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The Use of S-Benzylthiocarbonyl-α-amino Acids in the Papain-catalyzed Synthesis of Acylated Amino Acid Phenylhydrazides[1]

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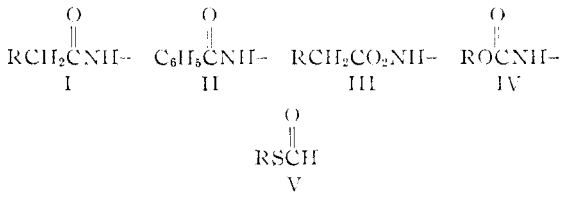
S-Benzylthiocarbonyl derivatives of a number of α-amino acids have been prepared. S-Benzylthiocarbonyl-D- and L-leucine and S-benzylthiocarbonyl-D- and L-methionine were used as substrates in the papain-catalyzed reaction with phenylhydrazine to form the phenylhydrazides. The D-phenylhydrazides were formed at a slower but not greatly different rate from the L-antipodes. The S-benzylthiocarbonyl group was cleaved from the amino acid derivatives with sodium in liquid ammonia, aqueous sodium hydroxide or lead acetate. Lead acetate reacted with S-benzylthiocarbonylglycylglycine ethyl ester to form ethyl hydantoin-3-acetate and with S-benzylthiocarbonylglycine and S-benzylthiomethionine phenylhydrazides to form the corresponding 2-phenyl-5-alkyl-3,6-dioxohexahydro-1,2,4-triazine.

Several years ago it was shown that the nature of the acyl group has a profound effect upon the stereochemical course of the papain-catalyzed reaction of an acylated α-amino acid with phenylhydrazine to form the corresponding phenylhydrazide. Niemann[2,3] found that for acylated D,L-phenylalanines, $RCONHCH(CH_2C_6H_5)\ CO_2H$, where R = CH_3– or C_6H_5–, the papain-catalyzed synthesis of the phenylhydrazide proceeds with almost complete stereochemical specificity for the L-antipode. However, when R = CH_3O–, C_2H_5O– or $C_6H_5CH_2O$–, this stereochemical specificity is lost and the D-phenylhydrazide is formed at a comparable rate with the L-antipode. In our laboratory[4,5] it was shown that when carboallyloxy-D,L-leucine is used as a substrate in the above reaction the D-phenylhydrazide is formed along with the L-antipode, but when benzylsulfonyl-α-amino acids were used as substrates, only the L-phenylhydrazides were formed.

Thus when the acyl amide group of the substrate has structures I, II or III, the enzyme is stereospecific, but when the substrate has structure IV, the stereospecificity of the enzyme is reduced. This raises the question as to whether this difference in stereospecificity is due to a steric effect or due to an electronic effect. A partial answer was sought by using substrates with structure V, as the sulfur



atom has the size of a methylene group but an electronic structure similar to oxygen.

Ehrensvard[6] suggested phenylthiocarbonyl chloride as a group-protecting reagent in peptide synthesis. This acid chloride reacted with α-amino acid esters to form phenylthiocarbonyl-α-amino esters, which were readily converted to phenylthiocarbonyl-α-amino acids by acidic hydrolysis. These latter compounds in the form of their acid chlorides were coupled with other α-amino acid esters to form phenylthiocarbonyl dipeptide esters. Ehrensvard reported that short heating with lead acetate in 70% ethanol removed the phenylthiocarbonyl blocking group from both the amino acid and dipeptide derivatives. However, Hofmann, *et al.*,[6,7] reported that the latter reaction led to the formation of hydantoin derivatives and not to dipeptide esters. Killonitsch, *et al.*,[8] showed that the phenylthiocarbonyl group could be removed from phenylthiocarbonyl dipeptides by oxidation with perbenzoic acid without forming a hydantoin. Niemann[9,10] showed that when phenylthiocarbonyl chloride reacted with phenylalanine under Schotten–Baumann conditions N,N-carbonyl-bisphenylalanine was formed in good yield and when N-monosubstituted S-phenylthiocarbamates were treated with base, the corresponding substituted ureas were formed.

Killonitsch, *et al.*,[8] also prepared a series of benzylthiocarbonyl derivatives of amino acids and dipeptides. Because these compounds were shown to be more stable in weakly basic solutions than the phenylthiocarbonyl derivatives and because of their similarity to the carbobenzoxy derivatives a series of S-benzylthiocarbonyl-α-amino acids were prepared and used in this study as substrates in the papain-catalyzed reaction with phenylhydrazine.

Discussion of Results.—The S-benzylthiocarbonyl-α-amino acids reacted with phenylhydrazine in the presence of papain to form S-benzylthiocarbonyl-α-amino acid phenylhydrazides. The reactions proceeded as readily as when the corresponding carbobenzoxy derivatives were used as substrates. Although the solutions were incubated for as much as 25 days, there was no appreciable cleavage of the S-benzylthiocarbonyl group. It was immediately apparent that the stereospecificity of the papain was lost to a marked degree when S-benzylthiocarbonyl-α-amino acids were used as substrates as shown in Table II. When S-benzylthiocarbonyl-D-amino acids were used as substrates the D-phenylhydrazides were formed as readily as

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(2) E. L. Bennett and C. Niemann, THIS JOURNAL, **70**, 2610 (1948).

(3) E. L. Bennett and C. Niemann, *ibid.*, **72**, 1698 (1950).

(4) H. B. Milne and C. M. Stevens, *ibid.*, **72**, 1742 (1950).

(5) H. B. Milne and Chi-Hsieh Peng, *ibid.*, **79**, 645 (1957).

(6) G. C. H. Ehrensvard, *Nature*, **159**, 500 (1947).

(7) A. Lindenmann, N. H. Khan and K. Hofmann, THIS JOURNAL, **74**, 476 (1952).

(8) J. Killonitsch, V. G. Abor, A. Hajor, *Nature*, **177**, 841 (1956); J. Killonitsch, *et al.*, *Chem. Ber.*, **89**, 2288 (1956).

(9) W. H. Schuller and C. Niemann, THIS JOURNAL, **75**, 3425 (1953).

(10) D. G. Crosby and C. Niemann, *ibid.*, **76**, 4458 (1954).

TABLE I

CRYSTALLINE BENZYLTHIOCARBONYL DERIVATIVES[a]

	Yield, %	M.p., °C.	Formula	Calcd. C, %	Calcd. H, %	Calcd. N, %	Calcd. S, %	Found C, %	Found H, %	Found N, %	Found S, %
NH_3	80	123.5–124.5	C_8H_9NOS	57.50	5.37	8.4	19.15	57.5	5.37	8.72	18.9
D,L-Leucine	42	96–97	$C_{14}H_{19}NO_3S$	59.79	6.76	4.98	11.40	59.5	6.65	4.74	11.2
Glycine	75	150.5–151[c]	$C_{10}H_{11}NO_3S$	53.33	4.89	6.22	14.22	53.3	4.85	6.20	14.10
D,L-Alanine	45	173–175[c]	$C_{11}H_{13}NO_3S$	55.23	5.44	5.86	13.40	55.3	5.29	5.73	13.24
L-Tyrosine (mono)	43	147–148	$C_{17}H_{17}NO_4S$	61.65	5.17	4.23	9.65	61.45	5.12	4.22	9.54
L-Alanine	37.8	123–125[c]	$C_{11}H_{13}NO_3S$	55.23	5.47	5.85	13.40	55.28	5.51	5.64	13.9
D,L-Threonine	47.5	95–98	$C_{12}H_{15}NO_4S$	53.51	5.61	5.20	11.91	53.72	5.19	5.25	11.6
Glycylglycine ethyl ester	35	110–111[b,c]	$C_{14}H_{18}N_2O_4S$	54.18	5.81	9.03	10.33	54.14	5.64	9.23	10.31

[a] The amino acid ethyl ester hydrochlorides used in the preparation of these derivatives were prepared by Jerry Still, a senior in chemistry. [b] The S-benzylthiocarbonylglycylglycine ethyl ester was prepared by Irwin Klundt, a senior in chemistry. [c] Killonitsch reported the following melting points for the S-benzylthiocarbonyl derivatives: glycine, 151–152°; D,L-alanine, 176–178°; L-alanine, 126–127°; glycylglycine ethyl ester, 117–119°.

TABLE II

PAPAIN-CATALYZED SYNTHESIS OF BENZYLTHIOCARBONYL AMINO ACID PHENYLHYDRAZIDES

S-Benzylthiocarbonyl derivative of	Weight substrate, g.	Phenylhydrazine hydrochloride, g.	Papain, g.	Time of incub., days	Yield, %	M.p., °C.	$[\alpha]^{20}D$
Glycine	1	0.7	0.7	2	100	185–186	...
D,L-Alanine	1	0.6	0.6	3	70	157–158	−85°
D,L-Leucine	2	1.1	2.0	4	44	105–107	−84
				7	50	115–136	−33.4
				14	52	140–144	− 2.9
				25	55	143–144	0
L-Leucine	0.6	1	0.5	2	91	120–121	−89
				4	100	120–121	−89
D-Leucine	0.6	1	0.5	4	9	120–121	+88
				25	40	120–121	+89
L-Methionine	8	4	3	1	80	133–134	−49
D-Methionine	8	4	3	4	6	133–134	+51
				25	35	133–134	+48

has been reported for carboallyloxy or carbethoxy-D-amino acids.[2-4] Thus it may be inferred that the observed differences in stereospecificity are due in part to the electronic character of the acyl blocking group and not entirely to steric effects.

The amino acids which have been used as substrates in reactions which this lack of stereospecificity has been reported, have been neutral amino acids. Smith, *et al.*,[11] have shown that in the papain-catalyzed hydrolysis of acylated α-amino acid amides and acylated α-amino acid ethyl esters, the most reactive substrates have been those derived from basic amino acids; for example, benzoyl-L-arginine amide and benzoyl-L-arginine ethyl ester. On the assumption that $k_1 = K_0/K_m$ the data indicated that the interaction between enzyme and substrate involved two titratable groups in the enzyme, an ionized carboxyl group and an un-ionized sulfhydryl group. Smith proposed that the reactive group at the active site of the enzyme involves a thio ester which is formed and maintained by the folding energy of the protein. The change in stereospecificity with changes in structure the α-acyl amide group suggests that in addition to an ionized carboxyl and an un-ionized sulfhydryl group there is a group on the enzyme which interacts with the α-acyl amide group of the substrate.

Cleavage of the S-Benzylthiocarbonyl Group.—The S-benzylthiocarbonyl group was cleaved by reduction with sodium in liquid ammonia, by reduction with Raney nickel, by heating with 3 *N* sodium hydroxide or by treatment with lead acetate. In each case the glycine was recovered in satisfactory yield. In the reaction of S-benzylthiocarbonylglycine with 3 *N* sodium hydroxide, a 76% yield of glycine was obtained. Apparently the S-benzylthiocarbonylamino acids do not form N,N′-carbonyl-bis-amino acids in the presence of base under the conditions reported by Niemann[9] for the S-phenylthiocarbonyl-α-amino acids.

As it has been reported[7] that S-phenylthiocarbonylglycylglycine ethyl ester reacted with lead acetate to form ethyl hydantoin-3-acetate in an 82% yield, S-benzylthiocarbonylglycylglycine ethyl ester was prepared and treated with lead acetate under similar conditions. Ethyl hydantoin-3-acetate, m.p. 118–120°, was isolated in 36% yield.

As Hofmann[7] reported that the reaction of phenylthiocarbonylglycine carbobenzoxyhydrazide with lead acetate led to the formation of 2-carbobenzoxy-3,6-dioxohexahydro-1,2,4-triazine and did not give glycine carbobenzoxyhydrazide, it was of interest to see if the S-benzylthiocarbonyl-α-amino acid phenylhydrazides would undergo the same reaction. Both S-benzylthiocarbonylglycine phenylhydrazide and S-benzylthiocarbonyl-L-methionine phenylhydrazide were refluxed in the presence of an equimolar quantity of lead acetated in ethanol. In each case a compound which analyzed correctly for the corresponding 2-phenyl-5-alkyl-3,6-dioxohexahydro-1,2,4-triazine was isolated in about 60%

(11) E. L. Smith, B. J. Finkle and A. Stockell, *Disc. Faraday Soc.*, **20**, 96 (1955); A. Stockell and E. L. Smith, *J. Biol. Chem.*, **227**, 1 (1957); E. L. Smith, V. J. Chavre and M. J. Parkes, *ibid.*, **230**, 283 (1958); E. L. Smith, *ibid.*, **233**, 1392 (1958).

yield. The compound formulated as 2-phenyl-3,6-dioxohexahydro-1,2,4-triazine reacted with acetyl chloride to form a diacetate of composition $C_{13}H_{13}N_3O_4$.

The infrared spectrum of the 2-phenyl-3,6-dioxohexahydro-1,2,4-triazine shows a characteristic NH stretching frequency at 3300 cm.$^{-1}$ and two carbonyl bands at 1780 and 1735 cm.$^{-1}$. The infrared spectrum of the diacetate does not show a peak in the 3300 cm.$^{-1}$ region but it does show carbonyl frequencies at 1760 and 1710 cm.$^{-1}$.

It was of interest to see if the phenylhydrazide group could be removed from S-benzylthiocarbonyl-α-amino acid phenylhydrazides using the method of Milne, *et al.*[12] S-Benzylthiocarbonyl glycine was recovered in 94% yield when S-benzylthiocarbonylglycine phenylhydrazide was refluxed with an acetone–water solution of ferric chloride.

The S-benzylthiocarbonyl group does not have the general utility of the carbobenzyloxy group because of the instability of the S-benzylthiocarbonyl group in basic solutions. However, because the group may be selectively removed from a peptide it should be an important addition to the blocking groups which are available for peptide synthesis.

The infrared spectra were determined for S-benzylthiocarbonyl amide, the S-benzylthiocarbonyl-α-amino acids and the S-benzylthiocarbonyl-α-amino acid phenylhydrazides. In each case there was a characteristic carbonyl stretching frequency at 1640 ± 5 cm.$^{-1}$.

Experimental

Papain.—Commercial papain (Nutritional Biochemical Corp.) was purified by the procedure of Grossman[13] and Bergmann and Fraenkel-Conrat[14] as modified by Bennett and Niemann.[2] After four successive treatments with hydrogen sulfide, followed by precipitation with methanol, the precipitate was lyophilized and a white powder was obtained.

Benzylthiocarbonyl Chloride.—The acid chloride was prepared by a modification of Arndt's[15] method. Aluminum chloride (0.5 g.) was dispersed in liquid phosgene (71.2 ml.). Benzyl mercaptan (117.4 ml.) was slowly added to the mixture. The reaction mixture was stirred mechanically during the additions of the reagent. After the addition of the benzyl mercaptan was complete, the solution was allowed to warm to room temperature and allowed to stand until the evolution of hydrogen chloride had ceased. At this time chloroform (300 ml.) was added and the aluminum chloride filtered from the solution. The chloroform was removed *in vacuo* at 40° and the material distilled. The fraction boiling at 138° (22 mm.) was collected. The product weighed 101.4 g. (54.4%).

Benzylthiocarbonyl Amide.—Benzylthiocarbonyl chloride (1 g.) was added to concentrated ammonium hydroxide (5 ml.). The resulting crystals were recrystallized from chloroform; yield 0.8 g. (90%), m.p. 123.5–124°.

Anal. Calcd. for C_8H_9NOS: C, 57.50; H, 5.37; N, 8.39; S, 19.15. Found: C, 57.50; H, 5.37; N, 8.72; S, 18.90.

Preparation of S-Benzylthiocarbonyl Derivatives of Amino Acids.—The ethyl ester hydrochloride of the chosen amino acid was dissolved in the minimum amount of chloroform to give a clear solution. Two equivalents of triethylamine was added and the reaction cooled in an ice-bath.[15] One equivalent of benzylthiocarbonyl chloride was slowly added while the mixture was stirred mechanically. The stirring was continued for 20 min. after the addition of the benzylthiocarbonyl chloride was complete.

At this time 200 ml. of dry ether was added and the triethylamine hydrochloride was filtered. The filtrate was evaporated under vacuum to give a viscous oil. This oil was refluxed for 15 min. with 50 ml. of 50:50 glacial acetic acid–concentrated hydrochloric acid. At the end of this time 100 ml. of distilled water was added and the solution cooled overnight in a refrigerator. The crystalline[16] benzylthiocarbonyl-α-amino acid was then filtered and recrystallized from the appropriate solvent. The results are shown in Table I.

Enzymatic Synthesis of Phenylhydrazides of S-Benzylthiocarbonyl-α-amino Acids.—These phenylhydrazides were prepared according to the method of Bergmann and Fraenkel-Conrat.[14] Some of the results are listed in Table II. The S-benzylthiocarbonyl-α-amino acids were dissolved in the minimum amount of a sodium acetate (18 g./100 ml.). Versene solution (1 g./100 ml.). To this solution was added 1 equivalent of phenylhydrazine hydrochloride and an equal weight of cysteine hydrochloride dissolved in 50 ml. of distilled water. The total volume of the solution was from 100–200 ml. for each gram of S-benzylthiocarbonyl-α-amino acid. After the *p*H of the solution had been adjusted to 4.7, papain was added, and nitrogen was bubbled through the solution for a few minutes. The flask was stoppered and the solution was incubated in a water-bath at 40°. At appropriate intervals the product was collected by filtration. The results of the enzymatic reactions are shown on Tables II and III.

Cleavage of S-Benzylthiocarbonylglycine. (a) With Sodium and Liquid Ammonia.—One gram of S-benzylthiocarbonylglycine was dissolved in 200 ml. of liquid ammonia. Sodium (0.28 g.) was slowly added in small pieces to a permanent blue. The ammonia was allowed to evaporate and the glycine converted to hippuric acid; yield 0.2 g. (25.4%), m.p. 188–189°, mixed melting point with authentic sample hippuric acid 188–189°.

(b) With Sodium Hydroxide.—A solution of S-benzylthiocarbonylglycine (0.5 g.) dissolved in 10 ml. of 3 *N* sodium hydroxide was heated on a steam-bath for 45 minutes. During this time the solution became slightly turbid. The solution was extracted with two 15-ml. portions of ether, acidified (congo red) and extracted with two 20-ml. portions of chloroform. The aqueous solution was then made basic to litmus and the volume reduced to 20 ml. The glycine was recovered as hippuric acid 0.3 g. (76%), m.p. 187–188°.

(c) With Lead Acetate.—The reaction was carried out according to the method Lindenmann, *et al.*,[7] used in the reaction of lead acetate with phenylthiocarbonylglycine. S-Benzylthiocarbonylglycine (4.4 mmoles) was added to a solution of 0.91 g. (2.4 mmoles) of lead acetate in 70 ml. of 70% ethanol and the mixture was heated for 10 min. at 75–80° on a steam-bath. The solution was cooled to room temperature and the yellow precipitate of lead benzylmercaptide was removed by filtration. The filtrate was evaporated to dryness and the glycine converted to hippuric acid; 0.3 g. (38%), m.p. 187–188°.

Reaction of S-Benzylthiocarbonyl-D,L-Leucine with Sodium Hydroxide.—In a large test-tube was placed 0.5 g. (0.0018 mole) of benzylthiocarbonyl-D,L-leucine with 10 ml. of 3 *N* sodium hydroxide solution. The solution was allowed to stand for 48 hr. (white solid formed). The mixture was extracted several times with ether. The aqueous solution was then brought to the isoelectric point and leucine was isolated. The crude yield was 0.2 g. (84.4%), and the yield of purified product was 0.15 g. (63.6%).

Reaction of S-Benzylthiocarbonylglycylglycine Ethyl Ester with Lead Acetate.—This reaction was carried out according to a method similar to that described by Lindenmann, *et al.*,[7] in the reaction of lead acetate with phenylthiocarbonylglycylglycine ethyl ester. To a warmed solution of 0.61 g. (0.0016 mole) of lead acetate in 50 ml. of 70% ethanol was added 0.93 g. (0.003 mole) of S-benzylthiocarbonylglycylglycine ethyl ester, and the mixture was heated at 75–80° on a hot water-bath for 10 min. After cooling to room temperature, the lead benzylmercaptan was filtered and the filtrate

(12) H. B. Milne, J. E. Halver, D. S. Ho and M. S. Mason, THIS JOURNAL, **79**, 637 (1957).

(13) W. Grossman, *Biochem. Z.*, **279**, 131 (1935).

(14) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).

(15) F. Arndt, E. Milde and G. Eckert, *Ber.*, **76B**, 1976 (1923).

(16) In the cases where the benzylthiocarbonyl-α-amino acid was not crystalline at this point, the solution was extracted with two 25 ml. portions of 5% sodium bicarbonate solution. The combined bicarbonate extracts were acidified with hydrochloric acid and cooled in a refrigerator. The crystalline benzylthiocarbonyl-α-amino acid then was filtered.

TABLE III

ANALYSES OF S-BENZYLTHIOCARBONYL AMINO ACID PHENYLHYDRAZIDES

S-Benzylthiocarbonyl phenylhydrazides of	Formula	Calcd., % C	H	N	S	Found, % C	H	N	S
Glycine	$C_{16}H_{17}N_3O_2S$	61.00	5.40	13.32	10.15	60.80	5.25	13.10	9.95
L-Alanine	$C_{17}H_{19}N_3O_2S$	62.00	5.78	12.75	9.75	62.0	5.83	12.70	9.71
L-Leucine	$C_{20}H_{25}N_3O_2S$	64.70	6.75	11.3	8.63	64.5	6.62	11.5	8.53

was saturated with hydrogen sulfide. The resulting lead sulfide was filtered and filtrate was evaporated to dryness *in vacuo*. The residue was dried in a vacuum desiccator overnight. The solid residue was recrystallized three times from chloroform–petroleum ether (30–60°). The yield of colorless needles (m.p. 118–120°) was 0.2 g. (35.9%) (reported[17] 118–120°), assumed to be ethyl hydantoin-3-acetate.

Anal. Calcd. for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05. Found: C, 45.30; H, 5.55; N, 15.10.

Reaction of S-Benzylthiocarbonylglycine Phenylhydrazide with Lead Acetate.—Lead acetate (10.0 g.) was dissolved in 1 l. of warm 95% ethanol. To this solution was added 15.2 g. of S-benzylthiocarbonylglycine phenylhydrazide. The temperature of the mixture was maintained at 75–80° until the suspension of lead benzyl-mercaptide settled (*ca.* 40 min.). The solution was then filtered and the filtrate was saturated with hydrogen sulfide. The resulting lead sulfide was removed by filtering through a mat of Filter-Cel. The filtrate was evaporated to dryness and the solid residue was recrystallized first from 95% ethanol then from chloroform to yield 6.05 g. (59.2%) of 2-phenyl-3,6-dioxohexahydro-1,2,4-triazine, m.p. 165.0–166.5°.

Anal. Calcd. for $C_9H_9N_3O_2$: C, 56.54; H, 4.75; N, 21.98. Found: C, 56.84; H, 4.63; N, 22.00.

The above triazine (0.5 g.) was heated with 20 g. of acetyl chloride. The mixture was evaporated on a steam-bath to form a brown oil which crystallized from benzene–petroleum ether yielding 0.22 g. of white crystals, m.p. 157–158°.

Anal. Calcd. for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.26. Found: C, 56.64; H, 4.82; N, 15.30.

Reaction of S-Benzylthiocarbonylmethionine Phenylhydrazide with Lead Acetate.—This reaction was carried out in the same manner as the above using 0.78 g. (0.002 mole) of S-benzylthiocarbonylmethionine phenylhydrazide, 0.42 g. (0.0011 mole) of lead acetate and 40 ml. of 70% ethanol. The reaction mixture was heated on a hot water-bath for 8 min. The crude product was recrystallized three times from ethanol–water. The yield of triazine (m.p. 122–123.5°) was 0.4 g. (75.5%).

Anal. Calcd. for $C_{12}H_{15}N_3O_2S$: C, 54.32; H, 5.69; N, 15.84; S, 12.08. Found: C, 54.48; H, 5.84; N, 15.61; S, 12.20.

Reaction of S-Benzylthiocarbonylglycine Phenylhydrazide with Ferric Chloride.—A solution of 16 g. of ferric chloride hexahydrate dissolved in 25 ml. of distilled water was added to a solution of 8 g. of S-benzylthiocarbonylglycine phenylhydrazide dissolved in 30 ml. of acetone. After the addition was complete, the solution was refluxed for 3 hr. The solution was distilled until 25 ml. of the acetone had been removed. The solution was then cooled overnight in a refrigerator. The resulting crystals were filtered and recrystallized from chloroform; yield 5.1 g. (94%), m.p. 151–152°, mixed melting point with an authentic sample of S-benzylthiocarbonylglycine 151–152°.

(17) R. Locquin and V. Cerchez, *Bull. soc. chim.*, **49**, 309 (1939).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XXXVI. Alkylating Agents Derived from 5-Aminouracil

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Two monofunctional alkylating agents, 5-(2-chloroethylamino)-uracil (V) and 5-[2-chloroethyl)-ethylamino]-uracil (VIII), were synthesized for comparison of effectiveness as antitumor compounds with difunctional alkylating agents. An unknown material, obtained *via* the hydroxyethylation of 5-(ethylamino)-uracil (III), was identified as 3-(2-chloroethyl)-5-[(2-chloroethyl)-ethylamino]-uracil (XIIa) by comparison of its ultraviolet spectra with those of the previously unknown compounds, 5-[bis-(2-chloroethyl)-amino]-3-methyluracil (XVIII) and 5-[bis-(2-chloroethyl)-amino]-1-methyluracil (XXVI).

Recently the hypothesis was put forward that alkylating agents consist of a carrier and the alkylating group and that differences in effects and side effects on tumors might be related to the differences in the carrier group.[2] More recently this hypothesis was expanded into a rationale for the design of specific irreversible enzyme inhibitors.[3] This rationale proposed that substrates, properly substituted by an alkylating group, could fit the specific enzyme site for the substrate, then replace a nearby active hydrogen by alkylation, thus resulting in specific irreversible inactivation of the enzyme.

Recent synthetic work in the nitrogen mustard field has resulted in several promising anticancer compounds, such as phenylalanine mustard (sarcolysin),[4] *m*-phenylalanine mustard,[3a,5] chlorambucil,[6] uracil mustard[7] and benzimidazole mustard.[8] All of the above compounds are so-called "two-

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(3) (a) H. F. Gram, C. W. Mosher and B. R. Baker, THIS JOURNAL, **81**, 3103 (1959); (b) B. R. Baker, Cancer Chemotherapy Reports No. 4, p. 1 (1959), a publication of the National Cancer Institute.

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