Antiradiation Compounds V

α-Amino Acid Esters of 2-Mercaptoethylamine

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A series of *S*-α-amino acid esters of 2-mercaptoethylamine (MEA) has been prepared in an attempt to obtain less toxic and longer-acting derivatives of MEA with radiation-protective properties. The method of preparation consisted of a dry fusion of the amino acid chloride hydrochloride with MEA hydrochloride. It was found that the thioesters liberate MEA on acid hydrolysis; hydrolysis constants have been determined.

BETA - MERCAPTOETHYLAMINE (cysteamine, MEA), one of the more effective of the radiation-protective compounds in animals (1), has shown toxic symptoms in mice (2) and dogs (3) with doses close to those necessary for radioprotection. In humans, its use in moderate doses (200–400 mg. i.v.) over periods of several weeks without causing unfavorable effects has been reported (4). To provide less toxic and longer-acting derivatives of MEA, thioesters with α-amino acids have therefore been prepared. The α-amino acids themselves, except for cysteine which gives good protection against ionizing radiation, have shown but slight radioprotective action (5). Previously, we have prepared α-amino acid derivatives to lower the toxicity of bis-(4-aminophenyl)sulfone (6) and various nitro and halogenated benzene compounds with antibacterial activity (7). Acylation of MEA with aliphatic acids has already been shown to lower the intraperitoneal toxicity of MEA (8).

It was anticipated that the α-aminoacyl thioesters of MEA would liberate MEA *in vivo* over a period of time, or that possibly the thioesters would function as more active thiolation agents *in vivo* than the mercaptan. In regard to the hydrolysis of thioesters of MEA, however, some question exists concerning the nature of the hydrolysis products that might be expected *in vivo*. Wieland (9) has shown that in strong alkali the acyl group shifts to the nitrogen exclusively, giving amides which generally show little or no radioprotective capacity. Chromatographic tests conducted at the Walter Reed Army Institute of Research have shown that at least three species are present after hydrolysis of *S*-acetyl MEA at pH 7.4 (10), whereas Purdy (11) has found that only straight hydrolysis to MEA occurs on hydrolysis of this compound over a wide pH range in dilute solutions.

The method of preparation of the α-amino acid thioesters was patterned after that of Wieland (9), which consisted of a dry fusion of the α-amino acid chloride hydrochloride (12) and MEA hydrochloride. An excess of MEA hydrochloride was more effective in obtaining products of good purity than an excess of the acid chloride as recommended by Wieland. With heat labile acid chlorides, such as those from L-proline and DL-methionine, the reaction proceeded satisfactorily at room temperature. Infrared absorption spectra of the compounds showed no absorption band near 2550 $cm.^{-1}$ attributable to a free mercapto group, nor did their aqueous solutions reduce iodine solution until after standing for varying periods of time. Physical properties of the thioesters prepared are recorded in Table I.

Hydrolysis rate constants were determined for the thioesters in acetate buffer at pH 3.7 and a temperature of 15°. In alkaline solution, the esters undergo an immediate shift to the *N*-acyl derivatives (9), and in neutral solution the hydrolysis was too rapid for accurate measurements; therefore, the comparison was made in acid solution. The liberated thiol was titrated with standard iodine solution. Kuhn *et al.* (13) have shown that in 90% acetic acid thiol esters are oxidized readily to disulfides with iodine in stoichiometric amount, using very small quantities of material and without excluding atmospheric oxygen. Values for k were obtained from the slope of the plot of log C (concentration of unhydrolyzed ester) *versus* time. The data agreed with first-order kinetics, as would be expected from acid hydrolysis of esters, and the k values are listed in Table II. Representative data for a hydrolysis are presented in Table III.

The following sequence of hydrolytic reaction rates in decreasing order was: L-prolyl > glycyl >

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TABLE I.—α-AMINO ACID ESTERS OF MEA

$$R\text{—}\overset{O}{\overset{\|}{C}}\text{—}SCH_2CH_2NH_2\cdot 2HCl$$

R	M.p., °C.	Yield, %	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	N, % Calcd.	N, % Found	S, % Calcd.	S, % Found
NH_2CH_2	175–179 dec.[a]	55	$C_4H_{12}Cl_2N_2OS$	23.19	23.06	5.84	5.99	13.53	13.85	15.47	16.19
DL-$CH_3CH(NH_2)$	187–189.5 dec.	21	$C_4H_{14}Cl_2N_2OS$	27.15	26.89	6.38	6.27	12.67	12.52	14.50	14.85
DL-$CH_3CH_2CH(NH_2)$	192–196 dec.	32	$C_5H_{16}Cl_2N_2OS$	30.64	30.87	6.86	7.15	11.91	12.19	13.63	13.93
DL-$(CH_3)_2CHCH(NH_2)$	190–194 dec.[b]	30	$C_7H_{18}Cl_2N_2OS$	33.74	33.03	7.28	7.18	11.24	11.51	12.87	13.31
L-$(CH_3)_2CHCH_2CH(NH_2)$	178–181 dec.	44	$C_8H_{20}Cl_2N_2OS$	36.48	36.61	7.65	7.38	10.70	10.80	12.17	12.26
DL-$C_4H_9CH_2CH(NH_2)$	184–186 dec.	26	$C_{11}H_{16}Cl_2N_2OS$	44.44	44.98	6.10	6.06	9.43	9.30	10.78	10.81
L-	178–184 dec.	27	$C_7H_{16}Cl_2N_2OS$	34.01	33.85	6.53	6.63	11.34	11.08	12.97	13.55
DL-$CH_3SCH_2CH_2CH(NH_2)$	168–172 dec.	25	$C_7H_{18}Cl_2N_2OS_2$	29.89	29.92	6.45	6.67	9.96	9.95	22.80	23.10

[a] Lit. (9) m.p. 184° dec. [b] Lit. (9) m.p. 197° dec.

TABLE II.—HYDROLYSIS RATE CONSTANTS OF MEA ESTERS[a]

Ester Dihydrochloride	$k \times 10^4$
S-L-Prolyl MEA	16.00
S-Glycyl MEA	14.40
S-DL-Methionyl MEA	6.86
S-DL-β-Phenylalanyl MEA	6.70
S-DL-Alanyl MEA	4.60
S-L-Leucyl MEA	2.56
S-DL-α-Amino-*n*-butyryl MEA	1.80
S-DL-Valyl MEA	0.23

[a] Observed at pH 3.7 and 15° in acetate buffer solution.

DL-methionyl > DL-β-phenylalanyl > DL-alanyl > L-leucyl > DL-α-aminobutyryl > DL-valyl. It is apparent that neither molecular weight nor chain length are determinant factors in the ease of hydrolysis of these esters.

A paper chromatographic determination of the hydrolysis products of one of the thioesters, the valyl derivative, was carried out in a butanol-formic acid-water system at 25°. Spots were developed with ninhydrin and ammonia, and after remaining overnight in this medium, *S*-valyl MEA liberated MEA (R_f = 0.23), the disulfide of MEA (R_f = 0.06), and *N*-valyl MEA (R_f = 0.52). Wieland (9) had previously observed these compounds in the same solvent system at an unspecified temperature, and listed corresponding R_f values of 0.24, 0.05, and 0.50. It is unlikely that the $S \rightarrow N$ acyl shift occurred at the low pH (3.7) of the hydrolysis rate determinations, but it appears quite probable that some *N*-acyl derivative would be formed *in vivo* or in the solutions (pH 7.4) used for antiradiation testing.

Antiradiation testing results have not yet become available for these compounds. Bonati and Nuvulone (14) have tested several *N*-substituted α-amino acid derivatives of MEA, however, and claimed that *N*-glutamyl cysteamine and *N*-cysteaminemonoacetic acid were as effective against 600 r of X-radiation as cysteamine itself. Tank (15) has also claimed that *S*-2-aminoisobutyryl cysteamine has no radioprotective ability.

EXPERIMENTAL

Melting points were taken on a Mel-temp apparatus and are uncorrected. Carbon, hydrogen, and nitrogen analyses were done by Weiler and Strauss, Oxford, England.

α-Amino Acid Chloride Hydrochlorides.—The following procedure is representative. To a suspension of 5.0 Gm. (0.038 mole) of L-leucine in 100 ml. of reagent grade acetyl chloride was added 8.0 Gm. (0.038 mole) of phosphorus pentachloride. The suspension was shaken for 2 hours at room temperature. An additional 1.5 Gm. of phosphorus pentachloride was added, and the shaking period

TABLE III.—HYDROLYSIS OF *S*-DL-α-AMINO-*n*-BUTYRYL MEA·2HCl[a]

t, Sec.	0.01 *N* I_2 Consumed	SH, %	*S*-Acyl, %	C, mole/L.	Log *C*
0	0	0	100	0.0150	$\bar{2}$.1761
620	2.30	15.34	86.67	0.0127	$\bar{2}$.1038
1825	5.60	37.34	62.66	0.0094	$\bar{3}$.9731
3660	8.22	54.81	45.19	0.0068	$\bar{3}$.8312
6870	10.37	69.14	30.86	0.0046	$\bar{3}$.6656
10,170	11.30	75.34	24.66	0.0037	$\bar{3}$.5682

[a] Observed at pH 3.7 and 15° in acetate buffer solution.

was continued for 1.5 hours. The resulting white solid was collected in a sintered-glass crucible and washed several times with anhydrous ether. It was fused immediately with cysteamine hydrochloride as described below. The yield of L-leucyl chloride hydrochloride was 3.4 Gm. (47% of theoretical). Modifications were followed in the case of DL-valine (16), DL-alanine (17), L-proline (18), and DL-methionine (19).

Thioesters of Cysteamine Hydrochloride.—The following procedure is representative. In a mortar placed in a drying oven maintained at 80–90°, 4.5 Gm. (0.04 mole) of cysteamine hydrochloride (Chemicals Procurement Laboratories, Inc.) was melted. Then 3.4 Gm. (0.018 mole) of L-leucyl chloride hydrochloride was added all at once. The mass was triturated constantly; a large amount of hydrogen chloride was evolved. When hydrogen chloride evolution had ceased, the mortar was removed from the oven, and a small amount of absolute ethanol was added to the hot mass. The mass was triturated with the hot alcohol to remove excess MEA hydrochloride and the mortar was placed in an ice bath. After being cooled, a white solid crystallized. This was collected and washed several times with absolute ethanol. The residue was dissolved in a minimum amount of ice-cold 2*N* hydrochloric acid, and the solution was filtered into 150 ml. of acetone. A white solid crystallized and was isolated in a sintered-glass crucible; it was washed several times with absolute ethanol and stored in a desiccator over Drierite. The yield was 2.0 Gm. (44% of theoretical).

The ester was extremely water soluble, and its aqueous solution did not immediately decolorize iodine solution, but in approximately 30 minutes the solution was decolorized. The ester darkened at 174° and melted at 178–181° with decomposition.

Anal.—Calcd. for $C_8H_{20}Cl_2N_2OS$; C, 36.48; H, 7.65; N, 10.70; S, 12.17. Found: C, 36.61; H, 7.38; N, 10.80; S, 12.26.

Determination of Hydrolysis Rate Constants.—The following procedure is representative. A buffer solution having a pH of 3.7 was prepared by mixing equal volumes of 1 *N* hydrochloric acid solution and 1.25 *N* sodium acetate solution. Throughout the hydrolysis the temperature was maintained at 15 ± 0.1°. To 100.00 ml. of buffer solution contained in a stoppered iodine flask was added 0.3528 Gm. (0.0150 mole) of *S*-DL-α-amino-*n*-butyryl cysteamine dihydrochloride. At exactly determined times, aliquot portions were removed and added to 25 ml. of 2 *N* hydrochloric acid solution to attain a pH of 1 and stop further hydrolysis. Twice the theoretical amount of iodine was added to this acid solution; after 1 minute, the volume was doubled by the addition of distilled water. The excess iodine was back titrated with standard sodium thiosulfate solution. A *k* value was obtained by determining the slope of the straight line from the plot of log *C versus* time and multiplying this value by 2.303. The data from this determination are shown in Table III.

Chromatographic Analysis.—*S*-DL-Valyl cysteamine dihydrochloride (5 mg./ml.) in phosphate buffer solution (pH 7.4) was placed on Whatman No. 1 paper, dried, and allowed to remain in contact with *n*-butanol-85% formic acid-water (75:15:10 parts by volume) overnight at 25°. Spots were developed by treating with ninhydrin in *n*-butanol, drying, and suspending over ammonia. R_f values were taken at the center of the spots. Authentic samples of cysteamine and its disulfide (cystamine) (8) gave R_f values of 0.23 and 0.06, respectively. *N*-Valyl cysteamine hydrochloride was obtained by making strongly alkaline an aqueous solution of *S*-valyl cysteamine dihydrochloride followed by acidification (9).

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