H_{19}N_2O_6PS_2 \cdot 0.5H_2O$

[a] *Anal.* C, H, N, P, and S for all compds except **2n**, which gave satisfactory results for C, H, N, and S. [b] The Na salt from which **16c** was prepd was pptd from the reaction soln with Me_2CO instead of EtOH.

to room temp, and DMF (one-half the vol of H_2O used) was added. Pulverized N-substd bromoalkylamine·HBr (equimolar with Na_3PSO_3) was added, and the mixt was stirred until the $AgNO_3$ test for PSO_3^{3-} was negative.[19] Except for the prepns which led to **2e**, **2k**, and **2n**, the addn of EtOH (to cause pptn of the desired Na salt) followed, although **2b**, **2g**, **26c**, **26d**, **32d**, and the Na salt from which **32e** was derived had partially sepd from the reaction mixt. The ppt was collected, washed (EtOH, then Et_2O), and suction dried. The Na salts **2b**, **2g**, **26c**, **26d**, and **32d** were dissolved in the required vol of H_2O at 25°, then repptd by addn of EtOH, collected, washed as above, and air-dried. Compds **2e**, **2k**, and **2n** crystd directly from the reaction soln safter the addn of AcOH in small excess (approx 1 ml in a 10-mmole run). The collected products were washed (cold H_2O, EtOH, then Et_2O) and dried *in vacuo* (25–30°, P_2O_5). Compds **2i**, **2p**, **16c**, and **32e** were similarly obtained after H_2O solns of the EtOH-pptd Na salts were treated with AcOH.

2-[2-(2-Phthalimidoethylamino)ethyl]-2-thiopseudourea (2c)·2HBr.—A soln of 15.0 mmoles each of **1a** (5.67 g) and thiourea (1.14 g) in EtOH was refluxed 30 min. A test portion of the soln dild with EtOAc was chilled, stirred, and scratched to give seed crystals, which, when added to the reaction soln, caused crystn of pure **2c**·2HBr, mp 220–222° dec, in 44% yield (3.02 g). *Anal.* ($C_{13}H_{16}N_4O_2S \cdot 2HBr$) C, H, N, S.

2-[3-(3-Phthalimidopropylamino)propyl]-2-thiopseudourea (21)·2HBr·H_2O.—A stirred mixt of 12.3 mmoles each of **1e** (5.00 g) and thiourea (0.940 g) in EtOH (150 ml) was refluxed until soln occurred (15 min), then distd until 100 ml had been collected. The cooled residual soln deposited cryst product, which was collected and recrystd from EtOH. The dried (*in vacuo*, 77°, P_2O_5) sample underwent a wt increase when exposed to ambient condns of the lab (~60% rel humidity) and eventually came to const wt; yield 62% (3.84 g), mp 98–100°. *Anal.* ($C_{15}H_{20}N_4O_2S \cdot 2HBr \cdot H_2O$) C, H, N, S.

2-[4-(3-Oxo-1,2-benzisothiazolin-2-yl)butylamino]ethanethiol S,S-Dioxide·HBr (33).—A mixt of **32e**·0.5H_2O (4.24 g, 10.5 mmoles) and 3 *N* HBr (50 ml) was stirred at 70° until soln occurred. The soln was allowed to cool, and **33** sepd as long colorless needles. The collected material was washed (Et_2O), air-dried, and recrystd from MeOH–Et_2O to give **33**·0.5H_2O, mp 185–187°, in 76% yield (3.04 g). *Anal.* ($C_{13}H_{18}N_2O_3S_2 \cdot HBr \cdot 0.5H_2O$) C, H, N, S, SH.

Acknowledgments.—The authors are indebted to Dr. D. P. Jacobus and Dr. T. R. Sweeney for antiradiation data and to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for spectral data.

(19) S. Åkerfeldt, *Acta Chem. Scand.*, **16**, 1897 (1962).

Terminal Dicarboximido Analogs of *S*-2-(ω-Aminoalkylamino)ethyl Dihydrogen Phosphorothioates and Related Compounds as Potential Antiradiation Agents. 2. Succinimides, Glutarimides, and *cis*-1,2-Cyclohexanedicarboximides[1]

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Terminal dicarboximido analogs of highly radioprotective *S*-2-(ω-aminoalkylamino)ethyl dihydrogen phosphorothioates and related compounds were prepared from succinimide, glutarimide, glutethimide, and *cis*-1,2-cyclohexanedicarboximide *via* 3-substituted 2-oxazolidinones. A novel method was developed for the preparation of thiosulfates in this series in which N-substituted 2-bromoethylamine intermediates were treated with MgS_2O_3 in MeOH, but such treatment of *N*-[2-(2-bromoethylamino)ethyl]glutarimide·HBr (**5a**) resulted in an unexpected condensation and formation of the bicyclic betaine thiosulfate **7**. A departure from the general reaction scheme was also encountered in the formation of *N,N'*-(iminodiethylene)bis(*cis*-1,3-cyclohexanedicarboximide) (**14**) in the preparation of 3-[2-(*cis*-1,2-cyclohexanedicarboximido)ethyl]-2-oxazolidinone (**11a**). Of the series of thiosulfates and phosphorothioates prepared, only *S*-2-(2-succinimidoethylamino)ethyl sodium hydrogen phosphorothioate (**3b**) trihydrate and *S*-2-[2-(*cis*-1,2-cyclohexanedicarboximido)ethylamino]ethyl sodium hydrogen phosphorothioate (**13b**) tetrahydrate showed good radioprotective activity in mice.

Terminal substitution by aliphatic dicarboximido groups in the synthesis of analogs of a radioprotective series of *S*-2-(ω-aminoalkylamino)ethyl dihydrogen phosphorothioates[2] was accomplished by methods based on those described in the preceding paper[3] for the introduction of phthalimido and related groups. The key reaction was again the selective HBr cleavage of 3-substituted 2-oxazolidinones.

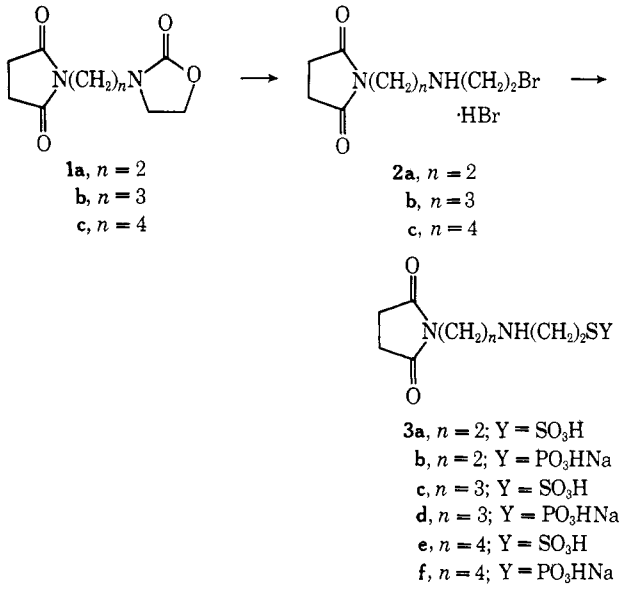
In the preparation of succinimido analogs (Scheme I), conversions of the bromides **2a,b,c** into the corresponding thiosulfates **3a,c,e** were effected in MeOH at room

(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contracts Nos. DA-49-193-MD-2028 and DADA17-69-C-9033.

(2) J. R. Piper, C. R. Stringfellow, Jr., R. D. Elliott, and T. P. Johnston, *J. Med. Chem.*, **12**, 236 (1969).

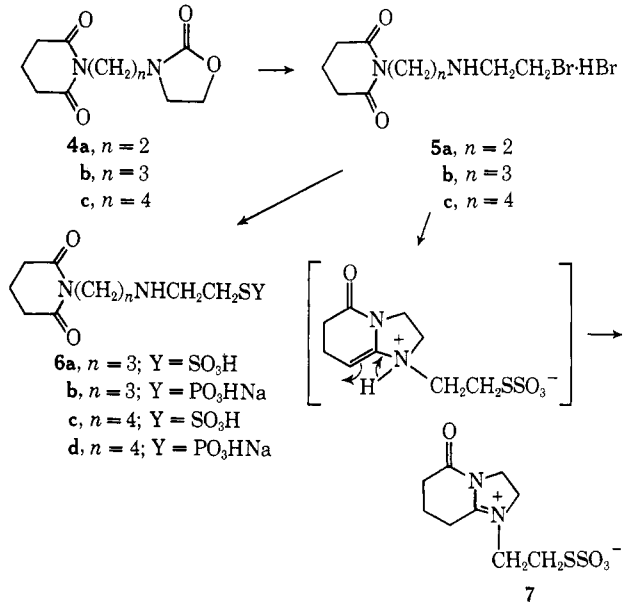
(3) J. R. Piper, C. R. Stringfellow, Jr., R. D. Elliott, and T. P. Johnston, *ibid.*, **14**, 345 (1971).

SCHEME I



temp by treatment with magnesium thiosulfate: the products crystallized directly from the reaction mixtures leaving the coproduct $MgBr_2$ in solution. This method is limited to MeOH-soluble reactants and, preferably, to MeOH-insoluble products, but promises to have considerable utility, especially in the preparation of highly water-soluble thiosulfates.

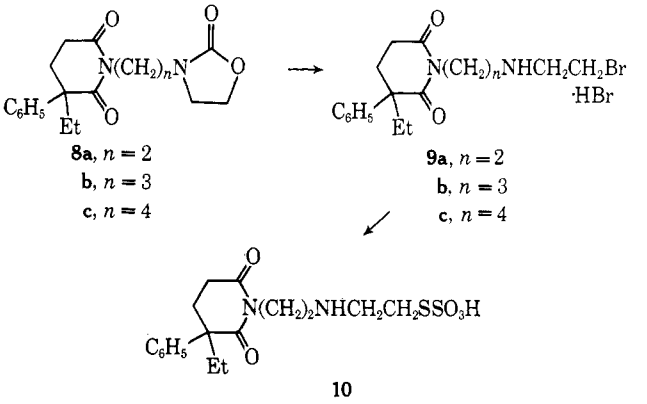
SCHEME II



Difficulties due principally to incomplete alkylation in the conversion of glutarimide to the oxazolidinones **4a–c** (Scheme II) were eventually overcome by raising the reaction temp to 130–140°, which was permitted by the use of *N,N*-dimethylacetamide (DMAC) as solvent instead of DMF. A novel and unexpected condensation occurred in the reaction of the bromide **5a** with MgS_2O_3 in MeOH; the product was assigned the thiosulfate betaine structure **7** on the basis of elemental analysis and spectral data. Its ir spectrum did not show the CO absorption characteristic of an N-substituted glutarimide. Its pmr spectrum was consistent with an arrangement of 7 CH_2 groups as in **7** and eliminated the intermediate structure (Scheme II) that required a vinyl proton and an NH proton exchangeable with D_2O. A deliquescent phosphorothioate that was eventually derived from **5a** could not be satisfactorily characterized.

The derivation of a series of thiosulfates and phosphorothioates from glutethimide was also attempted (Scheme III), but isolation and characterization in the

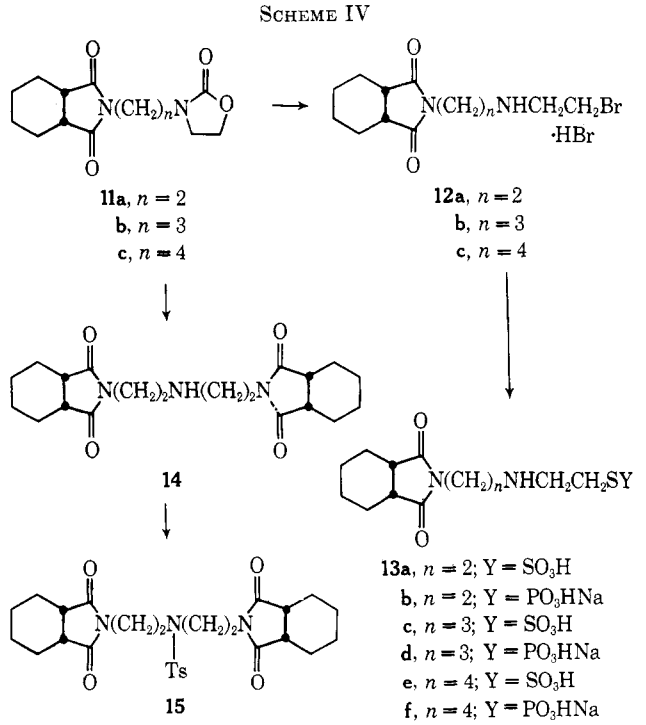
SCHEME III



final step was successful only in the case of the thiosulfate **10**.

Alkylations in DMAC provided the oxazolidinones **11a–c** from which the *cis*-1,2-cyclohexanedicarboximido analogs **13a–f** were derived (Scheme IV). An

SCHEME IV



alkylation at 95–100° in DMF, however, was preferred in the preparation of **11a**, since alkylation at 130–135° in DMAC afforded a low yield in addition to an appreciable yield of the by-product **14**, which was recognized as such after isolation of the corresponding hydrobromide in an attempted cleavage with dry HBr in AcOH.

The identity of **14** was confirmed by conversion of the hydrobromide into the *p*-toluenesulfonamide **15**. The formation of **14** was attributed to a ring-opening attack by *cis*-1,2-cyclohexanedicarboximide, as its anion, on the oxazolidinone function of **11a** with concomitant loss of CO_2.

These compounds were tested in mice for radioprotective activity at the Walter Reed Army Institute of Research by previously described methods.[4] Two phosphorothioates (**3b**·$3H_2O$ and **13b**·$4H_2O$) showed good activity, and one (**3d**·$3H_2O$) showed fair activity. Other phosphorothioates (**6b**·$4H_2O$, **6d**·$5H_2O$, and **13f**·$2.5H_2O$) in the series showed slight activity (see Table I). On the other hand, none of the thiosulfates were even slightly protective. It is apparent, however, that the most active compounds of this type, phthalimido,[3] succinimido, or *cis*-1,2-cyclohexanedicarboximido, do not compare at all favorably with the corresponding, highly active amino compounds.[2]

TABLE I

RADIOPROTECTIVE ACTIVITY OF SUCCINIMIDO AND RELATED ANALOGS OF *S*-2-(ω-AMINOALKYLAMINO)ETHYL DIHYDROGEN PHOSPHOROTHIOATES[a]

Compd no.	Approx LD_{50}, mg/kg	Drug dose, mg/kg[b]	Drug admin-irradiation interval, min	30-day survival, %[c]
3b·$3H_2O$	550	300	15	73
		150	15	13
3d·$3H_2O$	700	440	15	40
		220	15	13
6b·$4H_2O$	150	80	15	13
		40	15	0
6d·$5H_2O$	125	60	15	7
		30	15	0
13b·$4H_2O$	>390	200	15	53
		200	30	60, 87
		200	60	33, 60
		200	90	13
		100	30	0
	>900 (po)	600	30	47
		300	15	7, 40
		300	30	7, 20, 0
		300	60	7, 20
13f·$2.5H_2O$	150	80	15	7
		40	15	0

[a] Compds tested in mice against lethal radiation (950 R, γ rays). [b] Compd injected ip (unless designated po, *per os*) as 0.8–3.0% soln in H_2O (**3b,d**; **13b,** (po) **13f**) or physiological saline soln (**6b,d**; **13b**), pH unadjusted, in designated time before irradiation. [c] No 30-day survival among control mice.

Experimental Section[5]

3-(ω-Succinimidoalkyl)-2-oxazolidinones (1a–c).—A stirred mixt of 0.100 mole each of succinimide (9.91 g) and the appropriate 3-(ω-chloroalkyl)-2-oxazolidinone in DMF (100 ml) contg K_2CO_3 (20.0 g, 0.145 mole) was gradually heated to 100°, maintained at 90–100° for 2 hr, cooled, and filtered. DMF was removed by distn *in vacuo*, and the residue was purified by crystn from the solvent listed in Table II.

TABLE II

3-(ω-DICARBOXIMIDOALKYL)-2-OXAZOLIDINONES

No.	Recrystn solvent	Yield, %	Mp, °C	Formula[a]
1a	EtOH	86	102–104	$C_9H_{12}N_2O_4$
1b	C_6H_6	96	89–90	$C_{10}H_{14}N_2O_4$
1c	EtOH	76	88–89	$C_{11}H_{16}N_2O_4$
4c	EtOAc	38	83–84	$C_{12}H_{18}N_2O_4$
11a	EtOAc	86	88–90	$C_{13}H_{18}N_2O_4$

[a] *Anal.* C, H, N.

3-(ω-Glutarimidoalkyl)-2-oxazolidinones (4a–c) were prepd from glutarimide in a manner like that described above for the prepns of **1a–c** except that the prepns of **4b** and **4c** were carried out in DMAC at 130–135° (4-hr reaction time). Only **4c** (Table II) was obtained in cryst form. Oily **4a** and **4b** were, however, used successfully in conversions into **5a** and **5b**.

3-[ω-(2-Ethyl-2-phenylglutarimido)alkyl]-2-oxazolidinones (8a–c).—Alkylation of 2-ethyl-2-phenylglutarimide in essentially the manner described for the prepns of **1a–c** gave **8a–c** as oils. Crude **8a** and **8c** were readily converted into pure **9a** and **9c**, resp; but the attempted conversion of crude **8b** failed to provide cryst **9b**.

3-[2-(*cis*-1,2-Cyclohexanedicarboximido)ethyl]-2-oxazolidinone (11a). Method A. In DMAC with Accompanying Formation of *N,N'*-(Iminodiethylene)bis(*cis*-1,2-cyclohexanedicarboximide) (14).—A stirred mixt of *cis*-1,2-cyclohexanedicarboximide (25.0 g, 0.163 mole), 3-(2-chloroethyl)-2-oxazolidinone (24.4 g, 0.163 mole), K_2CO_3 (33.8 g, 0.245 mole), and DMAC (250 ml) was heated at 130–135° for 4 hr, cooled, and filtered. Removal of DMAC by distn *in vacuo* left an oily product mixt.

Isolation of 11a.—The oil was successively dissolved for decolorization and clarification in EtOH, C_6H_6, and EtOAc; solvents were removed from the EtOH and C_6H_6 solns by evapn under reduced pressure. Addn of 30–60° ligroin to the EtOAc soln caused pptn of partially purified **11a**; yield 26% (11.4 g), mp 78–80°. Pure **11a** was later obtained by method B.

Isolation of Crude 14.—Removal of solvents from the ligroin–EtOAc filtrate left a noncrystallizable orange oil. The oil was repptd from EtOAc (50 ml) by the addn of 30–60° ligroin (200 ml), and crude oily **14** was sepd and dried *in vacuo;* wt 12.3 g. *Anal.* ($C_{20}H_{29}N_3O_4$) H; C: calcd, 63.97; found, 61.78. Treatment of crude **14** (12.1 g) with 30% dry HBr–AcOH (50 ml) and dilu of the soln with Et_2O gave cryst material; wt 7.40 g, mp 194–198°. Successive recrystns from MeOH–Et_2O and EtOH gave product (5.61 g) with mp 206–207°. Spectral evidence (ir, pmr) indicated this material to be **14**·HBr, which was not analytically pure. *Anal.* ($C_{20}H_{29}N_3O_4$·HBr) H, N; C: calcd, 52.63; found, 52.12; Br: calcd, 17.51; found, 18.55. The pure tosyl deriv of **14**, ***N,N'*-bis[2-(*cis*-1,2-cyclohexanedicarboximido)ethyl]-*p*-toluenesulfonamide (15),** was prepd by dropwise addn of a soln of TsCl (0.42 g, 2.2 mmoles) in DMF (5 ml) to a stirred mixt of **14**·HBr (1.00 g, 2.2 mmoles), K_2CO_3 (0.64 g, 4.6 mmoles), and DMF (5 ml) followed by a 1-hr stirring period at 25–30°. Dilu with H_2O (75 ml) caused sepn of cryst **15**, which was recrystd from EtOH; yield 46% (0.53 g), mp 155–157°. *Anal.* ($C_{27}H_{35}N_3O_6S$) C, H, N, S. Compd **14** was then formed by treatment of pure **11a** (see method B) with 1,2-cyclohexanedicarboximide in DMAC containing K_2CO_3 at 130–135° for 4 hr. A work-up like that described above to obtain crude **14** led to an oily product, which, when tosylated, gave cryst **15** identical (ir spectra, mp, mmp) with the sample derived from the by-product formed along with **11a** by method A.

Method B. Improved Preparation of 11a with Exclusion of 14.—A stirred mixt of *cis*-1,2-cyclohexanedicarboximide (15.3 g, 0.100 mole), 3-(2-chloroethyl)-2-oxazolidinone (15.8 g, 0.106 mole), K_2CO_3 (20.0 g, 0.145 mole), and DMF (100 ml) was heated at 95–100° for 2.5 hr, cooled, and filtered. DMF was removed by distn *in vacuo*. The yellow residual oil was dissolved in EtOAc (75 ml). Addn of 30–60° ligroin to the clarified EtOAc soln caused pptn of cryst **11a** (see Table II).

***N*-[2-(2-Bromoethylamino)ethyl]-*cis*-1,2-cyclohexanedicarboximide·HBr (12a).**—The following description is typical of the

(4) L. Field, A. Ferreti, R. Crenshaw, and T. Owen, *J. Med. Chem.*, **7**, 39 (1964).

(5) Melting points were determined with a Mel-Temp apparatus unless noted otherwise. Ir spectra were determined with Perkin-Elmer Models 521 and 621 spectrophotometers, pmr spectra with a Varian A-60A spectrometer with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal reference. Chemical shifts for complex multiplets are recorded as the approx centers. Microanalyses were performed for the most part by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, anal. results obtained for those elements were within 0.4% of the calcd values.

method used for prepg the N-substd 2-bromoethylamine hydrobromides of Table III. A soln of **11a** (10.0 g, 37.5 mmoles) in

TABLE III

N-[ω-(2-BROMOETHYLAMINO)ALKYL]DICARBOXIMIDE HYDROBROMIDES

No.	Recrystn solvent[a]	Yield, %	Mp, °C	Formula	Analyses
2a	A	90	175–177	$C_8H_{13}BrN_2O_2 \cdot HBr$	C, H, Br, N
2b	A	91	146–148	$C_9H_{15}BrN_2O_2 \cdot HBr$	C, H, Br, N
2c	A, B	35	125–126	$C_{10}H_{17}BrN_2O_2 \cdot HBr$	C, H, Br
5a	A	40[b]	155–156	$C_9H_{15}BrN_2O_2 \cdot HBr$	C, H, Br
5b	A	64[b]	140–142	$C_{10}H_{17}BrN_2O_2 \cdot HBr$	C, H, Br
5c	C	57	144–145	$C_{11}H_{19}BrN_2O_2 \cdot HBr$	C, H, Br, N
9a	A, D	73[b]	191–193	$C_{17}H_{23}BrN_2O_2 \cdot HBr$	C, H, N
9c	E, F	31[b]	145–147	$C_{19}H_{27}BrN_2O_2 \cdot HBr$	C, H, N
12a	A	64[c]	165–166	$C_{13}H_{19}BrN_2O_2 \cdot HBr$	C, H, N
12b	A	48[b]	151–152	$C_{13}H_{21}BrN_2O_2 \cdot HBr$	C, H, N
12c	A	22[b]	128–129	$C_{14}H_{23}BrN_2O_2 \cdot HBr$	C, H, Br, N

[a] A, MeOH–Et_2O; B, MeOH; C, EtOH; D, MeCN; E, EtOH–Et_2O; F, H_2O. [b] Overall yield for 2 steps. [c] Prepn of this compd given in text as typical example.

30% dry HBr–AcOH (50 ml) was kept at 25–30° for 18–20 hr. Addn of Et_2O caused pptn of cryst **12a**, which was collected, washed with Et_2O, and recrystd from MeOH–Et_2O.

***S*-2-(ω-Dicarboximidoalkylamino)ethyl Hydrogen Thiosulfates (Table IV).**—Procedures for the prepn of this group of compds

TABLE IV

S-SUBSTITUTED HYDROGEN THIOSULFATES

No.	Yield, %	Mp, °C (dec)	Formula[a]
3a	87	191–194	$C_8H_{14}N_2O_5S_2$
3c	77	175–178	$C_9H_{16}N_2O_5S_2$
3e	81	176–179	$C_{10}H_{18}N_2O_5S_2$
6a	38	197–198	$C_{10}H_{18}N_2O_5S_2$
6b	44	189–191	$C_{11}H_{20}N_2O_5S_2$
10	93	187–188	$C_{17}H_{24}N_2O_5S_2$
13a	87	196–197	$C_{12}H_{20}N_2O_5S_2$
13c	48	169–171	$C_{13}H_{22}N_2O_5S_2$
13e	67	185–186	$C_{14}H_{24}N_2O_5S_2$

[a] *Anal.* C, H, N, S.

are grouped below according to the dicarboximido function. Examples that required purification treatment that deviated from the general procedure are described separately.

A. *S*-2-(ω-Succinimidoalkylamino)ethyl Hydrogen Thiosulfates (3a, 3c, 3e).—A soln of 10.0 mmoles each of $MgS_2O_3 \cdot 6H_2O$ (2.44 g) and the appropriate **2** in MeOH (50 ml) was kept at 25–30° for 24 hr. The cryst ppt that formed was collected and dissolved in H_2O (5 ml). MeOH (50 ml) was added, and the filtered soln gradually deposited the pure product.

B. *S*-2-(3-Glutarimidopropylamino)ethyl Hydrogen Thiosulfate (6a).—A soln of 16.8 mmoles each of $MgS_2O_3 \cdot 6H_2O$ (4.09 g) and **5b** (6.00 g) in MeOH (50 ml) was refluxed 10 min, filtered while warm, and refrigd. Cryst product that sepd was collected, washed with MeOH, and dried *in vacuo;* crude yield 86% (4.47 g), mp 178–183°. This material was stirred with boiling MeOH (300 ml), and the portion that remained insol in the cooled mixt was collected and dried to give pure **6a**.

***S*-2-(4-Glutarimidobutylamino)ethyl Hydrogen Thiosulfate (6b).**—Treatment of **5c** (7.00 g, 18.8 mmoles) with $MgS_2O_3 \cdot 6H_2O$ (4.59 g, 18.8 mmoles) in MeOH (65 ml) as described for **6a** gave impure **6b** (3.4 g). This sample was stirred with boiling MeOH (50 ml); the mixt was cooled, and the collected insol portion (2.98 g) was finally obtained pure by repptn from H_2O (10 ml) by addn of EtOH (30 ml).

C. *S*-2-[2-(2-Ethyl-2-phenylglutarimido)ethylamino]ethyl Hydrogen Thiosulfate (10).—A soln of $Na_2S_2O_3 \cdot 5H_2O$ (1.50 g, 6.05 mmoles) in hot H_2O (15 ml) was added to a hot soln of **9a** (2.70 g, 6.03 mmoles) in H_2O (70 ml), and the resulting soln was boiled for 2–3 min. Cryst **10**, which sepd readily from the cooled soln, was collected, washed with cold H_2O, and dried *in vacuo* (78°, P_2O_5).

D. *S*-2-[ω-(*cis*-1,2-Cyclohexanedicarboximido)alkylamino]ethyl Hydrogen Thiosulfates (13a, 13c, 13e).—A soln of equimolar amts of the appropriate **12** and $MgS_2O_3 \cdot 6H_2O$ in MeOH (4 ml/mmole of **12**) was refluxed 10 min; isolation procedures for individual compds follow.

13a.—Crystn started during the reflux period. The mixt was allowed to cool, and the collected product was washed (MeOH) and dried *in vacuo* (25°, P_2O_5).

13c.—The soln was refrigd overnight, and the solid that formed was collected and recrystd from H_2O–MeOH.

13e.—Overnight refrign did not cause sepn of the product. The soln was evapd to dryness, and the solid residue was stirred with MeOH, collected, and recrystd from H_2O–EtOH.

Inner Salt from Thiosulfuric Acid *S*-Ester with 1-(2-Mercaptoethyl)-2,3,5,6,7,8-hexahydro-5-oxoimidazo[1,2-*a*]pyridin-1-ium Hydroxide (7).—The faintly turbid soln, obtained when 14.5 mmoles each of **5a** (5.00 g) and $MgS_2O_3 \cdot 6H_2O$ (3.55 g) were dissolved in MeOH (40 ml), was clarified by filtration, boiled for 10 min, and left to stand for 3 days. The cryst ppt that formed was collected and air dried: yield 44% (1.77 g), mp 185–187° dec; ir absorption (major bands, KBr disk) at 3000–2800, 1720, 1635, 1490, 1380, 1280, 1210, 1180, 1055, 610 cm^{-1}; pmr (D_2O) δ *ca.* 2.2 (m, 2, $CH_2CH_2CH_2$), *ca.* 2.8 (m, 2, CH_2CH_2CO), *ca.* 3.2 [m, 2, $CH_2CH_2C(=N^+)N$], *ca.* 3.5 (m, 2, $CH_2CH_2S_2O_3^-$), and *ca.* 4.0–4.3 (broad complex m, 6, $CH_2CH_2NCH_2CH_2N$). *Anal.* ($C_9H_{14}N_2O_4S_2$) C, H, N, S.

Procedures for the preparation of the ***S*-2-(ω-dicarboximidoalkylamino)ethyl sodium hydrogen phosphorothioate hydrates** of Table V are grouped below according to the dicarboximido function.

TABLE V

S-SUBSTITUTED SODIUM HYDROGEN PHOSPHOROTHIOATES

No.	Yield, %	Formula	Analyses
3b	75	$C_8H_{14}N_2NaO_5PS \cdot 3H_2O$	C, H, N, P, S
3d	87	$C_9H_{16}N_2NaO_5PS \cdot 3H_2O$	C, H, N, P, S
3f	70	$C_{10}H_{18}N_2NaO_5PS \cdot 3.5H_2O$	C, H, N, P, S
6b	66	$C_{10}H_{18}N_2NaO_5PS \cdot 4H_2O$	C, H, N, S
6d	81	$C_{11}H_{20}N_2NaO_5PS \cdot 5H_2O$	C, H, N, S
13b	71	$C_{12}H_{20}N_2NaO_5PS \cdot 4H_2O$	C, H, N, P, S
13d	49	$C_{13}H_{22}N_2NaO_5PS \cdot 3.5H_2O$	C, H, N, P, S
13f	60	$C_{14}H_{24}N_2NaO_5PS \cdot 2.5H_2O$	C, H, N, P, S

A. *S*-2-(2-Succinimidoethylamino)ethyl Sodium Hydrogen Phosphorothioate (3b).—Solid **2a** (6.60 g, 20.0 mmoles) was added to a stirred partial soln of Na_3PSO_3 (3.60 g, 20.0 mmoles) in H_2O (20 ml), and, after complete soln had occurred (10 min), DMF (10 ml) was added. The soln was kept at 25–30° for 2 hr and then added dropwise to rapidly stirred EtOH (300 ml). The ppt that formed was collected, washed with EtOH, and recrystd with the aid of refrign from EtOH (75 ml)-dild, Norit-treated H_2O (25 ml) soln. Cryst product was collected, washed with EtOH followed by Et_2O, and air-dried.

***S*-2-(3-Succinimidopropylamino)ethyl Sodium Hydrogen Phosphorothioate (3d).**—Treatment of **2b** with Na_3PSO_3 in the manner and on the scale described for homologous **3b** resulted in crystn of **3d** directly from the reaction soln following addn of DMF. Stirring was contd for 2 hr. EtOH (100 ml) was added, and the collected, EtOH-washed product was recrystd with refrign from EtOH (25 ml)-dild, Norit-treated H_2O (25 ml) soln. The collected product was air-dried.

***S*-2-(4-Succinimidobutylamino)ethyl Sodium Hydrogen Phosphorothioate (3f).**—Treatment of **2c** with Na_3PSO_3 as described for the prepn of **3b** gave **3f** (recrystd from H_2O–MeOH).

B. *S*-2-(ω-Glutarimidoalkylamino)ethyl sodium hydrogen phosphorothioates (6b, 6d) were prepd from the appropriate **5** and Na_3PSO_3 in the manner described for the prepn of **3b**. Pure **6b** was obtained after recrystn from H_2O–MeOH. Compd **6d** was not recrystd; the EtOH-pptd product was collected, washed with EtOH followed by Et_2O, and air-dried.

C. *S*-2-[2-(*cis*-1,2-Cyclohexanedicarboximido)ethylamino]ethyl Sodium Hydrogen Phosphorothioate (13b).—Na_3PSO_3 (3.60 g, 20.0 mmoles) was dissolved with stirring in H_2O (20 ml) at 40–45°. The soln was cooled with rapid stirring to about 10°

to give a partial soln of finely divided Na_3PSO_3. DMF (10 ml) was added, and the mixt was allowed to warm to 25°. Powdered **12a** (7.68 g, 20.0 mmoles) was then added in portions. Complete soln occurred after 10–15 min. Stirring was contd, and after 30–40 min cryst **13b** began separating. EtOH (500 ml) was added; and, after overnight refrign, the solid was collected, washed with EtOH, and recrystd twice from H_2O (30 ml)–EtOH (300 ml). The collected product, washed successively with EtOH and Et_2O, was air-dried and then allowed to equilibrate at const 50% relative humidity.

***S*-2-[3-(*cis*-1,2-Cyclohexanedicarboximido)propylamino]-ethyl sodium hydrogen phosphorothioate (13d)** was prepd from **12b** (15.0-mmole scale) as described above for the conversion of **12a** into **13b**. The product that pptd from the EtOH-dild reaction mixt was recrystd from refrigd H_2O (50 ml)–EtOH (50 ml) soln, air-dried, and equilibrated at 50% relative humidity.

***S*-2-[4-(*cis*-1,2-Cyclohexanedicarboximido)butylamino]ethyl Sodium Hydrogen Phosphorothioate (13f).**—Adaptation of the procedure described for the prepn of **13d** readily afforded **13f**.

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Antistaphylococcal and Antifibrinolytic Activities of ω-Amino Acids and Their L-Histidine Dipeptides[1,2]

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The relationship was investigated between the molecular structure and the antistaphylococcal and antifibrinolytic actions of ω-amino acids and their L-histidine dipeptides, of which δ-aminovaleryl-L-histidine (**9**) and ε-aminocaproyl-L-histidine (**10**) are newly synthesized. The antistaphylococcal properties were demonstrated through their protective effects against staphylococcal infections in mice. The antifibrinolytic activities were determined *in vitro* by measuring prolongation of lysis time of a fibrin clot. The order of antistaphylococcal potencies of these compounds was: (a) glycine (**1**) < β-alanine (**2**) < γ-aminobutyric acid (**3**) < ε-aminocaproic acid (**5**) < δ-aminovaleric acid (**4**) and (b) glycyl-L-histidine (**6**) < β-alanyl-L-histidine (**7**) < γ-aminobutyryl-L-histidine (**8**) < δ-aminovaleryl-L-histidine (**9**) < ε-aminocaproyl-L-histidine (**10**). Comparing a and b, the protective power of ω-aminoacyl-L-histidines was much higher than that of the corresponding ω-amino acids. The order of antifibrinolytic potencies of ω-amino acids was identical with that of antistaphylococcal action except that **4** < **5** in the former. Practically no antifibrinolytic activity of ω-aminoacyl-L-histidines was observed *in vitro* under the conditions we employed.

It was previously reported that, by a prophylactic procedure, homocarnosine (**8**) and carnosine (**7**) protected C3H/HeJ mice[3a,b] but only **8** protected Swiss albino mice[3b,c] from death by *Staphylococcus aureus* infections. In this work, a series of component ω-amino acids was also examined and compared with the peptides by a combined prophylactic–therapeutic procedure with Swiss albino mice.[4]

The compounds discussed in this paper are: ω-amino acids, $H_2N(CH_2)_nCOOH$, where $n = 1$, glycine (**1**); $n = 2$, β-alanine (**2**); $n = 3$, γ-aminobutyric acid (**3**); $n = 4$, δ-aminovaleric acid (**4**); $n = 5$, ε-aminocaproic acid (**5**); and ω-aminoacyl-L-histidines, $H_2N(CH_2)_nCO$-His, where $n = 1$, glycyl-L-histidine (**6**); $n = 2$, β-alanyl-L-histidine (**7**) (carnosine); $n = 3$, γ-aminobutyryl-L-histidine (**8**) (homocarnosine); $n = 4$, δ-aminovaleryl-L-histidine (**9**); $n = 5$, ε-aminocaproyl-L-histidine (**10**).

The results indicate that **4** and **5**, higher homologs of **3**, were more effective against staphylococcal infections than **3**. Comparing **8** with **3**, and also **7** with **2**, both histidine dipeptides were more potent than their component ω-amino acids. These two facts suggested to us that both **9** and **10** might have even higher activities than **8**, as verified by the data in Figure 1.

The mechanism of the antistaphylococcal action also has been considered because none of the ω-amino acids and ω-aminoacyl-L-histidines tested showed bactericidal or bacteriostatic effects *in vitro*. We have been especially interested in the possible relationship between antistaphylococcal and antifibrinolytic activities, since **4** and **5** were reported to have antifibrinolytic activity.[5]

Chemistry.—Two general synthetic procedures were used to prepare the ω-aminoacyl-L-histidines in this study. The first was the phthalyl method, a modification of the one described by Sheehan and Frank[6] and similar to that reported by Turner,[7] except for the final purification process, in which we used ion-exchange chromatography and the phenol–calcium hypochlorite color reaction for the isolation and detection of ω-amino acids and their histidine dipeptides. The carbobenzoxy method, used as the second procedure, was modified from Bergmann and Zervas,[8] Sifferd and duVigneaud,[9] and Pisano, *et al.*[10] The yields, melting points, specific

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