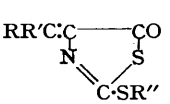
398. *Syntheses in the Penicillin Field. Part IX. A Synthesis of Some Penicillamine Analogues and Attempts to obtain New Types of Penicillins.*

By A. H. Cook, G. Harris, J. R. A. Pollock, and J. M. Swan.

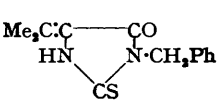
The method of synthesising β-phenylcysteine (Cook, Harris, and Heilbron, Studies in the Azole Series, Part V, *J.*, 1948, 1060) was applied to penicillamine (Cook, Harris, Heilbron, and Shaw, "The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 458). Improved methods of synthesis led to β-(*p*-chlorophenyl)cysteine and β-(*p*-methoxyphenyl)cysteine, which, like the β-phenyl compound, failed to afford penicillin-like activity under standardised conditions. Attempts to apply the synthesis of penicillin due to du Vigneaud and his co-workers to thiazolone instead of oxazolone derivatives were unsuccessful.

The condensation of 2-ethylthiothiazolin-5-one with acetone in place of benzaldehyde was expected to yield the thiazolinone (I; R = R′ = Me, R″ = Et). Reaction of *N*-dithiocarbethoxyglycine (II) with acetone and acetic anhydride gave only the 2-ethylthiothiazolin-5-one, characterised as *N*-dithiocarbethoxyglycine anilide, and as *N*-benzylthioureidoacetbenzylamide, but when sodium acetate was added to the mixture as a condensing agent the reaction proceeded as expected to give 2-ethylthio-4-*iso*propylidenethiazolin-5-one (I; R = R′ = Me, R″ = Et). The last compound was characterised by its reaction with benzylamine forming the thiohydantoin (III), a transformation analogous to that undergone by 2-ethylthio-4-benzylidene-



(I.)

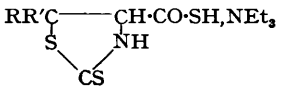
EtS·CS·NH·CH$_2$·CO$_2$H
(II.)

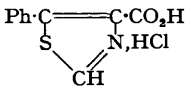


(III.)

thiazolin-5-one (Cook, Harris, and Heilbron, *J.*, 1948, 1060). The compound (I; R = R′ = Me, R″ = Et) with hydrogen sulphide in methanolic triethylamine gave the triethylammonium salt (IV; R = R′ = Me) in good yield. It had been found previously that the hydrolysis of (IV; R = Ph, R′ = H) or the thio-acid with concentrated hydrochloric acid proceeded anomalously. The product obtained did not give the ferric chloride, nitroprusside, or ninhydrin reaction expected for β-phenylcysteine, and the properties and analysis of the product prompt its formulation as 5-phenylthiazole-4-carboxylic acid hydrochloride (V), formed from the thiothiazolidone (IV; R = Ph, R′ = H) by elimination of the elements of hydrogen sulphide. Obviously the compound (IV; R = R′ = Me) could not undergo any comparable change, and treatment with hydrochloric acid gave 2-thio-5 : 5-dimethylthiazolidone-4-carboxylic acid which

was converted into penicillamine by reductive fission with aluminium and acid (Cook, Heilbron, and Shaw, CPS 311; " The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 458).


(IV.)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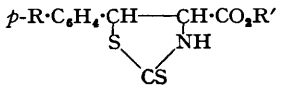
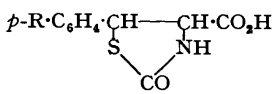

(VI.)

The synthesis of other amino-mercapto-acids by this route were not pursued since more convenient routes utilising the parent 2-mercaptothiazolin-5-one in place of the ethylthio-derivatives were devised (cf. Chatterjee, Cook, Heilbron, and Levy, *J.*, 1948, 1337), although the compounds (I; R = *p*-C_6H_4·NO_2, R′ = H, R″ = Et) and (I; R = *p*-C_6H_4·OAc, R′ = H, R″ = Et) were prepared as possible intermediates for such syntheses.

2-Mercaptothiazolin-5-one was found to condense readily with anisaldehyde, *p*-acetoxybenzaldehyde, and *p*-chlorobenzaldehyde, among others, in acetic acid containing a trace of morpholine to yield 2-mercapto-4-*p*-methoxybenzylidene-, -4-*p*-acetoxybenzylidene-, and -4-*p*-chlorobenzylidene-thiazolin-5-one (I; R = R″ = H, R′ = *p*-C_6H_4·OMe, *p*-C_6H_4·OAc, *p*-C_6H_4Cl respectively). These compounds were well characterised as their 2-methylthio-derivatives (I; R = H, R″ = SMe, R′ = *p*-C_6H_4·OMe, *p*-C_6H_4·OAc, *p*-C_6H_4Cl respectively) by reaction with methyl sulphate and alkali, and the chloro- and acetoxy-compounds were degraded with phosphorus and hydriodic acid to β-(*p*-chlorophenyl)alanine and tyrosine respectively (cf. Billimoria and Cook, *J.*, 1949, 2323).

Treatment of the thiazolinones (I; R = R″ = H, R′ = *p*-C_6H_4·OMe and *p*-C_6H_4Cl) with methanolic sodium hydroxide gave the acids (VI; R′ = H, R = OMe or Cl) and with methanolic sodium methoxide gave the methyl esters (VI; R′ = Me, R = OMe or Cl) as in previous similar experiments. The acetoxy-compound behaved abnormally with alkalis, however, and to obtain isomerisation of the arylidenethiazolinone to the 2-mercapto-Δ^2-thiazoline or the thiothiazolidone system recourse was had to the method used above for the thiazolinone (I; R = R′ = Me, R″ = Et). When this acetoxy-compound was treated with hydrogen sulphide in methanolic triethylamine the compound (IV; R = *p*-C_6H_4·OAc, R′ = H) was obtained in good yield, and this salt was converted into the carboxylic acid (VI; R = OH, R′ = H) by means of concentrated hydrochloric acid. Hydrolysis of the acids (VI; R′ = H, R = OH, OMe, or Cl) with concentrated hydrochloric acid by the method used for the preparation of β-phenylcysteine (Cook, Harris, and Heilbron, *loc. cit.*) was in no case found to lead to the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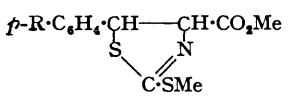
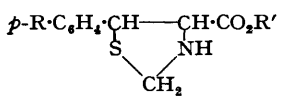

(VIII.)


(IX.)

amino-mercapto-acid, although the hydroxy-compound was isolated from the methoxy-compound in some instances. The difficulty encountered here may well have been caused by partial thiazole formation as encountered above. An effort to overcome these difficulties was made by preparing 5-*p*-methoxyphenyl-2-thiazolidone-4-carboxylic acid (VII; R = OMe) by the oxidation of (VI; R = OMe, R′ = H) with alkaline hydrogen peroxide. The compound (VII; R = H) had been shown to yield β-phenylcysteine on hydrolysis (Cook, Harris, and Heilbron, *loc. cit.*), but (VII; R = OMe) gave no amino-mercapto-acid on such treatment. Reductive degradation of (VI; R = OMe, R′ = H) with aluminium and acid (Cook, Heilbron, and Shaw, CPS 311; *op. cit.*, p. 458) or with tin and acid was likewise unsuccessful.

By this time the discovery of a new method of fission of the thiothiazolidone ring system had been found in another series of compounds containing the amide group in place of the CO_2R groups discussed here (cf. Cook, Hunter, and Pollock, *J.*, in press) and consisted essentially in methylating the 2-mercapto-group of the thiothiazolidones (such as VI) (which may be regarded as 2-mercapto-Δ^2-thiazolines) to give compounds of type (VIII) (cf. Cook, Harris, and Heilbron, *loc. cit*), which were then reduced with aluminium amalgam with loss of methanethiol to form the thiazolidines (IX). Fission of the latter compounds was readily accomplished with mercuric chloride to yield the required amino-mercapto-acids.

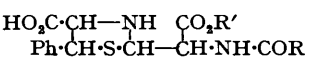
Application of the technique to the acids (VI; R = OMe or Cl, R′ = H), which were methylated with diazomethane, gave the methyl esters of the methylthio-compounds (VIII; R = OMe or Cl), reduction of which with aluminium amalgam in methanol gave the thiazolidines (IX; R′ = Me, R = OMe or Cl), the methoxy-compound being characterised as

its 3-acetyl derivative. Hydrolysis of (IX; R = OMe, R′ = Me) with hydrochloric acid gave no amino-mercapto-acid, but the acid (IX; R = OMe, R′ = H) isolated as its hydrochloride. Fission of this substance with boiling ethanolic mercuric chloride gave only the disulphide of β-(*p*-methoxyphenyl)cysteine, isolated as its oxalate, whereas fission of both (IX; R = OMe, R′ = Me) and (IX; R = Cl, R′ = Me) with hot aqueous mercuric chloride gave β-(*p*-methoxyphenyl)cysteine and β-(*p*-chlorophenyl)cysteine, respectively, as their hydrochlorides.

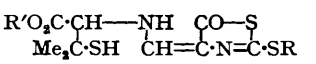
A series of three β-arylcysteines was thus available for attempts to obtain new penicillins.

Attempts to obtain Penicillin-like Materials from β-Phenylcysteines.—When this research was initiated, the thiazolidine-oxazolone structure (cf. *op. cit.*) was widely accepted as representing penicillin, and many attempts were made to obtain this, and later the thiazolidine β-lactam structure, by preparing azlactones from penicilloic acids, and by condensing ethoxymethyleneoxazolones with penicillamine. Accordingly, the preparation of penicilloic acid analogues (X) from β-phenylcysteine was undertaken.

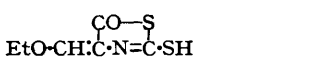
Condensation of β-phenylcysteine hydrochloride with ethyl α-formylphenaceturate and with α-formylhexoamidoacetate gave the thiazolidines (X; R′ = Et, R = CH_2Ph or *n*-C_5H_{11}), isolated first as the hydrochlorides. Hydrolysis of (X; R′ = Et, R = *n*-C_5H_{11}) with one equivalent of sodium hydroxide, isolating the product *via* its lead salt, gave the penicilloic acid analogue (X; R′ = H, R = *n*-C_5H_{11}). The phenylacetyl compounds were more intractable and attention was therefore concentrated on the hexoamido-compound. The attempted conversion of the last-named substance into an azlactone by various reagents comprising acetic anhydride alone and in admixture with pyridine or triethylamine at various temperatures for various times, carbon suboxide, acetic anhydride in methyl oxalate, and benzoyl chloride in pyridine gave no significantly bacteriostatic products (tested by the plate method).



(X.)



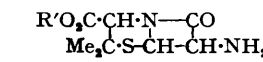
(XI.)



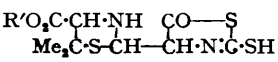
(XII.)

Some early attempts were made to condense β-phenylcysteine with 2-benzyl-4-ethoxymethylene(or -hydroxymethylene)benzyloxazolone in acetic anhydride–pyridine–sodium acetate or buffered dioxan solutions at room temperature or 60°, but again no active products were obtained. Later, it was shown by du Vigneaud and his co-workers (*J. Biol. Chem.*, 1948, **176**, 917) that penicillamine and other amino-mercapto-acids condensed with 2-benzyl-4-ethoxymethyleneoxazolone and other oxazolones to yield penicillins, albeit in very small yield. The application of their conditions to the condensation of β-phenylcysteine with 2-benzyl-4-ethoxymethyleneoxazolone, however, yielded no bacteriostatic product, and similar reactions with β-(*p*-methoxyphenyl)cysteine and β-(*p*-chlorophenyl)cysteine likewise failed to give bacteriostatic activity. Although the optimum conditions for penicillamine might not have been those for the β-arylcysteines with regard to these condensations, and optically inactive arylcysteines were used, it seems significant that these compounds gave rise to no detectable activity, particularly as β-benzyl-β-methylcysteine (Cook and Pollock, *J.*, in press) afforded activity on condensation with the above ethoxymethylene compound.

Attempts to extend the Above Type of Condensation.—It was shown previously (Cook, Harris, Heilbron, and Shaw, *J.*, 1948, 1056) that penicillamine or its methyl ester condensed readily with mercapto(or ethylmercapto)-2-benzyl-4-ethoxymethylenethiazolin-5-one to form analogues of penicillenic acid (XI; R = CH_2Ph or Et, R′ = H or Me), but that these compounds would not undergo closure of the thiazolidine ring. Experiments were also made on the condensation of 2-mercapto-4-ethoxymethylenethiazolin-5-one (XII) with penicillamine derivatives in order



(XIII.)

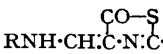


(XIV.)

to obtain a penicillenic acid analogue (XI; R = H, R′ = H or Me), which might be rearranged with loss of carbon disulphide to give the 6-aminothiazolidine-β-lactam structure (XIII), the parent structure of the penicillins, or a thiazolidinyl-thiazolinone structure (XIV), which might undergo self-acylation with loss of carbon disulphide to form (XIII). Presumably (XIV) would be an intermediate in the conversion of (XI; R = H) into (XIII), just as it may be supposed that the thiazolidine-oxazolone structure is intermediate in the transformation of penicillenic acid into penicillin (cf. du Vigneaud and his co-workers, *loc. cit.*).

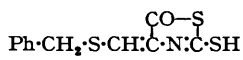
Model reactions showed that the penicillenic acid analogue would be the most likely product of such condensations. Thus, glycine ethyl ester and 2-mercapto-4-ethoxymethylenethiazolin-

5-one in aqueous acetone afforded 2-*mercapto*-4-(*carbethoxymethylaminomethylene*)*thiazolin*-5-*one* (XV; R = $EtO_2C{\cdot}CH_2$). In the same way, glycine sodium salt, DL-alanine methyl ester, and *S*-benzylpenicillamine sodium salt gave 2-mercapto-4-(carboxymethylaminomethylene)- (XV; R = $HO_2C{\cdot}CH_2$·), -4-(1′-carbomethoxypropylaminomethylene) (XV; R = $CHMe{\cdot}CO_2Me$), and



RNH·CH:C·N:C·SH

(XV.)



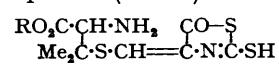
$Ph{\cdot}CH_2{\cdot}S{\cdot}CH{:}C{\cdot}N{:}C{\cdot}SH$

(XVI.)

-4-(1′-carboxy-2′-benzylthio-2′ : 2′-dimethylpropylaminomethylene)-thiazolin-5-one [XV; R = $Me_2C(S{\cdot}CH_2Ph){\cdot}CH{\cdot}CO_2H$] respectively. These compounds showed characteristic ultra-violet absorption maxima at 2450 and 3370—3460 A. Reaction between thiols and the ethoxymethylene compound appeared to be more sluggish, the latter reacting with toluene-ω-thiol only in the presence of a catalyst (triethylamine) to form two isomers of 2-mercapto-4-benzylthiomethylenethiazolin-5-one (XVI), presumably *cis*- and *trans*- about the exocyclic double bond.

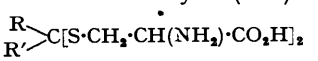
When penicillamine (as its sodium salt or methyl ester) was allowed to react with the sodium salt of (XII) in water, or with (XII) in ethanol, only amorphous, easily oxidised products were obtained. With penicillamine in ethanol, no reaction occurred, and in pyridine the product was an oil, whereas in aqueous Cellosolve the amorphous product was rapidly oxidised to a red tar. These difficulties were possibly caused in part by reaction of (XII) with the thiol group of penicillamine and, in part, by ring-opening of the thiazoline by the amino-group as experience had shown was feasible. Accordingly, reaction of (XV) with derivatives of 2 : 2 : 5 : 5-tetramethylthiazolidine-4-carboxylic acid was attempted, but the thiazolidine proved insufficiently basic to undergo the desired reaction in hot acetone, and, in the presence of triethylamine, only an amorphous product was obtained.

Another approach to (XIII) lay in attempts to obtain the compound (XVII), which on rearrangement *via* a seven-membered ring structure could give (XIII). The protective effect of salt formation on amino-groups appeared to be applicable to this case. Thus, Ratner and Clarke (*J. Amer. Chem. Soc.*, 1937, **59**, 200) in a study of the rate of formation of thiazolidine-4-carboxylic acid from cysteine and formaldehyde were able to show that in acid solution initial reaction occurred on the sulphur atom, and Armstrong and du Vigneaud (*J. Biol. Chem.*, 1948, **173**, 749) treated a variety of aldehydes and ketones with cysteine in 6N-hydrochloric acid to obtain the compounds (XVIII) instead of thiazolidines. The stability of (XII) to acids made



$RO_2C{\cdot}CH{\cdot}NH_2$; $Me_2C{\cdot}S{\cdot}CH{=}C{\cdot}N{:}C{\cdot}SH$; CO—S

(XVII.)



$R{>}C[S{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H]_2$; R′

(XVIII.)

the preparation of (XVII) seem feasible. However, when methyl penicillaminate hydrochloride and (XII) were refluxed together for long periods in chloroform, benzene, or ethyl acetate, or heated in anisole at 100°, only the unchanged reactants were recovered. At higher temperatures (refluxing anisole) decomposition occurred, and reaction in methanoliic hydrogen chloride or 6N-hydrochloric acid failed to bring about the desired condensation. Extension of such reactions to methyl *N*-acetylpenicillaminate or *N*-phenylacetylpenicillaminate, and *N*-carbobenzyloxypenicillamine was equally unsuccessful. Similar attempts to cause *N*-phenylthiocarbonylpenicillamine to react with oxazolones are described in a later paper.

Experimental.

Penicillamine Analogues.—*N*-Dithiocarbethoxyglycine (2·5 g.), acetone (2·0 g.), and acetic anhydride (6 c.c.) were heated on the steam-bath for 15 minutes, solvents were removed *in vacuo*, and the residue was distilled at 40° in a high vacuum, to give a pale yellow oil. A portion reacted vigorously with ethereal benzylamine. The product, recrystallised from chloroform–light petroleum, gave *N*-benzylthioureidoacetbenzylamide as needles, m. p. 145—146° (Found: C, 65·7; H, 6·1; N, 13·5. Calc. for $C_{17}H_{19}ON_3S$: C, 65·2; H, 6·1; N, 13·4%). A portion of the oil with ethereal aniline gave N-*dithiocarbethoxyglycine anilide*, which separated from chloroform as white laths, m. p. 159—160° (decomp.) (Found: C, 52·3; H, 5·8; N, 11·0. $C_{11}H_{14}ON_2S_2$ requires C, 52·0; H, 5·6; N, 11·0%).

N-Dithiocarbethoxyglycine (10 g.), acetone (26 g.), acetic anhydride (20 g.), and anhydrous sodium acetate (5 g.) were refluxed together for 3¾ hours. When the product was worked up by dissolution in ether and removal of acidic material with sodium hydrogen carbonate an oil was obtained, distillation of which gave 2-ethylthio-4-*iso*propylidenethiazolin-5-one (I; R = R′ = Me, R″ = Et) (5·5 g.), b. p. 110—115°/0·2 mm. (Found: N, 6·5. Calc. for $C_8H_{11}ONS_2$: N, 7·0%). With ethereal benzylamine, a portion of the oil gave 1-*benzyl*-4-iso*propylidene*-2-*thiohydantoin* (III), which crystallised from chloroform–light petroleum as white needles, m. p. 201° (Found: C, 63·4; H, 5·8. $C_{13}H_{14}ON_2S$ requires C, 63·4; H, 5·7%).

Triethylammonium 2-thio-5-phenylthiazolidone-4-thiocarboxylate (IV; R = H, R′ = Ph) (6·8 g.)

was heated for 16 hours at 90—100° with concentrated hydrochloric acid (35 c.c.) in a sealed tube. After cooling to 0°, the crystalline precipitate was removed, washed with *iso*propanol and water, and recrystallised several times from aqueous ethanol. *5-Phenylthiazole-4-carboxylic acid hydrochloride* (V) separated as clear, hexagonal plates, m. p. 225° (decomp.) (Found: C, 50·1; H, 3·7; N, 6·1. $C_{10}H_8NClS$ requires C, 49·8; H, 3·4; N, 5·8%). It gave no colour with ninhydrin or alkaline sodium nitroprusside, and a red precipitate with aqueous ferric chloride. From the filtrates, β-phenylcysteine hydrochloride was obtained (31% in one experiment). Hydrolysis of the free thiocarboxylic acid also gave a mixture of the thiazole and β-phenylcysteine.

N-Dithiocarbethoxyglycine (3·6 g.), *p*-hydroxybenzaldehyde, and acetic anhydride (15 c.c.) were heated together on the steam-bath for 10 minutes. Solvents were removed *in vacuo*, finally over potassium hydroxide. The sludge was treated with a small quantity of acetone, and the orange solid washed with *n*-amyl alcohol and with light petroleum, and recrystallised from acetone–water, *2-ethylthio-4-p-acetoxybenzylidenethiazolin-5-one* (I; R = H, R″ = Et, R′ = *p*-AcO·C_6H_4) (19%) separating as pale orange, rhombic plates, m. p. 108—110° (Found: C, 54·8; H, 4·5; N, 4·7. $C_{14}H_{13}O_3NS_2$ requires C, 54·7; H, 4·3; N, 4·6%). *N*-Dithiocarbethoxyglycine (1·8 g.), *p*-nitrobenzaldehyde (1·5 g.), and acetic anhydride (5 c.c.) were heated together at 100° for 3 minutes. The product (1·8 g.), which crystallised on cooling, was washed with light petroleum and ethanol and recrystallised from ethanol, *2-ethylthio-4-p-nitrobenzylidenethiazolin-5-one* (I; R = H, R″ = Et, R′ = *p*-NO_2·C_6H_4) separating as yellow needles, m. p. 161° (Found: C, 49·4; H, 3·7; N, 9·4. $C_{12}H_{10}O_3N_2S_2$ requires C, 49·0; H, 3·4; N, 9·5%). The filtrates gave a further 0·2 g.

β-(p-Methoxyphenyl)cysteine and Derivatives of β-(p-Hydroxyphenyl)cysteine.—*p*-Anisaldehyde (20 g.) was dissolved in boiling acetic acid (150 c.c.) containing 2-mercaptothiazolin-5-one (20 g.), and morpholine (10 drops) added. After 2 hours at room temperature, the yellow crystalline product (30 g.) was filtered off and washed with acetic acid and water; a further crop (2 g.) separated on dilution of the filtrate with water (500 g.). Recrystallised from acetic acid, *2-mercapto-4-p-methoxybenzylidenethiazolin-5-one* (I; R = R″ = H, R′ = *p*-C_6H_4·OMe) separated as yellow needles, m. p. 212° (decomp.) (Found: C, 52·2; H, 3·5; N, 5·8. $C_{11}H_9O_2NS_2$ requires C, 52·6; H, 3·6; N, 5·6%). This compound (1 g.) was taken up in 0·5N-potassium hydroxide (8 c.c.), methyl sulphate (0·5 g.) added, and the mixture shaken for 10 minutes. The yellow solid (1 g.) was recrystallised from a small volume of ethanol, *2-methylthio-4-p-methoxybenzylidenethiazolin-5-one* separating as long yellow needles, m. p. 124° (Found: N, 5·2. $C_{12}H_{11}O_2NS_2$ requires N, 5·3%).

2-Mercapto-4-*p*-methoxybenzylidenethiazolin-5-one (5 g.) was dissolved in hot methanolic sodium methoxide (1 g. of sodium per 30 c.c.) and kept for 1 hour. It was poured into ice-cold 2N-hydrochloric acid (150 c.c.), and the mixture kept for 1 hour. The gum was taken up in acetone and crystallised by the cautious addition of water at 0°. Next morning the product (2·8 g.) was recrystallised from acetone–water, *methyl 2-mercapto-5-p-methoxyphenylthiazoline-4-carboxylate* (VI; R = OMe, R′ = Me) forming pale yellow needles, m. p. 108° (Found: C, 50·7; H, 4·7. $C_{12}H_{13}O_3NS_2$ requires C, 50·9; H, 4·6%).

2-Mercapto-4-*p*-methoxybenzylidenethiazolin-5-one (37 g.) was added to a hot solution of sodium hydroxide (11 g.) in methanol (250 c.c.). After 2·5 hours at room temperature the solution was filtered through glass wool, diluted with water (1 l.), and acidified with concentrated hydrochloric acid. The oil crystallised when seeded. The product (28 g.) was recrystallised from acetone–water and boiling water, to give *2-mercapto-5-p-methoxyphenylthiazoline-4-carboxylic acid* as colourless, hexagonal plates, m. p. 149° (decomp.) (Found: C, 49·4; H, 4·3. $C_{11}H_{11}O_3NS_2$ requires C, 49·1; H, 4·1%).

Methyl 2-mercapto-5-*p*-methoxyphenylthiazoline-4-carboxylate (2 g.) in 10% aqueous sodium hydroxide (10 c.c.) was treated with 20-vol. hydrogen peroxide (10 c.c.). After 3 hours the solution was acidified with concentrated hydrochloric acid and the sticky solid crystallised from aqueous methanol (yield, 1·2 g.; m. p. 135°). Crystallised from water, 5-*p*-methoxyphenyl-2-thiazolidone-4-carboxylic acid separated as colourless rods, m. p. 138°.

β-(p-Chlorophenyl)cysteine.—*p*-Chlorobenzaldehyde (McEwen, *Org. Synth.*, Coll. Vol. II, 1943, p. 133 (18 g.) and 2-mercaptothiazolin-5-one (16 g.) were condensed in hot acetic acid (250 c.c.) containing morpholine (10 drops). *2-Mercapto-4-p-chlorobenzylidenethiazolin-5-one* (18 g.) was collected next morning, and recrystallised from acetic acid as orange needles, m. p. 250° (decomp.) (Found: C, 47·3; H, 2·6. $C_{10}H_6ONClS_2$ requires C, 47·0; H, 2·4%). This compound (1 g.) in 2N-sodium hydroxide (2·5 c.c.) was methylated by methyl sulphate (0·4 c.c.). The *2-methylthio*-derivative (1 g.) separated from methanol as golden needles, m. p. 145—146° (Found: C, 49·8; H, 3·0. $C_{11}H_8ONClS_2$ requires C, 48·9; H, 3·0%). The original mercaptothiazolinone (2 g.) in acetic acid (25 c.c.) and 40% hydriodic acid (10 c.c.) was heated under reflux for 4 hours with red phosphorus (2 g.). After cooling and filtration, the solution was evaporated *in vacuo*. The residue in water (15 c.c.) was repeatedly extracted with benzene to remove iodine. The aqueous layer was neutralised with aqueous ammonia (2%) and evaporated to dryness.

2-Mercapto-4-p-acetoxybenzylidenethiazolin-5-one (I; R = R″ = H, R′ = *p*-AcO·C_6H_4) (16 g.) separated from acetic acid as yellow needles, m. p. 180°, resolidifying and remelting at 196° (Found: C, 51·9; H, 3·1; N, 5·0. $C_{12}H_9O_3NS_2$ requires C, 51·6; H, 3·25; N, 5·0%). A portion (1 g.) in 0·5N-potassium hydroxide (9 c.c.) was methylated with methyl sulphate (0·5 g.). The yellow product (1 g.) was recrystallised from methanol, *2-methylthio-4-p-acetoxybenzylidenethiazolin-5-one* separating with m. p. 114° (Found: C, 53·6; H, 3·9. $C_{13}H_{11}O_3NS_2$ requires C, 53·25; H, 3·75%).

A suspension of 2-mercapto-4-*p*-acetoxybenzylidenethiazolin-5-one (5 g.) in methanol (20 c.c.) was treated with triethylamine (2 g.), dissolution occurring. Hydrogen sulphide was passed into the solution for 12 hours, and the liquid was then diluted with ether (250 c.c.) and seeded. The product (2·5 g.), m. p. 120—124°, was recrystallised from ethyl acetate, *triethylammonium 2-mercapto-5-p-acetoxyphenyl-Δ^2-thiazoline-4-thiocarboxylate* (IV; R = AcO, R′ = $NHEt_3$) separating as colourless prisms, m. p. 127—128° (decomp.) (Found: C, 52·7, 52·7; H, 6·4, 5·5; N, 6·9. $C_{18}H_{26}O_3N_2S_3$ requires C, 52·2; H, 6·3; N, 6·8%).

(*a*) 2-Mercapto-5-*p*-hydroxyphenylthiazoline-4-carboxyamide (1 g.) (Cook, Hunter, and Pollock, *J.*, in press) was refluxed for 1 hour with 2N-sodium hydroxide (10 c.c.), ammonia being evolved.

Acidification of the solution gave 2-*mercapto*-5-p-*hydroxyphenylthiazoline*-4-*carboxylic acid* (0·1 g.), which, recrystallised from water, separated as pale yellow, rhombic plates, m. p. 196° (Found: C, 47·5; H, 3·7. $C_{10}H_9O_3NS_2$ requires C, 47·1; H, 3·6%).

(*b*) The triethylammonium salt above (2 g.) in ethanol (5 c.c.) was treated with concentrated hydrochloric acid (2 c.c.). The solvent was removed and the gum extracted with a mixture of ethyl acetate (20 c.c.) and water (10 c.c.). The aqueous layer was re-extracted with ethyl acetate (10 c.c.) and the combined extracts were evaporated to give a yellow oil which was digested on the steam-bath for 3 hours with concentrated hydrochloric acid (10 c.c.). The solution was diluted to 30 c.c. with water and extracted with ethyl acetate (3 × 20 c.c.). The solution was filtered from amorphous material (probably the derived diketopiperazine), dried, and evaporated to small bulk and light petroleum (b. p. 60—80°) was cautiously added. The above acid (0·7 g.) separated as colourless plates containing ethyl acetate of crystallisation and having m. p. 122° (decomp.) (Found: C, 49·0, 48·9; H, 4·8, 4·8; N, 4·7. $C_{10}H_9O_3NS_2,C_4H_8O_2$ requires C, 49·0; H, 5·0; N, 4·1%). When kept at 100°/14 mm. for 18 hours it became sticky and, when recrystallised from hot water (charcoal), separated as plates, m. p. 190—194°, undepressed on admixture with the material obtained as recorded under (*a*).

2-Mercapto-5-*p*-methoxyphenylthiazoline-4-carboxylic acid (20 g.), suspended in ether (50 c.c.), was treated with diazomethane (10 g.) in ether (500 c.c.) slowly with stirring. After 2 hours ether was removed, leaving a viscous oil. The latter in methanol (1 l.) was reduced with aluminium amalgam (30 g.). After reaction was complete the hot solution was filtered and the alumina washed with hot methanol (2 × 250 c.c.). The filtrate was evaporated until cloudy and diluted with water. The product (6 g.) was recrystallised from aqueous methanol, giving *methyl* 5-p-*methoxyphenylthiazolidine*-4-*carboxylate* (IX; R = MeO, R′ = Me) as needles, m. p. 81° (Found: C, 56·7; H, 6·0; N, 4·8. $C_{12}H_{15}O_3NS$ requires C, 56·9; H, 6·0; N, 5·5%). Warming this with acetic anhydride gave the *acetyl* derivative, which crystallised from aqueous methanol as laths, m. p. 94—95° (Found: C, 56·8; H, 6·1. $C_{14}H_{17}O_4NS$ requires C, 57·0; H, 5·8%). Hydrolysis of the thiazolidine ester (0·5 g.) with 4N-hydrochloric acid (20 c.c.) during 13 hours, followed by evaporation to dryness and stirring of the residue with dry ether gave 5-p-*methoxyphenylthiazolidine*-4-*carboxylic acid hydrochloride* (0·4 g.) which recrystallised from methanol–ether, whereafter it had m. p. 193—196° (decomp.) (Found: C, 47·5; H, 5·4; N, 4·7. $C_{11}H_{14}O_3NClS$ requires C, 47·9; H, 5·1; N, 5·1%). The hydrochloride (0·3 g.) was dissolved in water (2 c.c.), and the product (0·2 g.) collected. Recrystallised from water the resultant free *acid* separated as prisms, m. p. 210° (decomp.) (Found: C, 55·1; H, 5·7; N, 6·3. $C_{11}H_{13}O_3NS$ requires C, 55·5; H, 5·9; N, 5·9%).

Methyl 5-*p*-methoxyphenylthiazolidine-4-carboxylate (2·5 g.) in methanol (10 c.c.) was added to mercuric chloride (5 g.) in methanol (300 c.c.). The clear mixture was refluxed for 1 hour, concentrated, and decomposed with hydrogen sulphide. Evaporation of the filtrate gave a yellow gum which was treated with hot acetone for 5 minutes, the solution evaporated, and the residue stirred with aqueous sodium hydrogen carbonate. The yellow oil was extracted into ether and dried, and oxalic acid added. After being kept overnight the product (0·7 g.) was separated and recrystallised from methanol–ether–light petroleum, *di*-(2-*amino*-2-*carbomethoxy*-1-p-*methoxyphenylethyl*) *disulphide di*(*hydrogen oxalate*) forming needles, m. p. 148° (Found: C, 47·0; H, 5·2; N, 3·8. $C_{25}H_{32}O_{14}N_2S_2$ requires C, 47·3; H, 4·9; N, 4·2%).

The technique described below for β-(*p*-chlorophenyl)cysteine was applied to the preparation of β-(p-*methoxyphenyl*)*cysteine hydrochloride* from methyl 5-*p*-methoxyphenylthiazolidine-4-carboxylate. The product was recrystallised by the slow addition of ether to a solution in dry ethanol; it separated as colourless prisms, m. p. 166° (Found: C, 45·0; H, 5·6; N, 4·9. $C_{10}H_{13}O_3NS,HCl$ requires C, 45·5; H, 5·4; N, 5·3%). It gave an indigo-blue colour with ferric chloride in neutral solution, and a red-purple colour with ninhydrin.

p-Acetoxybenzaldehyde (11 g.) (cf. Chattaway, *J.*, 1931, 2495) was added to a hot solution of 2-mercaptothiazolin-5-one. Piperidine (4 drops) was added, and after 2 hours the product was filtered off and washed with acetic acid, the residue stirred with water, and the product (0·8 g.) removed and recrystallised from water (charcoal). β-(*p*-Chlorophenyl)alanine separated as hexagonal tablets, m. p. 253° (decomp.) (Found: C, 54·3; H, 5·6; N, 6·7. Calc. for $C_9H_{10}O_2NCl$: C, 54·1; H, 5·0; N, 7·0%). The original mercaptothiazolinone (30 g.) was dissolved in hot methanolic potassium hydroxide (9 g./100 c.c.) and the solution kept for 0·5 hour. It was diluted to 500 c.c. and acidified. After 12 hours at 0° the aqueous layer was decanted and the oil dissolved in ethyl acetate (100°) and dried (solution *A*). A portion of *A* was extracted with aqueous sodium hydrogen carbonate, and the aqueous solution washed with ethyl acetate. Acidification gave an oil, extracted into ethyl acetate and dried. Addition of light petroleum (b. p. 40—60°) precipitated 2-*mercapto*-5-p-*chlorophenylthiazoline*-4-*carboxylic acid* (VI; R = Cl, R′ = H), which recrystallised as needles, m. p. 176° (Found: C, 43·8; H, 3·2; N, 5·3. $C_{10}H_8O_2NClS_2$ requires C, 43·9; H, 3·0; N, 5·1%). Solution *A* was treated with diazomethane (11 g.) in ether (300 c.c.). After 3 hours, evaporation of solvents gave a yellow oil, which was taken up in methanol (400 c.c.) and reduced in the usual way with aluminium amalgam (20 g.). The methanol filtrate was evaporated to small bulk and diluted with water (1 l.). The product (10·2 g.), m. p. 110°, was recrystallised from aqueous methanol to give *methyl* 5-p-*chlorophenylthiazolidine*-4-*carboxylate* (IX; R = Cl, R′ = Me) as needles, m. p. 114—115° (Found: C, 50·8; H, 4·7; N, 5·2. $C_{11}H_{12}O_2NClS$ requires C, 51·3; H, 4·7; N, 5·4%).

The above thiazolidine (1 g.) in ethanol (10 c.c.) was heated to boiling and diluted with hot water (100 c.c. at 60°). The milky liquid was added immediately to hot aqueous mercuric chloride (2·5 g./100 c.c.). Formaldehyde was evolved, a white curd being precipitaed. After cooling, the filtered curd was thoroughly washed, suspended in N-hydrochloric acid, and decomposed with hydrogen sulphide. The filtrate was evaporated to dryness and the residue, m. p. 170—173° (decomp.), recrystallised from ethanol–ether; β-(p-*chlorophenyl*)*cysteine hydrochloride* separated as colourless plates, m. p. 177° (decomp.) (Found: C, 39·7; H, 4·8; N, 5·1. $C_9H_{11}O_2NCl_2S$ requires C, 40·0; H, 4·1; N, 5·2%). It gave a purple colour with ferric chloride in neutral solution, and a red-purple colour with ninhydrin.

Attempts to obtain Penicillin-like Materials from β-Phenylcysteines.—(*a*) β-Phenylcysteine hydrochloride (2·3 g.) and ethyl α-formylhexoamidoacetate (2·6 g.) were heated for 10 minutes at 100° with a few drops of methanol, whereafter the melt gave no colour with ferric chloride. The resin was triturated with dry ether containing a little hydrogen chloride, and the white solid (2·8 g.) kept under dry ether until it no longer became sticky on exposure to air. Several recrystallisations from methanol–ether yielded *5-phenyl-2-hexoamidocarbethoxymethylthiazolidine-4-carboxylic acid hydrochloride* (X; R = C_5H_{11}, R′ = Et) as white needles, m. p. 179° (decomp.) (Found: C, 53·5; H, 6·6; N, 6·8. $C_{20}H_{29}O_5N_2ClS$ requires C, 54·0; H, 6·6; N, 6·5%). On dissolution of this in aqueous sodium hydrogen carbonate, acidification, and extraction with chloroform, *5-phenyl-2-hexoamidocarbethoxymethylthiazolidine-4-carboxylic acid* was obtained and had m. p. 135°, after recrystallisation from ethanol–water (Found: C, 56·6; H, 7·2; N, 6·3. $C_{20}H_{28}O_5N_2S,H_2O$ requires C, 56·3; H, 7·1; N, 6·6%). β-Phenylcysteine (1·0 g.) was heated for 12 minutes at 100° with ethyl α-formylphenaceturate (1·5 g.) and methanol (1 c.c.). After 4 minutes a few drops of methanolic hydrogen chloride were added and further heating conducted in a vacuum. The pale-orange resinous product was rendered solid as above, and the product (1·7 g.) dried *in vacuo* and purified by precipitation from chloroform–ether. *5-Phenyl-2-phenylacetamidocarbethoxymethylthiazolidine-4-carboxylic acid hydrochloride* (X; R = CH_2Ph, R′ = Et) was a white, amorphous solid, m. p. 100° (decomp.) (Found: C, 54·9; H, 5·5; N, 6·2. $C_{22}H_{25}O_5N_2ClS,H_2O$ requires C, 54·7; H, 5·6; N, 5·8%). The free *base* was obtained with chloroform and aqueous potassium acetate as a white powder, m. p. *ca.* 130° (decomp.) (Found: C, 62·5; H, 5·9; N, 6·7. $C_{22}H_{24}O_5N_2S$ requires C, 61·7; H, 5·7; N, 6·5%). It is probably a mixture of stereoisomers.

5-Phenyl-2-hexoamidocarbethoxymethylthiazolidine-4-carboxylic acid hydrochloride (3·9 g.) was dissolved in 0·509N-sodium hydroxide (52·5 c.c.) and kept for 23 hours. On filtration of the solution through charcoal, addition of acetic acid (1·58 g.) and aqueous lead acetate (30%; 12 c.c.) at 0°, a white precipitate (4·2 g.) was obtained. After being thoroughly washed, dried, and suspended in ethanol (30 c.c.) it was decomposed with hydrogen sulphide for 20 minutes. The lead sulphide was washed with ethanol (3 × 5 c.c.), and the filtrate evaporated *in vacuo*. The residue was dried and triturated with ether–light petroleum (1 : 1). The solid product (2·35 g.) had m. p. 102—104° (decomp.), and separated from chloroform–light petroleum; *5-phenyl-2-hexoamidocarboxymethylthiazolidine-4-carboxylic acid* had m. p. 108° (decomp.) (Found: C, 56·6; H, 6·4; N, 6·4. $C_{18}H_{24}O_5N_2S$ requires C, 56·8; H, 6·4; N, 7·4%). The foregoing acid (22 mg.), acetic anhydride (0·2 c.c.), and pyridine (0·1 c.c.) were heated together at 50° for 15 minutes, and solvents removed at 0·1 mm. (20 minutes). The residue was dissolved in 5% phosphate buffer (10 c.c.) at pH 7·0. It was inactive, as in a blank experiment. The same result was obtained when the acid was set aside with acetic anhydride and pyridine or triethylamine for 1·5 or 22 hours, or was heated with acetic anhydride alone, or in admixture with methyl oxalate, for 3 minutes. Keeping the acid with carbon suboxide or benzoyl chloride and pyridine for 20 minutes likewise gave inactive products. β-Phenylcysteine hydrochloride (22 mg.) was kept for 14 hours with aqueous sodium acetate (50 mg./1 c.c.) and 2-benzyl-4-ethoxymethyleneoxazolone (25 mg.) in dioxan (1·0 c.c.). The mixture was diluted with 5% phosphate buffer (20 c.c.) and assayed. It was inactive. Similarly, no activity was obtained by use of phenylcysteine hydrochloride and the sodium salt of 2-benzyl-4-hydroxymethyleneoxazolone in buffer or with the ethoxymethylene-oxazolone in acetic anhydride and pyridine overnight or at 60° for 10 minutes.

(*b*) *Further Condensations with 2-Benzyl-4-ethoxymethyleneoxazolone.*—(i) *β-Phenylcysteine.* β-Phenylcysteine hydrochloride (12·7 mg.), pyridine (2·5 c.c.), and the oxazolone (25·2 mg.) were heated at 100° for 30 minutes and then at 110° with a catalytic quantity of pyridine hydrochloride for 15 minutes. Solvents were removed at 0·1 mm. pressure, and the residue dissolved in acetone (6·4 c.c.), water (3·2 c.c.), and 5% phosphate buffer (4·2 c.c.) at pH 7·0. No activity was observed. β-Phenylcysteine hydrochloride (13·2 mg.), pyridine (2·5 c.c.), and the oxazolone (30·9 mg.) were heated at 100° for 30 minutes. Treatment as above gave an inactive solution. Repetition of the above experiments, and working up the residue from removal of solvents by shaking it with ether and buffer, likewise gave no activity. The amino-mercapto-acid itself was inactive.

(ii) *β-(p-Chlorophenyl)cysteine.* The hydrochloride of the mercapto-amino-acid (4·8 mg.) and 2-benzyl-4-ethoxymethylene-5-oxazolone (11·2 mg.) were heated together in dry pyridine (2 c.c.) at 100° for 0·5 hour. Pyridine hydrochloride (0·5 mg.) was added, the solution heated at 110—115° for 15 minutes and then chilled in ice, and pyridine removed in a vacuum at room temperature. The residue was dissolved in acetone (1 c.c.) and the solution diluted to 5 c.c. bulk with 5% phosphate buffer at pH 7·0.

(iii) *β-(p-Methoxyphenyl)cysteine.* The same method was used with mercapto-amino-acid hydrochloride (5 mg.) and the oxazolone (10 mg.).

In neither case (ii) nor case (iii) was antibiotic activity observed, although blanks with the mercapto-amino-acid hydrochlorides at concentrations of 1 mg./c.c. gave 6-mm. zones of inhibition of bacteria in the plate test.

Reactions with 2-Mercapto-4-ethoxymethylenethiazolin-5-one.—Glycine ether ester hydrochloride (0·347 g.) in acetone (5 c.c.) and 1·1N-potassium hydroxide (2·27 c.c.) was treated with the thiazolinone (0·472 g.) in warm acetone (20 c.c.). After this had been kept for 2·5 hours at room temperature, acetone (15 c.c.) was added and the mixture set aside overnight. The product (0·2 g.), m. p. 211° (decomp.), was filtered off and a further quantity (0·32 g.) obtained from the filtrate by rubbing with ether. Recrystallised from ethanol, *2-mercapto-4-carbethoxymethylaminomethylthiazolin-5-one* (XV; R = $CH_2{\cdot}CO_2Et$) separated as yellow plates or laths, m. p. 210° (decomp.) (Found: C, 38·5; H, 4·2; N, 11·3. $C_8H_{10}O_3N_2S_2$ requires C, 39·0; H, 4·1; N, 11·4%). Light absorption (ethanol): λ_{max} = 2450 and 3370 A. ($E^{1\%}_{1\,cm.}$ 400 and 570 respectively).

Glycine (0·187 g.) in acetone (5 c.c.) and water (30 c.c.) containing 1·1N-potassium hydroxide (2·27 c.c.) was treated with the thiazolinone (0·47 g.) in warm acetone (20 c.c.), and the mixture shaken for 2 hours and then set aside for 26 hours. 0·945N-Hydrochloric acid (2·65 c.c.) was added and the solution evaporated to small bulk *in vacuo*. The crystalline deposit (0·45 g.), m. p. 225° (decomp.), was

washed with water and recrystallised from acetic acid, *2-mercapto-4-carboxymethylaminomethylenethiazolin-5-one* (XV; R = $CH_2 \cdot CO_2H$) separating as sandy prisms, m. p. 222° (decomp.) (Found: C, 32·5; H, 3·1; N, 12·6. $C_6H_6O_3N_2S_2$ requires C, 33·0; H, 2·8; N, 12·8%). Light absorption (ethanol): $\lambda_{max.}$ = 2470 and 3390 A. ($E^{1\%}_{1\,cm.}$ 450 and 590 respectively).

DL-Alanine methyl ester (0·347 g.) in acetone (5 c.c.) and 1·1N-potassium hydroxide (2·27 c.c.) was treated as above with the thiazolinone (0·47 g.) in warm acetone (20 c.c.). *2-Mercapto-4-(1'-carbomethoxypropylaminomethylene)thiazolin-5-one* (XV; R = $CHMe \cdot CO_2Me$) (0·45 g.) recrystallised from ethanol as yellow prisms, m. p. 210—211° (decomp.) (Found: C, 38·9; H, 4·3; N, 11·2. $C_8H_{10}O_3N_2S_2$ requires C, 39·0; H, 4·1; N, 11·4%). Light absorption (ethanol): $\lambda_{max.}$ = 2450, 2500, and 3640 A. ($E^{1\%}_{1\,cm.}$ 470, 450, and 660 respectively).

The method for glycine above was used for *S*-benzylpenicillamine (0·597 g.) in 1·1N-potassium hydroxide (2·27 c.c.) and acetone (5 c.c.) with the thiazolinone (0·472 g.) in acetone (20 c.c.). *2-Mercapto-4-(1'-carboxy-2'-benzylthio-2' : 2'-dimethylpropylaminomethyl)thiazolin-5-one* [XV; R = $Me_2C(SCH_2Ph) \cdot CH \cdot CO_2H$] (0·95 g.) separated from acetone–water and acetic acid as yellow needles, m. p. 220° (decomp.) (Found: C, 50·2; H, 5·0. $C_{16}H_{18}O_3N_2S_3$ requires C, 50·2; H, 4·7%). Light absorption (ethanol): $\lambda_{max.}$ = 2450 and 3440 A. ($E^{1\%}_{1\,cm.}$ 390 and 490 respectively).

The thiazolinone (1·89 g.) and toluene-ω-thiol (1·24 g.) were heated at 100° for 5 minutes with triethylamine (1 c.c.). The red solution was shaken with chloroform and 2N-hydrochloric acid. The product (0·7 g.) was filtered off, and recrystallised from acetic acid and ethanol repeatedly, giving one *isomer* of *2-mercapto-4-benzylthiomethylenethiazolin-5-one* (XVI) as yellow needles, m. p. 185° (Found: C, 50·0; H, 3·3; N, 5·0; S, 35·7. $C_{11}H_9ONS_3$ requires C, 49·4; H, 3·4; N, 5·2; S, 36·0%). Light absorption (ethanol): $\lambda_{max.}$ = 2600, 2970, and 3650 A. ($E^{1\%}_{1\,cm.}$ 215, 195, and 500 respectively). The filtrate was separated, and the washed chloroform solution evaporated. Treatment of the residue with ether gave a crystalline precipitate (0·8 g.). Recrystallised from acetic acid and ethanol the second *isomer* formed prisms or needles, m. p. 143° (Found: C, 49·5; H, 3·4; N, 5·2. $C_{11}H_9ONS_3$ requires C, 49·4; H, 3·4; N, 5·2%). The m. p. of this was undepressed on admixture with its isomer, and crystallisation from acetic acid caused partial conversion into the higher-melting product.

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