Infrared studies on II and III showed C=C absorption at 1650 cm^{-1}, stronger in III. In the 1700–1735-cm^{-1} range, III has a doublet. Bands characteristic of II are at 1180 cm^{-1} and of III, at 1465, 1287, 1147, and 1075 cm^{-1}. For the acids IV and V, the C=C band was at 1647 cm^{-1}, stronger in V. Bands characteristic of IV are at 1082 and 858 cm^{-1} and for V, at 1145 and 1025 cm^{-1}.

Ultraviolet Irradiation of III.—A solution of III (100 mg) in 3 ml of ethanol in a stoppered quartz spectrophotometer cell was irradiated 6 in. from an unscreened Hanovia ultraviolet 325-w lamp for 4 hr in a hood. The alcohol was removed under vacuum and the residue was resolved by chromatography on silica gel (30 g) as described above. In this way there was obtained 38 mg of II and 57 mg of III. The identity of II was established by infrared spectra and hydrolysis to sarracinic acid.

Mikanecic Acid.—Compound V, the isomer of sarracinic acid, was heated with aqueous sodium hydroxide as described[7] for the conversion of sarracinic acid to mikanecic acid. After acidification, the aqueous solution was continuously extracted with ether for 6 hr. After drying and evaporation of the ether, the residue was recrystallized from aqueous acetone to give colorless crystals, mp 239–241° (lit.[7] mp 240°). A mixture melting point with VI prepared from sarracinic acid showed no depression.

Methyl 3-Hydroxy-2-bromopropanoate.—To a solution of 31 g (0.183 mole) of 2-bromo-3-hydroxypropanoic acid[15] in 150 ml of anhydrous ether, there was added dropwise with stirring at 5°, an ethereal solution of diazomethane (0.144 mole). After concentration by distillation to a volume of 200 ml, the ether solution was extracted with 10 ml of a saturated sodium bicarbonate solution, dried, and on evaporation of ether gave a 26-g residue which distilled at 58–60° (0.3 mm).

Anal. Calcd for $C_4H_7BrO_3$: C, 26.25; H, 3.86. Found: C, 25.69; H, 3.62.

Methyl 3-Acetoxy-2-bromopropanoate.—At 0° there was added dropwise with stirring 12.3 ml (0.17 mole) of freshly distilled acetyl chloride to 26.6 g (0.144 mole) of methyl 2-bromo-3-hydroxypropanoate in 25 ml of dry ether followed by 13.9 ml (0.17 mole) of anhydrous pyridine. After standing at room temperature for 30 hr, the mixture was poured into 500 ml of ice water and extracted with ether. The ether solution was dried and after removing the ether, the product was distilled at 74–75° (1.3 mm) in a 80% yield.

Anal. Calcd for $C_6H_9BrO_4$: C, 32.02; H, 4.03. Found: C, 32.29; H, 4.11.

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A Synthesis of L-Cystathionine and D-Allocystathionine[1]

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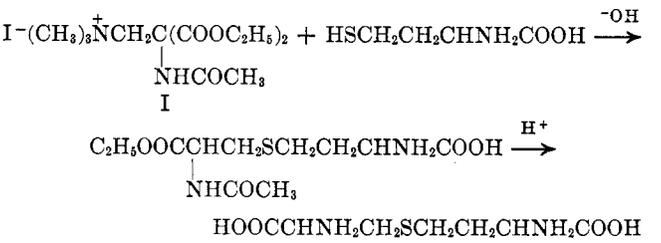
For metabolic studies with experimental animals, we needed convenient source of L-cystathionine. Diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide (I) was an intermediate available in the laboratory known to be useful for synthesizing a variety of β-substituted alanines. It had recently been employed in a condensation with $KC^{14}N$ to prepare 4-C^{14}-β-cyanoalanine, 4-C^{14}-aspartic acid, and 4-C^{14}-asparagine.[2-5] Its reaction with diethyl acetamidomalonate leads to 2,4-diaminoglutaric acid[6] and with sulfide ion and sodium benzylmercaptide, to lanthionine and S-benzylcysteine, respectively.[7,8] Condensation of I with the nucleophilic sulfur of homocysteine was therefore expected to be potentially capable of yielding cystathionine. The present Note reports a synthesis of a mixture of L-cystathionine and D-allocystathionine through this route, as shown in the diagram below.[9]



Also described are the separation of the formed diastereomeric mixture into L-cystathionine and D-allocystathionine in high yield, and a convenient method for analyzing such mixtures of diastereomers. This route is suitable for large-scale preparations of cystathionine. By substituting D-homocysteine, it should serve equally well to prepare D-cystathionine and L-allocystathionine. By employing I prepared from either labeled formaldehyde or labeled diethyl acetamidomalonate, the synthesis should yield L-cystathionine labeled with C^{14} in the cysteine moiety.

Compound I was prepared, in 50-g batches, by the method of Atkinson, *et al.*[7] Although its melting point differed significantly from that reported,[4] it had the expected elementary composition. Commercial L-methionine served as the other starting material. Other syntheses of cystathionine that use an L-homocysteinyl unit have frequently started from DL-methionine, which is converted to S-benzyl-DL-homocysteine and then resolved.[9b] For this, conversion of the S-benzylhomocysteine to an appropriate N-acyl derivative, followed by formation of diastereomeric salts,[10] or by stereospecific enzymatic anilide synthesis[9b,11] or hydrolysis,[12] has been required. In the present synthesis, S-benzyl-L-homocysteine was prepared directly from L-methionine[13] by refluxing with benzyl chloride and HCl, as described by Armstrong[14] for the preparation of S-benzyl-DL-homocysteine from DL-methionine. The crude S-benzyl-L-homocysteine was obtained on a 2-mole scale in 50% yield. After one reprecipitation, its optical rotation agreed with that of material prepared through the enzymatic synthesis of N-acetyl-S-benzyl-L-homocysteine anilide.[9b,11] The rotation, which

(1) This work was aided by U. S. Public Health Service grant NB 04316 and by Muscular Dystrophy Associations of America.

(2) C. Ressler, Y.-H. Giza, and S. N. Nigam, *J. Am. Chem. Soc.*, **85**, 2874 (1963), footnote 8.

(3) Y.-H. Giza and C. Ressler, unpublished results.

(4) R. O. Atkinson, *J. Chem. Soc.*, 3317 (1952).

(5) H. Hellmann and E. Folz, *Chem. Ber.*, **88**, 1944 (1955).

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(7) R. O. Atkinson, F. Poppelsdorf, and G. Williams, *J. Chem. Soc.*, 580 (1953).

(8) For reactions of I with other nucleophiles, see H. Hellmann and E. Folz, *Chem. Ber.*, **89**, 2000 (1956).

(9) For a review of other syntheses of cystathionine, see (a) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1961, pp 2683–2687; (b) A. Schöberl and G. Täuber, *Ann. Chem.*, **599**, 23 (1956); (c) A. Schöberl and J. Borchers, *Angew. Chem.*, **77**, 591 (1965).

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(12) S. M. Birnbaum and J. P. Greenstein, *Arch. Biochem. Biophys.*, **42**, 212 (1953).

(13) This route to S-benzyl-L-homocysteine has been noted briefly before but without detail and yield [W. Sakami, *Biochem. Prepn.*, **8**, 8 (1961)].

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was a little lower than the highest reported values,[12,15] did not change significantly on further recrystallization.

I was coupled in aqueous solution with an equimolar amount of L-homocysteine generated from the S-benzyl derivative by treatment with sodium in liquid ammonia.[10] The crude monoacetylcystathionine derivative obtained after removal of the solvent was triturated with acetone to remove sodium iodide. Hydrolysis in 2 *N* HCl afforded cystathionine in 90% yield, as determined by amino acid analysis of the crude hydrolysate. The product was crystallized from aqueous solution at pH 6 in 76% yield. It consisted of 43% L-cystathionine and 57% D-allocystathionine.

For determining the composition of this material and for following its fractionation into L-cystathionine and D-allocystathionine, polarimetric measurements could not be used since the optical rotations of both diastereomers are similar: L-, $[\alpha]^{20}D$ +23.7°;[16] and D-allo-, $[\alpha]^{21}D$ +24.5° (*c* 1, 1 *N* HCl).[17] Chromatography on columns of the cation resin Dowex 50 has been known to resolve several pairs of diastereomeric amino acids.[18] Blackburn and Schöberl[19] observed the separation in this way of mixtures of D-allo- and L-cystathionine and L-allo- and D-cystathionine, each into two peaks, but these were not characterized definitively. In other studies,[20] a commercial mixture of DL- and allocystathionine was observed to separate into two peaks under certain modified conditions on the automatic amino acid analyzer.[21] The chromatographic behavior on the automatic amino acid analyzer has now been established for the four authentic diastereomers. L-Allo- and D-allocystathionine are eluted together in the modified system as a single peak, followed by L- and D-cystathionine which also emerge as a single peak. Thus, the composition of a sample containing either L- or D-cystathionine mixed with either allo diastereomer can be determined quantitatively on the amino acid analyzer.

Previously reported attempts to fractionate L-cystathionine and D-allocystathionine include conversion to a mixture of the N,N′-dibenzoyl derivatives and separation of these by fractional crystallization. However, the rotations of the cystathionine products after debenzoylation were not reported.[9b,c] It is not certain that this route can yield optically pure diastereomers since McHale, Mamalis, and Green[22] found partial racemization on the acid hydrolysis of N,N′-dibenzoyl-D-cystathionine. Similarly, an attempted resolution of a mixture of L-allo- and D-cystathionine, by the papain-catalyzed synthesis of N,N′-dibenzoyl-L-allocystathionine monoanilide, gave L-allocystathionine with a low rotation[9b] apparently because of the presence of diastereomer.[19] Armstrong[23] resolved a mixture of L- and L-allocystathionine directly by fractional crystallization of the amino acids. His procedure (based on the lower solubility of L-allocystathionine in ammonia solution and the lower solubility of L-cystathionine in slightly acid solution of pH 4) after three crystallizations of the initially obtained fractions gives L-cystathionine and L-allocystathionine in 15 and 17% yield, respectively. An attempt was made to resolve the presently prepared mixture of D-allo- and L-cystathionine in this way. D-Allocystathionine appeared to be less soluble than the L-allocystathionine described, and it was readily obtained after two crystallizations in 76% yield and 96% stereochemical purity. However, the procedure was less suitable for freeing L-cystathionine completely from the diastereomer, since a fraction containing 82% L-cystathionine, obtained in 23% yield after one recrystallization, was not enriched by recrystallization at pH 4.

The fractionation procedure has been suitably adapted by reducing the volume of ethanol used in the initial fractionation step, and by carrying out the crystallization of L-cystathionine at pH 2 and at room temperature. A single crystallization of the initially obtained fractions now affords D-allocystathionine of 98% stereochemical purity and L-cystathionine of 96% stereochemial purity, each in 47% yield. If desired, a second crystallization gives the pure diastereomers which have the correct elementary analyses.

Experimental Section[24]

Amino Acid Analyses.[24]—Total crude yields of cystathionine in hydrolyzed mixtures were determined by analysis in system 1, in which all the diastereomers emerge as a single peak 7–8 ml after the indication of the pH change. Fractionation of the mixture of D-allo- and L-cystathionine was followed in system 2. In this system reference samples of L-allocystathionine and D-allocystathionine are eluted together as a single peak near 600 ml. Authentic L-cystathionine and D-cystathionine are eluted as a single peak directly after the allocystathionines, the broad peaks being separated by 29 ml at their apexes.

S-Benzyl-L-homocysteine.—The procedure described[14] for preparing S-benzyl-DL-homocysteine was followed except that L-methionine replaced the starting DL-methionine. The crude product, obtained on precipitation at pH 5 from acid solution with concentrated ammonia, was filtered off and washed thoroughly by resuspending it several times in water and filtering. The product was then washed on the filter until free of chloride ion. The total volume of water required was about 7 l. The solid was washed with ethanol and dried in air and then *in vacuo* over P_2O_5. A run with 298 g of L-methionine afforded 222 g (49.5%) of crude product melting at 240–242°.

The pulverized material (20.0 g) was suspended in 400 ml of hot water; concentrated HCl was added to effect solution. After clarification with charcoal, the hot solution was neutralized dropwise with concentrated ammonia with vigorous manual stirring. The mixture was chilled overnight and filtered, and the product was washed with water until free of chloride ion: wt 16.5 g, mp 236–241° dec, $[\alpha]^{26}D$ +22.9° (*c* 1, 1 *N* HCl); lit. mp 235°[17] and 241–242°;[11] $[\alpha]^{18}D$ + 22.4°,[9b] $[\alpha]^{22}D$ +23.7°,[11] $[\alpha]^{26}D$ +24–25°,[25] and $[\alpha]^{26}D$ +27.2°.[15] This product frequently contained a trace of impurity, insoluble in 1 *N* HCl, which was removed by allowing a solution of 1 g in 15 ml of 1 *N* HCl to stand at room

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(25) V. du Vigneaud and G. B. Brown, *Biochem. Prepn.*, **5**, 84 (1957).

temperature for 1 hr, followed by filtration and neutralization in the usual way, $[\alpha]^{24}D$ +22.1°. A subsequent recrystallization of 1 g of this material from 500 ml of water gave 0.75 g melting at 241–244° dec, $[\alpha]^{24}D$ +23.2°.

Diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide was prepared as described.[7] From two runs with 50 g of diethyl acetamidomalonate, the yield of crude product melting at 151–156° was 76%. One recrystallization from ethanol gave 124 g (65%), mp 159–160°. For analysis a sample was further recrystallized. It melted at 167.5–168.5°,[8] lit.[4] mp 384°.

Anal.[8] Calcd for $C_{13}H_{25}IN_2O_5$: C, 37.5; H, 6.05; N, 6.73. Found: C, 37.6; H, 6.02; N, 6.83.

L-Cystathionine and D-Allocystathionine Mixture.—Once-recrystallized S-benzyl-L-homocysteine (16 g, 0.071 mole) and sodium (3.8 g, 0.17 g-atom) were added alternately in portions to 300 ml of liquid ammonia. The openings on the three-necked flask were protected with drying tubes of Mallcosorb, and a stream of dry nitrogen was led slowly over the mixture. The final permanent blue color was just discharged with a few crystals of ammonium chloride which sometimes left a brown solution. The ammonia was evaporated under the stream of nitrogen and then *in vacuo* with a water pump. The vacuum was released under nitrogen, and the residue was taken up in 300 ml of freshly boiled water. To the solution was added diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide (I, 29.6 g, 0.071 mole, mp 159–160°), and the mixture was adjusted to pH 10 with air-free 2 *N* HCl if necessary. The solution was placed in an oil bath; the internal temperature was maintained at 80–85°. Introduction of nitrogen was continued. The solution was maintained at pH 8–9 with air-free 3 *N* LiOH as needed. Evolution of trimethylamine was followed by having an outlet tube dip into 75 ml of 1 *N* HCl. After 4 hr the yield of trimethylamine was 72% as determined by back-titration with 1 *N* NaOH (phenolphthalein). The reaction mixture (pH 6) gave a negative test for sulfhydryl and disulfide with nitroprusside reagent. It was filtered through a pad of Celite, and the clear solution was evaporated to dryness. The thick residue was allowed to stand under 500 ml of acetone in the cold overnight. The acetone was decanted, and the residue was triturated four times with 100 ml of acetone with gentle refluxing over a steam bath and decanting. A 1-ml sample of the last acetone triturate gave a negative test for iodide with acidic silver nitrate. The material was freed of residual acetone at the water pump and then taken up in 600 ml of 2 *N* HCl. The solution was refluxed for 2 hr. An aliquot placed on the amino acid analyzer showed cystathionine in 90% yield. The brown solution was concentrated to dryness, and the red solid residue was taken up in 50–75 ml of hot water. The resulting red solution was clarified with Darco (2–3 g). The pale yellow filtrate was concentrated to incipient precipitation (50 ml). The solid was dissolved by heating, and the solution was neutralized with 15–20 ml of concentrated ammonia to pH 6. Crystallization started from the hot solution. After standing at room temperature and chilling overnight, the white solid was filtered off and washed with cold water (about 200 ml) until free of chloride ion, then with cold absolute ethanol, and then dried (wt 10.4 g, 66%, $[\alpha]^{24}D$ +23.7°). Amino acid analysis showed 57% D-allocystathionine and 43% L-cystathionine. The material gave a negative cyanide–nitroprusside test for disulfide. The mother liquor and washings were concentrated to 75 ml, and two volumes of ethanol were added to incipient crystallization. After cooling, filtration, and washing, a second crop of 1.5 g was obtained (over-all yield 76%). Paper chromatography (ascending, Whatman No. 3MM, 70% phenol) showed for both crops a single ninhydrin-positive spot, R_F 0.28, agreeing with that of authentic DL- plus allocystathionine.

Fractionation of L-Cystathionine and D-Allocystathionine Mixture. 1A.—Cystathionine (9.4 g, 50% D-allo- and 50% L-cystathionine) was suspended in boiling water (100 ml) and dissolved by additon of concentrated ammonia (20 ml; pH of solution, 9.6). The solution was brought to turbidity by careful addition of hot ethanol (30 ml) and allowed to sit at room temperature whereupon crystallization began. After chilling overnight, the solid was collected by filtration and washed with cold water (wt 3.1 g, 66% of theoretical yield of D-allo-).

Amino acid analysis showed 89% D-allocystathionine and 11% L-cystathionine (quantitative recovery).

1B.—This material was recrystallized in the same manner from 30 ml of hot water with addition of 14 ml of concentrated aqueous ammonia and 15 ml of hot ethanol (wt 2.2 g, 47% of theoretical yield of D-allo-).

Amino acid analysis showed 98% D-allocystathionine and 2% L-cystathionine (quantitative recovery).

For elemental analysis, a sample was recrystallized again at pH 9.6 and then at pH 5 by reprecipitation with ammonia from acidic solution: $[\alpha]^{26}D$ +25.3° ± 1.4% (*c* 1, 1 *N* HCl), lit.[17] $[\alpha]^{21}D$ +24.5°.

Anal. Calcd for $C_7H_{14}N_2O_4S$: C, 37.8; H, 6.35. Found: C, 37.5; H, 6.44.

Amino acid analysis showed less than 0.5% L-cystathionine with a quantitative recovery of D-allocystathionine.

IIA.—The filtrate and washings from 1A were concentrated to dryness, and the residue was dissolved in 60 ml of hot water by addition of concentrated HCl (4 ml). After clarification with charcoal, the hot solution was neutralized dropwise at the pH meter with concentrated ammonia. At pH 2 crystallization began. The solid was filtered after standing overnight at room temperature and washed on the filter with water until free of chloride ion (wt 2.2 g, 47% of theoretical yield of L-cystathionine).

Amino acid analysis showed 96% L-cystathionine, 4% D-allocystathionine (quantitative recovery).

For elemental analysis, a similar sample was recrystallized at pH 2: $[\alpha]^{27}D$ +23.9° ± 2% (*c* 1, 1 *N* HCl), lit. $[\alpha]^{20}D$ +23.9[23] and +23.7°.[16]

Anal. Calcd for $C_7H_{14}N_2O_4S$: C, 37.8; H, 6.35; N, 12.6; S, 14.4. Found: C, 37.5; H, 6.50; N, 12.3; S, 14.4.

Amino acid analysis showed quantitative recovery of L-cystathionine with no detectable D-allocystathionine.

IIB.—Neutralization of the mother liquor of IIA to pH 5 gave 2.4 g of cystathionine (47% D-allo-, 53% L-). Recovery of cystathionine in the three fractions, IA and IIA and B, amounted to 82%.

Acknowledgment.—We thank Miss Christine Lauinger and Mr. Arnold Benjamin for the amino acid analyses.

Reaction of Pyrrolidine with P-1-Aziridinyl-N,N,N′,N′-tetramethylphosphonic Diamides

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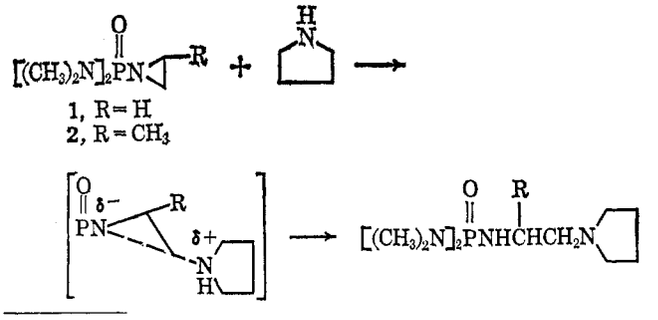
Recently Ham reported the bimolecular rate constants obtained from the reaction of 1-carboethoxyaziridine and substituted anilines in ethanol at 50°.[1] As part of our investigation of the chemistry of insect chemosterilants,[2] we wished to obtain comparable rate data for aziridines such as P-1-aziridinyl-N,N,N′,N′-tetramethylphosphonic diamide (**1**) and its 2-methyl analog (**2**). However, these compounds reacted



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