EXPERIMENTAL[7,8]

Ethyl p-[N,N-bis(2-hydroxyethyl)amino]benzoate (I). This was prepared by a modification of the procedure of Pan and co-workers.[5] To a chilled suspension of 80 g. (0.48 mole) of ethyl *p*-aminobenzoate in 640 ml. of 25% acetic acid, 100 g. of ethylene oxide was added with swirling. The mixture was stirred at room temperature for 20–25 hr. after which the clear solution was made slightly basic with sodium bicarbonate and extracted with ethyl acetate. After drying over anhydrous calcium sulfate and removing most of the solvent under reduced pressure, the product separated as white crystals on chilling. Recrystallization from benzene gave 68 g. (56%) of white leaflets, m.p. 93–94°.

p-[N,N-Bis(2-hydroxyethyl)amino]benzhydrazide (II). A mixture of 5 g. (0.02 mole) of I, 10 g. of 95% hydrazine, and 50 ml. of absolute ethanol was refluxed for 7 hr. On concentration of the solution the hydrazide (4.5 g., 95%) separated. After recrystallization from 95% ethanol, it melted at 141–142°.

Anal. Calcd. for $C_{11}H_{17}N_3O_3$: C, 55.23; H, 7.11; N, 17.57. Found: C, 54.84; H, 7.30; N, 17.66.

4-[N,N-Bis(2-hydroxyethyl)amino]benz-[4'-bis(2-chloroethyl)amino]benzylidene hydrazide (IV). A mixture of 4.5 g. (0.019 mole) of II and 4.6 g. (0.019 mole) of III[4] in 40 ml. of absolute ethanol was refluxed for 30 min. Separation of IV began within 10 min., and the yield was quantitative. Recrystallization from dimethylformamide–absolute ethanol gave yellow needles, m.p. 209–210°.

Anal. Calcd. for $C_{22}H_{28}Cl_2N_4O_3$: C, 56.58; H, 5.99; N, 11.99; Cl, 15.20. Found: C, 56.56; H, 6.04; N, 11.82; Cl, 15.34.

p-[N,N-Bis(2-chloroethyl)amino]benzoic acid. (VI). This was prepared essentially according to Pan and co-workers.[5] In view of the relative inaccessability of the original, details are given. Fifty milliliters of chilled phosphorus oxychloride was added slowly with swirling to 56 g. (0.221 mole) of I which was previously chilled in ice. The viscous mixture was heated on the steam bath until no further evolution of hydrogen chloride occurred. Excess phosphorus oxychloride was removed under reduced pressure, and the brown residue was refluxed with 200 ml. of concd. hydrochloric acid for 2 hr. After standing at room temperature overnight, the precipitate was collected and thoroughly washed with 50% ethanol. Recrystallization from 95% ethanol gave 33 g. (57%) of VI, m.p. 172–173°.

p-[N,N-Bis(2-chloroethyl)amino]benzhydrazide. (VIII). Twenty milliliters of purified thionyl chloride was added all at once to a solution of 9.6 g. (0.037 mole) of VI in 30 ml. of benzene, and the mixture was refluxed for 1 hr. After removal of the solvent at reduced pressure a quantitative yield of the acid chloride (VII), m.p. 88–94°, was obtained, reported m.p. 83–84°.[6] Recrystallization from benzene raised the m.p. to 94–96°.

A solution of crude VII in the minimum amount of dioxane was added dropwise to a solution of 20 g. of 95% hydrazine in 200 ml. of water. A white precipitate of VIII formed instantly and was collected. The yield was 10 g. (98%), m.p. 149–151.5°, after recrystallization from 95% ethanol.

Anal. Calcd. for $C_{11}H_{15}Cl_2N_3O$: C, 47.82; H, 5.43; N, 15.21; Cl, 25.72. Found: C, 47.82; H, 5.47; N, 15.22; Cl, 25.76.

4-[Bis(2-chloroethyl)amino]benz-[4'-bis(2-chloroethyl)-amino]benzylidene hydrazide. (V). A mixture of 4.1 g. (0.015 mole) of VIII and 4 g. (0.010 mole) of III in 100 ml. of 95% ethanol was heated on the steam bath for 20 min. A pale yellow precipitate appeared within 10 min. The crude product (6.3 g.), m.p. 225–227°, was collected and recrystallized from dimethylformamide–absolute alcohol.

Anal. Calcd. for $C_{22}H_{26}Cl_4N_4O$: C, 52.38; H, 5.15; N, 11.11; Cl, 28.18. Found: C, 52.30; H, 5.03; N, 10.98; Cl, 28.08.

Acknowledgment. We acknowledge the valuable assistance of James Hudson in the preparation of certain intermediates.

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(7) Melting points are uncorrected for stem exposure.

(8) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Mich.

[CONTRIBUTION FROM THE VEGETABLE LABORATORY, UNITED STATES DEPARTMENT OF AGRICULTURE]

Isolation of (+)*S*-Methyl-L-cysteine Sulfoxide and of (+)*S*-*n*-Propyl-L-cysteine Sulfoxide from Onions as their *N*-2,4-Dinitrophenyl Derivatives

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(+)*S*-Methyl-L-cysteine sulfoxide and (+)*S*-*n*-propyl-L-cysteine sulfoxide have been isolated from onions as the *N*-2,4-dinitrophenyl derivatives. Configurational identity was established by infrared spectra and by optical rotation. *N*-Dinitrophenyl derivatives of several *S*-alkyl cysteines and of the corresponding sulfoxides have been prepared and their optical rotations recorded.

Virtanen and Matikkala[2] have recently isolated *S*-methylcysteine sulfoxide and *S*-*n*-propylcysteine sulfoxide from Finnish onions, *Allium cepa*, without, however, establishing their configurations. By analogy with the occurrence of (+)*S*-methyl-L-cysteine sulfoxide[3] in *Brassica* species and of (+)*S*-allyl-L-cysteine sulfoxide[4] in the closely related garlic, *Allium sativum*, the two amino acids from the onion would be presumed to be the dextrorotatory sulfoxides derived from L-cysteine.

The present work confirms the presence of these two amino acids in commercial American onions

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(2) A. I. Virtanen and E. J. Matikkala, *Acta Chem. Scand.*, **13**, 1898 (1959).

(3) R. L. M. Synge and J. C. Wood, *Biochem. J.*, **64**, 252 (1956); C. J. Morris and J. F. Thompson, *J. Am. Chem. Soc.*, **78**, 1605 (1956).

(4) A. Stoll and E. Seebeck, *Experientia*, **3**, 114 (1947).

TABLE I

N-2,4-DINITROPHENYL DERIVATIVES OF L-AMINO ACID SULFIDES AND SULFOXIDES

2,4-Dinitrophenyl Derivative	M.P. (Dec.)	Calcd., %			Found, %			$[M]_D^{25}$ in Acetic Acid[a]
		C	H	N	C	H	N	
S-Methyl cysteine cyclohexylamine salt	181–182	48.0	6.04	14.0	47.7	5.91	13.9	−310
S-Ethyl cysteine cyclohexylamine salt	184–185	49.3	6.32	13.5	49.4	6.25	13.5	−332
S-Allyl cysteine cyclohexylamine salt	156–158	50.7	6.15	13.1	50.8	6.07	13.1	−509
S-*n*-Propyl cysteine cyclohexylamine salt	142–143	50.5	6.59	13.1	50.6	6.55	13.1	−309
(+)*S*-Methyl cysteine sulfoxide	161.5	37.9	3.50	13.2	38.0	3.48	13.2	−345
(−)*S*-Methyl cysteine sulfoxide cyclohexylamine salt	154–155	46.1	5.81	13.5	46.1	5.85	13.3	−862
(+)*S*-*n*-Propyl cysteine sulfoxide	155	41.7	4.38	12.2	41.9	4.34	12.2	−488
(−)*S*-*n*-Propyl cysteine sulfoxide	152–153	41.7	4.38	12.2	41.8	4.37	12.1	−771
(+)*S*-Allyl cysteine sulfoxide	150.5–151	42.0	3.82	12.2	42.3	3.95	12.0	−343
(−)*S*-Allyl cysteine sulfoxide	126–126.5	42.0	3.82	12.2	42.0	3.87	12.0	−804
(+) Methionine sulfoxide	215–215.5	39.9	3.96	12.7	40.0	4.10	12.6	−208
(−) Methionine sulfoxide	207–207.5	39.9	3.96	12.7	39.9	4.07	12.6	−582

[a] $[M]_D^{25} = \frac{[\alpha]_D^{25} \times \text{mol. wt.}}{100}$. Specific rotations were measured at concentrations of 0.4–1.0% in a 2-dm. tube.

and establishes their configuration as (+)*S*-methyl-L-cysteine sulfoxide and (+)*S*-propyl-L-cysteine sulfoxide. This was accomplished by isolation of the crystalline *N*-2,4-dinitrophenyl derivatives and comparison of their infrared spectra and specific rotations with those of the corresponding derivatives of the pure synthetic diastereomers. *S*-Allyl-L-cysteine sulfoxide and *S*-methyl-, *S*-propyl-, and *S*-allyl-L-cysteines[5] were shown to be absent by paper chromatography of the 2,4-dinitrophenyl derivatives. The neutral amino acid fraction from an onion extract (prepared under conditions to minimize enzymic action) was converted into a mixture of 2,4-dinitrophenylamino acids from which the two derivatives were separated by partition chromatography on buffered silicic acid with ethyl acetate. Basic and acidic amino acids and glutamine were removed prior to dinitrophenylation.

Although 2,4-dinitrophenyl derivatives have been widely used for end group determinations of proteins and peptides and for identifying amino acids in relatively simple mixtures, the technique has not been generally used in the separation of complex mixtures of amino acids. Rao and Sober[6] determined the molecular rotations of a number of 2,4-dinitrophenyl L-amino acids in acetic acid and in base and recommended the optical rotations for identification and determination of configuration. 2,4-Dinitrophenyl derivatives are particularly useful for isolating and identifying amino acid sulfoxides since they are easily crystallized, have high molecular rotations, and are readily separable by partition chromatography from the 2,4-dinitrophenyl derivatives of the other neutral amino acids. The technique is particularly recommended for the separation and identification of *S*-methylcysteine sulfoxide.

2,4-Dinitrophenyl derivatives of the pure *dextro* and *levo* diastereomers of *S*-methyl- and *S*-*n*-propyl-L-cysteine sulfoxides and of the L-methionine sulfoxides were prepared. In each case the crude sulfoxide was separated into its diastereomers by fractional crystallization before dinitrophenylation. *S*-Allyl-L-cysteine sulfoxide could not be separated into the two isomers, but the crude mixed sulfoxide was dinitrophenylated and then separated into the two 2,4-dinitrophenyl derivatives by fractional crystallization. By analogy with the behavior of the other sulfoxides, the more levorotatory 2,4-dinitrophenyl isomer is tentatively correlated with (−)*S*-allyl-L-cysteine sulfoxide and the less levorotatory compound with the natural dextrorotatory isomer. The 2,4-dinitrophenyl derivatives of *S*-methyl,[7] S-ethyl-, S-*n*-propyl, and S-allyl-L-cysteine were prepared and crystallized as their cyclohexylamine salts. As expected,[6] all rotations in acetic acid were negative. Table I lists analyses and rotations of these compounds.

EXPERIMENTAL

L-Cysteine hydrochloride, L-methionine, S-methyl-, and S-ethyl-L-cysteine were obtained from the California Biochemi-

(5) H. E. Renis and R. E. Henze [*Food Research*, **23**, 345 (1958)] identified *S*-propylcysteine sulfoxide and *S*-allylcysteine in mature onion bulbs by paper chromatography.

(6) K. R. Rao and H. A. Sober, *J. Am. Chem. Soc.*, **76**, 1328 (1954).

(7) 2,4-Dinitrophenyl-*S*-methyl-L-cysteine has been prepared and crystallized as its cyclohexylamine salt by Rinderknecht *et al.* [*Helv. Chim. Acta*, **41**, 1 (1958)].

cal Corp.[8] *S-n-Propyl-*[9] and *S-allyl-*L-*cysteine*[10] were synthesized.

The diastereomers of *S-methyl-*L-*cysteine sulfoxide* were prepared and had $[\alpha]_D^{25}$ +121 (1,2; *c*, 0.25 in water) and $[\alpha]_D^{25}$ −94.8 (1,2; *c*, 0.25 in water). Morris and Thompson[11] give the values +124 and −127.

The diastereomers of *S-n-propyl-*L-*cysteine sulfoxide* were synthesized[10] with $[\alpha]_D^{25}$ +25.0 (1,2; *c*, 1.25 in water) and $[\alpha]_D^{25}$ −30.9 (1,2; *c*, 1.25 in water). Stoll and Seebeck[10] report +31.7 and −28.2.

*S-Allyl-*L-*cysteine sulfoxide* was prepared as described by Stoll and Seebeck[10] but could not be separated into the isomers.

The dextro- and levorotatory sulfoxides of L-*methionine* were prepared. They had $[\alpha]_D^{25}$ +93 (1,2; *c*, 1 in water) and −69.5 (1,2; *c*, 1 in water). Lavine[12] gives +99 and −71.6.

Preparation of *2,4-dinitrophenyl-amino acids*. These were prepared by Sanger's Method[13] except that acetone-water (1:2) was used as the solvent.

The 2,4-dinitrophenyl-*S*-alkyl-L-cysteines were converted to their cyclohexylamine salts[7] which were crystallized from acetone or acetone-ether solutions. The 2,4-dinitrophenyl-*S*-alkyl-L-cysteine sulfoxides and the 2,4-dinitrophenyl-L-methionine sulfoxides were crystallized from methanol. ethanol, or ethyl acetate. 2,4-Dinitrophenyl(−)*S*-methyl-L-cysteine sulfoxide was more readily crystallized as its cyclohexylamine salt from acetone. In each case, the derivatives had constant rotations on repeated recrystallization.

*Dinitrophenylation of the mixed S-allyl-*L-*cysteine sulfoxides and separation into the isomeric sulfoxides*. A mixture of 7.0 g. of mixed *S*-allyl-L-cysteine sulfoxides, $[\alpha]_D^{25}$ −4.7 (water) was converted to the dinitrophenyl derivatives and the oily product was dissolved in 35 ml. of methanol. After 5 days at +3°, a yellow crystalline product was isolated, 0.84 g., $[\alpha]_D^{25}$ −108.1 (1,2; *c*, 0.62 in acetic acid). The mother liquor was concentrated *in vacuo* to 20 ml. and after a week at +3° yielded a second crystalline product, 1 08 g., $[\alpha]_D^{25}$ −228.9 (1,2; *c*, 0.93 in acetic acid). A third crop was obtained, 0.53 g., $[\alpha]_D^{25}$ −230.6.

The first crop was recrystallized by concentrating a solution in 25 ml. of acetone and 50 ml. of methanol to half volume to yield 0.58 g. of pale tiny yellow needles with $[\alpha]_D^{25}$ −100 in acetic acid, unchanged on repeated crystallization. The second and third crops were combined and recrystallized in the same manner to yield 0.77 g. as clusters of tiny yellow needles $[\alpha]_D^{26}$ −235.1 in acetic acid, essentially unchanged on repeated crystallization. Each of the isomers was occasionally obtained as amber prisms rather than needles but with unchanged rotations.

Preparation of onion extract. Ten kg. of freshly peeled onions[14] was rapidly chopped into enough boiling absolute ethanol to yield a final concentration of approximately 70% ethanol. The suspension was boiled for 10 min., disintegrated with a Waring Blendor, filtered, and the filtrate concentrated *in vacuo* to 6 l. Amino acids were adsorbed by passing the solution through a column, 56 × 460 mm. of Dowex 50-X4 (50–100 mesh) in the acid form. The resin was washed with 7 l. of water to remove sugars. The amino acids were then eluted from the column with 6 l. of 0.5*N* ammonium hydroxide. Ammonia and basic amino acids were then removed by passage through Amberlite (IRC-50 (acid form), 56 × 320 mm. The effluent from this resin was passed through Duolite A-4 (amino form) 56 × 320 mm. to remove acidic amino acids and acidic peptides.

The solution, *p*H 6.43, was reduced *in vacuo* to 500 ml. from which 1.15 g. of crystalline tyrosine was removed. The solution was then concentrated *in vacuo* to 100 ml., 18 ml. of 10*N* hydrochloric acid was added and the solution was further concentrated *in vacuo* to 60 ml., yielding a crystalline mush. After storage overnight at 3°, the crystals were removed by filtration and were washed successively with 20 ml. of cold 2*N* hydrochloric acid and 5 ml. of cold water. Nine grams of cycloalliun hydrochloride hydrate[15] was obtained.

The solution was then freed from mineral acid by treatment with Duolite A-4 (amino form), diluted to 600 ml. and the *p*H adjusted to 6.8 by the addition of sodium bicarbonate. To convert glutamine to pyrrolidone carboxylic acid, the solution was refluxed for 4 hr., and the dark solution again treated with Amberlite IRC-50 (acid form) and Duolite A-4 to remove pyrrolidonecarboxylic acid. The solution, now substantially free of basic and acidic amino acids and glutamine, was ready for dinitrophenylation.

Dinitrophenylation of neutral onion amino acids. One half of the solution, corresponding to 5 kg. of fresh onion, was diluted with water to 900 ml. Acetone, 450 ml., sodium bicarbonate, 45 g., and 40 g. of 1-fluoro-2,4-dinitrobenzene were added and the suspension was shaken mechanically in the dark at room temperature for 20 hr. The suspension was concentrated *in vacuo* to remove acetone, extracted twice with 500-ml. portions of ether and acidified to *p*H 2.0 with hydrochloric acid. The suspension containing the precipitated 2,4-dinitrophenylamino acids was extracted four times with 500-ml. portions of ethyl acetate, and the combined extracts were dried with sodium sulfate and reduced *in vacuo* to 100 ml. After standing in the refrigerator for 3 days, the solution deposited 1.93 g. of 2,4-dinitrophenyl-asparagine. The mother liquor yielded 15.5 g. of an amber gum which was redissolved in ethyl acetate and made to 150 ml.

*Isolation of 2,4-dinitrophenyl(+)S-methyl-*L-*cysteine sulfoxide*. Silicic acid, Mallinckrodt Analytical Reagent, was repeatedly washed by decantation with water until fines were removed and then dried in a forced draft oven at 84° for 64–72 hr. The dried silicic acid was then mixed with 67% of its weight of 0.25*M* phosphate buffer[16] adjusted to *p*H 6.18 and saturated with ethyl acetate. Columns were prepared by pouring a slurry of the buffered silicic acid in ethyl acetate (previously saturated with buffer) into the column followed by washing with two bed volumes of solvent.

A 25-ml. aliquot (*ca.* 2.6 g. of 2,4-dinitrophenylamino acids) was transferred to a column of 900 g. of buffered silicic acid, 56 × 570 mm., and the column developed with 2200 ml. of ethyl acetate. Most of the neutral 2,4-dinitrophenylamino acids were eluted rapidly as a series of poorly separated fast-moving bands. At the conclusion of the development, the column showed five major bands. A very sharp band, 2-cm. thick, had moved only 0.5 cm. from the top. This was not identified. A sharp intense band, 1 cm. below and 3.5-cm. thick, consisted of nearly pure 2,4-dinitrophenyl-*S*-methylcysteine sulfoxide. The corresponding propyl derivative was found in a weak band 3-cm. thick and approximately 3 cm. below the methyl derivative. The largest band, occupying one third of the column, was just below and was poorly separated from the band containing the propyl derivative. This was principally 2,4-dinitrophenyl-asparagine. The fifth or fastest moving band just below contained the 2,4-dinitrophenyl derivatives of asparagine and of serine. Chromatography was repeated with two additional 25-ml. aliquots of the stock solution (total equivalent to 2.5 kg. of onion) and the corresponding zones containing the 2,4-dinitrophenyl-*S*-methyl- and -*S*-propylcysteine sulf-

(8) Mention of commercial supplier by name does not imply endorsement over other products of similar purity.

(9) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **31**, 189 (1948).

(10) *Helv. Chim. Acta*, **34**, 481 (1951).

(11) C. J. Morris and J. F. Thompson, *J. Am. Chem. Soc.*, **78**, 1605 (1956).

(12) T. F. Lavine, *J. Biol. Chem.*, **169**, 477 (1947).

(13) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(14) An Improved Southport White Globe "Sunspice" donated by the Basic Vegetable Dehydration Co., Vacaville, Calif.

(15) A. I. Virtanen and E. J. Matikkala, *Acta Chem. Scand.*, **13**, 623 (1959).

(16) S. Blackburn, *Biochem. J.*, **45**, 579 (1949).

oxides were carved from the columns, extracted with 95% ethanol, and filtered. The filtrates, contained the desired derivatives, were concentrated *in vacuo* to dryness, suspended in 1*N* hydrochloric acid, and extracted into ethyl acetate. The ethyl acetate extracts were dried with sodium sulfate and concentrated *in vacuo* to dry solids.

The yellow crystalline solid isolated from the slower of the two bands was recrystallized from 15 ml. of ethanol at −20° to yield 151 mg. of bright yellow prisms. This was shown to be 2,4-dinitrophenyl(+)*S*-methyl-L-cysteine sulfoxide by specific rotation and by infrared, either of which distinguishes the compound from its diastereomer. $[\alpha]_D^{25}$ −111.6 (1,2; *c*, 0.33 in acetic acid). For the synthetic compound, $[\alpha]_D^{25}$ −108.8.

The infrared spectrum (0.96 mg. in 250-mg. potassium bromide pellet) was identical with that of the authentic compound with absorption (μ) at 5.75 (s), 6.15 (s), 6.25 (s), 6.55 (s), 6.65 (shoulder), 7.00 (s), 7.27 (shoulder), 7.45 (s), 7.60 (s), 7.70 (s), 7.98 (m), 8.64 (m), 8.98 (m), 9.38 (m), 9.97 (s), 10.28 (m), 10.40 (w), 10.55 (w), 10.73 (w), 11.96 (w), 12.10 (w), 13.0 (w), 13.40 (w), and 13.95 (w).

Very slow crystallization from ethanol frequently yields the derivative as dense opaque yellow spherules with significant differences in the infrared, particularly in the 10–11-μ region.

Isolation of 2,4-dinitrophenyl(+)S-propyl-L-cysteine sulfoxide. The solid from the band just below the methyl derivative contained, in addition to 2,4-dinitrophenyl-*S*-propyl-cysteine sulfoxide, 2,4-dinitrophenylasparagine and slower-moving unidentified material. Chromatography a second time on a 38 × 765 mm. column containing 500 g. of buffered silicic acid with ethyl acetate yielded three poorly separated zones. Repeated chromatography of the middle zone on a silicic acid column of the same size yielded one band from which 10.7 mg. of yellow prisms was obtained by crystallization from 0.5 ml. of ethyl acetate. The product usually separates first as needles which gradually change to prisms. The compound was identified as 2,4-dinitrophenyl(+)*S*-propyl-L-cysteine sulfoxide by optical rotation and by infrared, $[\alpha]_D^{25}$ −133 (1,0.5; *c*, 0.247 in acetic acid). The synthetic compound has $[\alpha]_D^{25}$ −141.2.

The infrared spectrum in a potassium bromide pellet (0.91 mg./250 mg.) was identical with that of the authentic compound from 3–15 μ with absorption (μ) at 5.15 (m), 5.83 (s), 6.15 (s), 6.29 (s), 6.64 (s), 7.02 (s), 7.45 (s), 7.60 (s), 7.74 (s), shoulder on 7.74, 8.03 (s), shoulder on 8.03, 8.10 (s), 8.63 (m), shoulder on 8.63, 8.76 (m), 8.98 (m), 9.40 (m), 10.25 (s), 10.44 (s), 10.80 (m), 11.60 (w), 11.96 (m), 12.06 (m), 12.30 (m), 13.06 (w), 13.41 (m), 13.70 (w), and 13.90 (m).

Infrared spectra were determined on a Beckman IR-5 recording spectrophotometer.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF TEXAS, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE]

The Synthesis of *N*-(6-Purinyl)amino Acids. Amino Acids with a Single Reactive Amino Group[1a]

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A group of *N*-(6-purinyl)amino acids has been prepared from the corresponding amino acid and 6-chloropurine in carbonate-bicarbonate buffer. Purification was achieved by chromatography on anion exchange resins with formic acid elution. Instability of some of the compounds in acid solution was the major difficulty encountered.

Adenylosuccinic acid was isolated by Carter and Cohen[2] as the product of the enzymatic reaction of adenosine-5′-phosphate with fumaric acid. The aglycone of this compound, *N*-(6-purinyl)aspartic acid, was synthesized by Carter as part of the proof of structure.[3] Adenylosuccinic acid or the aglycone has subsequently been identified from a number of sources (yeast,[2] *E. coli*, mammalian liver, cod liver, human urine, and *Neurospora*[4]) and is also involved in other biological systems.[5,6]

The present study was undertaken to extend the series of *N*-(6-purinyl)amino acids started by Carter[3] and to make these compounds available in quantities sufficient for antitumor screening. Three methods of synthesis have been proposed.[7,8] Carter[3] utilized the reaction of 6-chloropurine with the free amino acid. In addition to the purine deriv-

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