460. *New Syntheses of Cystine.*

By J. C. Crawhall and D. F. Elliott.

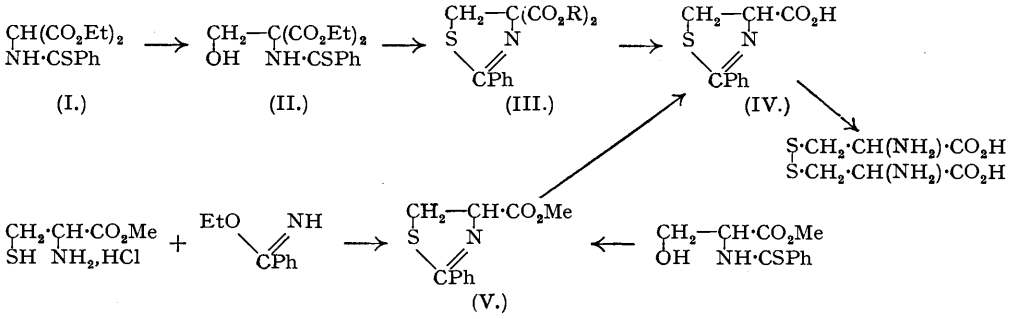
Serine * was converted into *N*-thiobenzoylserine methyl ester which gave methyl 2-phenyl-Δ^2-thiazoline-4-carboxylate on treatment with thionyl chloride. Hydrolysis of the thiazoline derivative yielded cystine. This method of replacing a hydroxyl group by a thiol group was utilised in the synthesis of cystine from aminomalonic ester. This ester was converted into thiobenzamidomalonic ester and into ethyl *N*-dithiocarbobenzyloxyaminomalonate. These substances were condensed with formaldehyde, and the products converted by the action of thionyl chloride into thiazolines from which cystine was obtained.

It was recently found that replacement of the β-hydroxyl group by a thiol group occurred when *N*-thiobenzoylserine methyl ester was treated with thionyl chloride (Elliott, *Nature*, 1948, **162**, 658). The good yield obtained and the simplicity of the procedure prompted a more detailed investigation of the reaction for synthetic purposes. Cystine has now been synthesised from aminomalonic ester by two related routes, this replacement reaction being used at an intermediate stage. The β-carbon atom of the cystine molecule was introduced by aldol condensation of formaldehyde with a thioacylaminomalonic ester, a reaction which gave excellent yields as in the case of the synthesis of serine from acetamidomalonic ester described by King (*J. Amer. Chem. Soc.*, 1947, **69**, 2738). This method of introducing a carbon atom is also valuable because it provides a convenient route to cystine containing a radioactive β-carbon atom.

Aminomalonic ester was conveniently purified and stored as its hydrochloride from which thiobenzamidomalonic ester (I) was prepared by reaction with (thiobenzoylthio)acetic acid and aqueous alkali according to Holmberg's method (Thé Svedberg Anniversary Volume, p. 299, Uppsala : Almquist and Wiksellis, 1944). It was later found more convenient to carry out this reaction in pyridine with use of triethylamine as a base. Addition of formaldehyde

* All substances containing asymmetric carbon atoms described in this paper were the DL-isomers.

to the thiobenzoyl compound (I) proceeded smoothly to give ethyl α-carbethoxy-β-hydroxy-α-thiobenzamidopropionate (II) in almost theoretical yield. In this condensation pyridine was used as a solvent and catalyst, a method due to Arnstein (*Nature*, 1949, **164**, 361) which is more convenient, when dealing with substances sparingly soluble in water, than the aqueous medium used by King (*loc. cit.*). When the thiobenzoyl compound (II) was added to ice-cold thionyl

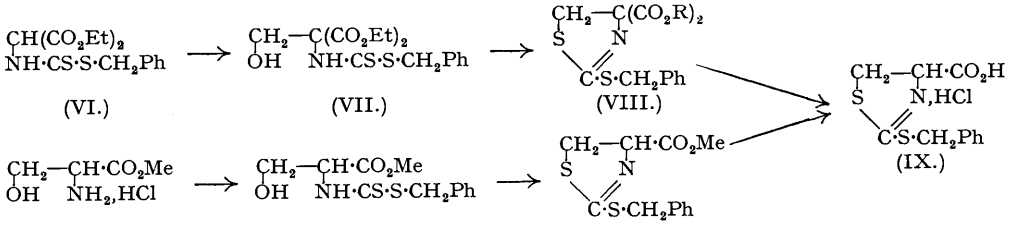


chloride an immediate liberation of gas took place, but it was found that the yield of 4 : 4-dicarbethoxy-2-phenyl-Δ^2-thiazoline (III) was greatly reduced if the reaction mixture was not kept for 30 minutes after the reaction had apparently ceased. It is possible that an intermediate chlorosulphinic ester was produced and cyclised relatively slowly to the thiazoline (III). Indirect evidence in favour of the formation of a compound of this type in related cyclisation reactions was obtained during some experiments on the synthesis of threonine (Elliott, *J.*, 1949, 589). The thiazoline ester (III) appeared to be very feebly basic, a fact which might be explained by the proximity of the carbethoxy-groups to the nitrogen atom, and no solid derivatives were obtained from it. Hydrolysis of the ester groups occurred very rapidly when alcoholic sodium hydroxide was added to the thiazoline ester, and produced a precipitate of the disodium salt (III; R = Na) of the corresponding dicarboxylic acid. The free acid was evidently very unstable because treatment of the disodium salt with dilute acid yielded 2-phenyl-Δ^2-thiazoline-4-carboxylic acid (IV) with spontaneous liberation of carbon dioxide. The structure assigned to the acid (IV) was confirmed by its synthesis, as indicated in the preceding scheme, by two independent routes: (1) from thiobenzoylserine methyl ester as already briefly described by Elliott (*loc. cit.*); (2) from cystine methyl ester hydrochloride by condensation with ethyl benzimidate (compare Jacobsen, *Compt. rend. Trav. Lab. Carlsberg, Sér. Chim.*, 1947, **26**, No. 1) followed by mild alkaline hydrolysis. Acid hydrolysis of the thiazoline ester (V) or the acid (IV) followed by oxidation gave inactive cystine in good yield.

The preparation of (thiobenzoylthio)acetic acid from benzotrichloride by Holmberg's method (*Arkiv Kemi, Min. Geol.*, 1944, **17**, *A*, No. 23) was inconvenient because of the long time required, and we were unable to achieve the yield claimed by Holmberg. By a modified procedure it was possible to prepare the substance in 40% yield in one day. As the starting materials used in the preparation are cheap and accessible, the low yield obtained is not a serious disadvantage; other methods of preparing thiobenzamidomalonic ester were, however, investigated. These included reaction of benzamidomalonic ester with phosphorus pentasulphide and potassium polysulphide (compare Kindler, *Annalen*, 1923, **431**, 187), which gave some of the desired substance though its isolation was difficult, reaction of thiobenzamide with aminomalonic ester, which failed, and reaction of benzaldehyde and sulphur with aminomalonic ester (Kindler, *loc. cit.*), which also failed. The reaction of sulphur with benzylideneaminomalonic ester by Kindler's method (*loc. cit.*) was not investigated because the latter substance was obtained in poor yield. When this work had been completed a publication by Kjaer (*Acta Chem. Scand.*, 1950, **4**, 1347) appeared describing the preparation of (thiobenzoylthio)-acetic acid in 51·5% yield from bromobenzene by a Grignard reaction followed by condensation of the dithiobenzoic acid with sodium chloroacetate.

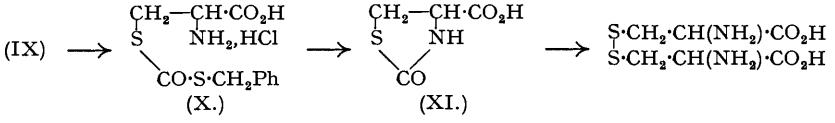
The initial difficulty experienced in the preparation of (thiobenzoylthio)acetic acid led to an investigation of another thioacyl derivative of aminomalonic ester. Cook, Harris, Heilbron, and Shaw (*J.*, 1948, 1056) prepared ethyl *N*-dithiocarbobenzyloxyaminomalonate (VI) by reaction of aminomalonic ester with carbon disulphide followed by alkylation with benzyl chloride in the presence of potassium hydroxide. This method did not give a good yield and it was found preferable to carry out both stages in pyridine solution in the presence of triethylamine. Condensation of the malonic ester derivative (VI) with formaldehyde gave an excellent

yield of ethyl α-dithiocarbobenzyloxyamino-α-hydroxymethylmalonate (VII), which was cyclised with thionyl chloride in the usual way. The product, 2-benzylthio-4 : 4-dicarbethoxy-Δ^2-thiazoline (VIII), like the related 2-phenyl-substituted thiazoline (III), did not form solid derivatives; it behaved similarly to (III) on alkaline hydrolysis also, giving the disodium salt (VIII; R = Na). Spontaneous decarboxylation occurred on acidification of the disodium



salt (VIII; R = Na), yielding an oily acid which formed a crystalline hydrochloride (IX). This substance was synthesised from serine methyl ester hydrochloride by a related series of reactions, as indicated in the scheme above, and its structure thereby confirmed.

The behaviour of the hydrochloride (IX) on acid hydrolysis was unexpected; no cystine was formed, the product being a crystalline substance which appeared to have resulted by addition of the elements of water to the starting material. This substance was assigned the structure (X) for three reasons : (1) it contained no free thiol groups; (2) it appeared to be the hydrochloride of a base, because it contained ionic chlorine, gave a ninhydrin reaction, and yielded a formyl derivative containing no chlorine; (3) above pH 7 it liberated toluene-ω-thiol and produced 2-ketothiazolidine-4-carboxylic acid (XI). *S*-Thiolcarbobenzyloxycysteine hydrochloride (X) was very resistant to acid hydrolysis, remaining largely unchanged after being heated for several hours with concentrated hydrochloric acid in a sealed tube at 160°. This resistance was later found to be attributable partly to the sparing solubility of the hydrochloride in concentrated hydrochloric acid; it was soluble in 6N-hydrochloric acid at the boiling point and slow hydrolysis occurred. The stability of compound (X) might result from the inhibition of the approach of hydrogen ions to the carbonyl group caused by the positively charged nitrogen atom in the vicinity (compare Neuberger and Moggridge, *J.*, 1938, 745;

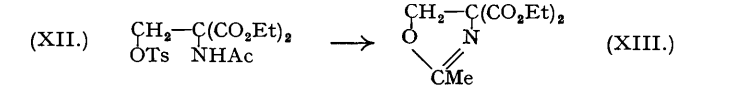


Synge, *Biochem. J.*, 1939, **33**, 1924). Attempts to bring about fission of the ester linkages by reaction of compound (X) with mercury salts under various conditions were not successful. An attempt to reduce the thiazoline (VIII; R = Et) with aluminium amalgam to a thiazolidine which would have been susceptible to mercuric chloride fission (Bentley, Catch, Cook, Heilbron, and Shaw, C.P.S. 267, September 15, 1944; " The Chemistry of Penicillin," p. 922, Princeton University Press, 1949) was also unsuccessful. The conversion of (X) into 2-ketothiazolidine-4-carboxylic acid (XI) under the influence of dilute alkali is probably analogous to the well-known rearrangement of *O*- to *N*-acylamino-alcohols under the same conditions. The reactions exemplified by the compounds (IX)—(XI) appear to be general for 2-alkylthiothiazolines and are being further studied.

In the synthesis of cystine by this method it was found preferable to proceed through compound (XI) rather than to carry out prolonged hydrolysis of the *s*-dithiocarbonic acid derivative (X). Acid hydrolysis of (XI), which was formed in quantitative yield from (X), occurred relatively rapidly. Although this route to cystine appears to be more involved than the synthesis from thiobenzamidomalonic ester, it is the more convenient method, it being possible to proceed from the disodium salt (VIII; R = Na) to cystine without isolation of any of the intermediates, whereas a very poor yield of cystine was obtained from the disodium salt (III) when intermediate isolation of the acid (IV) was omitted.

An unsuccessful attempt was made to synthesise cystine by Chapman and Owen's method (*J.*, 1950, 579) for preparing thiols from alcohols. Ethyl α-acetamido-α-carbethoxy-β-hydroxypropionate was converted into the corresponding toluene-*p*-sulphonic ester (XII), and when this reacted with potassium thiolacetate, formation of potassium toluene-*p*-sulphonate occurred,

but no substance containing a thiol group was detected after hydrolysis of the reaction product. It is possible that an oxazoline (XIII) was formed by the mechanism previously proposed for the cyclisation of *N*-benzoyl-*O*-toluene-*p*-sulphonyl*allo*threonine ethyl ester (Attenburrow, Elliott, and Penny, *J.*, 1948, 310) in the presence of potassium acetate, the only difference in the present example being the presence of thiolacetate ion instead of acetate ion. It is of



(XII.) (XIII.)

interest that Fry (*J. Org. Chem.*, 1950, **15**, 438) succeeded in opening the ring in 2-phenyl-Δ^2-oxazoline-4-carboxylic acid by the action of thiolbenzoic acid in pyridine to give *NS*-dibenzoylcysteine. Evidently a reaction of this kind did not occur in the present case although an excess of potassium thiolacetate was used.

Experimental.

(M. p.s are uncorrected.)

Aminomalonic Ester Hydrochloride.—The free base was prepared by Snyder and Smith's method (*J. Amer. Chem. Soc.*, 1944, **66**, 350) and the hydrochloride was formed by dissolution of the base in a large volume of dry ether and saturation with anhydrous hydrogen chloride (Levene and Schormüller, *J. Biol. Chem.*, 1934, **106**, 601); yield, 125 g. from 200 g. of malonic ester.

(Thiobenzoylthio)acetic Acid.—This compound has been prepared from benzotrichloride by Holmberg (*loc. cit.*). In our hands only a 40% yield could be obtained (cf. Kjaer, *loc. cit.*). Various attempts to improve this yield included the use of sodium sulphide instead of potassium sulphide in the reaction with benzotrichloride, the addition of alcoholic instead of aqueous sodium chloroacetate to the crude alkali salt of dithiobenzoic acid, and carrying out the reactions at the b. p.s of the solvents or under nitrogen. No improvement in yield could be effected, but the following method gave a 40% yield in 1 day instead of the 3 days necessary in Holmberg's method. A solution of potassium hydroxide pellets (7·5 g.) in ethanol (50 ml.) was divided into halves and one half was saturated with hydrogen sulphide which had been dried by passage through calcium chloride; the other half was then added to the first portion in a three-necked flask fitted with reflux condenser, nitrogen inlet, and dropping-funnel. A slow stream of nitrogen was passed through, the temperature was raised to 30°, and benzotrichloride (4·05 ml.; 5·6 g.) added dropwise at such a rate that the temperature did not rise above 60°. The mixture was then allowed to cool and remained for 2 hours at room temperature. Monochloroacetic acid (4 g.) was dissolved in water (25 ml.) and neutralised by addition of solid sodium carbonate, and the solution was added to the previous solution under nitrogen, and the mixture well shaken and then quickly raised to the b. p.; as soon as the colour change from brown to red had occurred (2—5 mins.) the solution was rapidly cooled (further heating caused considerable decomposition). The solution was diluted with water (100 ml.), acidified with concentrated hydrochloric acid, and extracted with benzene. The benzene layer was extracted with sodium carbonate solution, the sodium carbonate layer was acidified with concentrated hydrochloric acid and extracted with benzene, and the benzene layer evaporated to 10 ml. To this solution was added petroleum (b. p. 60—80°, 100 ml.), giving an instant precipitate of bright red plates (2·52 g., 41% based on benzotrichloride), m. p. 125°.

Thiobenzamidomalonic Ester.—(*a*) Aminomalonic ester hydrochloride (10 g.) was dissolved in pyridine (30 ml.), triethylamine (1 mol., 4·8 g.) and (thiobenzoylthio)acetic acid (11 g., 1·1 mol.) were then added, and the solution was shaken until the solid had dissolved. It was set aside overnight and then poured into excess of 2N-sulphuric acid containing some ice, and the mixture extracted with ether. The ethereal layer was extracted with dilute sodium hydrogen carbonate to remove excess of (thiobenzoylthio)acetic acid, leaving an olive-green ethereal solution which was dried (Na_2SO_4). Evaporation of the ether left an oil which solidified when scratched after addition of petroleum (b. p. 60—80°). The yield of *ethyl thiobenzamidomalonate*, m. p. 57°, was 11·6 g. (83%). Recrystallisation from ligroin raised the m. p. to 62° (Found: C, 56·8; H, 6·0; N, 5·0. $C_{14}H_{17}O_4NS$ requires C, 56·9; H, 5·8; N, 4·7%).

(*b*) Benzamidomalonic ester (4·0 g.) (Redemann and Dunn, *J. Biol. Chem.*, 1939, **130**, 341) was placed in a three-necked flask fitted with a stirrer and condenser closed by a calcium chloride tube. Dry xylene (30 ml.), phosphorus pentasulphide (0·96 g.), and liver of sulphur (1·52 g.) were added, and the temperature raised to 80—90° for 4 hours (cf. Kindler, *loc. cit.*). The red xylene solution was decanted from the undissolved material to which more dry xylene (20 ml.) was added, and heating and stirring at 90° were continued for 30 minutes. The xylene solution was again decanted and combined with the first extract, and the solution evaporated to dryness, leaving an oil which partly solidified. This was dissolved in aqueous alcohol, and after 4 days, well-defined needles (1·3 g.) of thiobenzamidomalonic ester had separated out; it had m. p. 61°, mixed m. p. with ester from (*a*) 61°.

Benzylideneaminomalonic Ester.—Benzaldehyde (3·2 g.) was added to aminomalonic ester (5 g.) in dry benzene (50 ml.), and the mixture refluxed for 4 hours, a Dean and Stark apparatus being used for the separation of the water formed. The solution, which smelt strongly of benzaldehyde, was extracted with concentrated aqueous sodium hydrogen sulphite. The benzene layer was evaporated to dryness, leaving the *diethyl* ester as an oil which had solidified after 3 days (4·2 g.), m. p. 96—101° (Found: N, 5·3. $C_{14}H_{17}O_4N$ requires N, 5·3%).

Ethyl α-Carbethoxy-β-hydroxy-α-thiobenzamidopropionate.—Thiobenzamidomalonic ester (4·42 g.) was dissolved in pyridine (4 ml.) and 39% aqueous formaldehyde (1·1 ml., 1 mol.) solution was delivered from a pipette under the surface of the solution, which was then left at 0° for 24 hours. The solution

was poured into a mixture of water and ether, and concentrated hydrochloric acid added until the aqueous layer was acid to Congo-red. The aqueous layer was thoroughly extracted with ether, and the ethereal layer washed with sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated, leaving an oil which crystallised on cooling; the solid (4·53 g., 93%) had m. p. 106°. Recrystallisation from ligroin gave pure *ethyl α-carbethoxy-β-hydroxy-α-thiobenzamidopropionate* in the form of needles, m. p. 110° (Found: C, 55·4; H, 5·8; N, 4·1; S, 9·5. $C_{15}H_{19}O_5NS$ requires C, 55·4; H, 5·9; N, 4·3; S, 9·8%).

4 : 4-*Dicarbethoxy*-2-*phenyl*-Δ²-*thiazoline*.—The foregoing ester (11·2 g.) was added in portions during 15 minutes to thionyl chloride (30 ml.; purified as described in *Org. Synth.*, Coll. Vol. II, p. 570) cooled in ice. After one hour at 0° the thionyl chloride was distilled off in a vacuum at 35°, the residual oil poured into a suspension of sodium hydrogen carbonate (50 g.) in water (100 ml.), and the mixture extracted with ether. The ethereal layer was dried and evaporated. The residual oil (10·15 g., 96%) was distilled and had b. p. 170°/0·05 mm. 4 : 4-*Dicarbethoxy*-2-*phenyl*-Δ²-*thiazoline* was a very weak base, forming neither a picrate nor a chloroplatinate (Found: C, 58·8; H, 5·6; N, 4·6. $C_{15}H_{17}O_4NS$ requires C, 58·7; H, 5·5; N, 4·6%).

Disodium salt. This thiazoline (10·1 g.) was dissolved in ethanol (20 ml.), and a N-solution of sodium hydroxide in ethanol (31 ml., 2·2 mols.) added with shaking. The transient yellow colour at first produced was followed rapidly by a white precipitate. After 15 minutes, the microcrystalline *disodium* salt (7·35 g., 76%) was separated by centrifugation and dried in a vacuum desiccator (Found: N, 4·7; Na, 15·9. $C_{11}H_6O_4NSNa$ requires N, 4·7; Na, 15·7%).

2-*Phenyl*-Δ²-*thiazoline*-4-*carboxylic Acid*.—(*a*) The foregoing disodium salt (2·4 g.) was covered with ethyl acetate, and N-hydrochloric acid (21 ml.) dropped into it with shaking until all the solid had dissolved. The ethyl acetate layer was washed three times with distilled water, dried, and evaporated. The residual oil (1 g., 60%) solidified, and was washed with ether and recrystallised from ethanol, giving 2-*phenyl*-Δ²-*thiazoline*-4-*carboxylic acid*, m. p. 125—126°; mixed m. p., with a sample prepared as in (*b*), 123°.

(*b*) Methyl 2-phenyl-Δ²-thiazoline-4-carboxylate (see below) (9 g.) was suspended in water (45 ml.) containing N-sodium hydroxide (45 ml., 1·1 mols.), and the mixture warmed to 50° until all the solid had dissolved. On cooling, the sodium salt of the corresponding acid crystallised and was dissolved by addition of water (20 ml.). N-Hydrochloric acid (45 ml.) was added, precipitating an oil which was extracted into ethyl acetate. The solvent was evaporated, and the crystalline acid (7 g.) purified from benzene–petroleum (b. p. 60—80°) (4 : 1). The acid (6 g.) had m. p. 122° (Found: C, 57·8; H, 4·6; N, 6·7; S, 15·7. $C_{10}H_9O_2NS$ requires C, 58·0; H, 4·4; N, 6·8; S, 15·5%).

Thiobenzoylserine Methyl Ester.—Serine methyl ester hydrochloride, prepared in the usual way from serine (0·5 g.), was dissolved in dry pyridine (3 ml.), and triethylamine (1·5 g., 3 mol.) added followed by (thiobenzoylthio)acetic acid (1·1 g.; 1·1 mols.) in pyridine (5 ml.). The mixture was left overnight and then poured into excess of 2N-sulphuric acid covered with a layer of ether. The ethereal layer was separated, the aqueous layer extracted again with ether, and the combined extracts were washed with sodium hydrogen carbonate, dried, and evaporated. The residue rapidly crystallised; the solid (0·86 g., 76% based on serine) had m. p. 84—85°. After recrystallisation from benzene the *thiobenzoylserine methyl ester* had m. p. 88° (Found: N, 5·8. $C_{12}H_{13}O_3NS$ requires N, 5·9%). This compound was also prepared in 63% yield from serine methyl ester hydrochloride by using aqueous sodium hydroxide as a base, according to Holmberg's method (*loc. cit.*).

Methyl 2-*Phenyl*-Δ²-*thiazoline*-4-*carboxylate*.—(*a*) Thiobenzoylserine methyl ester (12·6 g.) was added in portions during 20 minutes to purified thionyl chloride (30 ml.) cooled in ice. After 30 minutes, the thionyl chloride was distilled off in a vacuum at 35°. The residue of *methyl* 2-*phenyl*-Δ²-*thiazoline*-4-*carboxylate hydrochloride* (yield 93%) was crystallised from chloroform–ether and had m. p. 113—114° (Found: N, 5·55. $C_{11}H_{12}O_2NClS$ requires N, 5·4%). To prepare the free base, the same amount of crude hydrochloride was covered with a layer of ether and hydrated sodium acetate (5·94 g., 2 mols.) in water (20 ml.) was added, the mixture shaken, and the ethereal layer separated. After being washed with sodium hydrogen carbonate solution, the ethereal layer was dried and passed through a short column of alumina to remove impurities. The eluate was evaporated to dryness, leaving a crystalline solid, m. p. 67—68° (7·4 g., 64% based on thiobenzoylserine methyl ester). After recrystallisation from ligroin *methyl* 2-*phenyl*-Δ²-*thiazoline*-4-*carboxylate* had m. p. 69° (Found: N, 6·0; S, 14·0. $C_{11}H_{11}O_2NS$ requires N, 6·3; S, 14·5%).

(*b*) Cysteine (20 g.) was suspended in dry methanol (150 ml.), and a stream of nitrogen passed through the solution. A rapid stream of dry hydrogen chloride was passed in until the solution boiled. It was then cooled, saturated with hydrogen chloride, and left overnight. The methanol was evaporated under reduced pressure in an atmosphere of nitrogen, the residue was dissolved in distilled water (40 ml.) with nitrogen passing through, and aqueous ammonia (*d* 0·88) added until the pH was raised to 3. Ethyl benzimidate (Elliott, *Biochem. J.*, 1949, **45**, 429) (30 g.) in ether (100 ml.) was then added, and the mixture shaken overnight. The two layers had emulsified next day, so more water was added and the layers were separated by centrifugation. The aqueous layer was extracted with ether, and the ethereal layers combined, dried, and evaporated. The residual oil was distilled under reduced pressure. The lower-boiling fraction was discarded and the fraction, b. p. 140°/0·05 mm., was collected. This solidified and was crystallised from ligroin; the solid (20 g.) had m. p. 68°. The mixed m. p. with the thiazoline methyl ester prepared as in (*a*) was 68°.

Cystine.—2-Phenyl-Δ²-thiazoline-4-carboxylic acid or its methyl ester (1 g.) was refluxed with 3N-hydrochloric acid (25 ml.) for 4 hours, and the solution cooled and extracted twice with ether. The aqueous layer was evaporated to dryness, and the residual oil dissolved in N-hydrochloric acid (5 ml.) and titrated at 0° with 0·1N-iodine solution; 42·5 ml. were required (calc.: 48·5 ml.). The slight excess of iodine was removed with sodium thiosulphate solution, the pH was adjusted to 5 with sodium

hydroxide, and hydrated sodium acetate (0·2 g.) added. The solution then had pH 5·7. After two days at 0° the inactive cystine (0·32 g.) was filtered off. The aqueous filtrate was evaporated to dryness and after extraction with alcohol the residue was dissolved in hot water (15 ml.) which on cooling gave a further quantity of cystine (0·08 g.). Microbiological assay showed that this material had 50% of the growth-promoting activity of L-cystine for *Leuconostoc mesenteroides* P. 60.

Ethyl N-*Dithiocarbobenzyloxyaminomalonate* (cf. Cook *et al., loc. cit.*).—Aminomalonic ester hydrochloride (3·7 g.) was dissolved in pyridine (12 ml.) which had been dried by distillation over sodium hydroxide, and triethylamine (3·46 g.) was added. Instantaneous precipitation of triethylamine hydrochloride occurred. Carbon disulphide (2 ml., 2 mols.) was added dropwise with shaking at 0°, and the solution kept at 0° for 2 hours. Benzyl chloride (2 ml., 1 mol.) was then added, and the solution kept at 0° overnight. The solution was poured into water and acidified with concentrated hydrochloric acid, and the oil extracted with ether. The ether layer was washed with sodium hydrogen carbonate solution, dried, and evaporated, leaving an oil which solidified when scratched; yield 5·2 g. (88%), m. p. 70°.

Isolation of the Intermediate Triethylamine Salt of N-*Dicarbethoxymethyldithiocarbamic Acid.*—Aminomalonic ester (3 g.) was mixed with triethylamine (1·73 g.) and cooled to −4° and carbon disulphide (1 ml.) was added dropwise with vigorous shaking. The resulting oil was cooled to −60° and scratched vigorously under anhydrous ether. The colourless needles, m. p. 50° (decomp.), of the *triethylamine* salt were rapidly filtered off and washed thoroughly with cold anhydrous ether to remove colouring matter (Found : C, 48·0; H, 8·0; S, 17·9; N, 7·9. $C_{14}H_{28}O_4N_2S_2$ requires C, 47·6; H, 7·9; S, 18·2; N, 7·9%).

Ethyl α-Dithiocarbobenzyloxyamino-α-hydroxymethylmalonate.—Ethyl *N*-dithiocarbobenzyloxyaminomalonate (7·3 g.) was dissolved in dry pyridine (7 ml.), aqueous formaldehyde solution (36·7%; 3·0 ml., 1·1 mols.) added, and the solution set aside overnight, then poured into water and acidified with concentrated hydrochloric acid, liberating an oil which was extracted into ether. The ethereal layer was washed with sodium hydrogen carbonate solution, dried, and evaporated. The residual oil crystallised when scratched after addition of petroleum. The solid (6·83 g., 86%) had m. p. 80°, raised by repeated recrystallisations from benzene–petroleum (b. p. 60—80°) (1 : 3) or from aqueous alcohol to 89° (Found : C, 50·7; H, 5·4; N, 3·7. $C_{16}H_{21}O_5NS_2$ requires C, 51·4; H, 5·6; N, 3·8%). The crude *ester* was suitable for the next stage.

2-Benzylthio-4 : 4-dicarbethoxy-Δ²-thiazoline and Disodium 2-Benzylthio-Δ²-thiazoline-4 : 4-dicarboxylate.—The preceding ester (15 g.) was added during 15 minutes to purified thionyl chloride (30 ml.), cooled in ice, and the reaction mixture kept at 0° for 1 hour. Excess of thionyl chloride was removed under reduced pressure at 35°, and the residual oil was poured into a suspension of sodium carbonate (70 g.) in water (150 ml.) covered with a layer of ether. The aqueous layer was thoroughly extracted with ether, and the combined extracts were dried and evaporated. The yield of 2-benzylthio-4 : 4-dicarbethoxy-Δ²-thiazoline was 13·45 g. (95%). Like the corresponding 2-phenyl derivative, this compound did not form a picrate and no attempt was made at distillation. It was used in the crude state for the next stage.

The crude diethyl ester (2·6 g.) was dissolved in alcohol (7 ml.), and N-alcoholic sodium hydroxide (9·4 ml., 2·1 mols.) added, producing a copious white precipitate within a few minutes. The solid was removed by centrifugation, washed with alcohol and ether by centrifugation, and dried in a vacuum desiccator (yield 1·7 g., 65%). *Disodium 2-benzylthio-Δ²-thiazoline-4 : 4-dicarboxylate* was purified by precipitation from an aqueous solution with ethanol (Found : N, 3·95; S, 17·4; Na, 14·8. $C_{12}H_9O_4NS_2Na_2$ requires N, 4·1; S, 18·8; Na, 13·5%).

S-*Thiolcarbobenzyloxycysteine Hydrochloride* (X).—The foregoing disodium salt (26 g.) was treated with 6N-hydrochloric acid (130 ml.). When effervescence had ceased the solution was heated under reflux for 30 minutes and cooled at 0° for 2 hours, and the coarse white precipitate (18 g., 77%), m. p. 173° (decomp.), filtered off. It was crystallised from 6N-hydrochloric acid, giving the pure *hydrochloride*, m. p. 183° (decomp.) (Found : C, 43·1; H, 4·7; N, 4·8. $C_{11}H_{14}O_3NClS$ requires C, 43·0; H, 4·6; N, 4·6%). The amino-acid when run on a paper chromatogram in phenol gave a weak spot, R_F 0·72. It was found that complete hydrolysis of this substance required at least 70 hours at the b. p. in the presence of 6N-hydrochloric acid.

N-*Formyl*-S-*thiolcarbobenzyloxycysteine.*—The above cysteine derivative (0·31 g.) was suspended in 90% formic acid (1·5 ml.) containing hydrated sodium acetate (0·2 g.), and the solution warmed to 40°. Acetic anhydride (0·5 ml.) was then added dropwise, and after the addition the solution was kept at 80° for 30 minutes. The solvent was then evaporated, and the residue leached with ether, giving the N-*formyl* compound in crystalline plates, m. p. 145—150°, raised by recrystallisation from glacial acetic acid to 152° (mixed m. p. with starting material, m. p. 174°, was 150°) (Found : C, 48·1; H, 4·4; N, 5·0. $C_{12}H_{13}O_4NS$ requires C, 48·6; H, 4·4; N, 4·7%).

2-Ketothiazolidine-4-carboxylic Acid.—The hydrochloride (X) (1 g.), suspended in alcohol (30 ml.), was treated with N-sodium hydroxide (9·8 ml., 3 mols.) and after 30 minutes the solution was acidified with N-hydrochloric acid (9·8 ml.) and evaporated to dryness. The last traces of toluene-ω-thiol were removed by a second evaporation after addition of a small quantity of water. The residue was extracted repeatedly with ether and the combined extracts were evaporated to dryness, leaving a crystalline solid (0·48 g., 100%), m. p. 146—148°. It was recrystallised from ethyl acetate, giving pure *2-ketothiazolidine-4-carboxylic acid*, m. p. 155° (Found : N, 9·5. $C_4H_5O_3NS$ requires N, 9·5%), which was sparingly soluble in ether and more soluble in acetone. Acetone extraction of the crude product described above did not give satisfactory results.

The thiazolidone derivative was soluble in water, giving a solution which reacted acid to Congo-red paper, effervesced on addition of sodium hydrogen carbonate, and did not give a blue colour with ninhydrin. Hydrolysis experiments showed that for complete hydrolysis to cysteine it was necessary

to heat the thiazolidone derivative with excess of 6N-hydrochloric acid for 20 hours under reflux. Carbon dioxide was liberated during hydrolysis in an amount equivalent to the sulph-hydryl content of the solution. For preparative purposes isolation of (IX), (X), and (XI) can be omitted as shown below.

Cystine.—Disodium 2-benzylthio-Δ^2-thiazoline-4 : 4-dicarboxylate (2 g.) was heated under reflux for 2 hours with N-hydrochloric acid (17·5 ml.), the solution was allowed to cool, and 3N-sodium hydroxide solution added until the solution was strongly alkaline. It was then set aside for 20 minutes, acidified with concentrated hydrochloric acid (50 ml.), and heated under reflux for 20 hours. This solution was evaporated to dryness to remove the toluene-ω-thiol present. The residue was thoroughly extracted with alcohol, and the extract oxidised with N-iodine solution, neutralised with pyridine, and kept at 0° for 2 hours. The yield of inactive cystine was 0·30 g. (42·5%). This material was found to be pure inactive cystine by microbiological assay and by paper chromatography.

Methyl 2-benzylthio-Δ^2-thiazoline-4-carboxylate Hydrochloride.—Serine methyl ester hydrochloride (3·68 g.) was dissolved in pyridine (16 ml.), the solution cooled to 0° in ice, and triethylamine (4·74 ml., 2 mols.) added. This was followed by dropwise addition of carbon disulphide (2 ml.). The reaction mixture was left at 0° for 4 hours, then benzyl chloride (2 ml.) was added, and the solution kept at 0° overnight. The product was poured into a mixture of ether and water, and the aqueous layer was acidified with concentrated hydrochloric acid and extracted again with ether. The ethereal extract was washed with dilute hydrochloric acid followed by sodium hydrogen carbonate solution, dried, and evaporated. The residual oil, which could not be crystallised, was added dropwise to purified thionyl chloride (2 ml.) cooled in ice, and the mixture kept for 30 minutes at 0°. The thionyl chloride was distilled off under reduced pressure at 35° and dry ether was added to the residual oil, which solidified when scratched. *Methyl 2-benzylthio-Δ^2-thiazoline-4-carboxylate hydrochloride* had m. p. 113° (Found : N, 5·0. $C_{12}H_{14}O_2NClS_2$ requires N, 4·6%).

2-Benzylthio-Δ^2-thiazoline-4-carboxylic Acid Hydrochloride.—(*a*) The foregoing ester was suspended in water (this caused some dissociation of the hydrochloride), and the pH adjusted to 7 with N-sodium hydroxide. The oil was extracted with ether, the ethereal extract evaporated, the residual oil dissolved in alcohol, and N-alcoholic sodium hydroxide (6·2 ml.) added. The mixture was kept for 2 days. The solution was evaporated to dryness, N-hydrochloric acid (10 ml.) added, and the solution extracted thoroughly with benzene (this was possible because of the feebly basic properties of the thiazoline-carboxylic acid). The benzene was evaporated, and the residual oil triturated with 6N-hydrochloric acid (2 ml.). The oil rapidly crystallised and *2-benzylthio-Δ^2-thiazoline-4-carboxylic acid hydrochloride*, m. p. 163°, separated (Found : N, 4·9; S, 22·3. $C_{11}H_{12}O_2NClS_2$ requires N, 4·7; S, 22·2%).

(*b*) Disodium 2-benzylthio-Δ^2-thiazoline-4 : 4-dicarboxylate (1 g.) was treated with 6N-hydrochloric acid (5 ml.), liberating an oil with the evolution of carbon dioxide. The oil solidified when scratched. The yield was 0·51 g. of the hydrochloride, m. p. 157°, raised by crystallisation from glacial acetic acid to 165°.

Comparison of the two samples of the acid hydrochloride was best carried out by converting them both into the sparingly soluble *S*-thiolcarbobenzyloxycysteine hydrochloride by heating under reflux for 10 minutes with 6N-hydrochloric acid. The cysteine derivative prepared from the disodium salt had m. p. 183°, that derived from serine had m. p. 184°, and the mixed m. p. was 183° (all m. p.s with decomp.).

*Ethyl α-Acetamido-α-carbethoxy-β-toluene-*p*-sulphonyloxypropionate.*—A solution of ethyl α-acetamido-α-carbethoxy-β-hydroxypropionate (King, *loc. cit.*; 1 g.) in anhydrous pyridine (10 ml.) was cooled to −5°, toluene-*p*-sulphonyl chloride (0·85 g.; 10% excess) added, and the mixture shaken until the solid had dissolved and then set aside overnight at −2° (compare Tipson, *J. Org. Chem.*, 1944, **9**, 235). Water (1 ml.) was then added dropwise with cooling at 0°, followed by an additional 4 ml. in one portion. The mixture was then poured into 2N-sulphuric acid (100 ml.), and after thorough mixing the gum was allowed to settle, and the aqueous liquor decanted. The gum was dissolved in chloroform, and the solution shaken with 2N-sulphuric acid, then with sodium hydrogen carbonate solution, dried, and evaporated. The residual oil solidified on addition of petroleum (b. p. 40—60°). The yield of *ethyl α-acetamido-α-carbethoxy-β-toluene-*p*-sulphonyloxypropionate* (m. p. 78°) was 1·85 g. (57·5%); the m. p. was raised by recrystallisation from aqueous alcohol to 81° (Found : N, 3·5; S, 7·2. $C_{17}H_{23}O_8NS$ requires N, 3·5; S, 8·0%).

*Reaction of Ethyl α-Acetamido-α-carbethoxy-β-toluene-*p*-sulphonyloxypropionate with Potassium Thiolacetate.*—The toluene-*p*-sulphonyl derivative (1 g.) and potassium thiolacetate (1 g.) were dissolved in anhydrous ethanol (20 ml.), and the mixture refluxed for 40 minutes and then kept overnight. The potassium toluene-*p*-sulphonate (0·4 g.; 80%) was filtered off and washed with a little alcohol and then with ether. The filtrate was evaporated to dryness, the residue was extracted with anhydrous ether, and the extract evaporated to dryness, leaving an oil (0·66 g.) to which was added N-sodium hydroxide in ethanol (10·8 ml.), and the solution was set aside overnight. The precipitate was separated by centrifugation and washed with alcohol and ether. The dry solid was treated with excess of 3N-hydrochloric acid, which caused instantaneous liberation of carbon dioxide. Iodine titrations showed that neither this solution nor the solution obtained after prolonged boiling of the solid with dilute acid contained more than a trace of sulph-hydryl.

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