an oil which was purified by chromatography over acid-washed alumina: $n^{29}D$ 1.502; $[\alpha]^{31}D$ +24° (*c* 1.0); infrared bands at 1720, 1640, and 1620 cm^{-1}; nmr signals at 6.17 d (1.5) and 5.57 t (1, H_{13}), 4.72 d (2, 1.5) and 4.42 t (2, 2, H_{14}), 3.75 (methoxyl), and 0.74 ppm (C_{10}-methyl). These figures correspond closely to those reported[10] for methyl costate. Direct comparison of this material with an authentic sample of methyl costate established identity of infrared and nmr spectra. The two specimens were indistinguishable on tlc and had identical retention times on glpc.

Conversion of Ilicic Acid to Costic Acid.—To a solution of 1.50 g of methyl ilicate in 23 ml of pyridine was added dropwise, with cooling, 4.6 ml of phosphorus oxychloride. After 12 hr at room temperature, the mixture was poured into ice water and extracted with ether. The ether extracts were washed thoroughly with water, dilute acid, and water and were dried and evaporated *in vacuo*. The residue was chromatographed over acid-washed alumina and the product eluted with petroleum ether: wt 0.96 g, $n^{29}D$ 1.5055, $[\alpha]^{31}D$ +25.8° (*c* 2.145). Although the thin layer chromatogram of this material showed only one spot corresponding to that of authentic methyl costate, the nmr spectrum exhibited additional methyl singlets at 1.07 and 0.82 ppm corresponding to the C_{10}-methyl of the $\Delta^{4,5}$ and Δ^{3} isomers, respectively, an additional vinyl methyl singlet at 1.63 ppm ($\Delta^{4,5}$ and Δ^{3} isomers), and a vinyl proton multiplet at 5.33 ppm (Δ^{3} isomer). The intensity of these signals indicated a composition of *ca.* 70% methyl costate, 15% Δ^{3}, and 15% $\Delta^{4,5}$-isomer. Glc gave a main peak (*ca.* 75%) corresponding to methyl costate, with the minor peaks of the other two isomers almost superimposed.

The mixture of esters, wt 0.9 g, was hydrolyzed by refluxing with 25 ml of 6% methanolic potassium hydroxide for 1.5 hr. The mixture was concentrated at reduced pressure, diluted with water, acidified, and extracted with ether. The washed and dried ether extract was evaporated and the residual oil, wt 0.87 g, was chromatographed over 8 g of silicic acid. Benzene–petroleum ether (3:1) eluted semicrystalline material, wt 0.49 g, which after several recrystallizations from ethanol–water, melted at 88.5–89.5°, $[\alpha]^{30}D$ +28° (*c* 0.665), melting point undepressed on admixture of costic acid, infrared spectra superimposable.

The Configuration of (+)-S-(1-Propenyl)-L-Cysteine S-Oxide from *Allium cepa*

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Virtanen and Spåre[3] isolated from *Allium cepa* (onion) an amino acid which was shown to be the precursor of the lachrymatory property of the vegetable. The structure of this compound was established as (+)-S-(1-propenyl)-L-cysteine S-oxide[4] but the configuration of the olefinic double bond was left undecided. This report presents evidence based on nuclear magnetic resonance spectra that the compound is the *trans* isomer.

Our compound was isolated from commercial dehydrated onions and was shown to be identical with Virtanen's amino acid by elemental analysis, specific rotation, susceptibility to onion enzyme or to *Albizzia lophanta* C–S-lyase[5] to produce onion odor and lachrymatory properties, and particularly by the observation that the compound cyclizes in dilute ammonia to produce cycloalliin (3-methyl-1,4-thiazane-5-carboxylic acid S-oxide) in high yield. In the infrared, the compound shows strong absorption in the sulfoxide[6] region at 1025 and 1037 and also at 967 cm^{-1} suggesting a *trans* double bond.

The 60-Mc proton magnetic resonance (pmr) spectrum of the amino acid in deuterium oxide confirmed a *trans* configuration for the double bond. The ABX_3 multiplet at τ 3.4 arising from the olefinic protons clearly shows a 15–16 cps *trans* coupling between these protons, although an exact analysis of this complex multiplet was not attempted. To further strengthen this assignment a spectrum was also obtained at 100 Mc/sec. The additional separation of the two protons at the higher frequency makes the interpretation of the *trans* coupling unequivocal.

Two crystalline derivatives, the N-2,4-dinitrophenyl- and the N-2,4,6-trinitrophenylpropenylcysteine S-oxides were prepared. The pmr spectra of these compounds in deuterated dimethyl sulfoxide also showed a *trans* coupling of the olefinic protons (J = 16 cps).

Experimental Section

The pmr spectra were obtained on Varian A-60 and HR-100 spectrophotometers. Tetramethylsilane was the internal standard with deuterated dimethyl sulfoxide and sodium 3-(trimethylsilyl)-1-propanesulfonate was the internal standard with deuterium oxide. Infrared spectra were obtained with a Perkin-Elmer Model 237 instrument.

Isolation of (+)-S-(1-Propenyl)-L-cysteine S-Oxide.—Commercially dehydrated white onion powder (2 kg) was slowly added to 16 l. of boiling distilled water with vigorous stirring. The mixture was then allowed to stand for 4 min and 5 kg of ice was added. The slurry was filtered and the filtrate was adjusted to pH 4 with acetic acid. Approximately 20% of the juice was poured through a column of Dowex 50-X4 (H^+) (7 × 30 cm) and the column then eluted with 0.1 *M* sodium acetate adjusted to pH 6.5 with acetic acid. As soon as ninhydrin-positive material appeared in the eluate, 250-ml aliquots were collected. These were tested for the presence of the propenylcysteine sulfoxide by adding onion enzyme and organoleptically detecting the lachrymator. The amino acid emerges with the acidic amino acids and is completely eluted by the time a characteristic brown pigment emerges from the column. The remaining onion juice was treated with ion exchanger in the same manner and the combined fractions containing the amino acid precursor were passed through a second column of Dowex 50-X4 (H^+) (2.5 × 50 cm), and the amino acids were eluted with 0.05 *N* sodium hydroxide at 30 ml/hr and collected in 100-ml aliquots.

The fractions which contained the precursor were combined and passed through a column of Dowex 2-X8 (2 × 15 cm) in the acetate form to remove acidic amino acids. The lachrymatory precursor was not absorbed. Finally, the eluate was absorbed in a column of Dowex 50-X4 (2.5 × 50 cm); the amino acids were eluted with 0.05 *N* ammonium hydroxide. Fractions (50 ml) were collected and those that were chromatographically homogeneous on paper were combined, adjusted to pH 6.5 with acetic acid, and taken to dryness *in vacuo*. Several recrystallizations of the solid residue from aqueous acetone yielded the pure amino acid in a yield of 3 g from 2 kg of onion powder.

The compound decomposed sharply at 153° (lit.[3] dec pt 146–148°) and yielded one spot on paper chromatography with butanol–acetic acid–water (63:10:27) at 25°; relative R_f with respect to alanine 1.45; $[\alpha]^{26}D$ +74.9° (*c* 6.2, water) (lit.[3] value +74°).

Anal. Calcd for $C_6H_{11}NO_3S$: C, 40.66; H, 6.39; N, 7.90. Found: C, 40.8; H, 6.39; N, 7.84.

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Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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(3) A. I. Virtanen and C. G. Spåre, *Suomen Kemistilehti*, **B34**, 72 (1961); **B35**, 28 (1962).

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The infrared spectrum (potassium bromide disk) showed strong absorption at 1025 and 1037 cm^{-1} (sulfoxide region) and at 967 cm^{-1}.

Cyclization of Propenylcysteine Sulfoxide to Cycloalliin.—A solution of 120 mg of the amino acid in 80 ml of 1 *N* ammonium hydroxide was allowed to stand 4 days when it was concentrated *in vacuo* to a crystalline solid. The solid was dissolved in 5 ml of water, 5 ml of 2 *N* hydrochloric acid was added, and the solution was concentrated *in vacuo* at <25° to *ca.* 2 ml. The resulting crystalline suspension, after storage at 0° for 4 hr, was filtered and the product was washed with 0.5 ml of ice water and 2 ml of acetone. A yield of 102 mg of prisms was obtained. An additional 36 mg was obtained from the mother liquor by precipitation with acetone. The cycloalliin hydrochloride hydrate melted at 204–206° dec.

Anal. Calcd for $C_6H_{11}NOS \cdot HCl \cdot H_2O$: C, 31.09; H, 6.09; N, 6.04; S, 13.84. Found: C, 31.2; H, 5.94; N, 6.04; S, 14.0.

It was shown to be identical by infrared (KBr disk) and by paper chromatography with a sample of cycloalliin isolated from fresh onions.

N-2,4-Dinitrophenyl-S-(1-propenyl)-L-cysteine S-Oxide.—A solution containing 137 mg of the amino acid, 0.3 ml of 1-fluoro-2,4-dinitrobenzene, and 600 mg of sodium bicarbonate in 25 ml of aqueous acetone (1:1) was stirred overnight in the dark at room temperature. The solution was concentrated *in vacuo* to remove acetone, 20 ml of water was added, and the mixture was extracted with ether to remove excess reagent. Acidification of the aqueous solution with cold 3 *N* hydrochloric acid yielded a crystalline precipitate, 259 mg. Two crystallizations from acetone yielded the pure derivative, 173 mg, dec pt 147–147.5°, $[\alpha]^{25}D$ −218° (*c* 0.6, acetic acid).

Anal. Calcd for $C_{12}H_{13}N_3O_7S$: C, 41.98; H, 3.82. Found: C, 42.2; H, 3.84.

N-2,4,6-Trinitrophenyl Derivative.—A solution of 255 mg of the amino acid and 524 mg of sodium 2,4,6-trinitrobenzenesulfonate was stirred for 1 hr at 25° and the pH maintained at 6.5–7.0 by the careful addition of 4% sodium carbonate solution. The deep red solution was allowed to stand at room temperature for 1 hr, cooled in an ice bath, and acidified with cold 3 *N* hydrochloric acid. The resulting yellow precipitate was filtered, washed with 20 ml of ice water, and dried *in vacuo*, yield 455 mg. Three recrystallizations from acetone yielded 373 mg of canary yellow prisms, dec pt 130–131.5°.

Anal. Calcd for $C_{12}H_{12}N_4O_9S$: C, 37.12; H, 3.11. Found: C, 37.40; H, 3.28.

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A Synthetic Approach to Cephams

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A key intermediate in the total synthesis of penicillins, *t*-butyl-4-carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidine acetate, was prepared by the condensation of *t*-butyl phthalimidomalonaldehydate (**1**) with penicillamine.[2] This success (Scheme I) led to the possibility that *t*-butyl-4-carboxy-α-phthalimido-2-thiazane acetate (**3**) could be prepared by condensing **1** with DL-homocysteine (**2**). Compound **3** might then be used to prepare saturated analogs of cephalosporin C (cephams[3]).

In this laboratory **1** and **2** have been condensed under basic conditions to give a 30% yield of **3**. Only one isomer was obtained, mp 161–162°. The melting point indicates that it is identical with Eardley's isomer F,[4] mp 158–161°, which was stated to be formed together with isomer B under neutral conditions. Compound **3** is insoluble in dimethylformamide, acetone, ethanol, water, and pyridine. This insolubility is probably due to the dipolar ion structure.

Esterification of **3** with diazomethane generated the corresponding methyl ester **4** in 98% yield. The identity of this ester was determined by elemental analysis, infrared spectra, and nmr spectrum which confirmed the presence of an $-OCH_3$ at 3.8 ppm. By the action of hydrazine on **4**, the parent *t*-butyl-4-carbomethoxy-α-amino-2-thiazane acetate (**5**) was prepared in 25% yield.

The β-amino acid **6** is of special interest for the formation of the fused 1,3-thiazane β-lactam (**8**). A mixture of **6** and 2-phthalimidomethyl-4-carbomethoxythiazane hydrochloride (**7**) was obtained upon treatment of **4** with anhydrous hydrogen chloride at 0°. The major component of this mixture was **7**. Pure **6** was not isolated; attempted purification transformed the mixture into pure **7**. Treatment of **7** with pyridine gave a 95% yield of the free amine **9**. The acid **6** appears to be much more readily decarboxylated than the corresponding acid, 4-carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic acid hydrochloride, in the penicillin series. It has been reported that 2-phthalimido-4-carbomethoxy-5,5-dimethylthiazolidine hydrochloride is the principal product when *t*-butyl-4-carbomethoxy-α-phthalimido-5,5-dimethylthiazolidine is treated with anhydrous hydrogen chloride at temperatures above 90°; however, 2-phthalimido-4-carbomethoxy-5,5-dimethylthiazolidine hydrochloride is only a by-product at lower temperatures.[7]

Treatment of impure **6** with dicyclohexylcarbodiimide[8] afforded a complex mixture. An infrared spectrum of this mixture showed an absorption at 1760 cm^{-1}, which would be expected for a β-lactam. A tlc test for reactive β-lactams (using ammonia) was also positive. The complexity of the reaction mixture made it difficult to isolate the desired cepham **8** in pure form.

Wriston and Mackenzie have reported the synthesis of 1,3-thiazane-4-carboxylic acid (unreported yield) by condensing formaldehyde with homocysteine thiolactone hydrochloride (**10**) under basic conditions.[9] An attempted condensation of **1** with **10** at pH 8.0, however, produced a 63% yield of the Schiff base **11**.

(1) National Institutes of Health Predoctoral Fellow, 1963–1966.

(2) J. C. Sheehan and K. R. Henry-Logan, *J. Am. Chem. Soc.*, **81**, 3089 (1959).

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(4) After the completion of our work, a British patent came to our attention: S. Eardley, *et al.* (to Glaxo Group Ltd.), British Patent 985,966 (March 10, 1965), claim the preparation of *t*-butyl-4-carboxy-α-phthalimido-2-thiazane acetate (**3**) by condensing *t*-butyl phthalimidomalonaldehydate (**1**) with DL-homocysteine (**2**) under acidic or neutral conditions. By varying the conditions of the reaction they claimed to isolate four isomers, A, B, D, and F. It is surprising that the condensation can be run in neutral or acidic solutions since *t*-butyl esters are sensitive to hydrochloric acid[5] and homocysteine readily cyclizes to homocysteine thiolactone in neutral or acidic solutions.[6]

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