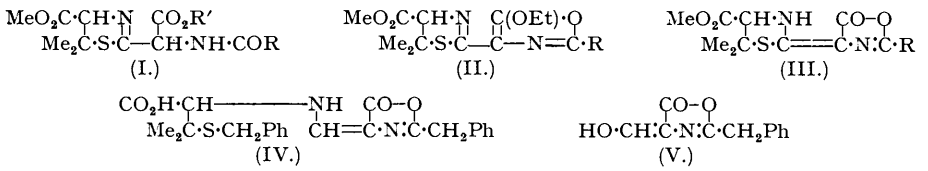
676. *Syntheses in the Penicillin Field. Part V. The Structure and Reduction of Some Thiazolinyloxalones.*†

By R. Bentley, A. H. Cook, and J. A. Elvidge.

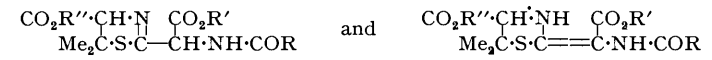
The structure of the previously described thiazolinyloxazolones is discussed in relation to simpler oxazolones, their precursors, and their degradation products. The formation of two isomeric *methyl derivatives* (VIII) from (III) is explained in the light of degradation to *N*-methylpenicillamine ester hydrochloride, characterised as the thiazolidine formed by condensation with acetone. This thiazolidine was also synthesised from 5 : 5-*dimethylthiazolidine*-2 : 4-*dicarboxylic acid*. Some attempts to reduce thiazolidylideneoxazolones are also described.

Comparison of the ultra-violet absorption spectra (Table I) of the compounds obtained by cyclising the dehydropenicilloate (I; R = *p*-$C_6H_4{\cdot}NO_2$) revealed increasing conjugation in passing from the thiazoline (I; R = *p*-$C_6H_4{\cdot}NO_2$, R′ = Et) through the 5-ethoxyoxazole (II) to the oxazolone (III) and suggested, therefore, that (III; R = *p*-$C_6H_4{\cdot}NO_2$) and not the alternative Δ^2-thiazolinyloxazolone (or Δ^2-thiazolinyl-hydroxyoxazole) formulation best represented the structure of the oxazolone.



Similarly, it appeared from the absorption spectra of the oxazolones derived from methyl *n*-amyl- and benzyl-dehydropenicilloates that the structures (III; R = *n*-C_5H_{11}) and (III; R = CH_2Ph), respectively, provided the best representation for these compounds. The positions and intensities of the absorption bands are to be compared with the data for the less complex oxazolones listed in Table I.

The formation of oxazolones from the dehydropenicilloates where other acylamido-acid esters give 5-alkoxyoxazoles suggested that the Δ^2-thiazoline formulation of the dehydropenicilloates (I) might be wrong. If these compounds possess the isomeric 2-thiazolidylidene structures, then cyclisation to conjugated (*i.e.*, stabilised) oxazolones would occasion no surprise. Certainly, the main ultra-violet absorption band of the dehydropenicilloates is at a considerably longer wave-length (*ca.* 2900 A.) than is to be expected for Δ^2-thiazolines, but at present little can be predicted regarding the absorption properties of the alternative systems :



In favour of the thiazolidylidene structure of the dehydropenicilloates must be cited the resistance to reduction by aluminium amalgam of these compounds and the corresponding nitriles (cf. Parts VII and VIII). On the other hand, the formation of an ethoxyoxazole in one instance

† A full description of the preparation of the thiazolinyloxazolones is given in " The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 857. The present account deals with work carried out largely after expiration of the co-operative wartime Anglo-American research on penicillin.

TABLE I.

Compound.	$\lambda_{max.}$, A.	$E^{1\%}_{1\ cm.}$
4-Carbomethoxy-5 : 5-dimethyl-2-*p*-nitrobenzamidocarbethoxymethylthiazoline (I; R = *p*-$C_6H_4{\cdot}NO_2$, R′ = Et)	2820 [2]	685
	2880 *	660
5-Ethoxy-2-*p*-nitrophenyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolinyl)oxazole (II; R = *p*-$C_6H_4{\cdot}NO_2$)	2510	400
	2800 * [2]	280
	3560	350
2-*p*-Nitrophenyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolidylidene)oxazolone (III; R = *p*-$C_6H_4{\cdot}NO_2$)	2510	360
	3220	260
	4020	820
	4120 *	800
4-Carbomethoxy-5 : 5-dimethyl-2-phenylacetamidocarbobenzyloxymethylthiazoline (I; R = R′ = CH_2Ph)	2910	470
4-Carbomethoxy-5 : 5-dimethyl-2-*n*-hexoamidocarbobenzyloxymethylthiazoline (I; R = C_5H_{11}, R′ = CH_2Ph)	2820	430
	2900	430
2-Benzyl-4-(4-carbomethoxy-5:5-dimethyl-2-thiazolidylidene)oxazolone (III; R = CH_2Ph)	2410	300
	3300	770
2-*n*-Amyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolidylidene)oxazolone (III; R = *n*-C_5H_{11})	2480	150
	3300	675
S-Benzyl benzylpenicillenic acid (IV) [1]	2400 [3]	177
	3225	660
2-Benzyl-4-hydroxymethyleneoxazol-5-one (V) [1]	2400 [4]	222
	3000	818

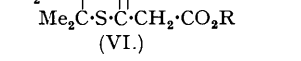
* Inflexion.

All measurements were made in chloroform unless stated otherwise. [1] Merck & Co., Inc., *M.* 12*c.*; *op. cit.*, p. 163. [2] In ethanol. [3] In methanol. [4] In 0·01N-sodium hydroxide.

(see later) and perhaps the *C*-nitrosation of the precursors (VI; R = Ph·CH_2 and Et) are facts which lend support to a Δ^2-thiazoline formulation for the dehydropenicilloates since the precursors absorb at the same wave-length. It may be, therefore, that tautomerism occurs.

In the case of the oxazolone products, on the other hand, available evidence (as already described) points solely to the thiazolidylidene structure. Further evidence for the correctness of this structure for the dehydropenicillins follows.

Treatment of the acid (VII; R = CH_2Ph) with ethereal hydrogen chloride yielded a hydrated hydrochloride which on basification regenerated (VII; R = CH_2Ph). The ester (III; R = CH_2Ph) also yielded a crystalline hydrochloride in contrast to the behaviour of the uncyclised precursors, the dehydropenicilloates. It seems that the basic centre is in the oxazolone ring since the NH group of the thiazolidine portion of (III) and (VIII) appears to be acidic as indicated by the reaction with diazomethane. When (VII; R = CH_2Ph or *n*-C_5H_{11}) was treated with one mole of diazomethane, (III; R = CH_2Ph or *n*-C_5H_{11}) was obtained. When, however, (VII; R = CH_2Ph) or (III; R = CH_2Ph) was treated with excess of diazomethane two isomeric dimethyl compounds were obtained (*op. cit.*). These compounds had similar absorption spectra both to one another and to the compounds (III; R = CH_2Ph) and (VII; R = CH_2Ph), and it



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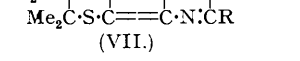

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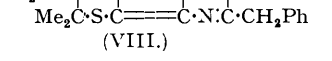


(VIII.)

appeared highly probable that they were the *cis*- and *trans*-forms of (VIII). Attempts to separate (III) or (VII) into isomers (*cis*- and *trans*-) were unsuccessful, although hydrated and unhydrated forms of (VII; R = CH_2Ph) were obtained and (III; R = CH_2Ph) formed two polymorphs,

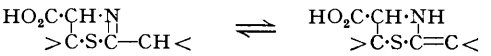
TABLE II.

Thiazolidylideneoxazolones.	$\lambda_{max.}$, A.	$E^{1\%}_{1\ cm.}$	Thiazolidylideneoxazolones.	$\lambda_{max.}$, A.	$E^{1\%}_{1\ cm.}$
(VII; R = *n*-C_5H_{11})	2370	145	(VII; R = CH_2Ph) hydrochloride hemihydrate	2420	185
	3290	800		2450 [1]	
				3300	630
(VII; R = CH_2Ph) anhydrous	2440	190			
	2480		(VIII) form B, m. p. 110—111°	2420	185
	3300	800		2440	
				3300	870
(VII; R = CH_2Ph) hemihydrate	2440	200			
	2490				
	3320	730			

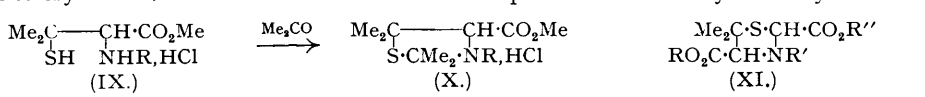
All measurements were made in chloroform unless stated otherwise. [1] In ethanol.

crystallising as prisms and needles, of which the latter tended to pass into the former on recrystallisation. The absorption spectra of the compounds described above are listed in Table II.

With ferric chloride in aqueous ethanol the acids (VII; R = CH_2Ph and n-C_5H_{11}), as well as the hemihydrate and hydrochloride of the former, gave Prussian-blue colours. Unlike the blue colour given by the mercapto-acids, penicillamine, cysteine, and thioglycollic acid, however, the colorations were persistent, and furthermore the acid (VII; R = CH_2Ph) was recovered largely unchanged after treatment for 60 minutes with bromine in chloroform. By polarography, the absence of a mercapto-group in the molecule was demonstrated conclusively. The esters (III; R = CH_2Ph and n-C_5H_{11}), (VIII), and all the previously described 4-carbomethoxythiazoline derivatives (see *op. cit.*, p. 886) produced no coloration with ferric chloride, whereas all the 4-carboxythiazolines including dehydropenicilloates did so. Thus it appears that this blue coloration is a characteristic of the thiazoline system, is not indicative of a free mercapto-group, and has no connection with the oxazolone portion of the molecules (III) and (VII).



By analogy with the hydrolytic scission of thiazolidines with mercuric chloride, similar degradation of compounds (III) and (VIII) should provide evidence for their structures. After treatment of the product of the interaction of (III; R = CH_2Ph) and methanolic mercuric chloride with hydrogen sulphide, penicillamine methyl ester hydrochloride (IX; R = H) was isolated. Similar degradation of (VIII) (m. p. 151—152°) furnished a water-soluble fraction which failed to crystallise. The latter material resembled penicillamine methyl ester hydrochloride in



giving a purple colour with ferric chloride in the presence of sodium hydrogen carbonate. Since this thiol reaction was no longer given after the gum had been heated with acetone it appeared that condensation had occurred. The crystalline product subsequently isolated differed from (X; R = H), however, and was regarded as the *N*-methylthiazolidine (X; R = Me), proof of its structure being provided by the following synthesis. Penicillamine hydrochloride was condensed with glyoxylic acid to give 5 : 5-*dimethylthiazolidine*-2 : 4-*dicarboxylic acid* (XI; R = R′ = R″ = H), which reacted with excess of diazomethane to yield dimethyl 3 : 5 : 5-trimethylthiazolidine-2 : 4-dicarboxylate (XI; R = R′ = R″ = Me) isolated as the known hydrochloride (Part I). Hydrolytic scission of the latter with methanolic mercuric chloride provided *N*-methylpenicillamine methyl ester hydrochloride (IX; R = Me) as a gum, which afforded the crystalline thiazolidine (X; R = Me), identical with the product obtained earlier. It should be mentioned that other possible routes to (IX; R = Me) and thence (X; R = Me) were unsuccessful. Thus the base corresponding to (X; R = H) did not react with diazomethane, and N-*toluene*-p-*sulphonyl*-S-*benzylpenicillamine* failed to undergo *N*-methylation in alkaline solution with methyl sulphate (cf. Cocker and Lapworth, *J.*, 1931, 1894) or methyl iodide (cf. Fischer and Lifschitz, *Ber.*, 1915, **48**, 360; du Vigneaud and Behrens, *J. Biol. Chem.*, 1937, **117**, 27). Similarly *N*-benzoyl-*S*-benzylpenicillamine failed to give the expected *N*-methyl derivative, and the attempted preparation of *N*-methylpenicillamine by the reaction of thiazolidines, derived from penicillamine or penicillamine methyl ester and formaldehyde, with sodium in ammonia (cf., Upjohn Co., *op. cit.*, p. 460; *U.* 18; *CPS.* 448 *) or methyl iodide in dioxan could not be effected.

The degradation of the high-melting form of (VIII) to *N*-methylpenicillamine methyl ester proved that the structure (VIII) and thence (III) was correct, and since the isomerides (VIII) have similar absorption spectra they must be regarded as having different geometric configurations.

Having obtained thiazolinyloxazolones by cyclisation of dehydropenicilloates, attempts were made to effect reduction in the hope that penicillins might be produced. Initially, the isolation of reduction products was not of primary importance and only antibacterial activity was sought.

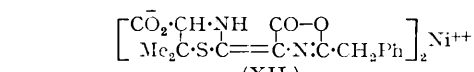
Repeated assay (plate method against *Staph. aureus*) showed (III; R = CH_2Ph) to possess small antibacterial activity (0·5—1·0 unit/mg.) in 50% aqueous acetone, in contrast to the acid (VII; R = CH_2Ph) which was virtually inactive in aqueous phosphate buffer at pH 7. The activity was not increased, however, by attempted reduction under a variety of conditions, which

* References to penicillin reports in this Series of papers are given in the form detailed in the preface to " The Chemistry of Penicillin."

included catalytic hydrogenation under pressure, treatment with aluminium amalgam in moist solvents, and reaction with magnesium and sulphur dioxide. Attempted reduction of (III; R = n-C_5H_{11}) or (VII; R = n-C_5H_{11}) likewise led to no enhanced antibiotic activity. The possibility of oxidising the thiazolinyloxazolones to the corresponding sulphones or sulphoxides was then briefly examined, in the hope that, by raising the sulphur atom to a higher state of oxidation, poisoning of hydrogenation catalysts would be obviated. The compound (III; R = CH_2Ph), however, appeared to be stable to hydrogen peroxide and sodium periodate, and other common oxidising agents seemed inadmissible.

When added to culture media, neither (VII; R = n-C_5H_{11}) nor (VII; R = CH_2Ph), at concentrations of 0·005—0·32%, produced any significant increase in the yields of penicillin given by surface growths of *Penicillium notatum* 1249. Thus in no sense did they behave as penicillin precursors.

Renewed efforts to effect catalytic hydrogenation of (III; R = CH_2Ph) afforded results which served to emphasise the stability of the thiazolinyloxazolone system. Thus, using a mixture of palladium on charcoal and barium sulphate (Mozingo *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 2092) and Raney nickel (Pavlic and Adkins, *ibid.*, 1946, **68**, 1471), hydrogenation at atmospheric pressure afforded a pale green *nickel* complex, together with a base which formed a crystalline *hydrochloride*. From the analytical data, light absorption, and properties, it appeared that these compounds were best represented as (XII) and (XIII), respectively. Hence, it was



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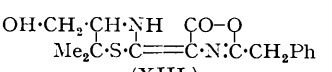

(XIII.)

concluded that, in order to effect reduction of the exocyclic double bond, conditions more vigorous than those required to effect hydrogenolytic attack at the penicillamine carbomethoxy-group would be necessary. The possibility of isolating a thiazolidyloxazolone or the required re-arrangement product, a thiazolidine-β-lactam, seemed very slight and new approaches to the problem of thiazolidyloxazolone and penicillin synthesis were therefore devised (see Parts VI, VII, and VIII).

Experimental.

Methylation of Thiazolidylideneoxazolones.—(*a*) 2-Benzyl-4-(4-carboxy-5 : 5-dimethyl-2-thiazolidylidene)oxazolone hemihydrate (215 mg.) in chloroform was treated slowly with 0·2N-diazomethane in ether (3·2 c.c.). After 30 minutes, the solution was extracted with aqueous sodium hydrogen carbonate, concentrated *in vacuo*, and then diluted with ether–light petroleum. Characteristic compact prisms (90 mg.) separated and were identified by mixed m. p. as 2-benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolidylidene)oxazolone. (*b*) The hemihydrate (200 mg.) dissolved with vigorous effervescence in excess of ethereal diazomethane. By evaporation of the solution *in vacuo* and addition of ether–light petroleum, two crystal crops were obtained: 90 mg., m. p. 149—150°, and 80 mg., m. p. 104—108°. From chloroform–light petroleum, form A of *2-benzyl-4-(4-carbomethoxy-3 : 5 : 5-trimethyl-2-thiazolidylidene)oxazolone* separated as prisms, m. p. 151—152° (Found: C, 59·8; H, 5·6; N, 7·9. $C_{18}H_{20}O_4N_2S$ requires C, 60·0; H, 5·6; N, 7·8%). Form B of the *oxazolone* crystallised from the same solvents as laths, m. p. 110—111° (Found: C, 59·8; H, 5·7; N, 7·6%). (*c*) A mixture of the same two isomers (separated as above) was also obtained on treating 2-benzyl-4-(4-carbomethoxy-5 : 5 : dimethylthiazolidylidene)oxazolone (200 mg.) in purified acetone (5 c.c.) with excess of ethereal diazomethane for 16 hours.

Degradation of Thiazolidylideneoxazolones.—(*a*) 2-Benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolidylidene)oxazolone (300 mg.) was dissolved in a 10% solution of mercuric chloride in methanol (25 c.c.). After 24 hours, a trace of precipitate was rejected, and hydrogen sulphide was passed into the solution, the filtrate from the mercuric sulphide then being evaporated *in vacuo* to leave a partly crystalline residue. Water (5 c.c.) was added: the insoluble portion which failed to crystallise had an odour of phenylacetic acid. The aqueous extract gave with 5% mercuric chloride a white precipitate which was dissolved in moist ethyl acetate and decomposed with hydrogen sulphide. Evaporation of the filtrate from this decomposition afforded needles, m. p. 167° (decomp.), identified as penicillamine methyl ester hydrochloride by mixed m. p. with an authentic specimen and by the ferric chloride test (transient purple coloration in the presence of aqueous sodium hydrogen carbonate). (*b*) 2-Benzyl-4-(4-carbomethoxy-3 : 5 : 5-trimethyl-2-thiazolidylidene)oxazolone (0·5 g.), m. p. 149—150°, was dissolved in a solution of mercuric chloride (3 g.) in dry methanol (30 c.c.). After 24 hours, hydrogen sulphide was passed through the solution, and the filtrate worked up, as previously, for the mercapto-amino-acid fragment. This formed an oil which gave a purple coloration with aqueous ferric chloride in the presence of sodium hydrogen carbonate, but not after first warming it with acetone. By evaporation of the acetone, a gum was obtained which slowly crystallised from chloroform–ether in prisms, m. p. 163° (decomp.), which depressed the m. p. of a specimen of 4-carbomethoxy-2 : 2 : 5 : 5-tetramethylthiazolidine hydrochloride [m. p. 160—161° (decomp.)] to *ca.* 135°. The material was identified by mixed m. p. with the thiazolidine hydrochloride prepared below.

Methyl 2 : 2 : 3 : 5 : 5-Pentamethylthiazolidine-4-carboxylate Hydrochloride.—Glyoxylic acid (Perkin, *J.*, 1877, **32**, 96) was added gradually to a hot solution of penicillamine hydrochloride (4 g.) in ethanol

(10 c.c.) until a positive ferric chloride reaction (indigo blue in aqueous solution) was no longer given. Evaporation of the solution then afforded an oil which crystallised when left under water. Purified by treatment of an aqueous sodium hydrogen carbonate (charcoal) solution with hydrochloric acid, 5 : 5-*dimethylthiazolidine*-2 : 4-*dicarboxylic acid* (1·8 g.) formed compact prisms, m. p. 205° (decomp.) (Found : C, 40·5; H, 5·4. $C_7H_{11}O_4NS$ requires C, 41·0; H, 5·4%). This acid with excess of ethereal diazomethane gave during 24 hours dimethyl 3 : 5 : 5-trimethylthiazolidine-2 : 4-dicarboxylate, isolated as the hydrochloride, m. p. 129—130° (Part I). This salt (1·2 g.) in methanol (5 c.c.) was warmed for 15 minutes with 5% aqueous mercuric chloride (sufficient to cause turbidity), and then treated with excess of the reagent (100 c.c.) and water (100 c.c.). After 2 hours the dense precipitate was suspended in moist ethyl acetate and decomposed with hydrogen sulphide, the filtrate solution filtered, and evaporated to a water-soluble oil which gave, in the presence of sodium hydrogen carbonate, a purple coloration with ferric chloride. After the oily *N*-methylpenicillamine methyl ester hydrochloride had been warmed with acetone (3 c.c.) this colour reaction was no longer given, and crystallisation occurred on spontaneous evaporation of the acetone. The methyl 2 : 2 : 3 : 5 : 5-pentamethylthiazolidine-4-carboxylate hydrochloride separated in prismatic needles, m. p. 164° (decomp).

N-*Toluene*-p-*sulphonyl*-S-*benzylpenicillamine*.—*S*-Benzylpenicillamine (24 g.), toluene-*p*-sulphonyl chloride (38 g.), and ether (100 c.c.) were shaken and 2N-sodium hydroxide (250 c.c.) was added in 4 portions, with cooling in ice. The aqueous layer was acidified with concentrated hydrochloric acid at 0° and the N-*toluene*-p-*sulphonyl*-S-*benzylpenicillamine* filtered off (yield : 32 g., 80%); it separated from ethanol–water in colourless prisms, m. p. 173° (Found : C, 58·2; H, 6·1; N, 3·9. $C_{19}H_{23}O_4NS_2$ requires C, 58·0; H, 5·9; N, 3·6%). Attempts to effect *N*-methylation failed.

Further Attempts to reduce (III; R = CH_2Ph).—2-Benzyl-4-(4-carbomethoxy-5 : 5-dimethyl-2-thiazolidylidene)oxazolone (0·5 g.) was shaken with Raney nickel (*ca.* 1 g.) (Pavlic and Adkins, *loc. cit.*), palladium–barium sulphate (0·2 g.), and palladium–norite (0·5 g.) (Mozingo *et al.*, *loc. cit.*) in hydrogen for 3·5 hours. After filtration and evaporation, the gum was dissolved in acetone, and a green solid obtained by spontaneous evaporation. Recrystallisation from chloroform–ether gave pale green hair-like needles, m. p. 225° (decomp.), of the *nickel* complex of 2-benzyl-4-(4-carboxy-5 : 5-dimethyl-2-thiazolidylidene)-oxazolone [Found : C, 53·4; H, 4·6; N, 7·7; S, 8·5. $(C_{16}H_{15}O_4N_2S)_2Ni$ requires C, 53·3; H, 4·2; N, 7·8; S, 8·9%]. Light absorption (in chloroform) : Maximum, 3300 A.; $E^{1\%}_{1\,cm.} = 360$. The filtrate from the nickel complex was evaporated, the residue dissolved in a small volume of ethanol, and ether (15 c.c.) added. Clarification and addition of ethereal hydrogen chloride gave crystals of 2-*benzyl*-4-(5 : 5-*dimethyl*-4-*hydroxymethyl*-2-*thiazolidylidene*)*oxazolone hydrochloride* which separated from ethyl acetate–ether in clumps of fine needles, m. p, 136° (Found : C, 54·2; H, 5·4; N, 8·0. $C_{16}H_{19}O_3N_2ClS$ requires C, 54·2; H, 5·4; N, 7·9%). Light absorption (in ethanol) : Maximum, 3300 A.; $E^{1\%}_{1\,cm.} = 650$.

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