Synthesis of the Diastereoisomers of *S*-(2-Carboxypropyl)-L-Cysteine and their *S*-Mono- and *SS*-Di-oxides

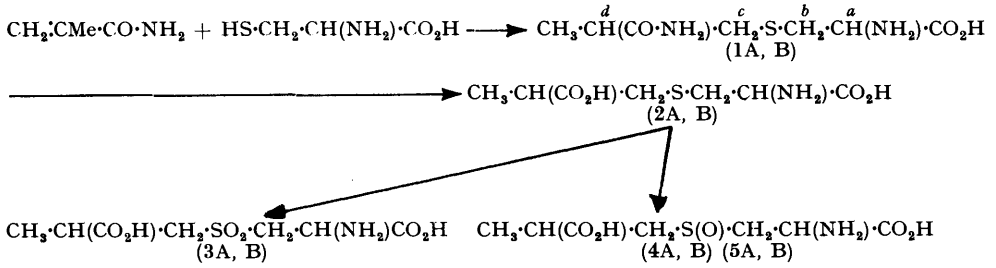
By **John F. Carson**, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710, U.S.A.

Addition of L-cysteine to methacrylamide followed by fractional crystallization yielded (+)- and (−)-*S*-(2-carbamoylpropyl)-L-cysteine, acidic hydrolysis of which gave the corresponding acids. These were converted into the corresponding two *N*-2,4-dinitrophenyl derivatives, two *SS*-dioxides, and four *S*-mono-oxides.

(−)-*S*-(2-CARBOXYPROPYL)-L-CYSTEINE (2A) occurs naturally as a component of the tripeptide *S*-(2-carboxypropyl)glutathione in the onion,[1] and in garlic.[2] Granroth [3] has shown that (2A) in the onion is enzymically decarboxylated with the introduction of a double bond, and is thus a biosynthetic precursor of *trans*-*S*-(prop-1-enyl)-L-cysteine. It was also observed [3] that introduction of a mixture of the diastereoisomers of ^{35}S-labelled 2-carboxypropylcysteine into the onion produced labelled *cis*- and *trans*-propenylcysteine. The (+)-*S*-oxide of the latter is the major flavour precursor of the onion.[4] (±)-2-Carboxypropyl-L-cysteine has been prepared by the reactions of β-bromoisobutyric acid or methacrylic acid with L-cysteine,[1] but in neither case was the mixture separated into the pure isomers.

This paper reports the synthesis of the naturally occurring (−)-isomer (2A) and also the (+)-isomer (2B). (3A and B) were prepared from the acids (2A and B) by an improved procedure involving oxidation with hydrogen peroxide in acetic acid–trifluoroacetic acid. Higher yields and purer products were obtained than with acetic acid–hydrogen peroxide.

The four diastereoisomeric *S*-oxides (4A), (5A), (4B), and (5B) were prepared by oxidation of the acids (2A and B) and were separated by fractional crystallization. Ordinarily for *S*-oxides of acyclic *S*-substituted cysteines, the two isomers have $[\alpha]_D$ values (H_2O) of opposite signs, with a large spread in the values and the *SS*-dioxides usually have rotations close to zero.[6] In the present case, the *SS*-dioxide (3A) and the *S*-oxides (4A) and (5A) of the (−)-acid had negative $[\alpha]_D$ values, and (3B), (4B), and (5B) derived from the (+)-acid had positive rotations, showing the dominant effect of the chiral centre attached to the side-chain carboxy-group.



Addition of L-cysteine to methacrylamide yielded a mixture of diastereoisomeric 2-carbamoylpropyl-L-cysteines. Fractional crystallization yielded the (−)-isomer (1A) (the less soluble product) and the (+)-isomer (1B). Acidic hydrolysis yielded the (−)-acid (2A) and the (+)-isomer (2B). Alternatively, the mixture of amides was hydrolysed directly and the isomeric acids were separated by fractional crystallization.

The *N*-2,4-dinitrophenyl (Dnp) derivatives of the (+)- and (−)-acids showed large negative optical rotations in agreement with the general rule that Dnp-L-amino-acids have negative rotations in acetic acid.[5] *S*-Substituted Dnp-L-cysteines in acetic acid have large negative rotations even in the presence of other chiral centres such as an *S*-oxide group.[6] The *SS*-dioxides

EXPERIMENTAL

I.r. spectra were obtained for KBr pellets with a Perkin-Elmer 237 spectrophotometer (maxima reported in cm^{-1}). N.m.r. spectra were obtained with a Varian HR100 instrument [reference DSS (sodium 3-trimethylsilylpropane-1-sulphonate) or TSP (sodium 3-trimethylsilylpropionate)]. Specific rotations were measured with a Bendix automatic polarimeter (series 1100) with a cell of 2 cm path length and incident light of wavelength 589 nm. Methacrylamide was prepared [7] from acetone cyanohydrin.

S-(2-*Carbamoylpropyl*)-L-*cysteine* (1A *and* B).—A solution of L-cysteine hydrochloride hydrate (20 g, 0.114 mol), methacrylamide (15 g, 0.176 mol), and sodium hydrogen carbonate (14 g) in water (800 ml) was heated under nitrogen for 4 h at 95 °C, cooled, treated with acetic acid (10 ml) and passed through a column of Dowex 50 resin

[1] A. I. Virtanen and E. J. Matikkala, *Z. physiol. Chem.*, 1960, **322**, 8.
[2] T. Suzuki, M. Sugii, and T. Kakimoto, *Chem. and Pharm. Bull. Japan*, 1962, **10**, 328.
[3] B. Granroth and A. I. Virtanen, *Acta Chem. Scand.*, 1967, **21**, 1654; B. Granroth, *Ann. Acad. Sci. Fennicae, Ser. A*, 1970, **154**.
[4] C. G. Spåre and A. I. Virtanen, *Acta Chem. Scand.*, 1963, **17**, 641; A. I. Virtanen, *Phytochem.*, 1965, **4**, 207.
[5] K. R. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, 1954, **76**, 1328.
[6] J. F. Carson and F. F. Wong, *J. Org. Chem.*, 1961, **26**, 4997. J. F. Carson and L. Boggs, *ibid.*, 1965, **30**, 895; 1966, **31**, 1634, 2862.
[7] E. C. Hornung, *Org. Synth.*, Coll. Vol. 3, 1955, p. 560.

(H^+) (500 cm^3). The resin was washed with water and the product eluted with 2N-ammonium hydroxide (2 l). Concentration of the eluate *in vacuo* yielded a solid which was redissolved in water (250 ml) and slowly passed through 200 cm^3 of Dowex 1 (acetate) to remove acidic impurities. The resin was washed with water (1 l); concentration of the effluent *in vacuo* yielded a mixture of diastereoisomeric amides as a white solid (21.0 g, 89%), $[\alpha]_D^{25}$ −22.9° (*c* 2.8 in H_2O). Slow crystallization (25 °C) from water (50 ml) and ethanol (75 ml) for 3 days yielded tufts of needles (7.63 g), $[\alpha]_D^{25}$ −51.4° (H_2O). The residue from the mother liquor on slow crystallization from water (25 ml) and ethanol (50 ml) yielded prisms (7.29 g), $[\alpha]_D^{25}$ +2.8° (H_2O). Four recrystallizations of the laevorotatory fraction from water–ethanol (1 : 2) yielded (−)-S-(2-*carbamoylpropyl*)-L-*cysteine* (1A), tiny needles (3.14 g), m.p. 200—202° (decomp.), $[\alpha]_D^{25}$ −75.3° (*c* 2.5 in H_2O), unchanged on repeated crystallization; $\nu_{max.}$ 1 600—1 650s (CO_2^- + amide), δ (D_2O; ref. TSP) 1.21 (3 H, d, CH_3),* 2.75 (3 H, d, *c* and *d*), 3.10 (2 H, m, *b*), and 3.90 (1 H, q, *a*) (irradiation at δ 2.77 caused collapse of the CH_3 doublet to a singlet) (Found: C, 40.9; H, 6.9; N, 13.6. $C_7H_{14}N_2O_3S$ requires C, 40.75; H, 6.85; N, 13.6%).

Four recrystallizations of the second fraction from water–ethanol (1 : 2) yielded prisms or blades (1.72 g) of the (+)-*amide* (1B), m.p. 191.5—193° (decomp.), $[\alpha]_D^{25}$ +46.3° (*c* 3 in H_2O); $\nu_{max.}$ 1 615s (CO_2^- + amide); n.m.r. data similar to those of the (−)-isomer (Found: C, 40.8; H, 6.9; N, 13.6%).

(−)-S-(2-*Carboxypropyl*)-L-*cysteine* (2A) *by Hydrolysis of the Amide* (1A).—A solution of (1A) (6.10 g, 0.0296 mol) with $[\alpha]_D$ −66° [92% (−)-isomer] in 2.5N-hydrochloric acid (400 ml) was heated in a boiling water bath for 8 h, then concentrated *in vacuo* to dryness. The residue was dissolved in water (100 ml) and passed through Dowex 1 (acetate) (300 cm^3). The ion exchanger was washed with water and developed with N-acetic acid (1 500 ml). Concentration of the eluate *in vacuo* yielded a crystalline solid (5.6 g, 92%). Two recrystallizations from water–acetone (1 : 2; 120 ml) gave the (−)-*acid* (2A) (4.0 g), m.p. 196—198° (decomp.), $[\alpha]_D^{25}$ −66.1° † (*c* 2.5 in H_2O) and −44.3° (*c* 2.4 in 2.5N-HCl); $\nu_{max.}$ 1 685 (CO_2H) and 1 600s (CO_2^-); δ (D_2O; ref. DSS) 1.25 (3 H, d, CH_3),* 2.80—3.20 (5 H, m), and 3.98 (1 H, q); δ (D_2O–NaOD; ref. TSP) 1.17 (3 H, d, CH_3), 2.40—3.20 (5 H, m), and 3.60—4.20 (1 H, unresolved) (Found: C, 40.5; H, 6.25; N, 6.7. $C_7H_{13}NO_4S$ requires C, 40.55; H, 6.3; N, 6.75%).

(+)-S-(2-*Carboxypropyl*)-L-*cysteine* (2B).—A solution of (1B) (5.65 g, 0.0274 mol) with $[\alpha]_D$ +37.7° [93% (+)-isomer] in 2.5N-hydrochloric acid (400 ml) was treated as described for the (−)-isomer. The crude product was crystallized from water (50 ml); yield 4.1 g, $[\alpha]_D$ +33.5° (H_2O). Recrystallization from water gave pure (+)-*acid* (2B), prisms, m.p. 199—200° (decomp.), $[\alpha]_D^{25}$ +35.8° (*c* 1.25 in H_2O) and +40.4° (*c* 2 in 2.5N-HCl); $\nu_{max.}$ 1 680s (CO_2H) and 1 600s (CO_2^-); δ (D_2O–NaOD; ref. TSP) 1.15 (3 H, d, CH_3), 2.05—3.00 (5 H, m), and 3.20—3.60 (1 H, unresolved) (Found: C, 40.6; H, 6.35; N, 6.75%).

Hydrolysis of (±)-S-(2-*Carbamoylpropyl*)-L-*cysteine and Isolation of the Acids* (2A *and* B).—From a mixture of (1A and B) (5.7 g), by acidic hydrolysis and purification as above, was obtained crude (±)-2-carboxypropyl-L-cysteine (5.0 g). Crystallization from water (40 ml) yielded material (3.43 g), $[\alpha]_D^{25}$ −22.3° (H_2O), and residue from the mother liquor on crystallization from aqueous acetone yielded material (1.41 g), $[\alpha]_D$ −44°. Five recrystallizations of the first fraction from water yielded the acid (2B) (0.53 g), $[\alpha]_D$ +34.4° (*c* 1.24 in H_2O). Three recrystallizations of the second fraction from aqueous acetone yielded the acid (2A) (0.39 g), $[\alpha]_D^{25}$ −60.1° (*c* 1.9 in H_2O).

The SS-*Dioxides* (3A *and* B).—A solution of the acid (2A) (0.60 g) in acetic acid (60 ml) and trifluoroacetic acid (15 ml) containing 30% hydrogen peroxide (12 ml) was kept for 24 h at 25 °C and then concentrated *in vacuo* to a solid. Trifluoroacetic acid was removed by passing an aqueous solution (50 ml) through 200 cm^3 of Dowex 1 (acetate). The resin was developed with N-acetic acid (800 ml) and the eluate evaporated *in vacuo* to a solid. Crystallization from water (15 ml) yielded blades (0.36 g). An additional 0.26 g was obtained from the mother liquor (ethanol–water, 2 : 1). Pure (−)-SS-*dioxide* (3A), decomposed sharply at 174° (gas evolved); $[\alpha]_D^{25}$ −24° (*c* 1.7 in H_2O); $\nu_{max.}$ 1 700m (CO_2H), 1 600s (CO_2^-), 1 290s (sulphone), and 1 130s (sulphone); δ (D_2O–NaOD; pH 6; ref. H_2O) 2.16 (3 H, d, CH_3), 2.31—2.44 (5 H, m), and 2.45—2.47 (1 H, q) (Found: C, 35.1; H, 5.5; N, 5.8. $C_7H_{13}NO_6S$ requires C, 35.15; H, 5.5; N, 5.85%).

Similarly from the acid (2B) (1.23 g) was obtained the SS-*dioxide* (3B) (1.11 g, 78%) as blades (from water), decomp. 176°, $[\alpha]_D^{25}$ +14° (*c* 1.97 in H_2O); $\nu_{max.}$ 1 700m (CO_2H), 1 620s (CO_2^-), 1 280s (sulphone), and 1 135s (sulphone); δ (D_2O–$CF_3{\cdot}CO_2D$; ref. H_2O) 2.13 (3 H, d, CH_3), 2.29—2.42 (5 H, m), and 2.46—2.47 (1 H, q) (Found: C, 35.2; H, 5.55; N, 5.9%).

The (−)-S-*Oxides* (4A) *and* (5A).—To a solution of the acid (2A) (5.16 g, 0.025 mol) in water (200 ml) was added 30% hydrogen peroxide (5 ml) in 4 equal portions over 4 h. After 18 h at 25 °C the solution was concentrated *in vacuo* to a solid, which was crystallized from water (100 ml) to yield prisms (1.77 g), $[\alpha]_D^{24}$ −24° (H_2O). Crystallization of the residue from the mother liquor from water (15 ml) yielded a mixture of prisms and needles (0.36 g). A third fraction was obtained from aqueous ethanol as needles (2.42 g), $[\alpha]_D$ −13° (H_2O). Recrystallization of the first fraction from water (75 ml) yielded the less soluble *sulphoxide* (4A), prisms (1.45 g), decomp. sharply at 181°; $[\alpha]_D$ −25° (*c* 0.85 in H_2O); $\nu_{max.}$ 1 700s (CO_2H), 1 600s (CO_2^-), and 990s (sulphoxide); ‡ ‡ δ (D_2O–$CF_3{\cdot}CO_2D$; ref. DSS) 1.39 (d, CH_3), 3.00—3.78 (5 H, m), and 4.62—4.78 (t, overlapped with internal standard) (Found: C, 37.5; H, 5.7; N, 6.3. $C_7H_{13}NO_5S$ requires C, 37.65; H, 5.85; N, 6.25%).

The last two fractions of sulphoxide were recrystallized from ethanol–water (2 : 3; 50 ml) to yield the pure more soluble *sulphoxide* (5A) as needles (1.64 g), decomp. 155°, $[\alpha]_D$ −12° (*c* 3 in H_2O), unchanged on recrystallization; $\nu_{max.}$ 1 700s (CO_2H), 1 625m (CO_2^-), and 975s (sulphoxide); ‡ ‡ δ (D_2O–$CF_3{\cdot}CO_2D$; ref. DSS) 1.38 (d, CH_3), 3.00—3.60 (5 H, m), and 4.43—4.58 (t) (Found: C, 37.8; H, 6.05; N, 6.3%).

The (+)-S-*Oxides* (4B) *and* (5B).—The acid (2B) (6.19 g,

* Additional lines observed due to virtual coupling with the protons *c*.

† Virtanen and Matikkala [1] report $[\alpha]_D^{21}$ −50.1° (H_2O) for their amino-acid isolated after hydrolysis of *S*-(2-carboxypropyl)-glutathione.

‡ The unusually low sulphoxide stretching frequencies of (4A) and (5A) are probably due to hydrogen bonding.

0.0299 mol) in water (400 ml) was oxidized with 30% hydrogen peroxide (6.4 ml) as above. The crude product, $[\alpha]_D$ +23° was crystallized slowly (3 days) from water (90 ml) at 25 °C to yield prisms and plates (1.52 g), $[\alpha]_D$ +21° (H_2O). The residue from the mother liquor on crystallization from water (50 ml) yielded prisms (1.6 g), $[\alpha]_D$ +27.6°. Slow crystallization of the first fraction from water (16 ml) yielded the *sulphoxide* (5B), plates (0.63 g), decomp. 162.5°, $[\alpha]_D^{25}$ +16° (*c* 2 in H_2O); $\nu_{max.}$ 1 680s (CO_2H), 1 625s (CO_2^-), and 1 020s (sulphoxide); δ (NaOD–D_2O; ref. TSP) 1.29 (3 H, d, CH_3), 2.60—3.40 (5 H, m), and 3.62—3.80 (1 H, t).

Recrystallization of the second fraction, $[\alpha]_D$ +27.6° from water (12 ml) (5 h at 25 °C) yielded the *sulphoxide* (4B) (1.16 g), $[\alpha]_D$ +29.0° (*c* 2.3 in H_2O), unchanged on repeated recrystallization, decomp. 170°; $\nu_{max.}$ 1 685s (CO_2H), 1 625s (CO_2^-), 1 020s (sulphoxide), and 1 010s (sulphoxide); δ (NaOD–D_2O; ref. TSP) 1.29 (3 H, d, CH_3), 2.60—3.35 (5 H, m), and 3.62—3.80 (1 H, t). The purity of these two diastereoisomeric sulphoxides was confirmed by n.m.r. spectra; elemental analyses agreed with calculated values.

(−)-S-(2-*Carboxypropyl*-N-(2,4-*dinitrophenyl*)-L-*cysteine*. —The acid (2A) (0.83 g) was treated with 1-fluoro-2,4-dinitrobenzene as previously described.[6] The resulting viscous oil was extracted with hot water (300 ml). The aqueous extract after refrigeration for several days deposited an amber gum gradually changing to yellow needles (0.62 g). An additional 0.43 g was obtained by concentrating the mother liquor. The derivative could not be crystallized from organic solvents. It had $[\alpha]_D^{25}$ −117° (*c* 1.5 in acetic acid) and melted over a range (107—118°) (Found: C, 41.7; H, 4.2; N, 11.1. Calc. for $C_{13}H_{15}N_3O_8S$: C, 41.8; H, 4.05; N, 11.25%).

(+)-S-(2-*Carboxypropyl*)-N-(2,4-*dinitrophenyl*)-L-*cysteine*. —This derivative was prepared from the acid (2B) (0.75 g) and crystallized from water as in the previous example to yield tiny needles (0.98 g), melting over a broad range (133—142°), $[\alpha]_D^{25}$ −92.6° (*c* 2 in acetic acid) (Found: C, 42.2; H, 4.2; N, 11.2%).

I thank R. Lundin for n.m.r. determinations, G. Secor for elemental analyses, and F. F. Wong for i.r. spectra.

[7/074 *Received*, 17*th January*, 1977]