679. *Syntheses in the Penicillin Field. Part VIII. Thiazolinyl-amino-oxazole Derivatives.*

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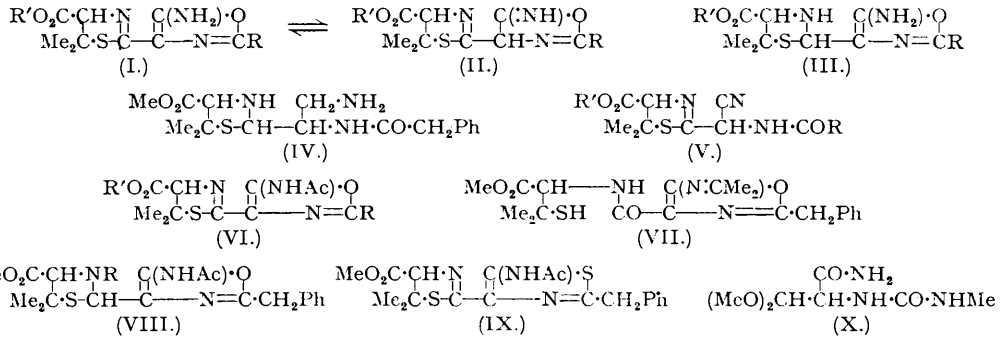
The attempted reduction of thiazolinyl-amino-oxazoles to the corresponding thiazolidines by mild reagents could not be effected although the reduction of an *acetyl* derivative was accomplished. The use of the resulting *thiazolidine* (VIII; R = H) in an attempted synthesis of penicillin led to a compound showing some antibiotic activity.

Part VII described the preparation and some properties of the tautomeric thiazolinyl-amino-oxazoles (I) and (II). The most attractive route to penicillins *via* compounds (I) appeared to be in reduction of the latter to the corresponding thiazolidinylamino-oxazoles (III), followed by the deamination of the amino-oxazole ring, perhaps by means of nitrous acid or nitrosyl chloride. Reduction of the base (II; R = CH_2Ph, R′ = Me) was first attempted by mild catalytic means using Raney nickel, palladium on barium sulphate, or a mixture of Raney nickel, palladium on barium sulphate, and palladium on charcoal, but (II) was recovered unchanged from these reactions.

With aluminium amalgam in moist ether, however, reduction of (II; R = CH_2Ph, R′ = Me) proceeded in an unexpected fashion. When an excess of aluminium amalgam was used, as for conversion of thiazolines into thiazolidines, the product, isolated as an amorphous *oxalate*, analysed as the thiazolidine (IV) instead of the required (III; R = CH_2Ph, R′ = Me). It was evident that reductive scission of the amino-oxazole ring had occurred, presumably by isomerisation to the acyclic form (V) followed by reduction of the cyano-group. Confirmation that the amino-oxazole ring would isomerise to the open-chain form under the conditions used for reduction was obtained by allowing (II; R = CH_2Ph, R′ = Me) to react with less aluminium amalgam. In this way reduction was avoided, and the product was (V; R = CH_2Ph, R′ = Me); this

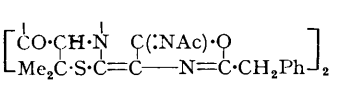


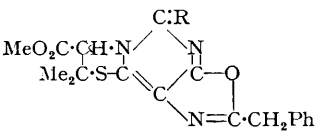
observation accords with the previous finding (Part VII) that (II; R = CH_2Ph, R′ = Me) readily undergoes ring-opening under mildly alkaline conditions, *e.g.*, with pyridine, and apparently the isomerisation is effected by the alumina formed during the reduction. The reduction of a cyano-group with aluminium amalgam is unusual, but is paralleled in a similar case, namely in the reduction of oximinomalonitrile which with aluminium amalgam yields αβ-diaminopropionitrile (unpublished observation). Similar reduction of the thiazolinyl-aminothiazole corresponding to (II; R = CH_2Ph, R′ = Me) (Part VII) gave only an unstable oil. Despite the ready ring-opening of (II) in pyridine, an *acetyl* compound (VI; R = CH_2Ph, R′ = Me) was obtained using pyridine–acetyl chloride or, in poor yield, by the action of hot acetic anhydride. In both reactions (V; R = CH_2Ph, R′ = Me) was obtained as a by-product, which formed no acetyl derivative under such conditions. The compounds (II; R = Ph, R′ = Me) and (II; R = *n*-C_5H_{11}, R′ = Me) also yielded *acetyl* derivatives which formed crystalline *hydrochlorides*, presumably by virtue of the tertiary nitrogen atom in the oxazole ring. The formation of such hydrochlorides is paralleled in the thiazolinyl-oxazolone series ("The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 857) and also in the related aminothiazole series, where ethyl 5-hexoamido-2-benzylthiazole-4-carboxylate forms a hydrochloride (J. A. Elvidge, Ph.D. Thesis, London, 1946). In the case of the hydrochlorides of the acetyl compounds (VI; R = Ph or *n*-C_5H_{11}, R′ = Me) the light absorption resembled that of the hydrochlorides of the parent amines (I; R = Ph or *n*-C_5H_{11}, R′ = Me) (see Table), and

differed from that of the bases, suggesting tautomerism similar to that encountered previously, *viz.*, (I) ⇌ (II) (Part VII). Treatment of the acetyl compound (VI; R = CH_2Ph, R′ = Me) with dilute hydrochloric acid in acetone apparently resulted in hydrolysis of the thiazoline ring as well as removal of the acetyl group for the product had ultra-violet absorption and analysis compatible with structure (VII). The acetyl compound (VI; R = CH_2Ph, R′ = Me) was unaffected by Raney nickel and hydrogen in ethanol, but treatment with aluminium amalgam gave the thiazolidine (VIII; R = H) isolated as crystalline *hydrochloride*, and as the *methylcarbamyl* derivative (VIII; R = NHMe·CO) formed from the oily base and methyl *iso*cyanate. A similar reduction of the *thiazolinyl acetamidothiazole* (IX) to a thiazolidine was unsuccessful, as were attempts to reduce the corresponding 5-aminothiazole, or the phenyl compound (II; R = Ph, R′ = Me).

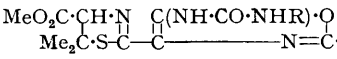
The successful reduction of (VI; R = CH_2Ph, R′ = Me) to (VIII) presented the possibility of obtaining, by deacetylation, the thiazolidine (III; R = CH_2Ph, R′ = Me). Attempts to accomplish the deacetylation with dry methanolic hydrogen chloride, however, led to extensive breakdown of the compound (VIII), the main product isolated being an unstable oil, which reacted with methyl *iso*cyanate to give (X). It thus appears that (VIII) undergoes scission of both the thiazolidine and acetamido-oxazole rings with methanolic hydrogen chloride, the thiazolidine portion giving rise to the dimethyl acetal grouping of (X), and the acetamido-oxazole ring being split hydrolytically to an α-amino-amide. The use of other deacetylation reagents was inadmissible since alkalis led to ring-opening of the amino-oxazole ring and hydrogen halides in other alcohols caused trans-esterification.

Meanwhile, similar experiments with (I; R = CH_2Ph, R′ = H) were carried out. The reduction of this compound with aluminium amalgam was not effected, and acetylation under the conditions described above for the corresponding ester yielded only the neutral *diketopiperazine* (XI). Treatment of the hydrochloride of (I; R = CH_2Ph, R′ = H) with diphenylketen, in the hope of removing hydrogen chloride and acylating in one step, yielded only a neutral oil. The formation of (XI) presented evidence that the thiazolidylidene structure

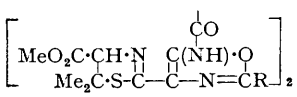
(XI.) 

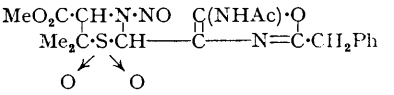


(XII.)



(XIII.)

(XIV.) 



(XV.)

could participate in the tautomerism of the thiazolinyl-amino-oxazoles, and further evidence for this was obtained when (II; R = CH_2Ph, R′ = Me) reacted with methyl or phenyl *iso*cyanate. The *iso*cyanates first reacted with the amino- or imino-group of the oxazole ring in (II), the products undergoing immediate cyclodehydration to yield the N-*methyl*- and N-*phenyl*-compounds (XII; R = NMe) and (XII; R = NPh) which showed light absorption in agreement with their formulation as purine analogues (see Table). This reaction with *iso*cyanates was originally intended to yield the ureido-derivatives (XIII; R = Me or Ph) for possible conversion into penicillin by means of nitrous acid.

A similar route to penicillin involved the preparation of the urea (XIII; R = H), but (II; R = CH_2Ph, R′ = Me) failed to react with cyanic acid, nitrourea, or urethane, and the reaction of (II; R = CH_2Ph, R′ = Me) or (II; R = Ph, R′ = Me) with carbonyl chloride and ammonia led only to the isolation of the *bis-compound* (XIV; R = Ph). Similarly, the reaction of (II; R = CH_2Ph, R′ = Me) with benzyl or ethyl chloroformate did not lead to crystalline products, the materials isolated consisting largely of (II) or (V), depending upon whether pyridine was used in the reaction or not. The compound (II; R = CH_2Ph, R′ = Me) did not react with carbon disulphide and potassium carbonate to yield a dithiocarbamate. The use of phenyl chlorothiolformate (Ehrensvard, *Nature*, 1947, **159**, 500) as an acylating agent for the protection of the amino-oxazole ring in (II; R = CH_2Ph, R′ = Me) yielded no crystalline acyl compound even when reaction was effected in the presence of triethylamine or pyridine as promoter. However, (II; R = Ph, R′ = Me) with phenyl chlorothiolformate

afforded the desired acyl compound which spontaneously eliminated thiophenol to yield a *purine* analogue (XII; R = O). This prevented the use of the required acyl compound for reduction to a thiazolidine, to be followed by removal of the phenylthioformyl group by known mild methods.

In view of the failure to isolate the compound (III; R = CH_2Ph, R′ = Me), a number of attempts to utilise the acetyl derivative (VIII; R = H) directly for the synthesis of penicillin were made. Reaction of this derivative with one mole of nitrous acid by treatment of the hydrochloride with sodium nitrite resulted only in the recovery of starting material, but interaction with excess of nitrous acid in aqueous ethanolic solution yielded a product (which gave a positive Liebermann reaction) tentatively formulated as (XV), *N*-nitrosation having apparently been accompanied by oxidation. This product was completely inactive against *Staph. aureus*.

Since nitrosyl chloride reacts with acylamido-compounds to yield *N*-nitroso-compounds, tautomeric with diazoacetates, the reaction with the thiazolidine (VIII; R = H) was attempted in the hope that the acylamido-group would be attacked, and that the intermediate obtained on working up in aqueous media, for which thiazolidinyloxazolone, triazine, or keten structures were possible, would lead to penicillin. The compound (VIII; R = H) was therefore treated with an acetic anhydride solution of nitrosyl chloride–pyridine or nitrosyl chloride–potassium acetate under various conditions. In this way products having some antibacterial activity were obtained (0·5 unit/mg., based on weight of ester taken). The most favourable conditions were: treatment with pyridine and two equivalents of nitrosyl chloride, followed by brief heating at 70° in the presence of pyridine hydrochloride. It is of interest that the acetyl compound (VI; R = CH_2Ph, R′ = Me) failed to react with nitrosyl chloride in acetic anhydride–potassium acetate under conditions which yielded *N*-nitrosoacetanilide from acetanilide in good yield.

In view of the relatively unpromising aspect of the routes to penicillin outlined above, attention was turned to other methods of synthesis, which will be described in future papers.

Absorption spectra.

Compound.	$\lambda_{max.}$, A.	$E^{1\%}_{1\,cm.}$
Thiazolidine.		
(IV, oxalate)	end absorption [1]	
Thiazolinyloxazoles		
(VI; R = CH_2Ph, R′ = Me)	2270 [1]	630
	2450 *	240
(VI; R = Ph, R′ = Me)	2810 [2]	700
	2900	700
	2990	700
(VI; R = Ph, R′ = Me) hydrochloride	2820 [2]	570
	2900	570
	3590	260
(VI; R = *n*-C_5H_{11}, R′ = Me), hydrochloride	2310 [1]	210
	2690	250
	3360	140
Oxazoles.		
(VII)	2450 [1]	190
	3040	420
(VII), hydrochloride	end absorption [1]	
Thiazolinylthiazole.		
(IX)	2280 [1]	510
	2680	300
	2980	250
	3090	275
Diketopiperazine.		
(XI)	2440 [2]	660
	2940	375
	3560	340
Dihydropyrimidines.		
(XII; R = NMe)	2880 [2]	480
	2950	450
(XII; R = NPh)	2910 [2]	770
Urea.		
(XIV; R = Ph)	2580 [2]	520
	2800	350
	2910	350
	3510	840
	3660	820

* Inflection. [1] Measured in ethanol. [2] Measured in chloroform.

Experimental.

Reaction of 5-Imino-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazoline with Aluminium Amalgam.—The compound (II; R = CH_2Ph, R′ = Me) (1·0 g.) in ether (150 c.c.) was reduced with aluminium amalgam (1 g.) and water (2 c.c.) in ethanol (10 c.c.) during 40 hours. After filtration, the filtrate and washings (ethanol, 20 c.c.) were concentrated by evaporation in a vacuum to a pale yellow oil, which was dissolved in ethyl acetate and poured into ethereal oxalic acid. The white hygroscopic solid *compound* was reprecipitated from acetic acid by ether, and washed with dry ether (Found: C, 52·6; H, 6·7%; equiv., 210. $C_{19}H_{27}O_7N_3S$ requires C, 51·7; H, 6·2%; equiv., 213·5). The base was inactive against *S. aureus*, and was also inactive after treatment with sodium nitrite in presence of phosphoric acid.

The compound (II; R = CH_2Ph, R′ = Me) (0·5 g.) was refluxed in ether (50 c.c.) with aluminium

amalgam (0·16 g.), while water (0·4 c.c.) was added slowly (2—3 hours). Refluxing was continued for 17—18 hours, and after filtration the filtrate and ethereal washings were evaporated in a vacuum. The residual oil crystallised on treatment with dry ether and was recrystallised from ethanol–water, whereupon (V; R = CH_2Ph, R′ = Me) separated as laths, m. p. 100° (after being washed with ether). The material showed ultra-violet absorption maximum at 2700 A. ($E^{1\%}_{1\,cm.}$ = 550), and with ethereal hydrogen chloride yielded a precipitate, m. p 157° (decomp.), undepressed on admixture with the hydrochloride (I; R = CH_2Ph, R′ = Me); the same hydrochloride was obtained from the ethereal filtrates.

5-Acetamido-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole (VI; R = CH_2Ph, R′ = Me).—(*a*) The base (II; R = CH_2Ph, R′ = Me) (0·43 g.) was heated on the steam-bath with acetic anhydride (5 c.c.) for 15 minutes. Removal of excess of reagent under reduced pressure yielded a yellow oil, which crystallised from ethanol–water, and was recrystallised from ethyl acetate–light petroleum (b. p. 60—80°), giving *5-acetamido-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)-oxazole* as needles, m. p. 118° (Found: C, 58·5, 59·0; H, 5·4, 5·4. $C_{19}H_{21}O_4N_3S$ requires C, 58·9; H, 5·5%), depressed on admixture with either starting material or (V; R = CH_2Ph, R′ = Me). The product was insoluble in ethanol but soluble in ether, in which solvent it yielded no permanent precipitate with hydrogen chloride. The aqueous ethanolic filtrates from the crude acetamido-compound deposited (V; R = CH_2Ph, R′ = Me), m. p. 108—111° undepressed on admixture with authentic material.

(*b*) The hydrochloride of (I; R = CH_2Ph, R′ = Me) (9 g.) was added to an ice-cold mixture of pyridine (44 g.), dry chloroform (160 c.c.), and acetyl chloride (4·6 c.c.), and after 17 hours the solution was evaporated to dryness in a vacuum at 40—50°. The residue was treated with water (100 c.c.) and ethyl acetate (150 c.c.), and the ester-phase washed with 2N-hydrochloric acid, sodium hydrogen carbonate solution, and water. Evaporation of the ethyl acetate gave a red oil which with aqueous ethanol afforded crystals (5·0 g.); recrystallised from ethyl acetate–light petroleum (b. p. 60—80°) the acetyl compound (4·5 g.) had m. p. 117—118°.

5-Acetamido-2-phenyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole.—The amino-oxazole (II; R = Ph, R′ = Me) (0·5 g.) was added to a mixture of pyridine (5 c.c.), chloroform (19 c.c.), and acetyl chloride (0·30 c.c.) at 0°. After 20 hours, evaporation of the solvents in a vacuum and working up as above yielded an oil, which was dissolved in ether, filtered, and treated with hydrogen chloride. The precipitate (0·45 g.) was washed with ether and warm ethyl acetate, and recrystallised from chloroform–ether whereupon *5-acetamido-2-phenyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole hydrochloride* separated as tablets, m. p. 155° (decomp.) (Found: C, 52·2; H, 4·9; N, 9·9; Cl, 8·8. $C_{18}H_{20}O_4N_3ClS$ requires C, 52·7; H, 4·9; N, 10·2; Cl, 8·65%). With chloroform (25 c.c.) and sodium hydrogen carbonate solution the hydrochloride (0·25 g.) gave an oil which was taken up in ether. The solution was filtered and evaporated, and the residue, which crystallised on trituration with ether, was recrystallised from ethyl acetate–light petroleum to give *5-acetamido-2-phenyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole* as white blades, m. p. 132—133° (Found: C, 58·6; H, 5·3; N, 11·3. $C_{18}H_{18}O_4N_2S$ requires C, 58·1; H, 4·9; N, 11·3%).

5-Acetamido-2-n-amyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole.—A solution of the hydrochloride of (I; R = n-C_5H_{11}, R′ = Me) (0·41 g.) in dry chloroform (15 c.c.) was left with acetyl chloride (0·20 c.c.) in pyridine (4 c.c.) at 0° for 18 hours. The oil, obtained in the usual way, was treated in ethyl acetate (3 c.c.) and ether (6 c.c.) with excess of ethereal hydrogen chloride, and the precipitate stirred with ether. The ethereal filtrate was evaporated, and the residue was stirred with fresh ether, the final ethereal filtrate being allowed to evaporate spontaneously, whereupon granular crystals, m. p. 113—114° (decomp.), were deposited. Recrystallised from ether (containing a little chloroform) and light petroleum, *5-acetamido-2-n-amyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole hydrochloride* separated as colourless prisms, m. p. 118° (decomp.) (Found: C, 50·9; H, 6·65. $C_{17}H_{26}O_4N_3ClS$ requires C, 50·5; H, 6·5%).

5-Acetamido-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)thiazole (IX).—*5-Amino-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)thiazole* (1·25 g.) was heated on the steam-bath for 0·75 hour with acetic anhydride (10 c.c.). Evaporation yielded an oil which soon solidified. Recrystallised from ethanol–water, *5-acetamido-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)thiazole* separated as white tablets or laths, m. p. 137° (Found: C, 56·6; H, 5·1; N, 10·5. $C_{19}H_{21}O_3N_3S_2$ requires C, 56·6; H, 5·2; N, 10·4%).

Attempted Deacetylation of (VI; R = CH_2Ph, R′ = Me).—The acetyl compound (150 mg.) in acetone (4 c.c.) was left for 20 hours with 2N-hydrochloric acid (4 c.c.). Acetone was removed under reduced pressure, the aqueous residue was extracted with chloroform, and the extract was washed with sodium hydrogen carbonate solution and evaporated to yield an oil, which crystallised under ethyl acetate–light petroleum. From ethyl acetate–light petroleum the *anil* (VII) separated as wedge-shaped prisms, m. p. 82—83° (Found: C, 56·5; H, 6·35; N, 10·3. $C_{20}H_{25}O_4N_3S,H_2O$ requires C, 56·7; H, 6·4; N, 9·9%).

Reduction of (VI; R = CH_2Ph, R′ = Me).—The compound (3·8 g.) in ether (700 c.c.) was reduced with aluminium amalgam (4·0 g.) and water (8·0 c.c.) in ethanol (60 c.c.) during 23 hours. The ethereal filtrate and washings were evaporated in a vacuum, and the residual oil treated with ethereal hydrogen chloride. The ether was decanted from the gummy precipitate, and the latter dissolved in ethyl acetate (50 c.c.). On being scratched, the product (1·8 g.) crystallised. Several crystallisations from ethyl acetate yielded *5-acetamido-2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolidinyl)oxazole hydrochloride* (VIII; R = H,HCl), m. p. 141—143° (decomp.) (Found: C, 52·8; H, 5·8; N, 9·9; Cl, 8·9. $C_{19}H_{24}O_4N_3ClS$ requires C, 53·6; H, 5·7; N, 9·9; Cl, 8·3%). The hydrochloride (150 mg.) was shaken with ethyl acetate and sodium hydrogen carbonate solution, and the dried organic layer evaporated to a yellow oil, which was left with excess of methyl *iso*cyanate for 29 hours. Evaporation of the mixture, and treatment of the residual oil with ether yielded, on spontaneous evaporation, crystalline material which was recrystallised from ethyl acetate–light petroleum giving *5-acetamido-2-benzyl-4-(3-methylcarbamyl-4-carbomethoxy-5 : 5-dimethyl-2-thiazolidinyl)oxazole* (VIII; R = NHMe·CO) as white needles, m. p. 196—197° (decomp.) (Found: C, 56·7; H, 5·95; N, 12·8. $C_{21}H_{26}O_5N_4S$ requires C, 56·5; H, 5·9; N, 12·6%).

Attempted Deacetylation of the Thiazolidinyl-5-acetamido-oxazole (VIII; R = H).—A solution of the hydrochloride (VIII; R = H,HCl) in dry methanol (5 c.c.) was saturated at 0° with hydrogen chloride. After 5 minutes the solution was evaporated at room temperature. A portion of the residue showed no significant antibacterial activity when tested against *Staph. aureus* in acetone–phosphate buffer solution (pH 7), either before or after treatment with nitrosyl chloride–acetic anhydride–pyridine or nitrosyl chloride–acetic anhydride–potassium acetate (fused). The remainder of the residue was shaken with ether and sodium hydrogen carbonate solution, the dried ethereal solution was evaporated, and the residue was treated with excess of methyl *iso*cyanate overnight. Removal of the excess of *iso*cyanate and treatment of the residue in warm dry ether with charcoal, followed by evaporation, gave N-*methyl*-N′-(1-*carbamyl*-2 : 2-*dimethoxyethyl*)*urea* (X) which separated from ethyl acetate–light petroleum as prismatic needles, m. p. 126° (Found : C, 41·5; H, 7·65. $C_7H_{15}O_4N_3$ requires C, 41·0; H, 7·4%). The aqueous sodium hydrogen carbonate layer gave a blue colour with ferric chloride solution, presumably due to penicillamine methyl ester.

Action of Acetyl Chloride on 5-Amino-2-benzyl-4-(4-carboxy-5 : 5-dimethyl-2-thiazolinyl)oxazole.—The acid hydrochloride (as I; R = CH_2Ph, R′ = H) (0·5 g.) was added to a mixture of pyridine (4 c.c.), chloroform (10 c.c.), and acetyl chloride (0·3 c.c.). The solvents were evaporated, and the residue was treated with ethyl acetate and 2N-hydrochloric acid. The washed and dried ethyl acetate layer gave a crystalline residue on evaporation. Recrystallised from ethanol, the *diketopiperazine* (XI) separated as white needles, m. p. 179° (Found : C, 60·3; H, 4·9. $C_{18}H_{17}O_3N_3S$ requires C, 60·9; H, 4·8%).

Reactions of Thiazolinyl-amino-oxazoles.—(a) *With* isocyanates. The base (II; R = CH_2Ph, R′ = Me) reacted with excess of methyl *iso*cyanate to give the *compound* (XII; R = Me) (90%) which was washed with ether, and recrystallised from ethanol. It had m. p. 218—219° (Found : C, 59·0; H, 4·9; N, 14·6. $C_{19}H_{20}O_3N_4S$ requires C, 59·3; H, 5·2; N, 14·6%). In another experiment, the product at first had m. p. 178° (decomp.), resolidifying, and remelting at 210°, but it reverted to (XII; R = Me) on crystallisation; it may have been the *ureido*-compound (XIII; R = Me).

The *phenyl* compound (XII; R = Ph) was obtained in similar fashion and, on recrystallisation from *n*-butanol, separated as micro-needles, m. p. 258° (decomp.) (Found : C, 64·0; H, 4·9; N, 12·9. $C_{24}H_{22}O_3N_4S$ requires C, 64·6; H, 5·0; N, 12·6%).

(b) *With carbonyl chloride.* The base (II; R = Ph, R′ = Me) (200 mg.) in toluene (5 c.c.) was treated with a solution of carbonyl chloride in toluene (10 c.c.; 12·5% w/v), and the precipitated hydrochloride (I; R = Ph, R′ = Me) collected after 4 hours and rejected. The filtrate was evaporated to small bulk and the residue treated with liquid ammonia. After evaporation of ammonia, the residue was shaken with chloroform and water, and the dried chloroform solution concentrated under reduced pressure to an oil, which with ether changed to a crystalline powder (30 mg.), m. p. 229° (decomp.). Recrystallised from chloroform–light petroleum (charcoal), NN′-*bis*-[2-*phenyl*-4-(4-*carbomethoxy*-5 : 5-*dimethyl*-2-*thiazolinyl*)*oxazol*-5-*yl*]*urea* separated as white, hexagonal tablets, m. p. 230° (decomp.) (Found : C, 57·8; H, 4·3. $C_{33}H_{32}O_7N_6S_2$ requires C, 57·5; H, 4·7%).

(c) *With phenyl chlorothiolformate.* The base (II; R = Ph, R′ = Me) (500 mg.) was left with phenyl chlorothiolformate (130 mg.) in ethyl acetate (20 c.c.) for 20 hours at room temperature and 48 hours at 0°. The solution was decanted from the hydrochloride of (II; R = Ph, R′ = Me), m. p. 220° (decomp.), and evaporated to an oil which was triturated with several portions of ether. The residue gave crystalline material from ethanol–water, and evaporation of the ethereal solutions yielded an oil, which gave more of the solid on being left with fresh ether. From ethanol the *compound* (XV) separated as white plates, m. p. 227—228° (decomp.) (Found : C, 57·5; H, 4·2; N, 11·8. $C_{17}H_{15}O_4N_3S$ requires C, 57·1; H, 4·2; N, 11·8%).

Reagents,					Antibiotic activity.	
3·6% w/w NOCl in Ac_2O, c.c.	Pyridine, c.c.	KOAc, mg.	Ac_2O, c.c.	Activation treatment.	Diam. of inhibition zone, mm.	Units/mg.*
0·11	—	49	1·0		6	0·03
0·11	1·0	—	—		10	0·1
0·22	—	6·3	1·0		5	—
0·22	1·0	—	—		10	0·1
0·22	1·0	—	—	Pyridine + trace of pyridinium chloride at 70—80°/0·5 hour	15	0·5
0·22	1·0	—	—	,, ,, ,,	12	0·3
0·22	—	50	1·0	,, ,, ,, and then at 110°/10 minutes	4	—

* As methyl benzylpenicillinate which has *ca.* one-fortieth of the activity of benzylpenicillin.

Treatment of the Thiazolidinyl-acetamido-oxazole (VIII; R = H) *with Nitrous Acid.*—The hydrochloride of (VIII; R = H) (150 mg.) in acetic acid (0·2 c.c.) and ethanol (5 c.c.) was treated with sodium nitrite (200 mg.) in water (5 c.c.) and the solution kept at 0° for 18 hours. Solvents were removed under reduced pressure, and the residual material shaken with chloroform and water. Evaporation of the dry chloroform solution gave a gum which crystallised under ether. From ethyl acetate–light petroleum (charcoal), 5-*acetamido*-2-*benzyl*-4-(3-*nitroso*-4-*carbomethoxy*-5 : 5-*dimethyl*-2-*thiazolidinyl*)-*oxazole* SS-*dioxide* separated as rosettes of rectangular plates, m. p. 147—148° (decomp.) (Found : C, 51·0; H, 5·15; N, 12·1. $C_{19}H_{22}O_7N_4S$ requires C, 50·7; H, 4·9; N, 12·4%). The compound gave a positive Liebermann reaction.

Attempted Conversion of (VIII; R = H,HCl) *into Methyl Benzylpenicillinate.*—The thiazolidine hydrochloride (25 mg. portions) was treated with nitrosyl chloride in acetic anhydride in the presence either of dry pyridine or of powdered anhydrous potassium acetate. After any activation treatment,

the solution was evaporated to dryness at 0·001—0·1 mm. pressure, and the residue taken up in 5% aqueous phosphate buffer (pH 7) and alcohol-free ether. The ether was separated, dried (Na_2SO_4), and concentrated under reduced pressure (without external heating) to 1—2 c.c. Benzene (2—3 c.c.) was added, the ether was removed under reduced pressure, and the residual benzene solution finally lyophilised at 0·01—0·1 mm. pressure. The residue was dissolved in 2 : 1 : 1-acetone–water–5% aqueous phosphate buffer (pH 7), and the solution (12 mg./c.c.) tested by the plate method using *Staph. aureus*.

Blank experiments in which either (VIII; R = H,HCl) or nitrosyl chloride was omitted led to no inhibition of growth of the test organism.

Similar experiments with the crude acid, obtained by treatment of the ester (VIII; R = H,HCl) with sodium hydroxide, did not yield any antibacterial activity, but since the acidic material failed to give (VIII; R = H) when treated with diazomethane this was not significant.

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