446. *Syntheses from Phthalimido-Acids. Part X.* Derivatives of* DL-*Penicillamine.*

By F. E. KING, J. W. CLARK-LEWIS, and W. A. SWINDIN.

In model experiments for a synthesis of the DL-penicillamine analogue (penithione) of glutathione, *S*-benzyl-DL-penicillaminylglycine ethyl ester was prepared *via* the phthaloyl derivative and converted into phthaloyl-γ-DL-glutamyl-*S*-benzyl-DL-penicillaminylglycine ethyl ester from which the amorphous *S*-benzylpenithione was obtained. Two other dipeptide derivatives, *viz.*, glycyl-*S*-benzyl-DL-penicillamine and phthaloyl-γ-DL-glutamyl-*S*-benzyl-DL-penicillamine, are also described.

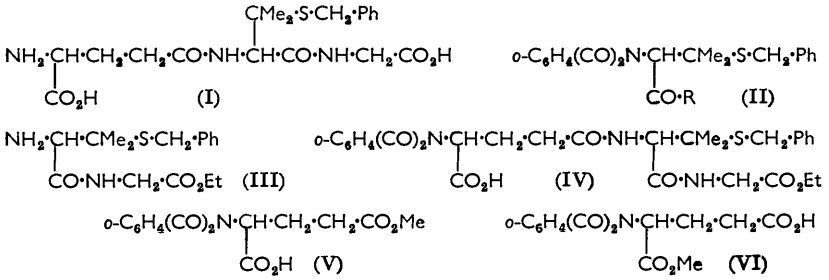
EXPLORATORY experiments with phthaloyl derivatives, similar to those having glutathione as their objective,* have been carried out with DL-penicillamine in a parallel investigation directed towards the synthesis of the penicillamine analogue of glutathione. The *S*-benzyl derivative (I) of γ-DL-glutamyl-DL-penicillaminylglycine was obtained in five steps from *S*-benzylpenicillamine (17% over-all), but the synthesis suffers from defects discussed earlier for that of glutathione,[1] and it was subsequently discontinued in favour of an examination of the thiazolidine route as described in the following paper.

S-Benzyl-*N*-phthaloyl-DL-penicillamine (II; R = OH) was newly prepared and characterised as the methyl and ethyl esters (II; R = OMe or OEt). The acid chloride gave the amide and anilide (II; R = NH_2 or NHPh) with ammonia and aniline respectively, but

* Part IX, *J.*, 1957, 886.

[1] King, Clark-Lewis, and Wade, *J.*, 1957, 880.

reacted with two molecules of the more basic benzylamine to give the bisbenzylamide of *S*-benzyl-*N*-*o*-carboxybenzoyl-DL-penicillamine in high yield. Under similar conditions, however, glycine ethyl ester gave the desired *S*-benzyl-*N*-phthaloyl-DL-penicillaminylglycine ethyl ester (II; R = $NH{\cdot}CH_2{\cdot}CO_2Et$) (65%), and the ester was hydrolysed to the protected dipeptide (II; R = $NH{\cdot}CH_2{\cdot}CO_2H$) by alkaline hydrolysis and subsequent treatment with acid.



The phthaloyl group was removed from *S*-benzyl-*N*-phthaloyl-DL-penicillaminylglycine ethyl ester (II; R = $NH{\cdot}CH_2{\cdot}CO_2Et$) with aqueous hydrazine and the free amino-ester (III) gave the fully protected tripeptide (IV) when treated with phthaloyl-DL-glutamic anhydride. Difficulty was experienced in stepwise removal of the protecting groups, but simultaneous removal of the ester and phthaloyl residues was achieved by the method of Grassmann and Schulte-Uebbing,[2] which gave the *S*-benzyl-tripeptide (*S*-benzylpenithione) in 73% yield as a deliquescent amorphous powder with the correct elementary composition. Debenzylation [3] of this product with sodium and liquid ammonia gave a thiol which was precipitated with mercuric sulphate reagent.[4] An attempt to prepare the cuprous derivative of the thiol by the method described [3] for glutathione gave a water-soluble copper derivative, which was isolated by addition of ethanol. Although this derivative contained the expected proportion of copper neither the composition of the remainder nor that of the regenerated thiol corresponded to the expected formula.

S-Benzyl-DL-penicillamine gave phthaloyl-γ-DL-glutamyl-*S*-benzyl-DL-penicillamine when treated with phthaloyl-DL-glutamic anhydride, and with phthaloylglycyl chloride gave phthaloylglycyl-*S*-benzyl-DL-pencillamine (95%) which was converted into glycyl-*S*-benzyl-DL-penicillamine by removal of the phthaloyl group with hydrazine.

The ready formation of *N*-phthaloyl-γ-glutamyl derivatives by interaction of phthaloylglutamic anhydride with bases or with alcohols has been demonstrated in previous parts of this series, and this property has already been utilised in a synthesis of glutathione,[1] the most important of the natural γ-glutamyl peptides. The value of the anhydride in the preparation of esters has been illustrated for γ-methyl hydrogen phthaloyl-L-glutamate (V) obtained directly from methanol, and for γ-methyl hydrogen phthaloyl-L-glutamate (VI) prepared indirectly from the γ-benzyl ester.[5] The γ-methyl ester (V) differs in m. p. from that described by Tipson [6] and although Tipson's α-methyl ester resembles our ester (VI) in m. p. and optical rotation, its infrared absorption (curve 7) [6] is incompatible with the assigned structure; indeed the absorption near 3200 cm.$^{-1}$ indicates an amide and hence a phthalamic acid structure. Tipson's criticism of previous work [7] is thus probably ill-founded and his conclusions regarding the optical purity of Sheehan and Bolhofer's phthaloylglutamic anhydride overlook and merely confirm published results.[8]

[2] Grassmann and Schulte-Uebbing, *Chem. Ber.*, 1950, **83**, 244.
[3] du Vigneaud and Miller, *J. Biol. Chem.*, 1936, **116**, 469.
[4] Kendal, Mason, and McKenzie, *ibid.*, 1929, **84**, 669.
[5] King, Clark-Lewis, and Wade, *J.*, 1957, **886**.
[6] Tipson, *J. Org. Chem.*, 1956, **21**, 1353.
[7] King and Kidd, *J.*, 1949, **3315**.
[8] Clark-Lewis and Fruton, *J. Biol. Chem.*, 1954, **207**, 477.

Experimental

Racemic penicillamine was used throughout this work and the prefix DL has therefore been omitted below.

S-Benzyl-N-phthaloylpenicillamine (II; R = OH) *and its Methyl and Ethyl Ester.*—*S*-Benzylpenicillamine (10 g.), m. p. 199° (decomp.) [lit.,[9] m. p. 197—198° (decomp.)], prepared from *N*-acetylpenicillamine, was heated for 12 hr. with phthalic anhydride (12·4 g., 2 mol.) in boiling acetic acid (125 c.c.). The hot filtrate was diluted with boiling water until turbid and the product (10 g., 65%), m. p. 161—164°, was collected from the cold solution. Recrystallisation from aqueous ethanol or acetone gave *S-benzyl-N-phthaloylpenicillamine* in prisms, m. p. 164° (Found: C, 65·0; H, 5·5; N, 3·8. $C_{20}H_{19}O_4NS$ requires C, 65·0; H, 5·2; N, 3·8%). Diazomethane gave the *methyl ester*, prisms, m. p. 92° (from methanol) (Found: C, 65·7; H, 5·9; N, 3·9. $C_{21}H_{21}O_4NS$ requires C, 65·8; H, 5·5; N, 3·7%), and ethanolic hydrogen chloride at room temperature for 72 hr. gave the *ethyl ester* in plates (from ethanol), m. p. 103—104° (Found: C, 66·7; H, 5·4; N, 3·3. $C_{22}H_{23}O_4NS$ requires C, 66·5; H, 5·8; N, 3·5%).

S-Benzyl-N-phthaloylpenicillaminyl Chloride, Anilide, and Amide.—(a) *Acid chloride.* Phosphorus pentachloride (6 g., 1·06 mol.) was added to an ice-cold suspension of powdered *S*-benzyl-*N*-phthaloylpenicillamine (10 g., 1 mol.) in dry chloroform (40 c.c.) and the mixture was cooled in an ice–salt bath and shaken intermittently during 35—45 min. The yellow solution was filtered through glass wool, and the filtrate was evaporated under reduced pressure (bath-temp. 20—25°) and the viscous residue was washed by decantation with cold light petroleum (b. p. 40—60°; 2 × 10 c.c.) before dissolution in cold, dry chloroform (40 c.c.); the solution was stored in an ice–salt bath. The acid chloride was also prepared from the acid and thionyl chloride at room temperature, and with methanol gave the methyl ester, m. p. and mixed m. p. 92°.

(b) *Anilide* (II; R = NHPh). Aniline (0·6 c.c., an excess) in ether (6 c.c.) was added to a stirred solution of the acid chloride (from the acid, 1·9 g.) in cold chloroform (*ca.* 12 c.c.). Next day the solvent was removed and the residue was washed with dilute acid and water. *S-Benzyl-N-phthaloylpencillaminanilide* crystallised from acetone–ethanol in needles (0·93 g., 40%), m. p. 159° (Found: C, 70·4; H, 5·3; N, 6·1. $C_{26}H_{24}O_3N_2S$ requires C, 70·3; H, 5·4; N, 6·3%).

(c) *Amide* (II; R = NH_2). A chloroform (2 c.c.) solution of the acid chloride (from 0·25 g. of acid) was treated with anhydrous ethereal ammonia at 0°, and the mixture was kept at room temperature for several hours and filtered. Evaporation of the filtrate left a residue of *S-benzyl-N-phthaloylpenicillaminamide* which crystallised from ethanol–light petroleum in needles (0·174 g., 70%), m. p. 164° (Found: C, 64·9; H, 5·4; N, 7·5. $C_{20}H_{20}O_3N_2S$ requires C, 65·2; H, 5·5; N, 7·6%).

[*S-Benzyl-N-(o-N-benzylcarbamoylbenzoyl)penicillaminyl*]*benzylamine.*—Benzylamine (1·5 c.c.) in ether (3·5 c.c.) was added to the above acid chloride (from 1·5 g. of acid) in chloroform (10 c.c.) under conditions already described for the anilide. Recrystallisation of the neutral product from 90% ethanol gave rectangular plates of the *benzylamide*, m. p. 169° (Found: C, 72·2; H, 6·4; N, 7·7. $C_{34}H_{35}O_3N_3S$ requires C, 72·2; H, 6·2; N, 7·4%).

S-Benzyl-N-phthaloylpenicillaminylglycine and Ethyl Ester (II; R = $NH{\cdot}CH_2{\cdot}CO_2Et$).—A chloroform solution (40 c.c.) of the acid chloride, prepared as already described from *S*-benzyl-*N*-phthaloylpenicillamine (10 g.), was added slowly to a cooled and stirred solution of glycine ethyl ester (11 g., 4 mol.) in dry chloroform (60 c.c.). The mixture was kept at room temperature for several hours, diluted with dry ether, and filtered from glycine ethyl ester hydrochloride. The filtrate was washed with 0·1N-hydrochloric acid (2 × 100 c.c.) and water (2 × 50 c.c.), and the ethereal layer dried ($MgSO_4$) and evaporated. Crystallisation of the residue from ethanol–light petroleum (b. p. 60—80°) gave *S-benzyl-N-phthaloylpenicillaminylglycine ethyl ester* (8 g., 65%), m. p. 90—92° raised to 94—95° by recrystallisation from ethanol (Found: C, 63·7; H, 5·7; N, 6·2. $C_{24}H_{26}O_5N_2S$ requires C, 63·4; H, 5·8; N, 6·2%). 10N-Sodium hydroxide (1·5 c.c., 3·4 equiv.) was added to a solution of the ester (2 g.) in dry ethanol (40 c.c.) and when separation of the solid appeared to be complete (*ca.* 10 min.) the solvent was removed under reduced pressure. The residue was dissolved in water (15—20 c.c.) and acidified with concentrated hydrochloric acid, and the suspension then heated with sufficient ethanol to effect solution at the b. p. Crystallisation gave *S-benzyl-N-phthaloylpencillaminylglycine*

[9] " The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 462.

sesquihydrate in rectangular plates (1·24 g., 62%), m. p. 178° after sintering at 118—120° (Found: C, 58·6; H, 5·7; N, 6·2. $C_{22}H_{22}O_5N_2S,1{\cdot}5H_2O$ requires C, 58·3; H, 5·6; N, 6·2%).

S-*Benzylpenicillaminylglycine Ethyl Ester Hydrochloride* (III).—32·8% Aqueous hydrazine (2·16 c.c.) was added to a solution of the ethyl phthaloyl ester (10 g.) described in the preceding paragraph in a mixture of dioxan (50 c.c.) and ethanol (50 c.c.). A granular precipitate was formed when the solution was kept at room temperature and, after 65 hr., the suspension was acidified (Congo Red) with hydrochloric acid and kept at 0° for 14 hr. before collection of phthalhydrazide. Evaporation of the filtrate (bath-temp. 35—40°) left a pale yellow viscous residue which was shaken with a mixture of aqueous sodium hydrogen carbonate and ether. The ethereal layer was dried ($MgSO_4$) and then saturated with dry hydrogen chloride, and the amino-ester hydrochloride (4·75 g., 60%), m. p. 106—110°, was collected after 3—4 hr. Recrystallisation of the product from ethanol–ether or from propan-2-ol–light petroleum (b. p. 60—80°) gave S-*benzylpenicillaminylglycine ethyl ester hydrochloride* in colourless needles, m. p. 110—111° (Found: C, 52·2, 51·9; H, 7·0, 7·3; N, 7·5. $C_{16}H_{25}O_3N_2ClS,\frac{1}{2}H_2O$ requires C, 52·0; H, 7·1; N, 7·6%. Found, on material dried at 80°: C, 53·8; H, 7·2; N, 7·5; loss on drying, 2·5. $C_{16}H_{25}O_3N_2ClS$ requires C, 53·3; H, 7·0; N, 7·8; loss 2·5%). The corresponding *dioxopiperazine* crystallised from aqueous acetone in needles, m. p. 233°, insoluble in dilute hydrochloric acid and in aqueous sodium hydrogen carbonate (Found: C, 60·8; H, 7·1. $C_{14}H_{18}O_2N_2S$ requires C, 60·4; H, 6·5%).

S-*Benzylpenicillaminylglycine*.—The foregoing ethyl ester and 0·3N-hydrochloric acid (10 c.c. per g.) were heated to the b. p. and the filtered solution was treated with sodium acetate. Insoluble material was digested with aqueous ethanol and collected next day; crystallisation from water gave S-*benzylpenicillaminylglycine* in plates, m. p. 233—234° (decomp.) (Found: C, 56·6; H, 6·8; N, 9·4. $C_{14}H_{20}O_3N_2S$ requires C, 56·7; H, 6·8; N, 9·5%). A solution of the dipeptide (0·45 g.) in 2N-hydrochloric acid (0·8 c.c.) evaporated to dryness when stored in a vacuum over sodium hydroxide; recrystallisation of the residue from ethanol–ether gave S-*benzylpenicillaminylglycine hydrochloride*, plates, m. p. 225—226° (Found: C, 50·3; H, 6·3; N, 8·3. $C_{14}H_{20}O_3N_2S,HCl$ requires C, 50·5; H, 6·4; N, 8·4%).

DL-*Phthaloyl-γ-glutamyl*-S-*benzylpenicillaminylglycine and Ethyl Ester* (IV).—A solution of *S*-benzylpenicillaminylglycine ethyl ester hydrochloride (5 g.) and anhydrous sodium acetate (1·2 g., 1·06 equiv.) in glacial acetic acid (28 c.c.) was heated with DL-phthaloylglutamic anhydride (3·59 g., 1 equiv.) on a steam-bath for 10 min. and then kept at room temperature for 20 min., filtered, and evaporated under reduced pressure. The residue was shaken with aqueous sodium hydrogen carbonate and ether containing a little chloroform, and the aqueous layer was filtered through kieselguhr into stirred 5N-hydrochloric acid (25 c.c.), and the precipitate (7·3 g., 90%) was collected, washed with a little cold water, and dried over phosphoric oxide. DL-*Phthaloyl-γ-glutamyl*-S-*benzylpenicillaminylglycine ethyl ester hydrate* crystallised from ethanol in plates, m. p. 126° after softening at 118° (Found: C, 57·3; H, 5·9; N, 6·7. $C_{29}H_{33}O_8N_3S,1{\cdot}5H_2O$ requires C, 57·1; H, 5·9; N, 6·9%). The *α-methyl ester*, prepared with diazomethane, crystallised from methanol in needles, m. p. 166° (Found: C, 60·6; H, 6·3; N, 7·1. $C_{30}H_{35}O_8N_3S$ requires C, 60·3; H, 5·9; N, 7·0%). Aqueous 10N-sodium hydroxide was added gradually to a solution of the ethyl ester (1·1 g.) in ethanol (11 c.c.) until the first permanent blue colour was obtained with thymolphthalein as indicator. Addition of one further equivalent of 10N-sodium hydroxide (0·58 c.c.) precipitated a sodium salt which, after evaporation of the solution to dryness, was dissolved in water. The filtered solution was then acidified with 0·5N-hydrochloric acid (10 c.c.). DL-*Phthaloyl-γ-glutamyl*-S-*benzylpenicillaminylglycine* (0·2 g., 20%) crystallised from aqueous dioxan, or from ethanol containing a little dioxan, in needles, m. p. 230—232° (Found: C, 58·5; H, 5·2; N, 8·0. $C_{27}H_{29}O_8N_3S$ requires C, 58·4; H, 5·3; N, 7·6%). The phthaloyltripeptide (0·12 g., 14%) was also obtained by direct coupling of *S*-benzylpenicillaminylglycine hydrochloride and phthaloyl-DL-glutamic anhydride in acetic acid containing sodium acetate (1 mol.).

γ-DL-*Glutamyl*-S-*benzylpenicillaminylglycine* (I).—Hydrated phthaloyl-γ-DL-glutamyl-*S*-benzylpenicillaminylglycine ethyl ester (3·05 g.) in alcoholic 0·87N-potassium hydroxide (11·5 c.c.) was heated with 32·8% aqueous hydrazine hydrate [2] (1·24 g.) under reflux for 2 hr. and the solution was then evaporated under reduced pressure. A solution of the residue in water (25 c.c.) was adjusted to pH 6·0 with acetic acid, heated on a steam-bath for 1 hr., and then filtered from phthalhydrazide. Evaporation of the filtrate at 35—40° left a viscous solution which gave γ-DL-*glutamyl*-S-*benzylpenicillaminylglycine* (1·55 g., 73%) when diluted with

ethanol. The deliquescent amorphous tripeptide was reprecipitated from moist alcoholic solution by addition of dry ethanol, and it melted in a sealed capillary at 172—173° after sintering at 116° (Found: C, 53·6; H, 6·4; N, 9·8. $C_{19}H_{27}O_6N_3S$ requires C, 53·6; H, 6·4; N, 9·9%). Debenzylation of the tripeptide (0·88 g.) with sodium in liquid ammonia gave a product isolated as the mercury derivative (1·58 g.),[4] which was freed from mercury with hydrogen sulphide and then converted with cuprous oxide into an unidentified copper derivative (0·33 g.). This was precipitated as a pale green powder, m. p. 194° (decomp.), by addition of ethanol (Found: C, 24·4; H, 4·9; N, 7·7; S, 9·0; Cu, 16·0%). Removal of copper as the sulphide gave an amorphous product of indefinite m. p., *ca.* 178°, which failed to crystallise on slow evaporation of an aqueous-ethanolic solution (Found: C, 32·1, 32·7; H, 5·6, 5·6; N, 12·6%).

*Phthaloyl-γ-*DL*-glutamyl-*S*-benzylpenicillamine.*—A mixture of *S*-benzylpenicillamine (1 g.) and phthaloyl-DL-glutamic anhydride (1·08 g., 1 mol.) in dry dioxan (20 c.c.) was boiled for 20 min., filtered, and evaporated under reduced pressure. The residue separated from aqueous dioxan as a colourless solid (0·4 g., 19%), m. p. 130—132°, and recrystallisation from aqueous alcohol gave *phthaloyl-γ-*DL*-glutamyl-*S*-benzylpenicillamine dihydrate* in rectangular plates, m. p. 130—132° (decomp.) (Found: C, 56·1; H, 5·8; N, 5·3. $C_{25}H_{26}O_7N_2S,2H_2O$ requires C, 56·2; H, 5·7; N, 5·2%).

*Phthaloylglycyl-*S*-benzylpenicillamine.*—Phthaloylglycyl chloride (4 g., 1 mol.) in glacial acetic acid (20 c.c.) was added during 20 min. to a stirred solution of *S*-benzylpenicillamine (8·52 g., 2 mol.) in acetic acid (120 c.c.). The solution was stirred for 2 hr. and kept at room temperature for 4 hr. before evaporation under reduced pressure (bath-temp. 45°). The residue was treated with 2N-hydrochloric acid, and insoluble material crystallised from aqueous ethanol (7·19 g., 95%; m. p. 177—180°). Recrystallisation from ethanol–light petroleum gave *phthaloylglycyl-*S*-benzylpenicillamine* in hard prisms, m. p. 183° (Found: C, 62·0; H, 5·2; N, 6·9. $C_{22}H_{22}O_5N_2S$ requires C, 61·9; H, 5·2; N, 6·6%). *S*-Benzylpenicillamine (*ca.* 2 g.) was recovered by adding an excess of sodium acetate to the acid solution.

*Glycyl-*S*-benzylpenicillamine.*—The preceding phthaloyl compound (3 g., 1 mol.) was heated on a steam-bath under reflux with alcoholic 0·33N-potassium hydroxide (21 c.c., 1 equiv.) and 50% aqueous hydrazine hydrate (0·7 g., 1 equiv.). The solution soon became turbid and after 40 min. further ethanol (7 c.c.) was added and heating was continued for a total of 2 hr. before filtration. The solid residue (3·1 g.) and the filtrate were heated separately with 0·6N-hydrochloric acid (50 c.c.) at 50° for 5 min., and filtered when cold from phthalhydrazide. The combined filtrates were partially neutralised with sodium hydroxide before addition of saturated aqueous sodium acetate to precipitate the product which was collected and washed with hot ethanol (10—15 c.c.). *Glycyl-*S*-benzylpenicillamine* (1·68 g., 80%) crystallised in rectangular plates, m. p. 228° raised to 231° by recrystallisation from water (Found: C, 56·7; H, 6·8; N, 9·6. $C_{14}H_{20}O_3N_2S$ requires C, 56·7; H, 6·8; N, 9·5%).

The authors thank the Medical Research Council for a maintenance grant (to W. A. S.).

UNIVERSITY OF NOTTINGHAM. [*Received, February 2nd,* 1959.]