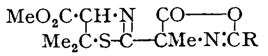
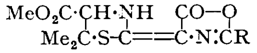
677. *Syntheses in the Penicillin Field. Part VI. Attempts to synthesise True Thiazolinyloxazolones.*

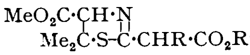
By A. H. Cook, J. A. Elvidge, A. R. Graham, and G. Harris.

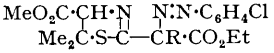
The compounds previously described as thiazolinyloxazolones were shown in Part V to be thiazolidylideneoxazolones (II) and thus not reducible to thiazolidyloxazolones or penicillins. The present work records attempts to obtain true Δ^2-thiazolinyloxazolones by the synthesis of the " methyldehydropenicillins " (I). Reduction of the compounds (I) would lead to methyl analogues of the penicillins, but the desired intermediates were not obtained.

Thiazolidylideneoxazolones (II) (Part V) were prepared, *via* the thiazoline (III; R = H, R′ = Et, or CH_2Ph), from penicillamine methyl ester and the imino-ethers derived from esters of cyanoacetic acid ("The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 858). An obvious route to the thiazoline (III; R = Me, R′ = Et), required for the preparation of (I) by a similar series of reactions, lay in the use of ethyl α-cyanopropionate. The preparation of this ester by treating the crystalline monosodio-derivative of ethyl cyanoacetate with methyl iodide in anhydrous ether was unsatisfactory. Reaction of the imino-ether, obtained from the product of this reaction, with penicillamine methyl ester yielded an oily thiazoline containing much of the compound (III; R = H, R = Et) in addition to (III; R = Me, R′ = Et), as was shown

(I.)

$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}N\quad CO{-\!-\!-}O}\\ \mathrm{\quad Me_2C{\cdot}S{-}C{-\!-}CMe{\cdot}N{:}CR}\end{array}$$

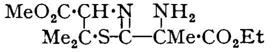
(II.)

$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}NH\quad CO{-}O}\\ \mathrm{\quad Me_2C{\cdot}S{-}C{=\!=}C{\cdot}N{:}CR}\end{array}$$

(III.)

$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}N}\\ \mathrm{\quad Me_2C{\cdot}S{-}C{\cdot}CHR{\cdot}CO_2R'}\end{array}$$

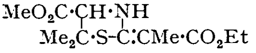
(IV.)

$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}N\quad N{:}N{\cdot}C_6H_4Cl}\\ \mathrm{\quad Me_2C{\cdot}S{-}C{-\!-}CR{\cdot}CO_2Et}\end{array}$$

by its reaction with *p*-chlorobenzenediazonium chloride to yield the *azo*-derivative (IV; R = H). This *azo*-derivative was also obtained from the pure thiazoline (III; R = H, R′ = Et) which failed to yield (III; R = Me, R′ = Et) when treated with sodium and methyl iodide. However, pure ethyl α-cyanopropionate, obtained *via* ethyl α-bromopropionate (Auwers and Bernhardi, *Ber.*, 1891, **24**, 2219; Steel, *J. Amer. Chem. Soc.*, 1931, **53**, 286), reacted satisfactorily with ethanol and dry hydrogen chloride to give the imino-ether, which was condensed successfully with penicillamine methyl ester to give the *thiazoline* (III; R = Me, R′ = Et). An attempt to couple this with *p*-chlorobenzenediazonium chloride failed to give the expected derivative (IV; R = Me), but furnished instead an unknown compound, m. p. 174—175°, isolated by chromatography. Attempts were then made to nitrosate (III; R = Me, R′ = Et) by means of nitrosyl chloride and potassium acetate in acetic anhydride, and by milder methods, but from these reactions only starting material was recovered and alternative routes to the compound (V) had to be devised. The inability of (III; R = Me, R′ = Et) to condense with *p*-chlorobenzenediazonium chloride or nitrosyl chloride, coupled with its ultra-violet absorption ($\lambda_{\max.}$, 2900 A.), suggests that it may possess the thiazolidylidene configuration (VI) which obviously cannot exhibit the malonic ester type of reactivity (cf. Part V).

(V.)



$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}N\quad NH_2}\\ \mathrm{Me_2C{\cdot}S{\cdot}C{-\!\!-}CMe{\cdot}CO_2Et}\end{array}$$

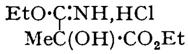


$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}NH}\\ \mathrm{Me_2C{\cdot}S{\cdot}C{:}CMe{\cdot}CO_2Et}\end{array}$$

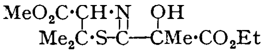
(VI.)

A further approach to (V) or its acyl derivatives involved the preparation of the *imino-ether hydrochloride* (VII) from the cyanohydrin of ethyl pyruvate. (VII) was condensed with penicillamine methyl ester in dry chloroform to give the *thiazoline* (VIII). This compound was of particular interest since it was the first example, in our hands, of a Δ^2-thiazoline which could not exist in the thiazolidylidene form. The dehydropenicilloates and their precursors, described previously (*op. cit.*, p. 857; Part V), had shown ultra-violet light absorption at 2800—2900 A. whereas compound (VIII) absorbed at 2400—2500 A. It appears highly probable, therefore, that the dehydropenicilloates, etc., exist in solution largely in the exocyclic double-bond form. This finding is in keeping with results obtained in the study of thiazolines containing simple substitutents at position 2 (Merck and Co., *M.* 25; *CPS.* 141) (cf. below).

(VII.)



$$\begin{array}{l}\mathrm{EtO{\cdot}C{:}NH,HCl}\\ \mathrm{MeC(OH){\cdot}CO_2Et}\end{array}$$

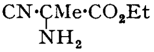


$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}N\quad OH}\\ \mathrm{Me_2C{\cdot}S{\cdot}C{-\!\!-}CMe{\cdot}CO_2Et}\end{array}$$

(VIII.)

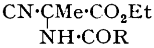
Attempts to cause the compound (VIII) to react directly with ethanolic ammonia failed to yield any product, and it was therefore treated with phosphorus tribromide and phosphorus pentachloride in chloroform. The desired halogenated products were not obtained however, and further reactions with ammonia or metallic derivatives of hexoamide or phenylacetamide were thus precluded.

The most promising approach to the preparation of methyldehydropenicilloates (XII) was then in another use of ethyl pyruvate cyanohydrin. Treatment of this compound with ethanolic ammonia gave the amino-nitrile (IX), characterised as the crystalline *oxalate* and the hydrated *hydrochloride*. Acylation of the crude amino-nitrile or its derivatives with benzoyl and with phenylacetyl chloride in a mixture of aqueous sodium hydrogen carbonate and ether yielded the *benzoyl* and *phenylacetyl* compounds (X; R = Ph) and (X; R = CH$_2$Ph), respectively. The yield of acyl derivatives obtained by direct acylation of the crude amino-nitrile was 25% (based on cyanohydrin), and attempts to increase it by increasing the proportion of ammonia to cyanohydrin gave unpromising results, probably due to reaction of the carbethoxy-group with ammonia. It was possible that the compounds (X) would react with alcohols to yield imino-ethers (XI; R′ = alkoxy) which might condense with penicillamine esters to give (XII), the required precursors of methyldehydropenicillins. An unexpected difficulty arose, however, in the conversion of the nitriles (X) into imino-ethers (XI). Numerous attempts to condense the carefully dried nitrile in a suitable dry solvent with dry ethanol and dry hydrogen chloride led only to excellent yields of *α-phenylacetamido-α-carbethoxypropionamide* (XIII; R = CO·NH$_2$). The use of dry methanol, *n*- or *iso*-propanol, or benzyl alcohol in place of ethanol gave the same



$$\begin{array}{l}\mathrm{CN{\cdot}CMe{\cdot}CO_2Et}\\ \mathrm{\quad NH_2}\end{array}$$

(IX.)



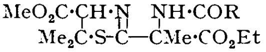
$$\begin{array}{l}\mathrm{CN{\cdot}CMe{\cdot}CO_2Et}\\ \mathrm{\quad NH{\cdot}COR}\end{array}$$

(X.)

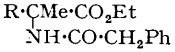
$$\begin{array}{l}\mathrm{HX\{HN{:}CR'{\cdot}CMe{\cdot}CO_2Et}\\ \mathrm{\qquad\qquad NH{\cdot}COR}\end{array}$$

(XI.)

(XII.)



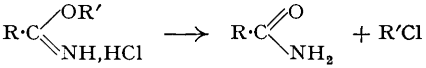
$$\begin{array}{l}\mathrm{MeO_2C{\cdot}CH{\cdot}N\quad NH{\cdot}COR}\\ \mathrm{Me_2C{\cdot}S{\cdot}C{-\!\!-}CMe{\cdot}CO_2Et}\end{array}$$



$$\begin{array}{l}\mathrm{R{\cdot}CMe{\cdot}CO_2Et}\\ \mathrm{NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

(XIII.)

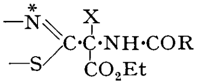
result, and in the last case benzyl chloride was identified as a reaction product. It is apparent that the desired imino-ether hydrochlorides, when formed, undergo a disproportionation reaction of the type shown by the imino-ethers derived from trichloroacetonitrile or nitroacetonitrile (Steinkopf, *Ber.*, 1909, **42**, 619), with the formation of the corresponding amide and halide, thus:



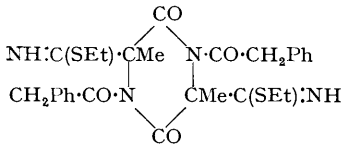
$$\mathrm{R{\cdot}C}\begin{matrix}\mathrm{OR'}\\ \mathrm{NH,HCl}\end{matrix} \longrightarrow \mathrm{R{\cdot}C}\begin{matrix}\mathrm{O}\\ \mathrm{NH_2}\end{matrix} + \mathrm{R'Cl}$$

The amide (XIII; R = CO·NH$_2$) readily reacted with ethanolic hydrogen chloride to give the corresponding ester, *ethyl α-phenylacetamido-α-methylmalonate* (XIII; R = CO$_2$Et).

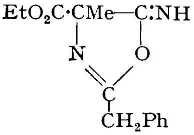
In contrast to the imino-ethers (XI; R′ = alkoxy), the *ethyl imino-thioether* (XI; R = CH$_2$Ph, R′ = SEt), prepared from (X; R = CH$_2$Ph) with ethanethiol (dried over calcium) and excess of dry hydrogen chloride, displayed considerable stability as the crystalline free base. The ultra-violet light absorption (at 2420 A.) of the base constituted an interesting confirmation of the light absorption to be expected of true Δ^2-thiazolines since it possesses the annexed absorbing system which, as in (VIII), is a fixed form as the group X (Me, OH, respectively) is unable to migrate to the nitrogen atom N*. The conclusion (see above) that the dehydropenicilloates and their precursors (where X = H) exist largely in the isomeric 2-thiazolidylidene form is thus further supported. Attempts to prepare other imino-thioethers, *e.g.*, from thioglycollic acid by the method of Condo, Hinkel, Fasseno, and Shriner (*J. Amer. Chem. Soc.*, 1937, **59**, 230), had meanwhile failed.



The stability of the compound (XI; R = CH$_2$Ph, R′ = SEt), which initially seemed so advantageous, soon appeared to be as great a hindrance as the instability of the corresponding imino-ethers. Thus the attempted condensation of the compound with penicillamine methyl ester, to give the thiazoline (XII; R = CH$_2$Ph), did not proceed either under conditions previously employed for imino-ethers (see, for example, *op. cit.*, p. 857) or under more vigorous treatment including fusing the reactants, heating them in xylene or pyridine, and refluxing them in acetic acid. Nor could the condensation be induced by hydrogen chloride in chloroform, or pyridine in chloroform. In most of the experiments the imino-thioether was recovered unchanged; the action of pyridine or acetic acid, however, yielded a *compound*, m. p. 181—182°, which was formulated as the diketopiperazine (XIV). This compound was not obtained by the action of pyridine alone, but required the presence of penicillamine hydrochloride as catalyst. The reaction with hydrogen chloride in chloroform yielded a product, which is best formulated as the *imino-oxazoline* (XV) [isomeric with (X; R = CH$_2$Ph)], formed in a manner analogous to the conversion of acylamido-nitriles into 5-amino-oxazoles (cf. *op. cit.*, p. 700; I.C.I., Ltd., *CPS*, 346,634; Parts VII and VIII).



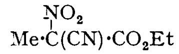
(XIV.)



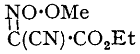
(XV.)

Attention was then turned to the methyl imino-thioether (XI; R = CH$_2$Ph, R′ = SMe) in the hope that it might prove more reactive than the ethyl compound. Accordingly the nitrile (X) was converted (by a method described in Part III) into the *thioamide* (XIII; R = CS·NH$_2$) which reacted with methyl iodide in chloroform at 0° to give the *methyl imino-thioether hydriodide* (XI; R = CH$_2$Ph, R′ = SMe), a crystalline solid, m. p. 110° (decomp.), which evolved methanethiol on being warmed with aqueous alkali. Reactions of the thioamide with methyl iodide at 50—60° or with chloroacetic acid or acetyl chloride led to decomposition. Treatment of the compound (XI; R = CH$_2$Ph, R′ = SMe) with penicillamine methyl ester failed to yield the desired thiazoline (XII), and attempted condensation of the parent thioamide with penicillamine methyl ester by fusing the reactants was likewise ineffective. The utilisation of an imino-halide derived from (X; R = CH$_2$Ph) for condensation with penicillamine methyl ester was also studied, but reaction of (X; R = CH$_2$Ph) with dry hydrogen chloride or hydrogen bromide in the presence of penicillamine ester in chloroform led only to the recovery of starting materials or of unidentified oils showing no characteristic light absorption. Similar reactions in the presence of one mole of an alcohol in the hope of obtaining the thiazoline (XII) *via* the formation *in situ* of imino-ethers also failed.

(XVI.)



$\mathrm{Me{\cdot}\overset{NO_2}{C}(CN){\cdot}CO_2Et}$

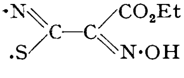


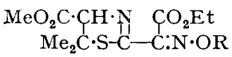
$\mathrm{\overset{NO{\cdot}OMe}{\overset{\|}{C}}(CN){\cdot}CO_2Et}$

(XVII.)

The possibility of preparing (XII) *via* imino-ethers, thioamides, etc., derived from (XVI), was also examined. The oxidation of nitrosomalonic ester to nitromalonic ester and the *C*-methylation of the latter with methyl iodide and silver oxide have been described (Ley and Hantzsch, *Ber.*, 1906, **39**, 3155), together with the preparation of the potassium salt of ethyl nitrocyanoacetate from the corresponding nitroso-compound (Conrad and Schulze, *Ber.*, 1909, **42**, 737). Since, however, free ethyl nitrocyanoacetate is not readily obtained from its salts (Ulpiani, *Gazzetta*, 1912, **42**, 395), methylation was effected by converting the unreactive, available potassium derivative into the silver salt and treating that with methyl iodide. *Ethyl α-nitro-α-cyanopropionate* (XVI) was thus obtained as a crystalline solid (yield, 40%), together with the corresponding *methyl nitro*-ester (XVII), an oil which exploded on attempted distillation. Such behaviour is characteristic of nitro-esters of the type $\mathrm{R{\cdot}CH{:}NO{\cdot}OMe}$ which decompose according to the scheme $\mathrm{R{\cdot}CH{:}NO{\cdot}OMe \xrightarrow{70-90^\circ} R{\cdot}CH{:}NO{\cdot}H + H{\cdot}CHO}$ (Arndt and Rose, *J.*, 1935, 1). Incidentally, it was found that the direct nitration of ethyl α-cyanopropionate using fuming nitric acid, as in the case of malonic ester (Franchimont and Klobbie, *Rec. Trav. chim.*, 1889, **8**, 283), or cupric nitrate in acetic anhydride, as in the case of ethyl acetoacetate (Menke, *ibid.*, 1925, **44**, 145), yielded only the unchanged cyano-ester. Attempts to prepare an imino-ether or orthoester from (XVI) unfortunately failed, so that the condensation with penicillamine ester in the normal manner was not possible. Direct interaction of the nitrile with penicillamine methyl ester in hot glycol monomethyl ether or fusion of the reagents yielded no crystalline product, and an attempt to form the thioamide from (XVI) with hydrogen sulphide led to partial reduction. Direct reduction of (XVI) with hydrogen and palladium on charcoal yielded ammonium formate.

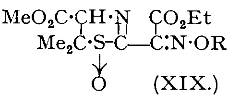
In view of the numerous failures with compound (XVI) an attempt was made to apply the experience gained from its preparation to the oximino-thiazoline (XVIII; R = H), an intermediate which had been used in the preparation of the dehydropenicillins (*op. cit.*, p. 858; Part V). It was hoped that (V) might be obtained by *C*-methylation and reduction of the nitro-compound corresponding to (XVIII; R = H), *viz.*, (XVIII; R = OH). Treatment of (XVIII; R = H) with hydrogen peroxide in various solvents, including acetone, dioxan, and acetone–aqueous sodium hydrogen carbonate, at various temperatures yielded only unchanged material, whereas the use of the oxidising agent in acetic acid led to the formation of penicillaminic acid methyl ester (*op. cit.*, p. 460). Oxidation with neutral potassium permanganate in acetone yielded only intractable oils, which did not give crystalline methyl derivatives with methyl bromide or methyl iodide under various conditions. Corresponding oxidation with potassium permanganate and excess of sodium carbonate in aqueous acetone proceeded smoothly, however, to yield a *product* containing one oxygen atom more than (XVIII; R = H). This product was a white, crystalline solid, which, like (XVIII; R = H), yielded brilliant colours in various solvents, *e.g.*, purple in chloroform, and blue-green in ethanol or acetone. Like (XVIII; R = H) also, it gave a chocolate-brown coloration with ethanolic cupric acetate and an orange-red coloration with ethanolic ferric chloride. In addition, its ultra-violet light absorption (Maxima at 2450 and 3370 A.; $E^{1\%}_{1\,\mathrm{cm.}} = 300$ and 100, respectively) corresponded closely to that of the oximino-compound (XVIII; R = H) (Maxima at 2440, 2510, and 3380 A.; $E^{1\%}_{1\,\mathrm{cm.}} = 250$, 250, and 75, respectively) when determined in chloroform solution. Whilst this evidence suggested that the oxidation product still contained the system shown and might therefore have been the 1-oxide (XIX; R = H), obviously the (desired) structure (XVIII; R = OH) for the product was not at this stage excluded. (These formulæ are written in the thiazoline form to account for the strong ultra-violet absorption band at 2400—2500 A.) Methylation of the oxidation product yielded





$\mathrm{\begin{array}{l}MeO_2C{\cdot}CH{\cdot}N\ \ CO_2Et\\ Me_2C{\cdot}S{\cdot}C{=\!=}C{:}N{\cdot}OR\end{array}}$

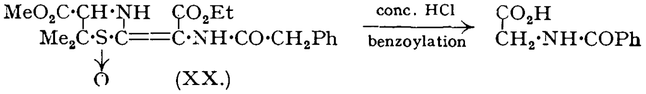
(XVIII.)



$\mathrm{\begin{array}{l}MeO_2C{\cdot}CH{\cdot}N\ \ CO_2Et\\ Me_2C{\cdot}\underset{\downarrow\atop O}{S}{\cdot}C{=\!=}C{:}N{\cdot}OR\end{array}}$

(XIX.)

a crystalline *methyl* derivative which gave no coloration on melting, or in solvents, or with cupric acetate, and which showed ultra-violet absorption at 2450 A. ($E^{1\%}_{1\,\mathrm{cm.}} = 360$); either of the structures (XVIII; R = OMe) and (XIX; R = Me) thus seemed applicable. Reduction of the methyl derivative with Raney nickel or stannous chloride did not yield crystalline products, but aluminium amalgam or, much better, hydrogen sulphide under pressure yielded a base which formed a crystalline oxalate and was characterised as its *phenylacetyl* derivative. The analysis of the latter showed that the extra oxygen atom was still present in the molecule, the 1-oxide structure (XX), written in the thiazolidylidene form to account for the ultra-violet absorption



$$\begin{array}{l} \text{MeO}_2\text{C·CH·NH}\quad\text{CO}_2\text{Et} \\ \text{Me}_2\text{C·S·C}{=\!=}\text{C·NH·CO·CH}_2\text{Ph} \\ \quad\;\;\downarrow \\ \quad\;\;\text{O} \end{array} \xrightarrow[\text{benzoylation}]{\text{conc. HCl}} \begin{array}{l} \text{CO}_2\text{H} \\ \text{CH}_2\text{·NH·COPh} \end{array}$$

(XX.)

maximum at 2860 A. ($E_{1\,\text{cm.}}^{1\%} = 430$), consequently being indicated. It followed that the precursors had the oxide structures (XIX; R = H) and (XIX; R = Me). That the phenylacetyl compound (XX) [and therefore the methylated precursor (XIX; R = Me)] was not, in fact, a *C*-methyl compound was proved conclusively by its hydrolysis with concentrated hydrochloric acid, followed by benzoylation of the amino-acid fraction, to yield hippuric acid and not benzoylalanine.

In view of these difficulties in obtaining the "methyldehydropenicillins," attention was turned in part to other approaches to true thiazolinyloxazolones. These experiments are described in Parts VII and VIII.

EXPERIMENTAL.

Preparation of Thiazolines.—*p*-Chloroaniline (0·66 g.) in 2N-hydrochloric acid (9 c.c.) was diazotised at 0° with sodium nitrite (0·4 g.) in water (4 c.c.). The solution was diluted with 1 : 2 aqueous ethanol (40 c.c.), sodium acetate (5 g.) was added, and the solution was vigorously stirred at 0° whilst a solution of methyl 5 : 5-dimethyl-2-carbethoxymethylthiazoline-4-carboxylate (1·02 g.) (Part III) in ethanol (10 c.c.) was slowly added. After being stirred for 1 hour the solid was filtered off and well washed with water (yield, 0·7 g.). Recrystallised from ethanol, *methyl* 5 : 5-*dimethyl*-2-p-*chlorobenzeneazocarbethoxymethylthiazoline*-4-*carboxylate* separated as yellow needles, m. p. 137—138° (Found : C, 51·4; H, 5·1; N, 10·4. $C_{17}H_{20}O_4N_3ClS$ requires C, 51·3; H, 5·1; N, 10·6%). Light absorption (in ethanol) : Maxima, 2420, 3070, and 3770 A.; $E_{1\,\text{cm.}}^{1\%} = 450$, 100, and 820.

A mixture of ethyl α-cyanopropionate (7·0 g.) (Steele, *J. Amer. Chem. Soc.*, 1931, **53**, 286) and dry ethanol (2·55 g.) was saturated with dry hydrogen chloride at 0° and set aside overnight. After being washed with dry ether the crystalline mass of *α-carbethoxpropionimino ethyl ether hydrochloride* (9·4 g.; 81%) had m. p. 91—92° (decomp.) (Found : N, 6·7. $C_8H_{15}O_3N,HCl$ requires N, 6·7%). A chloroform solution (200 c.c.) of penicillamine methyl ester, prepared from the hydrochloride (25 g.), was dried (Na_2SO_4) and concentrated to half-bulk. The above imino-ether hydrochloride (21 g.) was added, and the flask stoppered, shaken to effect dissolution, and set aside overnight. Ammonium chloride slowly separated. The mixture was washed 3 times with water, dried, concentrated, and distilled twice *in vacuo* to yield *methyl* 5 : 5-*dimethyl*-2-*α-carbethoxyethylthiazoline*-4-*carboxylate* (18 g., 65%) as a yellowish, somewhat viscous oil, b. p. 103—108°/0·0001 mm., n_D^{22} 1·5298 (Found : C, 52·4; H, 7·2; N, 5·5. $C_{12}H_{19}O_4NS$ requires C, 52·7; H, 7·0; N, 5·1%). After *ca.* 1 week, partial solidification occurred. The solid could be crystallised from aqueous ethanol, whereupon colourless plates, m. p. 36—38°, were obtained, but they slowly reverted to a mixture of solid and liquid when kept either at 0° or at room temperature in a vacuum desiccator.

Potassium cyanide (*ca.* 0·5 g.) was added in several portions to a mixture of ethyl pyruvate (50 g.) and anhydrous hydrogen cyanide (50 c.c.), causing a moderately vigorous reaction. The mixture was kept at room temperature overnight, concentrated sulphuric acid (1 c.c.) was then added, and the mixture was distilled *in vacuo*. The fraction, b. p. 87—97°/12 mm. (mainly 95—97°/12 mm.; n_D^{23} 1·4200), was collected (52·2 g., 84%) (cf. Ultee, *Ber.*, 1906, **39**, 1858, who gives b. p. 105°/19 mm., n_D^{17} 1·42435), and consisted of ethyl pyruvate cyanohydrin. A mixture of the cyanohydrin (6·7 g.) and ethanol (2·16 g.; dried over magnesium ethoxide) was saturated with dry hydrogen chloride at 0°. Next day a colourless mass had separated, which was washed with dry ether and gave *α-hydroxy-α-carbethoxypropionimino ethyl ether hydrochloride* as a hygroscopic solid (19·6 g., 91%), m. p. 104—105° (decomp.) (Found : N, 6·2. $C_8H_{15}O_4N,HCl$ requires N, 6·2%). A chloroform solution (100 c.c.) of penicillamine methyl ester, obtained from the hydrochloride (10 g.), was dried (Na_2SO_4) and concentrated to half-bulk *in vacuo*. The imino-ether hydrochloride (9·5 g.) was added, and the flask stoppered and shaken to effect dissolution. After 2 days the mixture was washed thoroughly with water, dried, concentrated, and distilled *in vacuo*. The fraction, b. p. 100—120°/0·001 mm., was redistilled and *methyl* 5 : 5-*dimethyl*-2-*α-carbethoxy-α-hydroxethyl*-Δ^2-*thiazoline*-4-*carboxylate* (VIII) was obtained as a very pale yellow oil, b. p. 108—109°/0·0005 mm., n_D^{24} 1·4870 (Found : C, 49·8; H, 6·9; N, 5·2. $C_{12}H_{19}O_5NS$ requires C, 49·8; H, 6·6; N, 4·8%). Light absorption (in ethanol) : Maxima, 2410 and 2510 A.; $E_{1\,\text{cm.}}^{1\%} = 1130$.

Products derived from α-Amino-α-carbethoxypropionitrile.—A mixture of ethyl pyruvate cyanohydrin (7 g.) and ethanolic ammonia (14 c.c.; 10% w/v) was set aside in a stoppered bottle at room temperature for 16 hours. The solvent and excess of ammonia were removed *in vacuo* below 30°, the residual oil was dissolved in a little ether, and ethereal hydrogen chloride was added. A pinkish oil was precipitated immediately, which gave a sticky solid after much manipulation. Crystallisation from acetone–ether gave colourless laths (1·9 g.), m. p. 92—93°. After they had been dried for 2 hours at 56° a white powder of *α-amino-α-carbethoxypropionitrile hydrochloride monohydrate*, m. p. 137°, was obtained (Found : C, 37·1; H, 6·9; N, 14·7. $C_6H_{11}O_2N_2Cl,H_2O$ requires C, 36·6; H, 6·6; N, 14·2%). The amine hydrochloride was benzoylated in aqueous sodium hydrogen carbonate, the resulting gum was extracted with chloroform, and the extract washed well with sodium hydrogen carbonate solution, dried, and concentrated. The gum obtained solidified readily and, on recrystallisation from dilute aqueous ethanol, *α-benzamido-α-carbethoxypropionitrile* formed colourless laths, m. p. 147° (Found : C, 63·2 ; H, 6·0 ; N, 10·9. $C_{13}H_{14}O_3N_2$ requires C, 63·4 ; H, 5·7 ; N, 11·4%).

Ethyl pyruvate cyanohydrin (20 g.) was treated with 8% ethanolic ammonia (30 c.c.) for 5 hours. The solution was concentrated *in vacuo* below 30°, and the residual oil added to a solution of sodium hydrogen carbonate (20 g.) in water (200 c.c.) covered with ether (10 c.c.). The whole was cooled in ice and stirred vigorously, and phenylacetyl chloride (12 g.) was added dropwise. A white solid separated in the ethereal layer, and when reaction was complete this solid was collected and washed with much water, followed by a little cold ether. *α-Phenylacetamido-α-carbethoxypropionitrile* (9 g., 25%) separated from aqueous ethanol as colourless needles, m. p. 141—142° (Found : C, 64·7 ; H, 6·2 ; N, 10·5. $C_{14}H_{16}O_3N_2$ requires C, 64·6 ; H, 6·2 ; N, 10·8%). A mixture of this nitrile (1·6 g.), carefully dried ethanol (0·3 g.), and chloroform (20 c.c. ; dried over P_2O_5) was cooled in ice, and saturated with dry hydrogen chloride. The clear solution obtained was set aside for 3 days and then concentrated *in vacuo* to a colourless resin. Addition of dry ether rapidly converted this into a powdery white solid which was quickly filtered off, washed with ether, and placed in a desiccator (yield, 1·5 g. ; m. p. 165—170°). Several recrystallisations from 95% ethanol gave colourless needles of *α-phenylacetamido-α-carbethoxypropionamide*, m. p. 177—178° (Found : C, 60·4 ; H, 6·7 ; N, 9·8. $C_{14}H_{18}O_4N_2$ requires C, 60·4 ; H, 6·5 ; N, 10·1%). A suspension of the amide (9 g.) in dry ethanol (50 c.c.), cooled in ice, was saturated with hydrogen chloride, whereupon the solid dissolved slowly. The solution was refluxed on a water-bath for 3 hours and poured into water (200 c.c.). An oil, which rapidly solidified, separated, and was collected. After crystallisation from aqueous ethanol, *ethyl α-phenylacetamido-α-methylmalonate* (5·9 g., 53%) formed colourless needles, m. p. 83—84° (Found : C, 62·5 ; H, 6·9 ; N, 4·7. $C_{16}H_{21}O_5N$ requires C, 62·5 ; H, 6·8 ; N, 4·6%).

A mixture of α-phenylacetamido-α-carbethoxypropionitrile (4·6 g.), in dry ether (100 c.c.), and ethanethiol (1·4 c.c. ; previously distilled from calcium) was saturated at 0° with dry hydrogen chloride. On being kept at 0° for 24 hours, the nitrile passed into solution. This was evaporated, the gummy residue redissolved in chloroform, and the solution shaken with aqueous sodium hydrogen carbonate, washed with water, and dried (Na_2SO_4). Evaporation of the solvent afforded *α-phenylacetamido-α-carbethoxypropionimino ethyl thioether* (3 g.), which crystallised from ethanol–water in thin parallelogram-shaped plates, m. p. 131—132° (Found : C, 60·2 ; H, 6·8 ; N, 8·7. $C_{16}H_{22}O_3N_2S$ requires C, 59·6 ; H, 6·8 ; N, 8·7%). Ethanol (20 c.c.), in which sodium (20 mg.) had been dissolved, was cooled to −10°, and saturated with hydrogen sulphide. α-Phenylacetamido-α-carbethoxypropionitrile (1 g.) was added and the mixture heated in a closed vessel at 50—60° for 6 hours. The solution was evaporated *in vacuo* and the residue crystallised from chloroform–ether to yield *α-phenylacetamido-α-carbethoxypropionothioamide* (0·85 g.) as cubic prisms, m. p. 144°, readily soluble in aqueous sodium hydroxide (Found : C, 57·0 ; H, 5·9 ; N, 9·3. $C_{14}H_{18}O_3N_2S$ requires C, 57·1 ; H, 6·1 ; N, 9·5%). This thioamide (1·65 g.) was treated with a large excess of methyl iodide (45 c.c.) in dry chloroform (30 c.c.), and the solution was kept for 3 days at 0°. The crystalline hygroscopic *product* (0·85 g.), m. p. 110° (decomp.), was washed with dry ether (Found : N, 7·4. $C_{15}H_{21}O_3N_2IS$ requires N, 6·4%. $C_{14}H_{18}O_3N_2S$ requires N, 9·5%). The product effervesced with aqueous ethanolic sodium hydrogen carbonate, and with nitric acid and silver nitrate yielded a yellow precipitate insoluble in ammonia. It gave a positive Beilstein test, and when it was warmed with 2N-sodium hydroxide methanethiol was evolved. It failed to yield a crystalline product on treatment with penicillamine methyl ester, although methanethiol was evolved.

Attempts to cause Penicillamine Ester to react with α-Phenylacetamido-α-carbethoxypropionimino Ethyl Thioether.—Penicillamine methyl ester hydrochloride (0·4 g. ; dried over P_2O_5) and the imino-thioether (0·5 g.) were dissolved in dry pyridine (10 c.c.), and the solution was refluxed for 1—3 hours. Pyridine was removed *in vacuo*, and the residue treated with ethyl acetate and water. The ethyl acetate was washed with 2N-hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried, and evaporated *in vacuo*. The residual oil crystallised when set aside in ether. The *diketopiperazine* (XIV) separated from ethanol–water or ethyl acetate as rectangular plates or tablets, m. p. 181—182° (Found : C, 59·0, 59·2 ; H, 6·0, 6·3 ; N, 9·9, 10·0 ; S, 11·3. $C_{26}H_{32}O_4N_4S_2,H_2O$ requires C, 59·0 ; H, 6·0 ; N, 9·8 ; S, 11·2%). Light absorption (in ethanol) : Maxima, 2410, 2450, 2660, and 3360 A. ; $E^{1\%}_{1\,\text{cm.}}$ = 640, 640, 490, and 490, respectively.

Penicillamine methyl ester hydrochloride (0·4 g. ; dried over P_2O_5) and the imino-thioether (0·5 g.) were dissolved in acetic acid (shaken with phosphoric oxide and redistilled), and the solution refluxed for 1·5 hours. The product finally had m. p. 181—182°, undepressed on admixture with the compound (XIV).

Dry penicillamine methyl ester hydrochloride (0·5 g.) and the imino-thioether (0·8 g.) were dissolved in chloroform (20 c.c. ; dried over P_2O_5 and distilled) and the solution saturated with dry hydrogen chloride at 0°, and set aside for 72 hours. The chloroform was removed *in vacuo*, and the residual oil treated with chloroform and aqueous sodium hydrogen carbonate. The chloroform layer was washed repeatedly with water, dried, and evaporated to yield an oil which partly crystallised on spontaneous evaporation of a solution in ether. The crystalline material was identified as unchanged imino-thioether (mixed m. p.), and the residual gum was again stirred with ether. After removal of insoluble material, the filtrate was evaporated, whereafter the residual oil slowly solidified (m. p. 94°). On recrystallisation from chloroform–light petroleum (b. p. 60—80°) *ethyl 5-imino-2-benzyl-4-methyloxazoline-4-carboxylate* (XV) separated as colourless, glistening laths, m. p. 100° (Found : C, 64·3 ; H, 6·0 ; N, 11·1. $C_{14}H_{16}O_3N_2$ requires C, 64·6 ; H, 6·2 ; N, 10·8%). Light absorption (in chloroform) : end absorption only.

Ethyl α-Nitro-α-cyanopropionate.—The silver salt of ethyl nitrocyanoacetate (28 g.) was prepared by adding aqueous silver nitrate to a hot solution of ethyl potassionitrocyanoacetate and then cooling the solution at once to 0°. The precipitate was washed with water, ethanol, and ether, and treated directly with methyl iodide (38 g.), ether being added to moderate the reaction. When reaction was complete, the solution was filtered and the silver iodide extracted with ether. Concentration of the filtrate and extract yielded a solid which separated as clusters of prisms (7·7 g.), m. p. 77—80°. Recrystallised from hot light petroleum (b. p. 60—80°) *ethyl α-nitro-α-cyanopropionate* separated as prisms, m. p. 80°

(Found: C, 41·7; H, 4·65; N, 16·7. $C_6H_8O_4N_2$ requires C, 41·9; H, 4·65; N, 16·3%). The ethereal filtrate yielded an oil (10·1 g.) which exploded on attempted distillation when the bath temperature reached 120°.

This nitro-cyanide (5 g.) was recovered unchanged after keeping overnight with dry ethanol (1·35 g.) and dry hydrogen chloride (1 g.) in dry ether. With excess of dry ethanolic hydrogen chloride, the cyanide (4·6 g.) gave ammonium chloride (0·8 g.) and an unidentified chloro-compound, b. p. 95°/9 mm. (Found: C, 43·1; H, 6·0; Cl, 18·7%).

The nitro-cyanide (3·6 g.) in anhydrous formic acid (40 c.c.) was hydrogenated at atmospheric pressure in the presence of 5% palladium–charcoal (1 g.). When 3 equivalents of hydrogen had been absorbed, the mixture was filtered and evaporated *in vacuo*, and an attempt was made to distil the residue. Deliquescent plates, m. p. 115°, identified as ammonium formate, sublimed rapidly.

Compound (XX).—Methyl 5 : 5-dimethyl-2-oximinocarbethoxymethylthiazoline-4-carboxylate (0·5 g.) was dissolved in acetic acid (5 c.c.), hydrogen peroxide (1 c.c.; 100-volume) was added, and the solution was heated to boiling. Evaporation *in vacuo* afforded a gum which was dissolved in ethanol; crystalline penicillaminic acid methyl ester, m. p. 220° (decomp.), soon crystallised as clusters of needles (cf. *S* 32, 6 *). The same product was obtained by conducting the oxidation in the cold. Methyl 5 : 5-dimethyl-2-oximinocarbethoxymethylthiazoline-4-carboxylate (2·0 g.) was dissolved in water (20 c.c.) containing sodium carbonate (3 g.) and acetone (5 c.c.). Potassium permanganate (1·0 g.) in water (30 c.c.) was then added. The solution became warm and reduction of the permanganate was rapid. After 10 minutes, the mixture was filtered, the filtrate cooled to 0°, acidified, and further clarified, and the filtrate extracted with chloroform (5 × 30 c.c.). The blue chloroform extract yielded on evaporation a purple gum, which set solid on addition of dry ether.

Methyl 5 : 5-*dimethyl*-2-*oximinocarbethoxymethylthiazoline*-4-*carboxylate* 1-*oxide* (XIX; R = H) (0·85 g., 40%) crystallised from chloroform–light petroleum (b. p. 60—80°) as white prisms, m. p. 164° (decomp.) (Found: C, 43·4; H, 5·2; N, 9·2. $C_{11}H_{16}O_6N_2S$ requires C, 43·4; H, 5·3; N, 9·2%). This compound in ethanol gave a chocolate-brown colour with cupric acetate and an orange colour with ferric chloride. In chloroform, more rapidly if warmed, a rich purple colour developed; solutions in ethyl acetate were bluish-green, in acetone green. Light absorption (in chloroform): Maxima, 2450 and 3370 A.; $E^{1\%}_{1\,\text{cm.}} = 300$ and 100.

This oxide (5·3 g.) was suspended in dry ether (30 c.c.) containing methyl iodide (36·0 g.), and silver oxide (5·0 g.) was added in portions to the refluxing solution during 15 minutes. After continued refluxing for 16 hours, the solution was filtered, and the solvent recovered by distillation. To the residue was added a chloroform extract of the silver halide–oxide mixture. Removal of the chloroform *in vacuo* then yielded a red oil, which soon crystallised; washed with ether the solid had m. p. 137—141° (yield, 2·7 g.). To effect complete purification, a portion was chromatographed in chloroform on alumina, and the *methyl* compound (XIX; R = Me) was then obtained from chloroform–light petroleum (b. p. 60—80°) as colourless, shining prisms, m. p. 146—147° (Found: C, 44·8; H, 5·8; N, 8·7. $C_{12}H_{18}O_6N_2S$ requires C, 45·3; H, 5·7; N, 8·8%). This compound did not colour on melting, and gave no coloration with solvents or alcoholic cupric acetate. Light absorption (in chloroform): Maxima, 2450 A.; $E^{1\%}_{1\,\text{cm.}} = 360$.

(i) The methyl compound (2·5 g.) in ether (80 c.c.) was added to aluminium amalgam (0·65 g.), and the solution was heated under reflux for 3·5 hours, water (1·9 g.) being added gradually during the first hour. The solution was filtered, and the dark-red filtrate treated with ethereal oxalic acid to precipitate a gummy oxalate which was washed by decantation with ether. (The decanted ether slowly deposited the crystalline oxalate, m. p. 105—110°.) A stirred mixture of the crude oxalate, ether (50 c.c.), and sodium hydrogen carbonate (5 g.) in water (100 c.c.) was treated with phenylacetyl chloride (1·5 g.). The dried ethereal layer, on evaporation, yielded a yellow oil, which partly crystallised when stored under ether–light petroleum (b. p. 60—80°). From ethyl acetate–light petroleum, *methyl* 5 : 5-*dimethyl*-2-*phenylacetamidocarbethoxymethylenethiazolidine*-4-*carboxylate* 1-*oxide* (XX) separated as colourless prisms, m. p. 107—108° (the m. p. appeared to vary with the rate of heating) (Found: N, 6·7. $C_{19}H_{24}O_6N_2S,H_2O$ requires N, 6·6%). Light absorption (in chloroform); Maxima, 2820 and 2910 A.; $E^{1\%}_{1\,\text{cm.}} = 750$).

(ii) A solution of the compound (XIX; R = Me) (14·7 g.) in methanol (150 c.c.) was added to ethanol (400 c.c.), previously saturated at −10° with hydrogen sulphide. After 16 hours at room temperature in a tightly stoppered bottle, the solution was cooled for 2 hours in ice, and the crystalline precipitate of sulphur (2·8 g.) removed. The filtrate was concentrated *in vacuo* in a stream of nitrogen, and the residual red oil was treated with ethereal oxalic acid giving a gummy precipitate which granulated under dry ether. The solid (7·9 g.) was then stirred for 1·25 hours with ether (100 c.c.), phenylacetyl chloride (4·0 g.), and excess of aqueous sodium hydrogen carbonate. The acyl compound which separated was collected, and washed well with water and ether (yield, 6·2 g.; m. p. 109°); the ethereal reaction-liquors on spontaneous evaporation deposited a further quantity (0·5 g.; m. p. 108—111°). Methyl 5 : 5-dimethyl-2-phenylacetamidocarbethoxymethylenethiazolidine-4-carboxylate 1-oxide crystallised from chloroform–light petroleum or ethanol–water as colourless prismatic laths, m. p. 115—118° (decomp.) (Found: C, 53·6; H, 6·2; N, 6·2. Light absorption (in chloroform); Maximum, 2860 A.; $E^{1\%}_{1\,\text{cm.}} = 450$. The compound showed a definite elevation of the m. p. (10—15°) on admixture with methyl 5 : 5-dimethyl-2-phenylacetamidocarbethoxymethylthiazoline-4-carboxylate.

The oxide (XX) (1·0 g.) was heated with concentrated hydrochloric acid (10 c.c.) in a sealed tube at 100° for 14 hours. The resulting mixture of oil and solution was evaporated to dryness *in vacuo*, and the residue treated with water and ether. The aqueous phase was re-extracted with ether, nearly neutralised with sodium hydrogen carbonate, and treated with mercuric chloride solution overnight (to precipitate any thiol). The small precipitate was rejected, and the solution was treated with hydrogen sulphide, filtered, and evaporated to dryness *in vacuo*. Redissolved in water (10 c.c.) the residue gave a strong violet coloration with the ninhydrin reagent, and a red coloration with ferric chloride. Sodium hydrogen carbonate (3·5 g.) and benzoyl chloride (1·5 g.) in portions were added with shaking. Shaking was

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* See footnote, p. 3218.

continued for 1·5 hours, and the solution was filtered, extracted with ether, and acidified. The precipitate was dried *in vacuo* and extracted with boiling light petroleum (3 × 25 c.c.). The residue had m. p. 181—184°, and on recrystallisation from hot water yielded shining needles (280 mg.), m. p. 187° undepressed in admixture with authentic hippuric acid.

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