

TABLE V
S-SUBSTITUTED SODIUM HYDROGEN PHOSPHOROTHIOATES AND
DIHYDROGEN PHOSPHOROTHIOATES

No.	ml of H ₂ O/ mmole of Na ₂ PSO ₃	Yield, %	Mp, °C dec	Formula ^a
2b	1.5	45		C ₁₂ H ₁₄ N ₂ NaO ₅ PS·6H ₂ O
2e	1.5	93	131-132	C ₁₃ H ₁₇ N ₂ O ₅ PS·H ₂ O
2g	5.0	77		C ₁₄ H ₁₈ N ₂ NaO ₅ PS·5H ₂ O
2i	4.2	45	148-150	C ₁₅ H ₂₁ N ₂ O ₅ PS
2k	2.3	44	158-160	C ₁₄ H ₁₉ N ₂ O ₅ PS·1.3H ₂ O
2n	3.5	56	151-152	C ₁₅ H ₂₁ N ₂ O ₅ PS
2p	3.5	43	179-180	C ₁₆ H ₂₃ N ₂ O ₅ PS
16c ^b	1.0	59	184-186	C ₁₆ H ₂₃ N ₂ O ₅ PS
26c	2.0	55		C ₁₄ H ₁₅ N ₂ NaO ₅ PS ₂ ·4.5H ₂ O
26d	2.0	70		C ₁₅ H ₂₀ N ₂ NaO ₅ PS ₂ ·5H ₂ O
32d	2.0	65		C ₁₇ H ₁₆ N ₂ NaO ₅ PS ₂ ·6.5H ₂ O
32e	2.0	89	182-183	C ₁₃ H ₁₉ N ₂ O ₆ PS ₂ ·0.5H ₂ O

^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 prep'd was ppt'd from the reaction soln with Me₂CO instead of EtOH.

to room temp, and DMF (one-half the vol of H₂O used) was added. Pulverized N-subst'd bromoalkylamine·HBr (equimolar with Na₂PSO₃) was added, and the mixt was stirred until the AgNO₃ test for PSO₃³⁻ 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 sep'd from the reaction mixt. The ppt was collected, washed (EtOH, then Et₂O), and suction dried. The Na salts **2b**, **2g**, **26c**, **26d**, and **32d** were dissolved in the required vol of H₂O at 25°, then rep'd by addn of EtOH, collected, washed as above, and air-dried. Compds

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

2e, **2k**, and **2n** crystd directly from the reaction soln after the addn of AcOH in small excess (approx 1 ml in a 10-mmole run). The collected products were washed (cold H₂O, EtOH, then Et₂O) and dried *in vacuo* (25-30°, P₂O₅). Compds **2i**, **2p**, **16c**, and **32e** were similarly obtained after H₂O 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. (C₁₃H₁₆N₄O₂S·2HBr) C, H, N, S.

2-[3-(3-Phthalimidopropylamino)propyl]-2-thiopseudourea (21)·2HBr·H₂O.—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 dist'd until 100 ml had been collected. The cooled residual soln deposited cryst product, which was collected and recryst'd from EtOH. The dried (*in vacuo*, 77°, P₂O₅) 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. (C₂₅H₂₀N₄O₈S·2HBr·H₂O) C, H, N, S.

2-[4-(3-Oxo-1,2-benzisothiazolin-2-yl)butylamino]ethanethiol S,S-Dioxide·HBr (33).—A mixt of **32e**·0.5H₂O (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** sep'd as long colorless needles. The collected material was washed (Et₂O), air-dried, and recryst'd from MeOH-Et₂O to give **33**·0.5H₂O, mp 185-187°, in 76% yield (3.04 g). Anal. (C₁₃H₁₈N₂O₃S₂·HBr·0.5H₂O) C, H, N, S, SH.

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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-Cyclohexanedicarboximides¹

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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 MgS₂O₃ 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 phosphorothioates² was accomplished by methods based

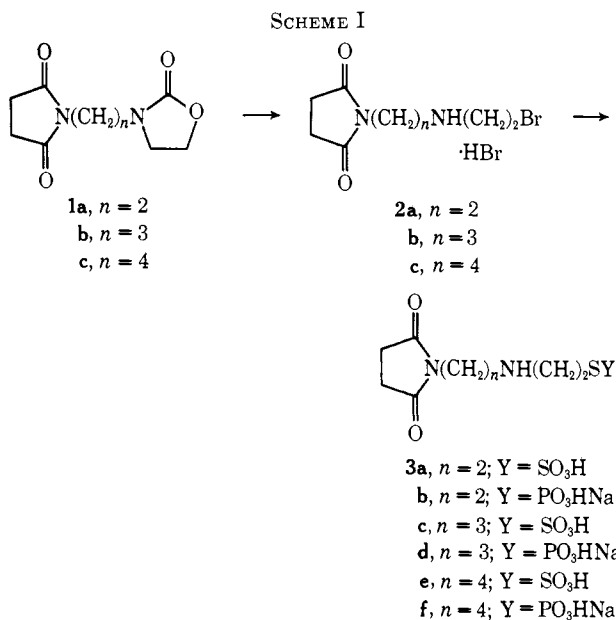
on those described in the preceding paper² for the introduction of phthalimido and related groups. The key reaction was again the selective HBr cleavage of 3-substituted 2-oxazolidinones.

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

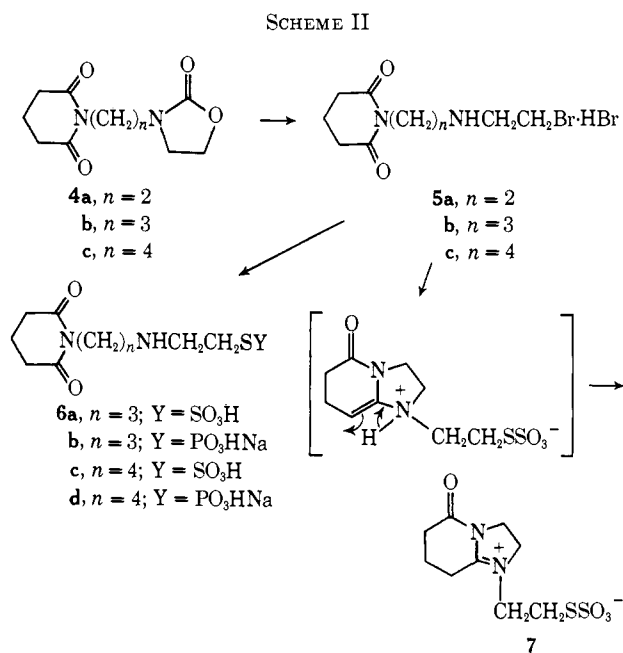
(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contracts Nos. DA-49-193-MD-2028 and DADA17-69-C-9033.

(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).



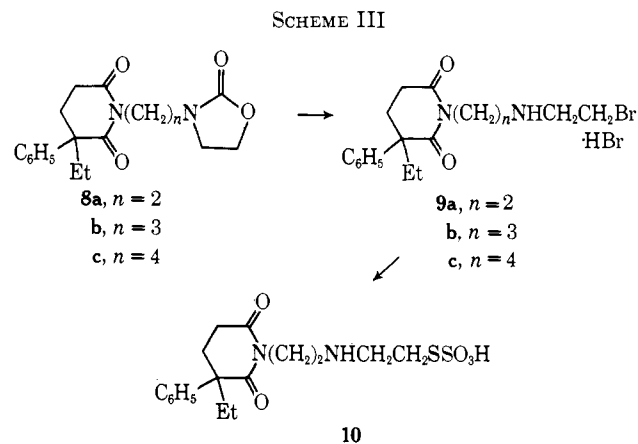
temp by treatment with magnesium thiosulfate: the products crystallized directly from the reaction mixtures leaving the coproduct MgBr_2 in solution. This method is limited to MeOH-soluble reactants and, preferably, to MeOH-insoluble products, but promises to have considerable utility, especially in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



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-sub-

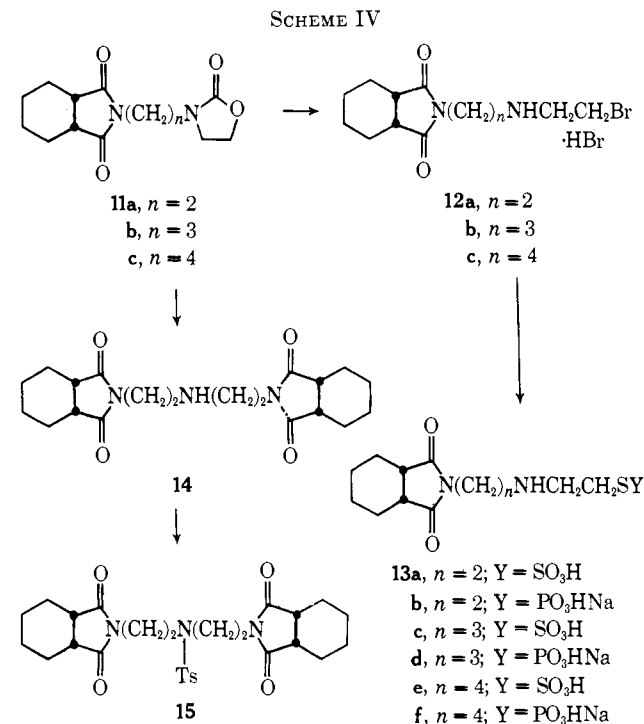
stituted glutarimide. Its pmr spectrum was consistent with an arrangement of 7 CH_2 groups as in **7** and eliminated the intermediate structure (Scheme II) that required a vinyl proton and an NH proton exchangeable with D_2O . A deliquescent phosphorothioate that was eventually derived from **5a** could 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



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



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 CO₂.

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**·3H₂O and **13b**·4H₂O) showed good activity, and one (**3d**·3H₂O) showed fair activity. Other phosphorothioates (**6b**·4H₂O, **6d**·5H₂O, and **13f**·2.5H₂O) in the series showed slight activity (see Table I). On the other hand, none of the thiosulfates

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 administration interval, min	30-day survival, % ^c	
3b·3H ₂ O	550	300	15	73	
		150	15	13	
3d·3H ₂ O	700	440	15	40	
		220	15	13	
6b·4H ₂ O	150	80	15	13	
		40	15	0	
6d·5H ₂ O	125	60	15	7	
		30	15	0	
13b·4H ₂ O	>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			
13f·2.5H ₂ O	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 H₂O (**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.

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

Experimental Section⁵

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

(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.

K₂CO₃ (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 ₉ H ₁₂ N ₂ O ₄
1b	C ₆ H ₆	96	89–90	C ₁₀ H ₁₄ N ₂ O ₄
1c	EtOH	76	88–89	C ₁₁ H ₁₆ N ₂ O ₄
4c	EtOAc	38	83–84	C ₁₂ H ₁₈ N ₂ O ₄
11a	EtOAc	86	88–90	C ₁₂ H ₁₈ N ₂ O 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 preps of **1a–c** except that the preps 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 preps 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), K₂CO₃ (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, C₆H₆, and EtOAc; solvents were removed from the EtOH and C₆H₆ 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 sep'd and dried *in vacuo*; wt 12.3 g. Anal. (C₂₀H₂₈N₂O₄) H, C: calcd, 63.97; found, 61.78. Treatment of crude **14** (12.1 g) with 30% dry HBr–AcOH (50 ml) and diln of the soln with Et₂O gave cryst material; wt 7.40 g, mp 194–198°. Successive recrystns from MeOH–Et₂O 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. (C₂₀H₂₈N₂O₄·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 prep'd 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), K₂CO₃ (0.64 g, 4.6 mmoles), and DMF (5 ml) followed by a 1-hr stirring period at 25–30°. Diln with H₂O (75 ml) caused sepn of cryst **15**, which was recrystd from EtOH; yield 46% (0.53 g), mp 155–157°. Anal. (C₂₇H₃₆N₂O₆S) C, H, N, S. Compd **14** was then formed by treatment of pure **11a** (see method B) with 1,2-cyclohexanedicarboximide in DMAC containing K₂CO₃ 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), K₂CO₃ (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). Cryst 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

method used for prep of the N-substituted 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.	Re-crystn solvent ^a	Yield, %	Mp, °C	Formula	Analyses
2a	A	90	175-177	C ₉ H ₁₃ BrN ₂ O ₂ ·HBr	C, H, Br, N
2b	A	91	146-148	C ₉ H ₁₃ BrN ₂ O ₂ ·HBr	C, H, Br, N
2c	A, B	35	125-126	C ₁₀ H ₁₇ BrN ₂ O ₂ ·HBr	C, H, Br
5a	A	40 ^b	155-156	C ₉ H ₁₃ BrN ₂ O ₂ ·HBr	C, H, Br
5b	A	64 ^b	140-142	C ₁₀ H ₁₇ BrN ₂ O ₂ ·HBr	C, H, Br
5c	C	57	144-145	C ₁₁ H ₁₉ BrN ₂ O ₂ ·HBr	C, H, Br, N
9a	A, D	73 ^b	191-193	C ₁₇ H ₂₃ BrN ₂ O ₂ ·HBr	C, H, N
9c	E, F	31 ^b	145-147	C ₁₉ H ₂₇ BrN ₂ O ₂ ·HBr	C, H, N
12a	A	64 ^c	165-166	C ₁₂ H ₁₉ BrN ₂ O ₂ ·HBr	C, H, N
12b	A	48 ^b	151-152	C ₁₃ H ₂₁ BrN ₂ O ₂ ·HBr	C, H, N
12c	A	22 ^b	128-129	C ₁₄ H ₂₃ BrN ₂ O ₂ ·HBr	C, H, Br, N

^a A, MeOH-Et₂O; B, MeOH; C, EtOH; D, MeCN; E, EtOH-Et₂O; F, H₂O. ^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 Et₂O caused pptn of cryst 12a, which was collected, washed with Et₂O, and recrystd from MeOH-Et₂O.

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

TABLE IV
S-SUBSTITUTED HYDROGEN THIOSULFATES

No.	Yield, %	Mp, °C (dec)	Formula ^a
3a	87	191-194	C ₈ H ₁₄ N ₂ O ₅ S ₂
3c	77	175-178	C ₉ H ₁₆ N ₂ O ₅ S ₂
3e	81	176-179	C ₁₀ H ₁₈ N ₂ O ₅ S ₂
6a	38	197-198	C ₁₀ H ₁₈ N ₂ O ₅ S ₂
6b	44	189-191	C ₁₁ H ₂₀ N ₂ O ₅ S ₂
10	93	187-188	C ₁₇ H ₂₄ N ₂ O ₅ S ₂
13a	87	196-197	C ₁₂ H ₂₀ N ₂ O ₅ S ₂
13c	48	169-171	C ₁₃ H ₂₂ N ₂ O ₅ S ₂
13e	67	185-186	C ₁₄ H ₂₄ N ₂ O ₅ S 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 MgS₂O₃·6H₂O (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 H₂O (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 MgS₂O₃·6H₂O (4.09 g) and 5b (6.00 g) in MeOH (50 ml) was refluxed 10 min, filtered while warm, and refigd. 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 MgS₂O₃·6H₂O (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 reppn from H₂O (10 ml) by addn of EtOH (30 ml).

C. S-2-[2-(2-Ethyl-2-phenylglutarimido)ethylamino]ethyl Hydrogen Thiosulfate (10).—A soln of Na₃S₂O₃·5H₂O (1.50 g, 6.05 mmoles) in hot H₂O (15 ml) was added to a hot soln of 9a (2.70 g, 6.03 mmoles) in H₂O (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 H₂O, and dried *in vacuo* (78°, P₂O₅).

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 MgS₂O₃·6H₂O in MeOH (4 ml/mole 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°, P₂O₅).

13c.—The soln was refigd overnight, and the solid that formed was collected and recrystd from H₂O-MeOH.

13e.—Overnight refign 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 H₂O-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 MgS₂O₃·6H₂O (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, 1250, 1210, 1180, 1055, 610 cm⁻¹; pmr (D₂O) δ ca. 2.2 (m, 2, CH₂CH₂CH₂), ca. 2.8 (m, 2, CH₂CH₂CO), ca. 3.2 [m, 2, CH₂CH₂C(=N⁺)N], ca. 3.5 (m, 2, CH₂CH₂S₂O₃⁻), and ca. 4.0-4.3 (broad complex m, 6, CH₂CH₂NCH₂CH₂N). Anal. (C₉H₁₄N₂O₅S₂) 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 ₈ H ₁₄ N ₂ NaO ₅ PS·3H ₂ O	C, H, N, P, S
3d	87	C ₉ H ₁₆ N ₂ NaO ₅ PS·3H ₂ O	C, H, N, P, S
3f	70	C ₁₀ H ₁₈ N ₂ NaO ₅ PS·3.5H ₂ O	C, H, N, P, S
6b	66	C ₁₀ H ₁₈ N ₂ NaO ₅ PS·4H ₂ O	C, H, N, S
6d	81	C ₁₁ H ₂₀ N ₂ NaO ₅ PS·5H ₂ O	C, H, N, S
13b	71	C ₁₂ H ₂₀ N ₂ NaO ₅ PS·4H ₂ O	C, H, N, P, S
13d	49	C ₁₃ H ₂₂ N ₂ NaO ₅ PS·3.5H ₂ O	C, H, N, P, S
13f	60	C ₁₄ H ₂₄ N ₂ NaO ₅ PS·2.5H ₂ O	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 Na₃PSO₃ (3.60 g, 20.0 mmoles) in H₂O (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 refign from EtOH (75 ml)-dild, Norit-treated H₂O (25 ml) soln. Cryst product was collected, washed with EtOH followed by Et₂O, and air-dried.

S-2-(3-Succinimidopropylamino)ethyl Sodium Hydrogen Phosphorothioate (3d).—Treatment of 2b with Na₃PSO₃ 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 refign from EtOH (25 ml)-dild, Norit-treated H₂O (25 ml) soln. The collected product was air-dried.

S-2-(4-Succinimidobutylamino)ethyl Sodium Hydrogen Phosphorothioate (3f).—Treatment of 2c with Na₃PSO₃ as described for the prep of 3b gave 3f (recrystd from H₂O-MeOH).

B. S-2-(ω-Glutarimidoalkylamino)ethyl sodium hydrogen phosphorothioates (6b, 6d) were prepd from the appropriate 5 and Na₃PSO₃ in the manner described for the prep of 3b. Pure 6b was obtained after recrystn from H₂O-MeOH. Compd 6d was not recrystd; the EtOH-pptd product was collected, washed with EtOH followed by Et₂O, and air-dried.

C. S-2-[2-(cis-1,2-Cyclohexanedicarboximido)ethylamino]ethyl Sodium Hydrogen Phosphorothioate (13b).—Na₃PSO₃ (3.60 g, 20.0 mmoles) was dissolved with stirring in H₂O (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, $\text{H}_2\text{N}(\text{CH}_2)_n\text{COOH}$, 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, $\text{H}_2\text{N}(\text{CH}_2)_n\text{CO-His}$, 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 com-

ponent 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 Frank⁶ 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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