307. *Studies in the Azole Series. Part XVI. Synthesis of a New Analogue of Penicillamine.*

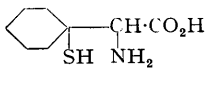
By J. D. Billimoria, A. H. Cook, and Sir Ian Heilbron.

2-Thio-5-thiazolidone (II) has been converted into an analogue (I) of penicillamine *via* the compounds (III) and (V). The acid (I), when condensed with 2-benzyl-4-ethoxymethylene-oxazolone, affords small antibacterial activities which are due, it is believed, to a product skeletally similar to the natural penicillins.

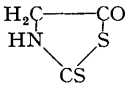
Part V of this series (Cook, Harris, and Heilbron, *J.*, 1948, 1060) described the synthesis of β-phenylcysteine which was obviously comparable with ββ-dimethylcysteine (penicillamine). However, β-phenylcysteine failed to give any significant antibiotic activity on attempted condensation with 2-benzyl-4-hydroxymethyleneoxazolone. The present paper describes the preparation and properties of an α-*amino*-β-*mercapto-acid* (I) more closely related to penicillamine.

2-Thio-5-thiazolidone (II) (Cook, Heilbron, and Levy, *J.*, 1948, 201) has been found to undergo facile condensation with aldehydes or ketones; thus, *cyclo*hexanone in an acid or basic medium

afforded 2-*thio*-4-cyclo*hexylidene*-5-*thiazolidone* (III) together with a by-product which the analytical evidence showed was derived by the condensation of two molecules of *cyclo*hexanone



(I.)



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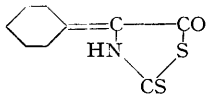
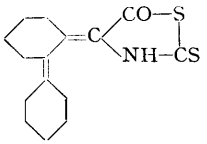
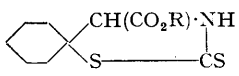


(III.)

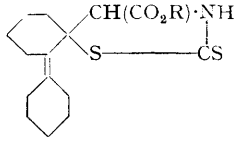
with one of the thiazolidone and which must be the *compound* (IV). When (III) was treated with an excess of alcoholic alkali it was converted by the formal addition of one molecule of water into an *acid* which, on the basis of similar transformations associated with syntheses of β-phenylcysteine and penicillamine (Cook, Harris, and Heilbron, *loc. cit.*), can only be (V; R = H).



(IV.)



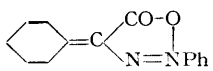
(V.)

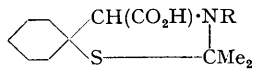


(VI.)

When only 1 mol. of sodium ethoxide was used in this transformation the product was the corresponding *ester* (V; R = Et) which was hydrolysed to the parent acid by means of alkali. The bis-condensation product (IV) underwent similar changes to give the *ester* and *acid* (VI; R = Et and H, respectively); of these the ester could be obtained directly from the mother-liquors obtained in the preparation of (III) by treatment with ethanol. The 2-thiothiazolidine (V; R = H or Et) was readily cleaved on heating with mineral acid to give the desired α-amino-β-mercapto-acid (I).

A synthesis of the latter acid *via* 4-cyclo*hexylidene*-2-*phenyl*-5-*oxazolone* (VII) was abandoned owing to the poor yield of condensation product obtained during azlactonisation of hippuric acid in the presence of *cyclo*hexanone.

(VII.) 



(VIII.)

Like other acids of its class, (I) gave an intense blue-violet coloration with ferric chloride, a sparingly soluble mercury derivative, and on condensing with acetone was converted into a *thiazolidine* (VIII; R = H) which afforded a characteristic N-*formyl* derivative (VIII; R = CHO).

The aminomercapto-acid (I) was condensed with 2-benzyl-4-ethoxymethylene-5-oxazolone under conditions similar to those which afforded penicillin from penicillamine (see, *e.g.*, du Vigneaud, Carpenter, Holby, Livermore, and Rachel, *Science*, 1946, **104**, 431). Small antibacterial activities equivalent to *ca.* 0·5 penicillin unit/mg. of aminomercapto-acid were constantly obtained. The active material could be extracted by organic solvents and it seems probable that the activity is due to a product with a constitution similar to that of the natural penicillins. Attempts are consequently being made to examine the behaviour in this respect of the optically active forms of the acid (I).

Experimental.

2-*Thio*-4-cyclo*hexylidene*-5-*thiazolidone* (III).—(*a*) 2-Thio-5-thiazolidone (Cook, Heilbron, and Levy, *loc. cit.*) (25 g.) was dissolved in warm *cyclo*hexanone (75 c.c.), the solution cooled to room temperature, and ethanol (0·2 c.c.) added. Dry hydrogen chloride was passed through the solution throughout. An exothermic reaction set in and after 2 hours the solution was cooled to 0°. The crystals were filtered off from the viscous solution, washed with ethanol, and recrystallised from acetic acid, giving the *thiazolidone* (III) (10 g.) as pale yellow needles, m. p. 225° (decomp.) (Found: C, 50·4; H, 5·15; N, 6·25. $C_9H_{11}ONS_2$ requires C, 50·7; H, 5·15; N, 6·55%).

(*b*) 2-Thio-5-thiazolidone (2 g.) was dissolved in dry *cyclo*hexanone (7 c.c.) at 140°, morpholine (1 drop) added, and the solution kept at room temperature. Morpholine (1 drop) was added to the solution every 24 hours during 5 days. The solution was diluted with acetic acid, cooled to 5°, and the crystalline condensation product filtered off and recrystallised from acetic acid, forming pale yellow needles (yield 1·8 g.), m. p. 225° (decomp.) undepressed by admixture with the above condensation product.

(*c*) 2-Thio-5-thiazolidone (10 g.) was dissolved in boiling acetic acid (100 c.c.), and *cyclo*hexanone diethylketal (12 c.c.) added followed by piperidine (2 drops). The solution was kept for 24 hours and the crystalline condensation product (11·5 g.) was filtered off; it had m. p. 225° (decomp.) and was identified with the preceding preparations.

2-*Thio*-4-*carbethoxy*-(2′-cyclo*hexylidene*-5 : 5-*pentamethylene*)*thiazolidine* (VI; R = Et).—The viscous filtrate from the above condensation (*a*) was diluted with ethanol, excess of *cyclo*hexanone, ethanol, and hydrogen chloride removed by heating in a vacuum, and the residual oil poured into a mixture of much light petroleum and water. After standing at 0° for 2 days a crystalline mass separated and was filtered off. The product crystallised from acetic acid as pale yellow needles, m. p. 125—126° (yield 8 g.). Analysis indicated that this was the rearranged *ester* of the 2-thiothiazolidine described below (Found: C, 60·1; H, 7·4; S, 18·5. $C_{17}H_{25}O_2NS_2$ requires C, 60·2; H, 7·4; S, 18·9%).

2-*Thio*-4-(2′-cyclo*hexylidene*)cyclo*hexylidene*-5-*thiazolidone* (IV).—2-Thio-5-thiazolidone (5 g.) was dissolved in warm *cyclo*hexanone, and dry hydrogen chloride passed through the solution for 6 hours. The solution was kept overnight, and small amounts of crystalline material (2-thio-4-*cyclo*hexylidene-5-thiazolidone) were filtered off. The mother-liquor was poured into a mixture of much light petroleum and water, and the solution kept for 2 days at 0°. The solid was filtered off and crystallised from ethanol–water. Recrystallisation from ethanol–water yielded pale yellow needles, m. p. 203—205° (decomp.), of the hydrated bis-condensation *product* (IV) (2 g.) (Found: C, 57·6; H, 6·75; N, 4·2. $C_{15}H_{19}ONS_2,H_2O$ requires C, 57·85; H, 6·95; N, 4·5%).

2-*Thio*-4-*carboxy*-(2′-*cyclohexylidene*-5 : 5-*pentamethylene*)*thiazolidine* (VI; R = H).—The above bis-condensation product (5 g.) was suspended in ethanol (20 c.c.), and to this was added an excess of alcoholic sodium ethoxide solution (from 2 g. of sodium). The solution was heated under reflux for 5 minutes, kept at room temp. for 1 hour, and poured on crushed ice (50 g.) and hydrochloric acid (50 g.). The precipitated *acid* (VI; R = H) was filtered off, dissolved in aqueous sodium hydrogen carbonate, and the solution acidified. The acid so obtained crystallised from acetic acid in yellow prisms (5 g.), m. p. 203—205° (decomp.) (Found: C, 57·7; H, 7·0; S, 20·3. $C_{15}H_{21}ONS_2$ requires C, 57·9; H, 6·75; S, 20·6%). Its ester (VI; R = Et) (1 g.) (described above) was heated under reflux for 15 minutes with aqueous alcoholic 5% sodium hydroxide (20 c.c.). After cooling, the solution was acidified, and the precipitated acid purified by solution in aqueous sodium hydrogen carbonate and reprecipitation with acid. The acid crystallised from acetic acid in yellow prisms, m. p. 203—205°, identical with the above preparation.

2-*Thio*-4-*carbethoxy*-5 : 5-*pentamethylenethiazolidine* (V; R = Et).—The thiazolidone (III) (above) (25 g.) was suspended in dry ethanol (125 c.c.) to which was added a saturated solution of alcoholic sodium ethoxide (from 4 g. of sodium). The thiazolidone immediately dissolved. The solution was heated under reflux for 3—4 minutes and poured on crushed ice (100 g.) and hydrochloric acid (100 g.), a gum separating. The solution was diluted with water, (1500 c.c.) and kept at 0° for 12 hours, whereupon the gum solidified. The solid was filtered off and a small portion sublimed in a high vacuum, a pale yellow crystalline sublimate, m. p. 136—138°, being obtained. The remaining solid was dissolved in acetone, and the solution treated with charcoal, and seeded with the sublimate; the *ester* crystallised, and recrystallisation from aqueous acetone gave almost colourless needles (27 g.) (Found: C, 50·75; H, 6·6; N, 5·85. $C_{11}H_{17}O_2NS_2$ requires C, 50·95; H, 6·6; N, 5·45%).

2-*Thio*-4-*carboxy*-5 : 5-*pentamethylenethiazolidine* (V; R = H).—2-Thio-4-*cyclo*hexylidenethiazolidone (2 g.) was heated under reflux for 4 minutes with ethanol (10 c.c.) and an excess of sodium ethoxide (from 2 g. of sodium). The product was poured on crushed ice and hydrochloric acid. The precipitated *acid* was purified by dissolving it in sodium hydrogen carbonate and reprecipitating it with acid, and crystallised from acetic acid as colourless needles, m. p. 203—205° (decomp.) (Found: C, 46·45; H, 5·65; N, 5·7. $C_9H_{13}O_2NS_2$ requires C, 46·75; H, 5·8; N, 6·0%). The same acid was obtained by heating the above ester (1 g.) under reflux with 5% aqueous alcoholic sodium hydroxide solution (20 c.c.) and purifying the crude acid in the usual manner.

α-*Amino*-β-*mercapto*-ββ-*pentamethylenepropionic Acid Hydrochloride*.—(*a*) The foregoing thiazolidine ester (V; R = Et) (2 g.) was heated with hydrochloric acid (20 c.c.) under pressure at 110° for 36 hours. The solution was evaporated to dryness in a vacuum under nitrogen. The gummy residue was dissolved in water, the solution filtered, and saturated aqueous mercuric chloride added until no further precipitation occurred. The white curdy precipitate of the mercury complex was suspended in ethyl acetate (20 c.c.), previously moistened with 2N-hydrochloric acid, and hydrogen sulphide passed through the solution. The solution was filtered from mercuric sulphide, and the filtrate, after expulsion of hydrogen sulphide, was saturated with hydrogen chloride and evaporated to dryness in a vacuum. The residual white solid crystallised from a small volume of warm acetic acid after addition of light petroleum as needles, m. p. 222—223° (decomp.).

(*b*) The thiothiazolidine ester (20 g.) was heated with concentrated hydrochloric acid (80 c.c.) (sealed vessel) at 140° for 24 hours, and the solution kept at room temperature for 24 hours; the amino-mercapto-acid *hydrochloride* crystallised out in thick needles (13 g.), m. p. 223—224° (frothing, decomp.). A portion was recrystallised from cold methanol by the addition of ether, and the m. p. was unchanged (Found: C, 42·5; H, 7·1; N, 6·1. $C_8H_{16}O_2NSCl$ requires C, 42·6; H, 7·1; N, 6·2%). The acid hydrochloride when neutralised with aqueous sodium hydrogen carbonate and treated with neutral ferric chloride solution gave an intense violet coloration.

4-*Carboxy*-5 : 5-*pentamethylene*-2 : 2-*dimethylthiazolidine Hydrochloride* (VIII; R = H).—The above acid (5 g.) was suspended in dry acetone (30 c.c.), acetone saturated with hydrogen chloride (2 drops) was added, and the mixture heated under reflux under nitrogen for 1 hour. The reaction proceeded throughout in suspension. After cooling to 0° for 1 hour the *thiazolidine hydrochloride* was filtered off as colourless micro-needles (6 g.), m. p. 215—217° (frothing). It recrystallised from acetic acid–ether in colourless needles with the same m. p. (Found: C, 50·0; H, 7·6; N, 5·4; Cl, 13·4; S, 11·6. $C_{11}H_{20}O_2NSCl$ requires C, 49·70; H, 7·6; N, 5·3; Cl, 13·5; S, 12·1%).

Formyl derivative. The above thiazolidine hydrochloride (9 g.) was added to pyridine (9 c.c.). The mixture was warmed for a few minutes and the excess of pyridine removed in a high vacuum at 0°. Without removal of the pyridine hydrochloride, the residual crust was dissolved in 98—100% formic acid (100 c.c.) and, with mechanical stirring and cooling in cold water, acetic anhydride (35 c.c.) was run in dropwise during 5 hours. Water (35 c.c.) was then run in dropwise with stirring, and the solution kept at room temperature for 12 hours. The N-*formyl* derivative separated in stout needles. The

crystals were filtered off, and the mother-liquor evaporated almost to dryness, a second crop being obtained; the total yield was 10·2 g. Recrystallisation from aqueous acetic acid gave silky needles, m. p. 201—202° (Found: C, 56·1; H, 7·4. $C_{12}H_{19}O_3NS$ requires C, 56·0; H, 7·45%).

The above *N*-formylthiazolidine (0·5 g.) was heated with 2N-hydrochloric acid (10 c.c.) under nitrogen for 1 hour. The solution was evaporated in a vacuum and the free amino-mercapto-acid hydrochloride, m. p. 223—224° (frothing), was obtained, undepressed when melted in admixture with the previously prepared sample.

Penicillin Condensation.—(*a*) The amino-mercapto-acid (11 mg.), pyridine (2·5 c.c.), and an excess of 2-benzyl-4-ethoxymethylene-5-oxazolone (22 mg.) were warmed on the water-bath for 0·5 hour. Dry pyridine hydrochloride (0·6 mg.) was then added, and the solution heated in an oil-bath for 10 minutes (external temp. 120°). The reaction mixture was rapidly chilled, and the pyridine removed in a high vacuum at 0°. The residue (*ca.* 0·2—0·5 c.c.) was buffered with 5% phosphate buffer (pH 7) (5 c.c.), and the total volume made up with buffer to 11 c.c. (Solution A). Three blank solutions, B, C, and D were prepared. (B) The amino-mercapto-acid (3 mg.) treated under the condensation conditions without the oxazolone (final volume, 3 c.c.). (C) The oxazolone (6 mg.) treated under the condensation conditions (final volume, 3 c.c.). (D) Pyridine (2·5 c.c.) and pyridine hydrochloride (0·6 mg.) treated under the condensation conditions (final volume, 11 c.c.).

Plate tests (*Staph. aureus*) indicated an inhibition zone of 6 mm. diam. corresponding to *ca.* 0·5 penicillin unit of activity per c.c. for solution A. Solutions B, C, and D showed no inhibition.

(*b*) The above condensation was repeated with the amino-mercapto-acid (200 mg.), pyridine (12 c.c.), and the oxazolone (210 mg.). The total buffer solution was made up to 30 c.c. Total activity 90 units (3 units per c.c.). The solution was cooled and acidified with 1N-phosphoric acid to pH 2·8 and rapidly extracted into ethyl acetate (50 c.c.). The ethyl acetate layer was separated and washed with a small quantity of cold water. The activity was then transferred by shaking with phosphate buffer solution (pH 7·2) (20 c.c.). The buffer solution gave an activity of 40 penicillin units.

4-cyclo*Hexylidene*-2-*phenyl*-5-*oxazolone* (VII).—Hippuric acid (40 g.) was powdered and mixed with finely powdered, freshly fused, sodium acetate (50 g.). The mixture was suspended in acetic anhydride (120 c.c.) and *cyclo*hexanone (22 c.c.) and heated for 1 hour on the steam-bath. The product was poured into crushed ice (200 g.) and light petroleum (400 c.c.). A gum separated which solidified on standing for some hours at 0°. The *oxazolone* (VII), filtered off and crystallised from aqueous ethanol, had m. p. 135—137°. A recrystallisation from aqueous ethanol yielded salmon-pink needles (5 g.), m. p. 135—137° (Found: C, 74·5; H, 6·2; N, 5·95. $C_{15}H_{15}O_2N$ requires C, 74·7; H, 6·2; N, 5·8%).

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