Reactive Acyl Dipeptides as Potential Penicillin Analogues. Part II.[1] *N*-(Acylglycyl)-5-oxo-pyrrolidine-2-carboxylic Acids and *N*-(Acylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylic Acids

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Reaction of acylglycine *p*-nitrophenyl esters with the *N*-sodio-derivative of benzyl 5-oxopyrrolidine-2-carboxylate gave, after hydrogenolysis, *N*-(acylglycyl)-5-oxopyrrolidine-2-carboxylic acids. *N*-(Acylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylic acids were similarly prepared from benzyl 5,5-dimethyl-2-oxothiazolidine-4-carboxylate. Both types of acid could be regarded, like penicillin, as acyl dipeptides containing a reactive peptide bond, but they exhibited only very slight antibacterial activity.

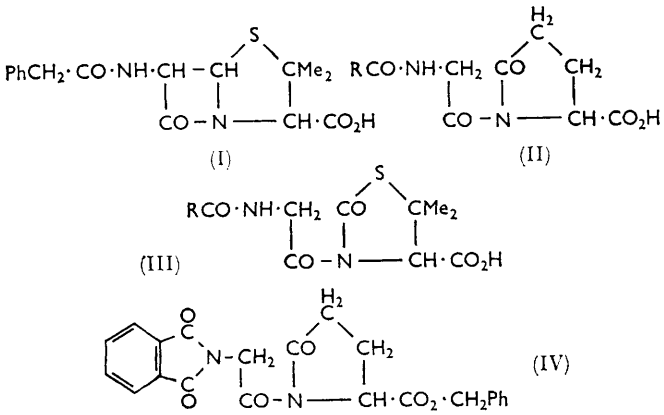
In Part I[1] the hypothesis was developed that acyl dipeptides containing an 'activated' peptide bond, for example one forming part of a diacylimide structure, might display antibacterial activity comparable to that of penicillin (I). The present Paper describes the preparation of two further groups of compounds of the desired type, namely *N*-(acylglycyl)-5-oxopyrrolidine-2-carboxylic acids (II) and *N*-(acylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylic acids (III).

Benzyl 5-oxopyrrolidine-2-carboxylate, prepared from DL-glutamic acid, was converted into the *N*-sodio-derivative by treating a solution in benzene with sodium hydride. Treatment with phthalimidoacetyl chloride readily gave benzyl 5-oxo-*N*-(phthalimidoacetyl)-pyrrolidine-2-carboxylate (IV) and hydrogenolysis over a palladium catalyst yielded the corresponding acid. The *N*-phenylacetylglycyl and *N*-phenoxyacetylglycyl derivatives of 5-oxopyrrolidine-2-carboxylic acid were prepared in similar fashion, except that the *N*-acylglycine *p*-nitrophenyl esters were more convenient acylating agents than the acid chlorides. The acids (II; R = $PhCH_2$ and $PhO{\cdot}CH_2$) were oils, but they gave pure crystalline salts with *NN'*-dibenzylethylenediamine.

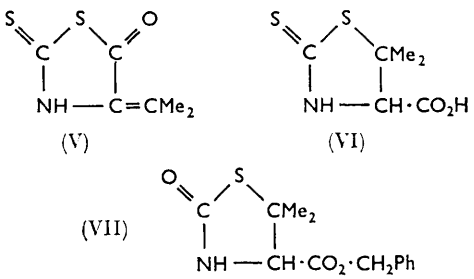
In order to increase the structural resemblance to

[1] Part I, M. J. Mardle, J. H. C. Nayler, D. W. Rustidge, and H. R. J. Waddington, preceding Paper.

penicillin (I) it was decided to introduce a sulphur atom and a *gem*-dimethyl grouping into the five-membered ring. 5,5-Dimethyl-2-thioxothiazolidine-4-carboxylic



acid (VI) was prepared readily by the action of methanolic potassium hydroxide on 4-isopropylidene-2-thioxo-5-thiazolidone (V).[2] When heated with benzyl alcohol and toluene in the presence of toluene-*p*-sulphonic acid compound (VI) gave benzyl 5,5-dimethyl-2-oxothiazolidine-4-carboxylate (VII), identical with that obtained by esterification of the known 5,5-dimethyl-2-oxothiazolidine-4-carboxylic acid.[3] When (VI) was esterified in the absence of toluene-*p*-sulphonic acid the thione sulphur atom was not replaced by oxygen.



The ester (VII) in benzene was stirred with sodium hydride to give the *N*-sodio-derivative, which on treatment with various *N*-acylglycine *p*-nitrophenyl esters readily underwent *N*-acylation with elimination of sodium *p*-nitrophenate. Catalytic hydrogenolysis of the benzyl esters gave the pure crystalline acids (III; R = $Me[CH_2]_4$ and $PhCH_2$·O) whilst their analogues (III; R = $PhCH_2$ and $PhO{\cdot}CH_2$) were more conveniently purified as the *NN'*-dibenzylethylenediamine salts. In an alternative approach to this type of compound the *N*-(benzyloxycarbonylglycyl) derivative of the ester (VII) was treated with hydrogen bromide in acetic acid to give benzyl *N*-glycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylate hydrobromide, and this with triethylamine and benzoyl chloride gave the benzyl ester of the acid (III; R = Ph).

That the diacylimide group in the acids of types (II) and (III) had acylating power was shown by reaction with cold neutral hydroxylamine reagent to give hydroxamic acids, detected colorimetrically as the ferric complexes, although the reaction was slightly slower than that with penicillins.[4] The acids or their salts were tested by Dr. G. N. Rolinson and Mr. R. Sutherland against a range of Gram-positive and Gram-negative bacteria *in vitro*. 5,5-Dimethyl-2-oxo-*N*(phenoxyacetylglycyl)thiazolidine-4-carboxylic acid (III; R = $PhO{\cdot}CH_2$) at a dilution of 1 in 2000 inhibited the growth of *Streptococcus viridans*, *Shigella flexneri*, and *Klebsiella pneumoniae*, whilst several other bacteria were inhibited by a 1 in 1000 dilution of the acid. This represents between one hundredth and one hundred-thousandth of the activity of benzylpenicillin depending on the test organism, and the other acids of types (II) and (III) were generally still less active.

EXPERIMENTAL

N-Acylglycine p-Nitrophenyl Esters.—Equimolecular quantities of phenylacetylglycine and *p*-nitrophenol in tetrahydrofuran were treated with *NN'*-dicyclohexylcarbodi-imide (1 mol.) in the same solvent and the solution was set aside overnight. Dicyclohexylurea was filtered off, the filtrate was evaporated *in vacuo*, and the residue gave p-*nitrophenyl phenylacetylglycinate* (86%), m. p. 140° (from ethanol) (Found: C, 61·0; H, 4·7; N, 9·0. $C_{16}H_{14}N_2O_5$ requires C, 61·1; H, 4·5; N, 8·9%). In similar fashion were prepared p-*nitrophenyl phenoxyacetylglycinate* (70%), m. p. 106° (from ethanol) (Found: C, 58·3; H, 4·5; N, 8·6. $C_{16}H_{14}N_2O_6$ requires C, 58·2; H, 4·3; N, 8·5%), and p-*nitrophenyl* N-*hexanoylglycinate* (26%), m. p. 97—98° (from ethyl acetate–light petroleum) (Found: C, 57·3; H, 6·5. $C_{14}H_{18}N_2O_5$ requires C, 57·1; H, 6·2%).

5-Oxo-N-(phthalimidoacetyl)pyrrolidine-2-carboxylic Acid.—Benzyl 5-oxopyrrolidine-2-carboxylate[5] (19·7 g., 0·09 mole) in dry benzene was stirred with sodium hydride (0·09 mole) until evolution of hydrogen ceased, then phthalimidoacetyl chloride[6] (20·1 g., 0·09 mole) in dry benzene (100 ml.) was added during 10 min. and the mixture was stirred for 16 hr. Sodium chloride was filtered off, the filtrate was evaporated under reduced pressure, and the residue (from ethanol) gave *benzyl 5-oxo-N-(phthalimidoacetyl)pyrrolidine-2-carboxylate* (60%), m. p. 167—169° (Found: C, 64·7; H, 4·8; N 7·0. $C_{22}H_{18}N_2O_6$ requires C, 65·0; H, 4·5; N, 6·9%). The benzyl ester in acetone was hydrogenated at room temperature and pressure over 10% palladium–charcoal until uptake of hydrogen ceased The product was triturated with ethanol to induce crystallisation and gave *5-oxo-N-(phthalimidoacetyl)pyrrolidine-2-carboxylic acid* (97%), m. p. 213° (from ethanol) (Found: C, 57·1; H, 4·1; N, 8·7. $C_{15}H_{12}N_2O_6$ requires C, 57·0; H, 3·8; N, 8·9%).

5-Oxo-N-phenylacetylglycylpyrrolidine-2-carboxylic Acid.—The *N*-sodio-derivative of benzyl 2-oxopyrrolidine-2-carboxylate was prepared as before and treated with

[2] R. Chatterjee, A. H. Cook, Sir Ian Heilbron, and A. L. Levy, *J. Chem. Soc.*, 1948, 1337.
[3] F. P. Doyle, D. O. Holland, P. Mamalis, and A. Norman, *J. Chem. Soc.*, 1958, 4605.
[4] J. H. Ford, *Ind. Eng. Chem. Analyt.*, 1947, **19**, 1004.
[5] J. H. Billman and J. L. Rendall, *J. Amer. Chem. Soc.*, 1944, **66**, 745.
[6] J. C. Sheehan and V. S. Frank, *J. Amer. Chem. Soc.*, 1949, **71**, 1856.

p-nitrophenyl phenylacetylglycinate (1 equiv.) in dry benzene. The mixture was stirred for 16 hr., and filtered to remove sodium *p*-nitrophenate, and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with sodium hydrogen carbonate solution until the washings were no longer yellow. The dried solution was evaporated and the residue gave *benzyl 5-oxo-N-phenylacetylglycylpyrrolidine-2-carboxylate* (40%), m. p. 115—116° (from ethyl acetate–light petroleum) (Found: C, 67·4; H, 6·0; N, 7·2. $C_{22}H_{22}N_2O_5$ requires C, 67·0; H, 5·6; N, 7·1%). Catalytic hydrogenation of the benzyl ester as before gave 5-oxo-*N*-phenylacetylglycylpyrrolidine-2-carboxylic acid as an oil which was therefore dissolved in sodium hydrogen carbonate solution and treated with the calculated quantity of a strong aqueous solution of *NN'*-dibenzylethylenediamine diacetate. The precipitate of *NN'-dibenzylethylenediamine 5-oxo-N-phenylacetylglycylpyrrolidine-2-carboxylate* (53%) had m. p. 181—182° (from ethanol) (Found: C, 65·5; H, 6·5; N, 9·5. $C_{46}H_{52}N_6O_{10}$ requires C, 65·1; H, 6·2; N, 9·9%).

5-Oxo-N-phenoxyacetylglycylpyrrolidine-2-carboxylic Acid.—This was prepared as described for the *N*-phenylacetylglycyl analogue except that purification of the intermediate benzyl ester was omitted. The product was isolated as the *NN'-dibenzylethylenediamine salt* (53%), m. p. 199—200° (from ethanol) (Found: C, 62·8; H, 6·1; N, 9·6. $C_{46}H_{52}N_6O_{12}$ requires C, 62·7; H, 6·0; N, 9·5%).

5,5-Dimethyl-2-thioxothiazolidine-4-carboxylic Acid.—2-Thioxo-5-thiazolidone [7] (107 g.) suspended in dry acetone (1 l.) was treated with benzylamine (6 ml.). The solid slowly dissolved and then yellow crystals commenced to separate. Next morning the product was collected and the filtrate was concentrated under reduced pressure to give a second crop, bringing the yield of 4-isopropylidene-2-thioxo-5-thiazolidone [8] to 83%. Treatment of this compound with potassium hydroxide in methanol [2,3] gave 5,5-dimethyl-2-thioxothiazolidine-4-carboxylic acid (74%).

Benzyl 5,5-Dimethyl-2-thioxothiazolidine-4-carboxylate.—A mixture of 5,5-dimethyl-2-thioxothiazolidine-4-carboxylic acid (40 g.), benzyl alcohol (160 ml.), and xylene (240 ml.) was refluxed for 6 hr. with azeotropic removal of water, then evaporated under reduced pressure. The residue was dissolved in ether, washed with sodium hydrogen carbonate solution, dried, and evaporated to an oil which crystallised on trituration with light petroleum and gave the *benzyl ester* (38%), m. p. 99—100° (from ether–light petroleum) (Found: C, 55·6; H, 5·5; N, 4·9; S, 23·0. $C_{13}H_{15}NO_2S_2$ requires C, 55·5; H, 5·4; N, 5·0; S, 22·8%).

Benzyl 5,5-Dimethyl-2-oxothiazolidine-4-carboxylate.—(*a*) A mixture of 5,5-dimethyl-2-thioxothiazolidine-4-carboxylic acid (46 g.), toluene-*p*-sulphonic acid (3 g.), benzyl alcohol (200 ml.), and toluene (250 ml.) was refluxed for 6 hr. with azeotropic removal of water, then worked up as described in the previous experiment to give the *benzyl ester* (46 g., 72%), m. p. 74° (from ether–light petroleum) (Found: C, 58·9; H, 5·7; N, 5·3; S, 11·8. $C_{13}H_{15}NO_3S$ requires C, 58·9; H, 5·7; N, 5·3; S, 12·1%).

(*b*) The same ester was obtained (32%) by azeotropic esterification of 5,5-dimethyl-2-oxothiazolidine-4-carboxylic acid [3] with benzyl alcohol (6 hr. reflux in xylene).

Benzyl N-(Acylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylates.—A solution of benzyl 5,5-dimethyl-2-oxothiazolidine-4-carboxylate (5·3 g., 0·02 mole) in dry benzene (100 ml.) was added to a stirred suspension of sodium hydride (0·02 mole) in dry benzene (100 ml.), whereupon brisk evolution of hydrogen ensued. After 3 hr. a solution of *p*-nitrophenyl phenylacetylglycinate (0·02 mole) in dry benzene (100 ml.) was added and stirring was continued for 16 hr. Sodium *p*-nitrophenate was filtered off, the filtrate was evaporated under reduced pressure, and the residue was crystallised by trituration with light petroleum. *Benzyl 5,5-dimethyl-2-oxo-N-phenylacetylglycylthiazolidine-4-carboxylate* (70%) had m. p. 123—124° (from ethyl acetate–light petroleum) (Found: C, 62·6; H, 5·5; N, 6·5; S, 7·2. $C_{23}H_{24}N_2O_5S$ requires C, 62·7; H, 5·5; N, 6·4; S, 7·3%).

Similar acylations with the appropriate *N*-acylglycine *p*-nitrophenyl esters gave *benzyl 5,5-dimethyl-2-oxo-N-phenoxyacetylglycylthiazolidine-4-carboxylate* (88%), m. p. 135—136° (from ethanol) (Found: C, 60·6; H, 5·2; N, 6·3; S, 7·3. $C_{23}H_{24}N_2O_6S$ requires C, 60·5; H, 5·3; N, 6·1; S, 7·0%), *benzyl N-(hexanoylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylate* (60%), m. p. 58—59° (from ether–light petroleum) (Found: C, 59·5; H, 6·8; N, 6·7; S, 8·0. $C_{21}H_{28}N_2O_5S$ requires C, 60·0; H, 6·7; N, 6·7; S, 7·6%), and *benzyl N-(benzyloxycarbonylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylate* (86%), m. p. 84—85° (from ethyl acetate–light petroleum) (Found: C, 60·4; H, 5·1; N, 6·3; S, 7·4. $C_{23}H_{24}N_2O_6S$ requires C, 60·5; H, 5·3; N, 6·1; S, 7·0%).

Benzyl N-Glycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylate Hydrobromide.—Benzyl *N*-(benzyloxycarbonylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylate (2·28 g.), when stirred with a 36% solution of dry hydrogen bromide (5 g.) in acetic acid, dissolved slowly with evolution of carbon dioxide. After 30 min. the solution was diluted with dry ether and set aside in the refrigerator. The resulting needles were collected, dried *in vacuo* (KOH), and gave the *hydrobromide* (1 g.), m. p. 171—172° (from ethanol–ether) (Found: C, 44·3; H, 5·0; Br, 20·3; N, 7·1; S, 7·7. $C_{15}H_{19}BrN_2O_4S$ requires C, 44·7; H, 4·8; Br, 19·8; N, 7·0; S, 7·9%).

Benzyl N-Benzoylglycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylate.—Triethylamine (0·4 ml.) was added to a suspension of benzyl *N*-glycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylate hydrobromide (0·6 g.) in dry acetone (20 ml.); dissolution was followed by precipitation of triethylamine hydrobromide. Benzoyl chloride (0·2 g.) was added and the mixture was stirred for 1 hr., set aside overnight, and filtered. Evaporation of the filtrate under reduced pressure left an oil which slowly solidified and gave *benzyl N-benzoylglycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylate* (0·37 g.), m. p. 111—112°, identical with the product from *p*-nitrophenyl benzoylglycinate and the sodio-derivative of benzyl 5,5-dimethyl-2-oxothiazolidine-4-carboxylate (Found: C, 62·0; H, 5·2; N, 6·7; S, 7·3. $C_{22}H_{22}N_2O_5S$ requires C, 61·9; H, 5·2; N, 6·6; S, 7·5%).

N-(Acylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylic Acids.—(*a*) Benzyl *N*-benzoylglycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylate (5·3 g.) in acetone (150 ml.) was hydrogenated at room temperature and pressure over 5% palladium–calcium carbonate (3 g.). The product was dissolved in ethyl acetate and extracted with sodium hydrogen carbonate solution. Acidification of the extracts gave *N-benzoylglycyl-5,5-dimethyl-2-oxothiazolidine-4-carboxylic acid* (2 g.), m. p. 204—205° (from aqueous methanol) (Found: C, 53·5; H, 5·2; N, 8·3; S, 9·9. $C_{15}H_{16}N_2O_5S$ requires C, 53·6; H, 4·8; N, 8·3; S, 9·5%).

(*b*) Similar hydrogenation of benzyl *N*-(hexanoylglycyl)-5,5-dimethyl-2-oxothiazolidine-4-carboxylate gave *N-*

[7] D. O. Holland, B.P. 689,243/1951.
[8] J. D. Billimoria and A. H. Cook, *J. Chem. Soc.*, 1949, 2323.

(*hexanoylglycyl*)5,5-*dimethyl*-2-*oxothiazolidine*-4-*carboxylic acid* (60%), m. p. 141—142° (from ethyl acetate–light petroleum) (Found: C, 51·0; H, 6·9; N, 8·9; S, 9·7. $C_{14}H_{22}N_2O_5S$ requires C, 50·9; H, 6·7; N, 8·5; S, 9·7%).

(*c*) Benzyl 5,5-dimethyl-2-oxo-*N*-phenylacetylglycylthiazolidine-4-carboxylate was hydrogenated similarly but the acidic product (80%) proved difficult to recrystallise. It was therefore dissolved in sodium hydrogen carbonate solution (3%; 1 mol.) and treated with a concentrated solution of *NN'*-dibenzylethylenediamine diacetate (0·5 mol.) to give an immediate precipitate of NN'-*dibenzylethylenediamine*-5,5-*dimethyl*-2-*oxo*-N-*phenylacetylglycylthiazolidine*-4-*carboxylate* (70%), m. p. 166° (from ethanol) (Found: C, 59·7; H, 6·0; N, 8·7; S, 7·0. $C_{48}H_{56}N_6O_{10}S_2,H_2O$ requires C, 60·1; H, 6·1; N, 8·8; S, 6·7%).

(*d*) 5,5-Dimethyl-2-oxo-*N*-phenoxyacetylglycylthiazolidine-4-carboxylic acid was prepared similarly and isolated as the *NN'-dibenzylethylenediamine salt* (60%), m. p. 180—181° (from ethanol) (Found: C, 58·6; H, 5·7; N, 8·4; S, 6·3. $C_{48}H_{56}N_6O_{12}S_2,0{\cdot}5H_2O$ requires C, 58·7; H, 5·8; N, 8·6; S, 6·5%).

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