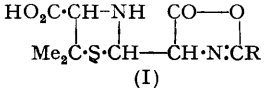
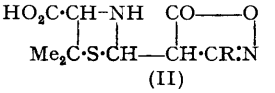
856. *Syntheses in the Penicillin Field. Part X.** 4-*Formyl*isooxazolones and Derived Thiazolidines.†

By A. H. Cook and G. Shaw.

*iso*Oxazolones (IV) are difficultly accessible and their direct formylation seems impracticable. The formyl compounds (III) are, however, obtained by treating readily available benzylidene*iso*oxazolones (VII) with diphenylformamidine and hydrolysing the resulting anilinomethylene-*iso*oxazolones (VIII). The formyl compounds behave as aldehydes and yield thiazolidines.

When penicillin was thought to possess the oxazolone-thiazolidine structure (I), synthesis of the analogous *iso*oxazolone-thiazolidines (II) was undertaken in the hope that these substances, in view of their spatial similarity to (I), might have useful antibacterial properties. The condensation of 4-formyl*iso*oxazolones (III) with penicillamine (ββ-dimethylcysteine) was accordingly investigated as a direct route to the required compounds.


(I)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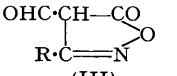
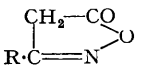

(III)

Initial experiments designed to prepare (III) involved formylation of 3-substituted *iso*oxazolones (IV) but a survey of the literature revealed that only one such authenticated compound (IV; R = Ph), prepared from ethyl benzoylacetate and hydroxylamine, was available (Dains and Griffin, *J. Amer. Chem. Soc.*, 1913, **35**, 959; Claisen and Zedel, *Ber.*, 1891, **24**, 140). Condensation of (IV; R = Ph) with ethyl formate and sodium, or with ethyl orthoformate and acetic anhydride, led in each case to the formation of a non-aldehydic yellow crystalline substance which must be the compound (V; R = Ph).

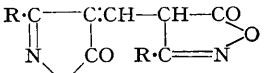
* Part IX, *J.*, 1950, 1947.

† Most of the work described in this paper was carried out during the war-time Anglo-American co-operative project on penicillin. None of the present work, however, is described in "The Chemistry of Penicillin," Princeton University Press, 1949.

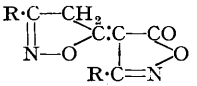
To ensure as close an approach as possible to the penicillin model it was desirable that the substituent R should be an alkyl or aralkyl group. A product of the reaction of ethyl acetoacetate and hydroxylamine (Hantzsch, *Ber.*, 1891, **24**, 498; Knorr and Reuter, *ibid.*, 1894, **27**, 1174; Wahl and Meyer, *Bull. Soc. chim.*, 1908, **3**, 952; Uhlenhuth, *Annalen*, 1897, **296**, 44) has been previously assigned the structure (IV; R = Me) but Donleavy



(IV)



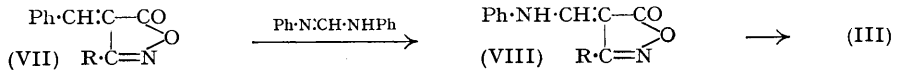
(V)



(VI)

and Gilbert (*J. Amer. Chem. Soc.*, 1937, **59**, 1072) have shown that the substance is in fact a bis-compound, probably (VI; R = Me). Moureau and Lezzenec (*Bull. Soc. chim.*, 1907, **1**, 1079) reported preparation of the ammonium salt of (IV; R = n-C_5H_{11}) from ethyl 3-keto-octanoate * and hydroxylamine in the presence of aqueous ammonia. This reaction was repeated and in addition a similar reaction was carried out with ethyl phenylacetoacetate and hydroxylamine, but in each case the compounds obtained appeared to be salts of (VI; R = n-C_5H_{11} and Ph·CH_2). The difficulties encountered in attempts to prepare (IV; R = n-C_5H_{11} and Ph·CH_2) and the failure of (IV; R = Ph) to undergo formylation by the usual procedures did not encourage further work along these lines, and attention was therefore turned to a survey of *iso*oxalones having a group in the 4-position capable of conversion into the formyl radical.

The most promising of such *iso*oxazolones appeared to be the anilinomethylene*iso*oxazolones (VIII) which were found to be readily obtainable in excellent yield by fusion of a mixture of the 4-benzylidene*iso*oxazolones (VII) with diphenylformamidine; the benzylidene derivatives were readily available from the reaction of benzaldehyde, hydroxylamine, and β-keto-esters (Dains and Griffin, *loc. cit.*). A few attempts were made to condense (VIII) with penicillamine and simple thiazolidines in the expectation that the desired analogues (II) would be obtained but these experiments were unsuccessful. It was soon found, however, that gentle hydrolysis of (VIII; R = Me, Ph, n-C_5H_{11}, and Ph·CH_2) with dilute (2%) sodium carbonate solution resulted in the liberation of aniline (cf. Ridi and Papini, *Gazzetta*, 1946, **76**, 376, who used potassium hydroxide), which was removed from the reaction mixture by distillation in steam, hydrolysis being deemed to be complete when the distillate failed to give a colour with sodium hypochlorite solution. When the ice-cold resulting solutions were acidified the formyl*iso*oxazolones (III; R = Me, Ph, n-C_5H_{11}, and Ph·CH_2) separated as crystalline solids. In addition small amounts of the bis-compounds (V) were isolated from the reaction mixtures except in the case (VIII; R = Me). It is noteworthy that more vigorous hydrolysis with N-sodium hydroxide gave the compounds (V) almost exclusively.



The formyl*iso*oxazolones, unlike the analogous hydroxymethyleneoxazolones, appeared to be true aldehydes as indicated by ready formation of 2 : 4-dinitrophenylhydrazones and reduction of ammoniacal silver nitrate. The aldehydes were also prone to self-condensation; thus a sample of (III; R = Ph) after being kept for three months was found to have been converted into (V; R = Ph), presumably with loss of formic acid. When the aldehydes (III; R = Me, Ph, n-C_5H_{11}, and Ph·CH_2) and penicillamine were brought together in ethanol or aqueous ethanol, the solutions soon failed to give ferric chloride and nitroprusside colour reactions of the thiol. From the solutions were isolated the required penicillin models (II; R = Me, Ph, n-C_5H_{11}, and Ph·CH_2) as pale yellow solids which could be precipitated from alkaline solution with acid; hydrolysis with warm dilute acid led to the re-formation of penicillamine and the formyl*iso*oxazolones. Assay of these *iso*oxazolone-thiazolidines (II) *in vitro* indicated that they were devoid of antibacterial activity.

* Geneva nomenclature, CO_2H = 1.

Experimental

The β-keto-esters employed were prepared by the Spassow method (Spassow, *Ber.*, 1937, **70**, 2381; Ogata *et al.*, *J. Pharm. Soc. Japan*, 1939, **59**, 105) by refluxing a mixture of ethyl acetoacetate, an acid chloride, and magnesium turnings in chloroform, followed by mild hydrolysis of the resultant diketo-ester with 2% aqueous ammonia. The yields were rarely greater than 50% but the method was less time-consuming than most others.

Reaction of Hydroxylamine with Ethyl Phenylacetoacetate.—The keto-ester (2 g.) and hydroxylamine hydrochloride (1 g.) were dissolved in concentrated aqueous ammonia (12 ml.), and the solution was set aside for 18 hours. The solvents were removed *in vacuo* over concentrated sulphuric acid, to leave a slightly sticky crystalline residue which, when triturated with dry ether, gave a colourless solid (0·4 g.). The solid separated from ethanol–benzene as needles, m. p. 185° (decomp.), and may be the *ammonium* salt of 3-benzyl-4-(3-benzyl*iso*oxazolin-5-ylidene)*iso*oxazol-5-one (VI; R = Ph·CH_2) (Found: C, 68·3; H, 5·5; N, 11·8. $C_{20}H_{20}O_3N_3$ requires C, 68·5; H, 5·7; N, 12·0%).

Reaction of 3-*Phenyl*iso*oxazol-5-one with Formylating Agents.*—The *iso*oxazolone (Dains and Griffin, *loc. cit.*; 1 g.), ethyl orthoformate (1 g.), and acetic anhydride (5 ml.) were warmed together on the steam-bath for 2 hours. The solvents were removed *in vacuo* and the resultant gum was dissolved in ethanol. Addition of water to the solution precipitated 4-(5-*keto*-3-*phenyl*iso*oxazolin*-4-*ylmethylene*)-3-*phenyl*iso*oxazol-5-one* (V; R = Ph) (0·8 g.) which separated from chloroform–light petroleum as yellow plates, m. p. 200° (Found: C, 68·6; H, 3·8; N, 8·3. $C_{19}H_{12}O_4N_2$ requires C, 68·7; H, 3·6; N, 8·4%). The same compound was obtained by treating 3-phenyl*iso*oxazol-5-one with ethyl formate and " molecular " sodium in benzene.

4-*Benzylidene- and* 4-*Anilinomethylene*-iso*oxazolones* (Dains and Griffin, *loc. cit.*).—The following compounds have not been described before: 3-*Benzyl*-4-*benzylidene*iso*oxazol-5-one* (VII; R = Ph·CH_2) separated from ethanol as pale yellow plates, m. p. 146—147° (Found: C, 77·2; H, 5·1; N, 5·3. $C_{17}H_{13}O_2N$ requires C, 77·5; H, 5·0; N, 5·3%). 4-*Anilinomethylene*-3-*benzyl*iso*oxazol-5-one* (VIII; R = Ph·CH_2) formed pale yellow plates (from benzene), m. p. 185—186° (decomp.) (Found: C, 73·6; H, 5·2; N, 9·8. $C_{17}H_{14}O_2N_2$ requires C, 73·4; H, 5·1; N, 10·0%). 3-n-*Amyl*-4-*benzylidene*iso*oxazol-5-one* (VII; R = *n*-C_5H_{11}) separated from ethanol as pale greenish-yellow sheaves, m. p. 76—77° (Found: C, 74·1; H, 7·1; N, 5·7. $C_{15}H_{17}O_2N$ requires C, 74·1; H, 7·1; N, 5·8%). 3-n-*Amyl*-4-*anilinomethylene*iso*oxazol-5-one* (VIII; R = *n*-C_5H_{11}) formed yellow plates, m. p. 86—87°, from ethyl acetate–light petroleum (Found: C, 69·85; H, 7·2; N, 10·75. $C_{15}H_{18}O_2N_2$ requires C, 69·75; H, 7·0; N, 10·85%).

*Formyl*iso*oxazolones* (III).—The general method for the preparation of these substances is illustrated as follows: 4-Anilinomethylene-3-benzyl*iso*oxazol-5-one (12 g.) was treated with 2% sodium carbonate solution (250 ml.), and the mixture distilled in steam until a test portion of the distillate failed to give a purple colour with aqueous sodium hypochlorite, indicating the absence of aniline; this required 1—2 hours. Acidification of the pale yellow solution with hydrochloric acid and cooling gave 3-benzyl-4-formyl*iso*oxazol-5-one *monohydrate* (III; R = Ph·CH_2) (6 g.) which separated from ethyl acetate–light petroleum as pale yellow plates, m. p. 80° (Found: C, 59·8; H, 5·1; N, 6·3. $C_{11}H_9O_3N,H_2O$ requires C, 59·7; H, 5·0; N, 6·3%). The hydrate readily lost water when warmed to 60° *in vacuo* to give 3-*benzyl*-4-*formyl*iso*oxazol-5-one* which crystallised from ethyl acetate–light petroleum as pale yellow prisms, m. p. 112—113° (decomp.) (Found: C, 65·1; H, 4·5; N, 6·8. $C_{11}H_9O_3N$ requires C, 65·0; H, 4·5; N, 6·9%). The 2 : 4-*dinitrophenylhydrazone*, prepared in aqueous-ethanolic sulphuric acid, crystallised from ethanol–water as yellow rosettes of needles, m. p. 150° (decomp.) (Found: C, 53·45; H, 3·5; N, 18·3. $C_{17}H_{13}O_6N_5$ requires C, 53·5; H, 3·4; N, 18·3%). Extraction of the filtrate from the above monohydrate preparation with chloroform (3 × 20 ml.) and evaporation of the solvent gave 3-*benzyl*-4-(3-*benzyl*-5-*keto*iso*oxazolin*-4-*ylmethylene*)iso*oxazol-5-one* (V; R = Ph·CH_2) (2 g.) which separated from chloroform–light petroleum as yellow plates, m. p. 154—155° (decomp.) (Found: C, 70·2; H, 4·7; N, 7·8. $C_{21}H_{16}O_4N_2$ requires C, 70·0; H, 4·5; N, 7·8%). The following substances were prepared in a similar manner: 4-*Formyl*-3-*phenyl*iso*oxazol-5-one monohydrate* formed pale brown needles (from ethyl acetate–light petroleum), m. p. 79—80° (Found: C, 58·0; H, 4·4; N, 6·65. $C_{10}H_9O_4N$ requires, C 58·0; H, 4·4; N, 6·8%); the anhydrous *aldehyde* (III; R = Ph), pale yellow prisms (from ethyl acetate–light petroleum), m. p. 178° (decomp. with darkening from 150°) (Found: C, 63·2; H, 3·9. $C_{10}H_7O_3N$ requires C, 63·45; H, 3·7%), gave a 2 : 4-*dinitrophenylhydrazone* which separated from dilute acetic acid as feathery orange needles, m. p. 167—168° (decomp.) (Found:

C, 51·6; H, 3·3; N, 18·7. $C_{16}H_{11}O_6N_5$ requires C, 52·0; H, 3·0; N, 19·0%). 3-*Phenyl*-4-(5-*keto*-3-*phenyl*iso*oxazolin*-4-*yl*)*methylene*iso*oxazol*-5-*one* formed yellow plates (from chloroform–light petroleum), m. p. 200° (decomp.), identical with the compound obtained from 3-phenyl*iso*oxazol-5-one and formylating agents. 3-n-*Amyl*-4-*formyl*iso*oxazol*-5-*one* (III; R = n-C_5H_{11}) crystallised in pale yellow needles, m. p. 45°, from ethanol–water (Found: C, 58·9; H, 7·0; N, 7·4. $C_9H_{13}O_3N$ requires C, 59·0; H, 7·2; N, 7·65%); its 2 : 4-*dinitrophenylhydrazone* formed scarlet needles, m. p. 135° (decomp.), from ethanol–water (Found: C, 49·4; H, 5·0; N, 19·2. $C_{15}H_{17}O_6N_5$ requires C, 49·6; H, 4·7; N, 19·3%). 3-n-*Amyl*-4-(3-n-*amyl*-5-*keto*iso*oxazolin*-4-*ylmethylene*)iso*oxazol*-5-*one* (V; R = n-C_5H_{11}) crystallised in yellow needles (from ethyl acetate–light petroleum), m. p. 60° (Found: C, 63·8; H, 7·6. $C_{17}H_{24}O_4N_2$ requires C, 63·7; H, 7·6%). 4-*Formyl*-3-*methyl*iso*oxazol*-5-*one* (III; R = Me) separated as pale brown needles, m. p. 143—144° (decomp.), from ethyl acetate–light petroleum (Found: C, 47·4; H, 4·2; N, 11·05. $C_5H_5O_3N$ requires C, 47·3; H, 4·0; N, 11·05%); this compound was soluble in water but could be extracted therefrom with *n*-butanol. Its 2 : 4-*dinitrophenylhydrazone* separated from methanol–acetic acid as scarlet prisms, m. p. 198° (decomp.) (Found: C, 43·1; H, 3·1; N, 22·75. $C_{11}H_9O_6N_5$ requires C, 43·0; H, 2·95; N, 22·8%).

*Reaction of the Formyl*iso*oxazolones with Penicillamine.*—2-(3-*Benzyl*-5-*keto*iso*oxazolin*-4-*yl*)-5 : 5-*dimethylthiazolidine*-4-*carboxylic Acid* (II; R = Ph·CH_2). 3-Benzyl-4-formyl*iso*oxazol-5-one (0·25 g.) and penicillamine hydrochloride (0·23 g.) were warmed in methanol (1·5 ml.) on the steam-bath for 2—3 minutes, after which a portion failed to give a blue colour with aqueous ferric chloride. The methanolic solution was added to water (10 ml.); the *thiazolidine* (0·3 g.) separated and was purified by dissolution in 2% sodium hydrogen carbonate solution, filtration, and precipitation with acid, to give a microcrystalline yellow powder, m. p. 163° (decomp.) (Found: C, 57·8; H, 5·25; N, 8·1. $C_{16}H_{18}O_4N_2S$ requires C, 57·5; H, 5·4; N, 8·4%). The thiazolidine (0·2 g.) was boiled with 2N-hydrochloric acid (2 ml.) for a short time; when cooled, the solution deposited 3-benzyl-4-formyl*iso*oxazol-5-one (0·05 g.), m. p. 80°, and the filtrate gave a deep blue colour with aqueous ferric chloride, typical of penicillamine. The following were prepared similarly: 2-(5-*Keto*-4-*methyl*iso*oxazolin*-4-*yl*)-5 : 5-*dimethylthiazolidine*-4-*carboxylic acid* (II; R = Me), a pale yellow powder, m. p. 170—171° (decomp.) (Found: C, 46·7; H, 5·7; N, 10·95. $C_{10}H_{14}O_4N_4S$ requires C, 46·5; H, 5·5; N, 10·85%). 2-(3-n-*Amyl*-5-*keto*iso*oxazolin*-4-*yl*)-5 : 5-*dimethylthiazolidine*-4-*carboxylic acid* (II; R = n-C_5H_{11}), a yellow powder, m. p. 141—142° (decomp.) (Found: C, 53·7; H, 7·0. $C_{14}H_{22}O_4N_2S$ requires C, 53·5; H, 7·05%). 2-(5-*Keto*-3-*phenyl*iso*oxazolin*-4-*yl*)-5 : 5-*dimethylthiazolidine*-4-*carboxylic acid* (II; R = Ph), a pale yellow powder, m. p. 124—125° (decomp.) (Found: C, 56·2; H, 5·1. $C_{15}H_{16}O_4N_2S$ requires C, 56·2; H, 5·0%).

We thank Sir Ian Heilbron, D.S.O., F.R.S., for his interest and encouragement and the Medical Research Council for a Senior Research Grant (to G. S.).

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