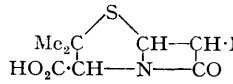
399. *Experiments on a Synthesis of Penicillin.*

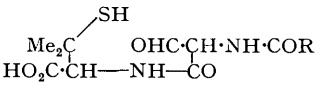
By J. W. Cornforth and H. T. Huang.

Various attempts are described to prepare *N*-(*n*-amylpenaldyl)penicillamine (II; R = n-C_5H_{11}). A novel oxazole synthesis incidental to this work is described.

Among the many schemes for synthesis of penicillins (I) there are some which involve intramolecular removal of water in the final stage. The possibility of dehydrating various penicilloic acid derivatives has been tried exhaustively and has led to no significant amount of antibiotic. Alternative intermediates are the *N*-penaldylpenicillamines (II), and this paper gives an account of some attempts to synthesise them.



(I.)



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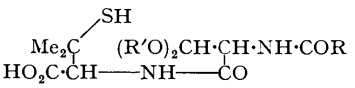


(III.)

Acetals of (II) (III; R = CH_2Ph, R′ = Me or Et) were prepared independently by several groups (Merck and Co.; University of Michigan; Squibb Institute; see "The Chemistry of Penicillin"); in all cases the azide of the appropriate penaldic acid acetal was condensed with penicillamine to give (III). Pyrolysis of (III; R = CH_2Ph, R′ = Me) was reported (Squibb Institute, *loc. cit.*) to give slight antibiotic activity but the result does not seem to be readily reproducible. Because others were engaged on this line, and because the acid hydrolysis of (III) to (II) seemed likely to be difficult, we explored other routes.

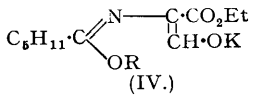
A synthesis of oxazoles by the action of acids on potassium enolates of the structure (IV) has been described (Cornforth and Cornforth, *J.*, 1947, 96). A theory of the mechanism of this change (Cornforth and Huang, this vol., p. 1960) led to the idea that in presence of alcohol a 5-ethoxyoxazoline (V; X = OEt) might be formed. If the carbethoxyl group in (V) could be modified to form a hydrazide, an azide, and finally an *N*-acylpenicillamine, it was expected that the final hydrolysis to (II) would be facile.

Potassium ethyl α-(1-ethoxyhexylideneamino)-β-hydroxyacrylate (IV; R = Et) on treatment with hydrogen chloride in ethanol gave a low-melting solid in good yield; this, however, was not the oxazoline but the diethyl acetal of ethyl *n*-amylpenaldate (VI; X = OEt; Chas. Pfizer and Co., "The Chemistry of Penicillin"). The structure was established by conversion into the 2 : 4-dinitrophenylhydrazone of ethyl *n*-amylpenaldate and by alkaline hydrolysis to *n*-amylpenaldic acid diethyl acetal (Cook, Elvidge, and Heilbron, "The Chemistry of Penicillin"; Chas. Pfizer and Co., *op. cit.*). Condensation of heximidomethyl ether with glycine ethyl ester hydrochloride gave *ethyl 1-methoxyhexylideneaminoacetate*, which was formylated to *potassium ethyl α-(1-methoxyhexylideneamino)-β-hydroxyacrylate* (IV; R = Me); the latter also gave (VI; X = OEt) with ethanolic hydrogen chloride. An attempt to obtain (V; X = OEt) by using less ethanol in the reaction with (IV; R = Et) only resulted in the formation of much ethyl 2-*n*-amyloxazole-4-carboxylate.

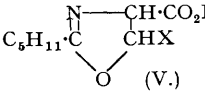
The ester (VI; X = OEt) being thus available, it was converted into the corresponding *hydrazide* and thence into the azide. Reaction of the latter with D-penicillamine gave the amorphous N-(*α-hexamido-ββ-diethoxypropionyl*)-D-*penicillamine* (III; R = n-C_5H_{11}, R′ = Et). Pyrolysis of this substance failed to give antibiotic activity.

The reaction of (IV; R = Et) with ethylthiol and dry hydrogen chloride was now studied. The product, obtained by distillation in satisfactory yield, was not the penaldic ester thioacetal (VI; X = SEt) but had the composition of the 5-ethylthio-oxazoline (V; X = SEt). The

corresponding acid was obtained on alkaline hydrolysis. The strong selective absorption of these two substances at 2800 A. left no doubt, however, that they were *ethyl α-hexamido-β-ethylthioacrylate* and the corresponding *acid* (VII; R = Et), respectively.



(IV.)



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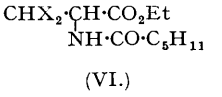
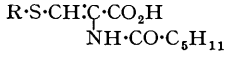


(VI.)

Ethyl α-hexamido-ββ-diethylthiopropionate (VI; X = SEt) was readily prepared from the unsaturated ester and ethylthiol by addition of a little sodium. The ester (VI; X = SEt) could be hydrolysed by alkali, but on acidification ethylthiol was lost, and the acid (VII; R = Et) formed. This accords with Posner's observations (*Ber.*, 1899, **32**, 2865; 1901, **34**, 2643) on the thioacetals of acetoacetic ester.

The use of mercuric chloride to regenerate carbonyl compounds from their thioacetals has produced good results in the acyclic sugar series (Levene and Meyer, *J. Biol. Chem.*, 1926, **69**, 175; Wolfrom, *J. Amer. Chem. Soc.*, 1929, **51**, 2188). The ester (VI; X = SEt) was cleaved by mercuric chloride–cadmium carbonate in aqueous acetone, ethyl *n*-amylpenaldate being isolated as the 2 : 4-dinitrophenylhydrazone. Under the same conditions the ethyl ester of (VII; R = Et) was recovered unchanged. Following this lead, we converted the ester (VI; X = SEt) by way of the *hydrazide* into the azide. The latter could be condensed with glycine to give N-(*α-hexamido-ββ-diethylthiopropionyl*)*glycine* (VIII; R = H) and with DL-α-amino-β-methoxy*iso*valeric acid to α-(*α-hexamido-ββ-diethylthiopropionamido*)-β-*methoxy*iso*valeric acid* (VIII; R = CMe_2·OMe); but with D- or DL-penicillamine the acidic product was a trace of intractable gum, and this way of preparing (II) *via* a thioacetal had to be abandoned.



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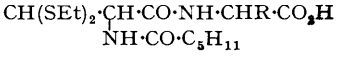
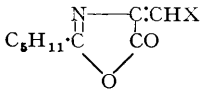


(VIII.)

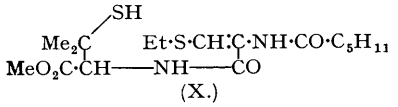


(IX.)

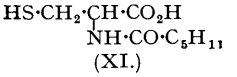
When the salt (IV; R = Et) was treated with benzylthiol and hydrogen chloride, a crystalline product could be obtained without distillation. The analysis and ultra-violet absorption spectrum indicated that this was *ethyl α-hexamido-β-benzylthioacrylate*. Alkaline hydrolysis gave the corresponding *acid* (VII; R = CH_2Ph).

The results outlined above are consistent with the view that an oxazoline of type (V) is the primary product when a salt such as (IV) reacts with hydrogen chloride in the presence of an alcohol or thiol. The oxazoline can undergo prototropic change to give an α-acylamidoacrylic ester, as with the thiols; when ethanol is used further reaction can take place, either before or after the prototropic change, to give a penaldic acetal of type (VI; X = OEt).

Availability of the acid (VII; R = Et) suggested a new line of attack. On treatment with acetic anhydride (less well with phosphorus pentachloride), the acid was smoothly dehydrated to *5-keto-2-n-amyl-4-ethylthiomethylene-4 : 5-dihydro-oxazole* (IX; X = SEt). Wellcome Institute workers (" The Chemistry of Penicillin," Chap. XXI) have shown that in alkylthiomethylene-oxazolones the 5-keto-group is usually the most reactive electrophilic centre; and here was no exception, for the oxazolone (IX; X = SEt) was decomposed by alkali to the parent acid (VII; R = Et), and by benzylamine and *p*-toluidine to the *benzylamide* and p-*toluidide* of this acid. Condensation with glycine ethyl ester was also achieved; it was found expedient to hydrolyse with alkali and isolate N-(*α-hexamido-β-ethylthioacrylyl*)*glycine*. With D-penicillamine methyl ester the usual difficulty was encountered and prolonged refluxing of the ester with the oxazolone (IX; X = SEt) in dry ether was the only procedure which furnished, in very low yield, a crystalline product. The ultra-violet absorption showed this to be N-(*α-hexamido-β-ethylthioacrylyl*)-D-*penicillamine methyl ester* (X). The preparation of (X) required a considerable quantity of the oxazolone (IX; X = SEt) and this was prepared by treatment of 5-keto-2-amyl-4-methoxymethylene-4 : 5-dihydro-oxazole (IX; X = OMe) with ethylthiol in pyridine (for preparation of the methoxymethyleneoxazolone, see Cornforth *et al.*, " The Chemistry of Penicillin," Chap. XXI). No success has yet attended our efforts to add ethylthiol to (X), or to induce intramolecular addition of the thiol group to the double bond.



(X.)

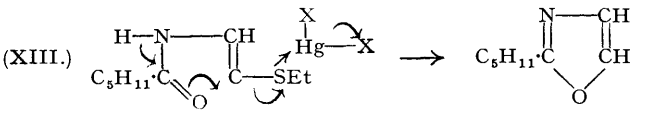


(XI.)

The benzylthio-acid (VII; R = CH_2Ph) readily yielded 5-*keto*-2-n-*amyl*-4-*benzylthiomethylene*-4 : 5-*dihydro-oxazole* (IX; X = S·CH_2Ph) with acetic anhydride. The oxazolone behaved like its ethylthio-analogue : the ring was opened by alkali and by benzylamine to give the acid (VII; R = CH_2Ph) and its *benzylamide* respectively. It was noted that 5-keto-2-phenyl-4-benzylthiomethylene-4 : 5-dihydro-oxazole on similar treatment with benzylamine gave the *benzylamide* of α-benzamido-β-benzylthioacrylic acid; this is in contrast to the behaviour of the same oxazolone with aniline, which converts it into the anilinomethyleneoxazolone (Barber, Sack, and Stickings, " The Chemistry of Penicillin," Chap. XXI.

It was hoped that a compound of type (X) would be available from (IX; X = S·CH_2Ph) and penicillamine methyl ester, when removal of the benzyl group with sodium and liquid ammonia might give the thioaldehyde corresponding to (II). A model reduction of the acid (VII; R = CH_2Ph) showed, however, that the double bond was also saturated, DL-N-*hexoylcysteine* (XI) being the only crystalline product isolated : and, as the condensation of the oxazolone with penicillamine methyl ester would give only gummy material, the work was abandoned at this point.

When mercuric chloride was added to a neutralised aqueous solution of α-hexamido-β-ethylthioacrylic acid (or its benzylthio-analogue) carbon dioxide was evolved, and on boiling, the characteristic smell of 2-amyloxazole (XII) appeared. The oxazole was isolated in poor yield as the picrate. In order to examine the mechanism of this change, β-ethylthiocrotonic acid, CH_3·C(SEt):CH·CO_2H (Posner *loc. cit.*), was treated with mercuric chloride under similar conditions. The products were carbon dioxide, acetone, and ethylthiomercuric chloride; no methylacetylene could be detected. This result makes it unlikely that (XII) is formed *via* the acetylene C_5H_{11}·CO·NH·C:CH, and a more likely mechanism is that of nucleophilic attack by the amide oxygen atom [in the intermediate complex (XIII)] upon the carbon atom bearing the alkylthio-group, removal of which is facilitated by the mercury.



β-*Ethylthiopropionic acid* was also treated with mercuric chloride; in this case no carbon dioxide was liberated.

Experimental.

Ethyl 1-*Methoxyhexylideneaminoacetate*.—Heximidomethyl ether was prepared in 55% yield by keeping equimolecular amounts of *n*-amyl cyanide, methanol, and hydrogen chloride in dry ether for three days at 0°. The crystalline hydrochloride was washed with ether and decomposed by aqueous potassium carbonate and ether, and the product distilled. A mixture of the imidoether (21 g.) and ether (20 c.c.) was shaken with a solution of glycine ethyl ester hydrochloride (24 g.) in water (20 c.c.) for one hour. The ethereal layer, after being washed with water, was dried and distilled, giving *ethyl α-methoxyhexylideneaminoacetate* (15 g.), a colourless oil, b. p. 85—90°/0·1 mm. (Found : C, 61·2; H, 9·6. $C_{11}H_{21}O_3N$ requires C, 61·4; H, 9·8%).

Potassium Ethyl α-(1-Methoxyhexylideneamino)-β-hydroxyacrylate (IV; R = Me).—Potassium (1·7 g.) was dissolved in a mixture of ethanol (6 c.c.) and ether (20 c.c.) under gentle reflux; the solution was diluted to 250 c.c. with ether. A cold mixture of the foregoing ester (10 g.) and ethyl formate (5 g.) was added at −5°, and this temperature then maintained for two hours. After being kept at 0° overnight, the pale pink *potassium* salt (10 g.) was collected and washed with ether; the filtrate and washings afforded an additional 1 g. after keeping for a day. The product was crystalline and deliquescent : it gave the characteristic ferric chloride test (Cornforth and Cornforth, *J.*, 1947, 101) (Found : N, 5·0. $C_{12}H_{20}O_4NK$ requires N, 5·0%). The same conditions were applied to prepare the ethoxy-analogue (IV; R = Et) in similar yield.

Action of Ethanol and Hydrogen Chloride on the Salt (IV; R = Et).—Potassium α-(1-ethoxyhexylideneamino)-β-hydroxyacrylate (15 g.) was added during 10 minutes to a stirred solution of hydrogen chloride (15 g.) in dry ethanol (150 c.c.) at −15°. After 2½ hours' stirring, the mixture was allowed to come slowly to room temperature and left overnight; the alcohol was removed at low pressure, and the remaining thick oil neutralised with cold aqueous potassium carbonate under ether. After three more extractions with ether the combined extract was dried and distilled, giving ethyl *n*-amylpenaldate diethyl acetyl (9 g.), b. p. 125°/0·1 mm., which solidified on cooling and had m. p. 50° after two recrystallisations from ethanol (Chas. Pfizer and Co., *op. cit.*, record m. p. 51°) (Found : C, 59·7; H, 9·5; N, 5·0. Calc. for $C_{15}H_{29}O_5N$: C, 59·4; H, 9·6; N, 4·6%). When this experiment was carried out in ether and with only 1½ equivs. of ethanol the main fraction had b. p. 95—100°/0·1 mm. and on saponification gave 2-amyloxazole-4-carboxylic acid, m. p. 94° undepressed by authentic material (Found : C, 58·8; H, 7·0. Calc. for $C_9H_{13}O_3N$: C, 58·9; H, 7·1%).

n-*Amylpenaldic Acid Diethylacetal* (VI; X = OEt).—Hydrolysis of the above ethyl ester acetal (2N-sodium hydroxide or 5% aqueous-alcoholic potassium hydroxide) gave the acid, slender needles, m. p. 80° (Found : OEt, 28·2; equiv., 302. Calc. for $C_{13}H_{25}O_5N,1·5H_2O$: OEt, 29·8%; equiv., 302). Other workers (*locc. cit.*) have recorded varying degrees of hydration. The anhydrous acid was obtained

by prolonged drying over phosphoric oxide in a vacuum, and recrystallised from light petroleum (b. p. 60—80°); m. p. 60—61° (Found : C, 56·5; H, 9·2. Calc. for $C_{13}H_{25}O_5N$: C, 56·7; H, 9·1%).

α-Hexamido-ββ-diethoxypropionylhydrazide.—Ethyl amylpenaldate diethyl acetal (2 g.) and hydrazine hydrate (2 c.c. of 50%) were treated with enough alcohol to effect mutual solution, and the mixture warmed for two hours at 60°, cooled, triturated and the solid collected. Two recrystallisations from aqueous ethanol gave the *hydrazide* (2 g.) in clusters of long silky needles, m. p. 161° (Found : C, 53·8; H, 9·2; N, 14·3. $C_{13}H_{27}O_4N_3$ requires C, 53·9; H, 9·3; N, 14·5%). The *benzylidene* derivative was prepared by adding benzaldehyde to an acid solution of the hydrazide; it separated from alcohol in long thin needles, m. p. 168·5° (Found : C, 63·7; H, 8·4; N, 11·1. $C_{20}H_{31}O_2N_3$ requires C, 63·7; H, 8·2; N, 11·1%).

N-(*α-Hexamido-ββ-diethoxypropionyl*)-D-*penicillamine* (III; R = *n*-C_5H_{11}; R′ = Et).—A solution of the above hydrazide (290 mg.) in N-hydrochloric acid (4 c.c.) at 0° was treated with a similarly cooled solution of sodium nitrite (90 mg.) in water (1 c.c.) with vigorous stirring. The thick oily drops of azide solidified on prolonged rubbing. The crude azide was collected, washed with a little cold water, and added at once to a solution of D-penicillamine hydrochloride (190 mg.) and anhydrous sodium carbonate (200 mg.) in water (3 c.c.) under carbon dioxide. The mixture was stirred for one hour then shaken vigorously for six hours, filtered, and the filtrate washed with ether, cooled to 0°, and brought to pH 4. The precipitated gum was collected by ether extraction and obtained as a white powder. It was purified by reprecipitation from aqueous sodium carbonate. The N-*acylpenicillamine* (200 mg.) was soluble in all common organic solvents and showed no tendency to crystallise. Its alkaline solution gave an intense red colour with sodium nitroprusside (Found : C, 53·5; H, 8·1; S, 7·7. $C_{18}H_{34}O_6N_2S$ requires C, 53·2; H, 8·4; S, 7·9%); $[\alpha]_D^{20°}$ +9·1° (*c* = 6 in ethanol). Pyrolysis of 5 mg. specimens was tried (*a*) at 120° for 10 minutes, then 150° for 10 minutes; (*b*) at 150° for 15 minutes. The pyrolysates after being taken into phosphate buffer showed no significant antibiotic activity against *S. aureus*.

Action of Ethylthiol and Hydrogen Chloride on the Salt (IV; R = Et).—Hydrogen chloride (15 g.) was passed during 20 minutes into a stirred suspension of potassium ethyl α-(α′-ethoxyhexylideneamino)-β-hydroxyacrylate (10 g.) in ether (100 c.c.) and ethylthiol (2·6 g.) at −5° under stringently anhydrous conditions. After 3 days (room temp.) the mixture was evaporated at low pressure, and the residual oil neutralised under ether with cold saturated aqueous potassium carbonate. Distillation of the ethereal extract gave a fraction (1 g.) boiling below 100°/14 mm., which was discarded; then a fraction (2 g.), b. p. 90—120°/0·1 mm., which gave 2-amyloxazole-4-carboxylic acid (m. p. and mixed m. p. 94°) on saponification. The main product (4·5 g.) boiled at 155—160°/0·1 mm. *Ethyl α-hexamido-β-ethylthioacrylate* was an oil, $n_D^{20°}$ 1·5149 (Found : C, 57·0; H, 8·6; N, 5·3; S, 11·7. $C_{13}H_{23}O_3NS$ requires C, 57·1; H, 8·4; N, 5·1; S, 11·7%). The absorption spectrum (in methanol) was taken on a freshly prepared, twice redistilled sample. There was a maximum at 2850 A. (ε, 10,620) and a minimum at 2440 A.

α-Hexamido-β-ethylthioacrylic Acid (VII; R = Et).—The foregoing ester (2 g.) was warmed with sodium hydroxide (20 c.c. of N) at 60° for 6 hours. The solution was washed with ether and acidified with N-hydrochloric acid. The *acid* (1·5 g.) was collected and dried in a vacuum over sodium hydroxide. Repeated crystallisation from benzene–light petroleum gave long, thin needles, m. p. 108° (Found : C, 53·6; H, 7·7; N, 5·7; S, 13·0. $C_{11}H_{19}O_3NS$ requires C, 53·8; H, 7·7; N, 5·7; S, 13·1%); λ max. 2800 A. (ε, 12,250); λ min. 2420 A. (in methanol).

Ethyl α-Hexamido-ββ-diethylthiopropionate (VI; X = SEt).—A small piece of freshly cut sodium was added to a solution of ethyl α-hexamido-β-ethylthioacrylate (2·5 g.) in ethylthiol (2·5 c.c.). After four days dry ether was added, and the solution filtered and evaporated. A solution of the residue in light petroleum (b. p. 60—80°) crystallised on evaporation at low pressure, giving the *diethylthioacetal* (2·5 g.) in long white needles, m. p. 40—45° raised to 50—51° after three recrystallisations from light petroleum (Found : C, 53·8; H, 8·7; N, 4·2; S, 18·0. $C_{15}H_{29}O_3NS_2$ requires C, 53·7; H, 8·7; N, 4·2; S, 19·1%). An attempt to prepare this substance from ethyl *n*-amylpenaldate with ethylthiol and hydrogen chloride led to an intractable product. When the diethylthioacetal ester (0·2 g.) was dissolved (½ hour at 100°) in N-sodium hydroxide (2 c.c.) and the solution was washed with ether and acidified to Congo-red, a strong odour of ethylthiol was noted and α-hexamido-β-ethylthioacrylic acid was precipitated (0·1 g.), m. p. and mixed m. p. 108° after recrystallisation from light petroleum (b. p. 60—80°).

Cleavage of Ethyl α-Hexamido-ββ-diethylthiopropionate with Mercuric Chloride.—A mixture of the thioacetal ester (180 mg.), mercuric chloride (360 mg.), cadmium carbonate (480 mg.), acetone (3 c.c.), and water (1·5 c.c.) was shaken mechanically for 24 hours. The precipitate was separated and washed with acetone and water. The filtrate, which gave a purple colour with alcoholic ferric chloride, was evaporated in the presence of a little cadmium carbonate, and the residue extracted with chloroform. Evaporation of the chloroform left an oil which was treated with an excess of 2 : 4-dinitrophenylhydrazine in 5N-hydrochloric acid. After 12 hours the yellow precipitate, m. p. 150°, was collected and recrystallised twice from ethanol and twice from ethyl acetate; final m. p. 166°, undepressed on admixture with authentic ethyl *n*-amylpenaldate 2 : 4-dinitrophenylhydrazone, m. p. 166—167°.

α-Hexamido-ββ-diethylthiopropionylhydrazide.—The thioacetal ester (VI; X = SEt) (1·8 g.) was warmed at 50° for 1 hour with hydrazine hydrate (0·8 c.c. of 50%) and ethanol (5 c.c.). Next day an equal volume of water was added. On very slow cooling of the warmed solution the *hydrazide* (1·6 g.) separated; soft silky needles, m. p. 148—149° unaffected by further crystallisation (Found : C, 48·7; H, 8·2; N, 13·4. $C_{13}H_{27}O_2N_3S_2$ requires C, 48·6; H, 8·4; N, 13·1%). The *benzylidene* derivative was prepared by shaking a solution in N-hydrochloric acid with benzaldehyde; slender needles from ethanol, m. p. 163° (Found : C, 58·5; H, 7·4. $C_{20}H_{31}O_2N_3S_2$ requires C, 58·7; H, 7·6%).

N-(*α-Hexamido-ββ-diethylthiopropionyl*)*glycine* (VIII; R = H).—An ice-chilled solution of sodium nitrite (43 mg.) in water (0·2 c.c.) was added to a stirred, similarly cooled solution of the above hydrazide (200 mg.) in N-hydrochloric acid (3 c.c.). The gummy azide was extracted with ether and obtained as an amorphous powder on evaporation. This was stirred with a solution of glycine (47 mg.) and potassium carbonate (88 mg.) in water (2 c.c.) for two hours. Next day the solution was filtered, and the filtrate

washed with ether, cooled, and acidified to Congo-red with 2N-hydrochloric acid. The *acylglycine* (44 mg., m. p. 145°) separated from aqueous ethanol in minute needles, m. p. 153° (Found: C, 48·6; H, 8·0; N, 7·7. $C_{15}H_{28}O_4N_2S_2,0{\cdot}5H_2O$ requires C, 48·3; H, 7·8; N, 7·5%).

α-(α-Hexamido-ββ-diethylthiopropionamido)-β-methoxyisovaleric Acid (VIII; R = CMe_2·OMe).—On repetition of the above experiment, using DL-α-amino-β-methoxy*iso*valeric acid (152 mg.) in place of glycine, the *acid* (VIII; R = CMe_2·OMe) was obtained (45 mg.); it crystallised from aqueous ethanol in microscopic rods, m. p. 118—120°, which were dried in a vacuum over sodium hydroxide (Found: C, 51·3; H, 8·0. $C_{19}H_{36}O_5N_2S_2,0{\cdot}5H_2O$ requires C, 51·2; H, 8·3%).

When the same conditions were applied in an attempt to condense the azide with D-penicillamine, a very low yield of yellow gum was obtained. When the reaction was carried out in homogeneous solution (aqueous dioxan), or by shaking the penicillamine solution with an ethereal solution of the azide, the result was no better.

Action of Benzylthiol and Hydrogen Chloride on the Salt (IV; R = Et).—Dry hydrogen chloride (20 g.) was passed during 20 minutes into a suspension of potassium ethyl α-(1-ethoxyhexylideneamino)-β-hydroxyacrylate (10 g.) in ether (100 c.c.) and benzylthiol (5·1 c.c.). The mixture was shaken frequently and kept at 0°. After two days at room temperature it was evaporated, and the residue washed with concentrated potassium carbonate solution and extracted with ether. The oil remaining after removal of the ether crystallised on being rubbed with light petroleum (b. p. 40—60°; 50 c.c.). The crude *ethyl α-hexamido-β-benzylthioacrylate* (5·52 g.; m. p. 65—75°; further 3·4 g. from mother-liquors) was recrystallised from light petroleum, b. p. 60—80°; clusters of fine needles, m. p. 77° (Found: C, 65·0; H, 7·5; N, 4·5; S, 9·6. $C_{18}H_{25}O_3NS$ requires C, 64·5; H, 7·5; N, 4·2; S, 9·6%). The ultra-violet absorption curve of a methanol solution showed a maximum at 2840 A. (ε, 18,000) and a minimum at 2460 A. The substance was unaffected by keeping with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid for three days.

α-Hexamido-β-benzylthioacrylic Acid (VII; R = CH_2Ph).—The crude ester (3 g.) was refluxed for 20 minutes with sodium hydroxide (30 c.c. of 2N). After being washed with ether, the solution was acidified (Congo-red), and the *acid* (2·1 g.) collected. Two recrystallisations from benzene gave long, thin needles, m. p. 161—161·5° (Found: C, 62·2; H, 6·6; N, 4·5; S, 10·0. $C_{16}H_{21}O_3NS$ requires C, 62·5; H, 6·9; N, 4·6; S, 10·4%); λ max. 2840 A. (ε, 13,500); λ min. 2440 A. (in methanol).

5-Keto-2-n-amyl-4-ethylthiomethylene-4 : 5-dihydro-oxazole (IX; X = SEt).—Ethylthiol (3·5 g.) was added to a solution of 5-keto-2-*n*-amyl-4-methoxymethylene-4 : 5-dihydro-oxazole (11 g.) in pyridine (40 c.c.). After 24 hours the pyridine was removed in a vacuum, and the residue distilled. The crude, pale yellow oxazolone, b. p. 100—110°/0·05 mm., was immediately warmed with 2N-sodium hydroxide until all was in solution. After working up in the usual manner, α-hexamido-β-ethylthioacrylic acid (10 g.) was collected. Two recrystallisations from benzene gave shining needles (8 g.), m. p. 108°.

The acid (4 g.) was dissolved in acetic anhydride (20 c.c.). After 20 minutes at 100° distillation at low pressure gave the *oxazolone* (IX; X = SEt) as a light yellow oil, b. p. 100—110°/0·05 mm. (3·6 g.); $n_D^{20°}$ 1·5510 (Found: C, 58·6; H, 7·6; N, 6·4; S, 14·3. $C_{11}H_{17}O_2NS$ requires C, 58·2; H, 7·5; N, 6·2; S, 14·1%). The dehydration could also be effected by warming the acid (0·45 g.) with phosphorus pentachloride (0·4 g.) in chloroform (2 c.c.). On distillation, decomposition (of a hydrochloride?) took place at 120°/0·1 mm. and the oxazolone (0·2 g.) distilled (Found: C, 58·6; H, 7·9%). Alkaline hydrolysis gave the acid (VII; R = Et).

Reaction of the Oxazolone (IX; X = SEt) *with Amines.*—(i) Benzylamine (2 drops) was added to a solution of the oxazolone (25 mg.) in dry ether (3 c.c.). After one hour at 0° the crystalline *α-hexamido-β-ethylthioacrylylbenzylamide* was collected and recrystallised from acetone–ether; m. p. 127—128° (Found: C, 64·1; H, 7·6; S, 9·1. $C_{18}H_{26}O_2N_2S$ requires C, 64·6; H, 7·8; S, 9·6%). (ii) The oxazolone was similarly treated with *p*-toluidine and left overnight. *α-Hexamido-β-ethylthioacrylyl-p-toluidide* formed tiny prisms, m. p. 104°, from acetone–ether (Found: C, 64·2; H, 7·7; S, 9·6. $C_{18}H_{26}O_2N_2S$ requires C, 64·6; H, 7·8; S, 9·6%). (iii) A mixture of the oxazolone (1·6 g.), glycine ethyl ester (0·8 g.), and pure dry ether (20 c.c.) was refluxed gently for 12 hours. After evaporation at low pressure the product was warmed with sodium hydroxide (20 c.c. of N) for 20 minutes, and the resulting solution, after being washed with ether, was acidified (Congo-red). After three days the partly crystalline product was collected and washed with ether. Recrystallisation thrice from benzene–ethanol (charcoal) gave shiny rhombs of N-(*α-hexamido-β-ethylthioacrylyl*)*glycine*, m. p. 160° (Found: C, 51·7; H, 7·4; N, 9·4. $C_{13}H_{22}O_4N_2S$ requires C, 51·7; H, 7·3; N, 9·3%). (iv) A mixture of the oxazolone (3·1 g.), D-penicillamine methyl ester (2·23 g.), and pure dry ether (50 c.c.) was refluxed for 20 hours, then set aside for half a day. An equal volume of light petroleum (b. p. 40—60°) was added, and the mixture kept at −30° for two hours. After centrifuging and washing twice with ether–light petroleum (1 : 1) the solid, which now appeared free from gum, was collected by means of dry ether; m. p. 128—129°, yield 200 mg. Recrystallisation from benzene–light petroleum (b. p. 40—50°) gave the N-(*α-hexamido-β-ethylthioacrylyl*)-D-*penicillamine* (X) in fine thin needles, m. p. 129° (Found: C, 52·3; H, 7·6; N, 7·1; S, 16·4. $C_{17}H_{30}O_4N_2S_2$ requires C, 52·3; H, 7·7; N, 7·2; S, 16·4%). The nitroprusside test for sulphydryl was negative, but a positive Feigl azide–iodine reaction was observed. The ultra-violet absorption curve showed a maximum at 2840 A. (ε, 16,000) and a minimum at 2430 A. (in methanol). From the mother-liquor an amorphous powder of the same composition could be separated (200 mg.); this softened and melted between 70° and 90°. The above procedure was the only one found to give crystalline material.

5-Keto-2-n-amyl-4-benzylthiomethylene-4 : 5-dihydro-oxazole (IX; X = S·CH_2Ph).—A mixture of benzylthiol (1·1 c.c.), 5-keto-2-*n*-amyl-4-methoxymethylene-4 : 5-dihydro-oxazole (1·7 g.), and pyridine (5 c.c.) was kept at room temperature and 20 mm. pressure (to remove methanol) for 8 hours; the pyridine was then removed over sulphuric acid in a vacuum. After being kept for 10 hours at 0° the viscous oil solidified and was recrystallised from ether–light petroleum (b. p. 40—60°). The *oxazolone* formed microscopic silky needles, m. p. 65—66° (Found: C, 66·5; H, 6·7; S, 11·4. $C_{16}H_{19}O_2NS$ requires C, 66·4; H, 6·6; S, 11·1%). The product was converted smoothly by aqueous alkali into the expected α-hexamido-β-benzylthioacrylic acid, m. p. and mixed m. p. 161·5°. When the oxazolone

(50 mg.) was kept for 18 hours with benzylamine (4 drops) in dry ether (5 c.c.), *α-hexamido-β-benzylthioacrylylbenzylamide* (40 mg.) separated in soft fibrous needles, m. p. 129—130° unaffected by recrystallisation from acetone–ether (Found : C, 69·2; H, 7·0. $C_{23}H_{28}O_2N_2S$ requires C, 69·6; H, 7·1%). In a similar manner *α-benzamido-β-benzylthioacrylylbenzylamide* was obtained from 5-keto-2-phenyl-4-benzylthiomethylene-4 : 5-dihydro-oxazole (m. p. 122—124°) and benzylamine in dry ether; the amide separated from acetone–ether in fine aggregated needles, m. p. 227° (Found : C, 71·2; H, 5·4. $C_{24}H_{22}O_2N_2S$ requires C, 71·6; H, 5·5%).

Action of Sodium in Liquid Ammonia on α-Hexamido-β-benzylthioacrylic Acid.—Sodium (0·35 g. required) was added in slices to a stirred solution of the acid (1·4 g.) in liquid ammonia (20 c.c.) until a permanent blue colour appeared. A little ammonium chloride was added, and the ammonia evaporated. An aqueous solution of the residue was washed with ether, acidified, and extracted with ether. The extract after treatment with charcoal was evaporated; the residual oil crystallised on trituration with light petroleum. The solid (0·1 g.) after two recrystallisations from benzene–light petroleum (b. p. 40—60°) gave DL-N-*hexoylcysteine*, long slender needles, m. p. 131—132°. The nitroprusside test was positive (Found : C, 49·2; H, 7·7; S, 14·2. $C_9H_{17}O_3NS$ requires C, 49·3; H, 7·8; S, 14·6%).

Action of Mercuric Chloride on α-Hexamido-β-ethylthioacrylic Acid.—To a neutral solution of the acid (1 g.) in 0·1N-sodium hydroxide, mercuric chloride (1·11 g.) was added, and the resulting suspension refluxed for one hour : evolution of carbon dioxide was complete in 10 minutes. Water (10 c.c.) was added, and the mixture distilled. The distillate was made alkaline and extracted with ether, which was then washed thrice with 5% sodium hydroxide, dried ($CaSO_4$) and evaporated. The residual 2-amyloxazole was converted into the picrate, m. p. and mixed m. p. 85° after crystallisation from ethanol.

Action of Mercuric Chloride on β-Ethylthiocrotonic Acid.—Mercuric chloride (0·93 g.) was added to a neutral solution of the acid (0·5 g.; m. p. 92°) in 0·1N-sodium hydroxide. The mixture was warmed to 60°; a stream of carbon dioxide-free air after passing through it was led through aqueous barium hydroxide, an acetylene test solution (Ilosvay, *Ber.*, 1899, **32**, 2698), and finally a solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. Carbon dioxide and acetone (2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 125°) were thus detected, but no methylacetylene. The reaction was completed by warming on a steam-bath, and the crystalline residual precipitate collected. This consisted of ethylthiomercuric chloride (Found : C, 8·1; H, 1·7. Calc. for C_2H_5ClSHg : C, 8·1; H, 1·7%).

β-Ethylthiopropionic Acid.—Sodium (4·25 g.) was added in slices to a solution of β-chloropropionic acid (10 g.) and ethylthiol (5·15 g.) in ethanol (20 c.c.). After refluxing for one hour, the acidic products were isolated by means of ether and fractionally distilled. β-Ethoxypropionic acid was thereby separated from the required *acid*, which had b. p. 135°/15 mm., $n_D^{20°}$ 1·4832 (Found : C, 45·1; H, 7·8; S, 23·5. $C_5H_{10}O_2S$ requires C, 44·7; H, 7·5; S, 23·9%).

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