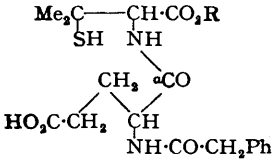
255. N-(N-*Phenylacetyl*-α-DL-*glutamyl*)-D-*penicillamine*.

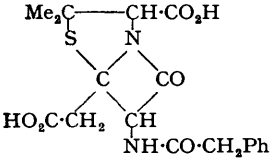
By WILSON BAKER and P. G. JONES.

N-Phenylacetyl-L-glutamic acid reacts with acetic anhydride to give an optically inactive anhydro-derivative, which is doubtless 2-benzyl-4-2′-carboxyethyloxazol-5-one (IV). The α-amide (V) and the α-hydrazide (VI) of *N*-phenylacetyl-DL-glutamic acid and the γ-methyl ester of *N*-(*N*-phenylacetyl-α-DL-glutamyl)-D-penicillamine (I) are formed when the anhydro-derivative is treated with ammonia, hydrazine, and D-penicillamine methyl ester respectively.

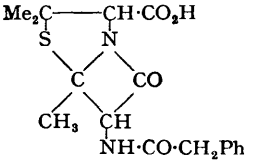
THIS paper describes the synthesis of the methyl ester of *N*-(*N*-phenylacetyl-α-DL-glutamyl)-D-penicillamine (I ; R = Me). The corresponding dicarboxylic acid was obtained but only partly purified. The object in preparing these compounds was to see if they could be converted by *Penicillium notatum* or *in vivo* into antibiotics related to benzylpenicillin. The possibility exists that the central methylene group of the glutamic acid residue might undergo biological oxidation to a carbonyl group, and then condense with the SH and NH groups of the penicillamine portion of the molecule to give either a carboxymethyl-benzylpenicillin (II) or, by decarboxylation of the intermediate stage, a methyl-benzylpenicillin (III).



(I.)



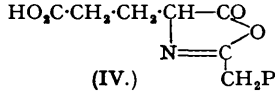
(II.)



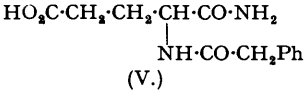
(III.)

No evidence is available to show if compounds of the type (II) or (III) would exhibit antibiotic activity, but a significant early observation made in the research laboratories of the Upjohn Company (Committee for Penicillin Synthesis Reports, C.P.S. 1944, No. 174) was that *N*-phenylacetyl-L-glutamic acid, when tested as a biological precursor of penicillin, gave preliminary results which were " sufficiently suggestive as to warrant further study." However, when compound (I ; R = H) and its methyl ester (I ; R = Me) were submitted to biological tests, no evidence of antibiotic activity was obtained.

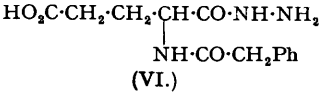
N-Phenylacetyl-L-glutamic acid, prepared by the action of phenylacetyl chloride on L-glutamic acid in alkaline solution, was converted by the action of acetic anhydride into a crystalline, optically inactive anhydro-derivative which is readily hydrolysed by cold, aqueous sodium hydrogen carbonate, giving *N*-phenylacetyl-DL-glutamic acid. Hydration also occurs on rapid titration in the cold in aqueous dioxan, two equivalents of alkali being neutralised. Since racemisation occurred, it is most probable that the anhydro-derivative is 2-benzyl-DL-4-2′-carboxyethyloxazol-5-one (IV). Treatment of (IV) with ammonia in chloroform gave an amide which, on this assumption, is the α-amide (V) of N-phenylacetyl-DL-glutamic acid.



(IV.)



(V.)



(VI.)

The α-hydrazide (VI) of *N*-phenylacetyl-DL-glutamic acid was prepared from the oxazolone (IV) by reaction with methyl alcohol and treatment of the resulting methyl ester with 96% hydrazine and also, more directly, by treating the oxazolone with hydrazine. Completely satisfactory analytical figures could not be obtained for this compound, but it readily yielded well-defined *iso*propylidene and benzylidene derivatives. The derived azide was condensed with D-pencillamine, yielding a non-crystalline product regarded as crude *N*-(*N*-phenylacetyl-α-DL-glutamyl)-D-penicillamine (I ; R = H).

The methyl ester of the hydrazide (VI) was prepared by the action of ethereal diazomethane, and characterised as *iso*propylidene and benzylidene derivatives. After conversion into the azide and condensation with D-penicillamine methyl ester, it gave a non-crystalline product which was not further investigated.

A more satisfactory result was obtained by the direct interaction of the oxazolone (IV) with D-penicillamine methyl ester. The product, the methyl ester of *N*-(*N*-phenylacetyl-α-DL-glutamyl)-D-penicillamine (I; R = Me) was again non-crystalline, but gave satisfactory analytical figures.

EXPERIMENTAL.

M. p.s are uncorrected. Analyses are by Drs. Weiler and Strauss, Oxford.

N-*Phenylacetyl*-L-*glutamic Acid*.—This compound was first prepared by Thierfelder and Sherwin (*Z. physiol. Chem.*, 1915, **94**, 1), who obtained it with some difficulty as a solid, m. p. 123°, and it was later prepared by Shiple and Sherwin (*J. Biol. Chem.*, 1922, **53**, 472) as an uncharacterised syrup; in neither case is any yield given. As prepared by the action of phenylacetyl chloride on L-glutamic acid in alkaline solution, the product is heterogeneous and suffers considerable loss on crystallisation. The yield of pure product obtained in the present work is 38%.

To a solution of L-glutamic acid (50 g.) in water (272 c.c.) containing sodium hydroxide (27·2 g.) was added slowly and simultaneously, with stirring and cooling in ice, phenylacetyl chloride (63 g., 1·2 mols.) and a solution of sodium hydroxide (19·6 g., 1·2 mols.) in water (98 c.c.). After 2 hours the solution was extracted with ether and acidified, and the precipitated oil was washed with benzene to remove phenylacetic acid, giving an oil (61·7 g.) which slowly crystallised in a desiccator. This sticky material was dissolved in three volumes of hot water, and the solution slowly deposited *N*-phenylacetyl-L-glutamic acid (34·5 g.; m. p. 123°) which after recrystallisation had m. p. 125—126°, $[\alpha]_D^{15}$ −21° (*c*, 1·0 in water) (Found: C, 58·9; H, 5·7. Calc. for $C_{13}H_{15}O_5N$: C, 58·9; H, 5·7%). The m. p. is lowered considerably by traces of water.

2-*Benzyl*-DL-4-2′-*carboxyethyloxazol*-5-*one* (IV).—*N*-Phenylacetyl-L-glutamic acid (5 g.) and acetic anhydride (15 c.c.) were heated on a water-bath for 15 minutes, and the excess of acetic anhydride removed under diminished pressure. Addition of benzene (30 c.c.) caused separation of crystals (3·4 g.), which were collected and recrystallised twice from benzene, giving 2-*benzyl*-DL-4-2′-*carboxyethyloxazol*-5-*one* as colourless needles, m. p. 114—115° (Found: C, 63·2; H, 5·4. $C_{13}H_{13}O_4N$ requires C, 63·1; H, 5·3%). This compound, when exposed to the air, undergoes change over a period of several days and the m. p. drops. Hydrolysis with cold aqueous sodium hydrogen carbonate gave *N*-phenylacetyl-DL-glutamic acid which separated from water in colourless crystals, m. p. 124—125°, having zero rotation. When mixed with *N*-phenylacetyl-L-glutamic acid it softened at 118° and melted between 123° and 125°.

α-*Amide* (V) *of* N-*Phenylacetyl*-DL-*glutamic Acid*.—The oxazolone (IV) (0·5 g.), dissolved in chloroform (15 c.c.), was treated with dry ammonia, a sticky mass being precipitated. After 12 hours the mixture was shaken with water (10 c.c.), and the aqueous layer acidified and extracted with ethyl acetate. The residue (0·34 g.) left after drying and removal of the solvent was crystallised 3 times from acetone–light petroleum (b. p. 60—80°), and the *amide* was obtained as needles, m. p. 144—146° (Found: C, 59·2; H, 6·0; N, 10·8. $C_{13}H_{16}O_4N_2$ requires C, 59·2; H, 6·1; N, 10·6%).

α-*Hydrazide* (VI) *of* N-*Phenylacetyl*-DL-*glutamic Acid*.—(a) *From the* α-*methyl ester of* N-*phenylacetyl*-DL-*glutamic acid*. The oxazolone (IV) (0·5 g.) was dissolved in anhydrous methanol and, after 12 hours, removal of the excess of alcohol gave an oil. This was dissolved in methanol (5 c.c.) and treated with 96% hydrazine hydrate (0·27 c.c.). After 24 hours the solution was rendered just acid to methyl-orange; and the hydrazide (VI) (0·23 g.) gradually separated. It crystallised from water in small needles, m. p. 181° (decomp.) (Found: C, 56·7; H, 6·3; N, 14·0. Calc. for $C_{13}H_{17}O_4N_3$: C, 55·9; H, 6·1; N, 15·0%).

(b) *From the oxazolone* (IV). The oxazolone (18·3 g.) was dissolved in 96% hydrazine hydrate (28 c.c.), then acidified to methyl-orange after 12 hours, and the solid collected and recrystallised from water, giving the hydrazide, m. p. 181° (11·8 g.).

The iso*propylidene* derivative of the hydrazide (VI) was prepared by dissolving the hydrazide (100 mg.) in acetone (3 c.c.), the crystals (74 mg.) being collected after 24 hours. It separated from light petroleum (b. p. 60—80°)–ethanol in compact crystals, m. p. 161—162° (Found: C, 60·2; H, 6·8. $C_{16}H_{21}O_4N_3$ requires C, 60·1; H, 6·6%). The *benzylidene* derivative of the hydrazide (VI) was prepared by shaking a solution of the hydrazide (200 mg.) in 0·5N-hydrochloric acid (5 c.c.) with benzaldehyde (0·11 c.c., 1·5 mols.), immediate separation of a precipitate occurring (260 mg.). The subtance separates from alcohol in microscopic crystals, m. p. 213—214° (Found: C, 65·2; H, 5·8. $C_{20}H_{21}O_4N_3$ requires C, 65·4; H, 5·7%).

Condensation of the α-*Azide of* N-*Phenylacetyl*-DL-*glutamic Acid with* D-*Penicillamine*. N-(N-*Phenylacetyl*-α-DL-*glutamyl*)-D-*penicillamine* (I; R = H).—To an ice-cooled, stirred solution of the hydrazide (VI) (1·6 g.) in 0·5N-hydrochloric acid (11·5 c.c.) was slowly added sodium nitrite (1·3 g., 1·2 mols.). After 15 minutes a slight excess (1·5 mols.) of potassium carbonate and a solution of D-penicillamine hydrochloride (1·7 g.) in dilute aqueous potassium carbonate (1 equiv.) were added, and the mixture was stirred for 45 minutes and then acidified with 2N-hydrochloric acid. The liberated oil was extracted into ethyl acetate, the extract washed and dried, the solvent evaporated at a low temperature, and the product finally dried in a vacuum, leaving a light, brittle mass (1·21 g.) (Found: C, 56·2; H, 6·2; N, 6·3. Calc. for $C_{18}H_{24}O_6N_2S$: C, 54·5; H, 6·1; N, 7·1%). With aqueous ferric chloride the compound gives a blue colour which rapidly fades.

Methyl Ester of α-*Hydrazide of* N-*Phenylacetyl*-DL-*glutamic Acid*.—The hydrazide (VI) (1 g.) in methanol (50 c.c.) was treated with an excess of ethereal diazomethane at 0°. After evolution of nitrogen had ceased, the solvents were removed under diminished pressure at room temperature, the residue was treated with a few c.c. of ether, and the solid collected (0·94 g.). This hydrazide was characterised by its condensation products with acetone and benzaldehyde, which were prepared as in the case of the

hydrazide (VI). The iso*propylidene* derivative separated from light petroleum (b. p. 60—80°)–acetone in compact crystals, m. p. 149° (Found : C, 61·3; H, 7·1. $C_{17}H_{23}O_4N_3$ requires C, 61·2; H, 6·9%). The *benzylidene* derivative separated from 50% ethanol as a microcrystalline powder, m. p. 176° (Found : C, 65·8; H, 6·2. $C_{21}H_{23}O_4N_3$ requires C, 66·1; H, 6·0%).

The methylated hydrazide was further converted into the azide which was allowed to react with D-penicillamine methyl ester in ethyl acetate solution. Condensation undoubtedly took place, but the product could not be obtained crystalline.

Condensation of the Oxazolone (IV) *with* D-*Penicillamine Methyl Ester. Methyl Ester of* N-(N-*Phenylacetyl-α-*DL-*glutamyl*)-D-*penicillamine* (I; R = Me).—A solution of free D-penicillamine methyl ester in chloroform prepared from the hydrochloride (0·3 g.) was added to a solution of the oxazolone (IV) (0·3 g.) in chloroform. Next morning the chloroform solution was well washed with dilute acid, then dried, and the solvent removed *in vacuo*, leaving an oil which set to a brittle glass which could not be crystallised (0·38 g.) (Found : C, 55·0; H, 6·7; S, 7·7. $C_{19}H_{26}O_6N_2S$ requires C, 55·6; H, 6·3; N, 6·8; S, 7·8%). The product gave no colour with ferric chloride.

Hydrolysis of this *methyl* ester of *N*-(*N*-phenylacetyl-α-DL-glutamyl)-D-penicillamine (40 mg.) was effected with N-sodium hydroxide (0·5 c.c.; 5 mols.) for ½ hour on the water-bath. After acidification and extraction with ethyl acetate, *N*-phenylacetyl-α-DL-glutamyl-D-penicillamine was obtained as a non-crystalline glass which gave a transient blue colour with aqueous ferric chloride (Found : equiv., 200. Calc. for $C_{18}H_{24}O_6N_2S$: equiv. as a dibasic acid, 198).

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