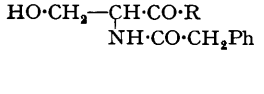
120. N-(N′-*Phenylacetylseryl*)-D-*penicillamines as Possible Biological Precursors of Benzylpenicillin.*

By WILSON BAKER and W. D. OLLIS.

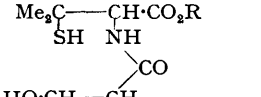
The dipeptides *N*-(*N*′-phenylacetyl-L-seryl)- and *N*-(*N*′-phenylacetyl-D-seryl)-D-penicillamine (II; R = H) have been synthesised; they do not stimulate biochemical penicillin production, and are not converted into penicillin *in vivo*. Reaction of *N*-phenylacetyl-DL-serylazide (I; R = N_3) with D-penicillamine methyl ester gave the diastereoisomeric *N*-(*N*′-phenylacetyl-L-seryl)- and *N*-(*N*′-phenylacetyl-D-seryl)-D-penicillamine methyl esters (II; R = Me) which were also prepared from the corresponding L- and D-seryl derivatives. Hydrolysis gave the related acids mentioned. Desulphurisation of these esters with Raney nickel gave *N*-(*N*′-phenylacetyl-L-seryl)- and *N*-(*N*′-phenylacetyl-D-seryl)-D-valine methyl ester respectively.

N-Phenylacetyl-DL-serylazide passes when heated into 4-phenylacetamidooxazolid-2-one (VII); the related D-serylazide gives the optically active oxazolidone.

MANY different substances have been tested as possible precursors of penicillin by adding them to the culture media and determining their effect, if any, on the penicillin production (see review by Behrens, "The Chemistry of Penicillin," Princeton Univ. Press, 1949, Chapter 19, p. 657; Behrens *et al.*, *J. Biol. Chem.*, 1948, **175**, 751, 765, 771, 793; *J. Amer. Chem. Soc.*, 1948, **70**, 2837, 2843, 2849). It is well established that stimulation of the production of benzylpenicillin occurs on addition of certain compounds containing the phenylacetyl group, or of compounds which can be converted biochemically into others containing this group. Experiments with nuclear substituted phenylacetic acids, and the formation therefrom of new biosynthetic pencillins, prove that phenylacetyl groups are incorporated into the penicillin molecules. Attempts to find the precursors of the rest of the penicillin molecule have been unsuccessful.



(I.)



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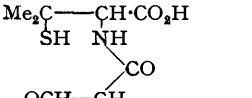
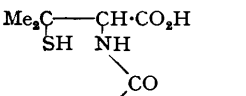


(III.)

Examination of the structure of benzylpenicillin (V) shows that it is closely related to the dipeptide *N*-(*N*′-phenylacetylseryl)-D-penicillamine (II; R = H), and the synthesis of the two diastereoisomeric forms of *N*-(*N*′-phenylacetylseryl)-D-penicillamine was undertaken because either of these substances might prove to be the biological precursor of benzylpenicillin. Oxidation of the primary alcohol group in (II; R = H) to the aldehyde (III) and subsequent cyclodehydration might give benzylpenicillin (V).



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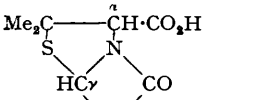

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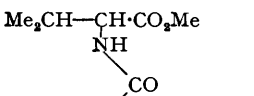


(VI.)

DL-Serine and phenylacetyl chloride gave *N*-phenylacetyl-DL-serine (I; R = OH) of which the methyl ester (I; R = OMe) was converted *via* *N*-phenylacetyl-DL-serylhydrazide (I; R = $NH{\cdot}NH_2$), characterised as its benzylidene derivative, into the corresponding azide (I; R = N_3). Reaction of this azide with D-penicillamine methyl ester in ethyl acetate solution (cf. Fruton, *J. Biol. Chem.*, 1942, **146**, 463) gave a mixture (*M*) of the two stereoisomeric forms of *N*-(*N*′-phenylacetylseryl)-D-penicillamine methyl ester (II; R = Me). This mixture

was separated into a crystalline solid (*A*) (m. p. 125°; $[\alpha]_D^{18}$ −13·0°), now known to be *N*-(*N'*-phenylacetyl-L-seryl)-D-penicillamine methyl ester, and a non-crystalline material (*B*) that was shown to consist mainly of *N*-(*N'*-phenylacetyl-D-seryl)-D-penicillamine methyl ester. These two products were identified by comparison with the substances obtained when the synthesis was repeated using first L-serine and then D-serine as the starting materials. From L-serine the synthesis proceeded smoothly and gave *N*-(*N'*-phenylacetyl-L-seryl)-D-penicillamine methyl ester (m. p. 123°). The slight discrepancy between this melting point and that of the compound obtained previously from fraction (*A*) is due to the fact that larger amounts of the latter compound were available and many recrystallisations were necessary before the final melting point of 125° was reached. That there is no difference between the crystalline methyl esters (II; R = Me) obtained from DL-serine and L-serine was proved by *X*-ray powder photographs, kindly taken by Dr. H. F. Kay and Mr. H. J. Wellard in the H. H. Wills Physical Laboratory of this University. They report as follows: " The sample, m. p. 125°, gives a powder pattern identical throughout the angular range for which reflections are observed (θ = 5—35°, using Co-K_α radiation) with that of *N*-(*N'*-phenylacetyl-L-seryl)-D-penicillamine methyl ester. It may therefore be presumed to be the latter substance." The material obtained from D-serine could not be crystallised. Comparison of its specific rotation, $[\alpha]_D^{14}$ +26·4°, with that of the non-crystalline material (*B*) ($[\alpha]_D^{20}$ +30·6°; $[\alpha]_D^{15}$ +28·7°) showed that the latter consisted mainly of *N*-(*N'*-phenylacetyl-D-seryl)-D-penicillamine methyl ester.

These identifications were further confirmed by desulphurisation with Raney nickel. *N*-(*N'*-Phenylacetyl-L-seryl)-D-penicillamine methyl ester (II; R = Me) gave *N*-(*N'*-phenylacetyl-L-seryl)-D-valine methyl ester (VI). Similarly, the non-crystalline D-seryl ester (II; R = Me) gave crystalline *N*-(*N'*-phenylacetyl-D-seryl)-D-valine methyl ester. Desulphurisation of part of fraction (*B*) gave mainly *N*-(*N'*-phenylacetyl-D-seryl)-D-valine methyl ester, but a very small amount of its diastereoisomeride was also isolated. Serine and valine, identified by paper chromatography, were formed by acid hydrolysis of these desulphurisation products.

The separation of the two diastereoisomerides formed by reaction of a D-amino-acid ester with the azide of a simple acylated racemic amino-acid does not appear to have been previously recorded. Polglase and Smith (*J. Amer. Chem. Soc.*, 1949, **71**, 3081; see Rydon, *Ann. Reports*, 1949, **46**, 195) have, however, shown that carbobenzyloxy-L-leucine azide and DL-alanine methyl ester gave a mixture from which the diastereoisomeric carbobenzyloxy-L-leucyl-D- and -L-alanine methyl esters were separated by crystallisation. Previously Baker and Ollis (*J.*, 1949, 345) treated the azide derived from one of the racemic forms of α-dimethoxymethyl-β-phenylacetamido-*n*-butyrylhydrazide (α-form) with D-penicillamine and isolated in a crystalline form one of the four possible stereoisomers of *N*-(α-dimethoxymethyl-β-phenylacetamido-*n*-butyryl)-D-penicillamine. Analogous resolutions have been recorded in the separation of the products of reduction of acetyldehydrophenylalanyl-L-leucine into acetyl-L- and acetyl-D-phenylalanyl-L-leucine (Behrens, Doherty, and Bergmann, *J. Biol. Chem.*, 1940, **136**, 65) and in the chromatographic separation of the two diastereoisomeric lactones produced from *N*-methyl-DL-valine and D(−)-α-bromo*iso*valeryl chloride (Cook, Cox, and Farmer, *J.*, 1949, 1028).

The stereochemistry of (II) is important when this substance is considered as a possible precursor of benzylpenicillin (V). Of the three asymmetric centres of penicillin, C$_\alpha$ and C$_\beta$ may be regarded as the α-carbon atoms of penicillamine, $Me_2C(SH)\cdot CH(NH_2)\cdot CO_2H$, and of *C*-formylglycine, $CHO\cdot CH(NH_2)\cdot CO_2H$, respectively. The D-configuration of penicillamine has been established (Merck and Co., Committee for Penicillin Synthesis Reports, C.P.S., **13**, 3; **14**, 1) by conversion into a derivative of D-valine, and Merck and Co. (C.P.S., **338**, 9, 23) have shown that one of the products obtained by Raney nickel desulphurisation of sodium benzylpenicillin was *N*-(*N'*-phenylacetyl-L-alanyl)-D-valine. These results prove that C$_\alpha$ and C$_\beta$ have D- and L-configurations respectively, and this was confirmed by *X*-ray investigation (Crowfoot, Bunn, Rogers-Low, and Turner-Jones; " The Chemistry of Penicillin," Chapter 11, p. 310).

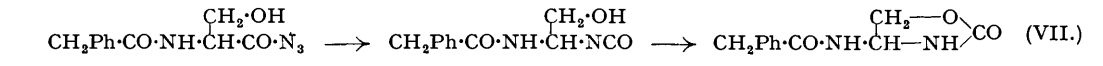
As far as the synthesis of penicillin is concerned, if the β-lactam ring and the thiazolidine ring are *cis* to one another, and if the intermediates are such that C$_\alpha$ and C$_\beta$ have the correct configuration, then there are only two possible configurations for the molecule that is formed.

It is possible, therefore, that if *N*-(*N'*-phenylacetyl-L-seryl)-D-penicillamine was oxidised to give (III), then this *N*-benzylpenaldyl-D-penicillamine might cyclise to give a product with the required configurations at C$_\alpha$ and C$_\beta$. *N*-(*N'*-Phenylacetyl-L-seryl)-D-penicillamine methyl ester (II; R = Me) was hydrolysed under mild conditions by aqueous alkali to the corresponding acid (II; R = H) which was kindly tested by Dr. E. P. Abraham of the Sir William Dunn School of Pathology, Oxford, as a possible precursor in the biosynthesis of penicillin. It was

found that it did not stimulate or increase the yield of penicillin produced by *P. notatum*, nor when injected into mice did it cause any antibiotic to be excreted in the urine.

N-(*N'*-Phenylacetyl-D-seryl)-D-penicillamine was also tested as a possible biological precursor because biochemical oxidation of the primary alcohol group of a D-seryl moiety might occur, although that of an L-seryl fragment might be unaffected. The conversion of *N*-(*N'*-phenylacetyl-D-seryl)-D-penicillamine (II; R = H) into (V) with the L-configuration for C_β would require an optical inversion This could take place if an equilibrium involving the enolic form (IV) was established. In order to test this hypothesis a mixture (mixture *M*; see above) of *N*-(*N'*-phenylacetyl-L-seryl)- and *N*-(*N'*-phenylacetyl-D-seryl)-D-penicillamine methyl esters was hydrolysed and the mixture of free acids was converted into sodium salts. Through the kindness of Sir Jack Drummond, of Boots Pure Drug Company, Nottingham, to whom we express our thanks, this substance was tested both as a precursor in normal penicillin production by *P. chrysogenum* Q.176, and as a precursor *in vivo* against streptococcal infection in mice. Both sets of experiments were negative.

During the course of this work an interesting example of the Curtius degradation has been encountered. When *N*-phenylacetyl-DL-seryl azide is heated, nitrogen is evolved and racemic 4-phenylacetamido-oxazolid-2-one (VII) is formed; this reaction involves the intermediate formation of an *iso*cyanate. The formation of similar cyclic urethanes has been noted by others (Schroeter, G.P. 220,852, "Friedländer," 1910—1912, **10**, 1309; Fruton, *J. Biol. Chem.*,



(VII.)

1942, **146**, 463; Newman, *J. Amer. Chem. Soc.*, 1949, **71**, 378). When the azide (I; R = N_3) derived from *N*-phenylacetyl-D-serylhydrazide is heated, the oxazolidone obtained is optically active ($[\alpha]_D^{20}$ +127°); no trace of the racemic compound was detected. This result is similar to the results obtained by others on the Curtius degradation of optically active azides (Kenyon *et al.*, *J.*, 1941, 263; 1946, 25) and shows that the rearrangement is intramolecular, since the asymmetric groups retain their configuration during migration. The high specific rotation of the active oxazolid-2-one is in agreement with the generalisation made by Neuberger ("Advances in Protein Chemistry," 1948, **4**, 305) that cyclic compounds derived from optically active amino-acids where the asymmetric carbon is part of a ring system have large optical rotatory powers.

Experimental.

(M. p.s are uncorr. Microanalyses are by Mr. W. M. Eno, Bristol, and Drs. Weiler and Strauss, Oxford).

N-*Phenylacetyl*-DL-*serine* (I; R = OH).—To a vigorously stirred solution of DL-serine (42 g.) in 2N-sodium hydroxide (300 c.c.) and water (100 c.c.) cooled in ice, phenylacetyl chloride (105 c.c.) and 2N-sodium hydroxide (920 c.c.) were added simultaneously during 2 hours so that the solution remained alkaline throughout. After being stirred for a further hour, the mixture was shaken with ether (500 c.c.), acidified with 2N-hydrochloric acid, and left overnight in a refrigerator. The solid was collected, dried, washed with cold benzene (350 c.c.), and crystallised from hot water, giving *N*-phenylacetyl-DL-serine (69 g.), m. p. 128°. Concentration of the mother-liquors and crystallisation of the product gave a further quantity (16 g.) of the acid (total yield, 96%). The united crops were recrystallised from benzene–ethanol and gave *N*-phenylacetyl-DL-serine as colourless, rhombic crystals, m. p. 131° (Found: C, 59·8; H, 6·0; N, 5·7. Calc. for $C_{11}H_{13}O_4N$: C, 59·2; H, 5·9; N, 6·3%). A less easy method for preparing this substance is given in "The Chemistry of Penicillin" (Chapter 4, p. 71).

N-*Phenylacetyl*-DL-*serine Methyl Ester* (I; R = OMe).—This esterification was carried out in a Soxhlet apparatus with anhydrous magnesium sulphate in the thimble. A mixture of *N*-phenylacetyl-DL-serine (22·3 g.), absolute methanol (200 c.c.), anhydrous benzene (50 c.c.), and concentrated sulphuric acid (2 c.c.) was boiled under reflux for 6 hours. After addition of water (1 c.c.), potassium hydrogen carbonate was added until the solution was neutral to Congo-red, and the solvents were removed under diminished pressure. The residue was dissolved in chloroform (150 c.c.), washed with water, and dried ($MgSO_4$), and removal of the chloroform then gave *N*-phenylacetyl-DL-serine methyl ester (22·4 g., 95%) as a colourless oil.

N-*Phenylacetyl*-DL-*seryl Hydrazide* (I; R = $NH{\cdot}NH_2$).—Hydrazine hydrate (96%; 9·6 c.c.) was added to *N*-phenylacetyl-DL-serine methyl ester (22·4 g.), a crystalline precipitate rapidly separating. After 20 hours, excess of hydrazine was removed under diminished pressure over concentrated sulphuric acid, and crystallisation of the residue (22·4 g., 100%) from aqueous ethanol gave *N-phenylacetyl*-DL-*serylhydrazide* (16·8 g.) as fine, white needles, m. p. 175° (Found: C, 56·1; H, 6·4; N, 18·2. $C_{11}H_{15}O_3N_3$ requires C, 55·7; H, 6·3; N, 17·7%).

Benzylidene derivative. Benzaldehyde (0·4 c.c.) in ethanol (1·5 c.c.) was added to a solution of this hydrazide (0·5 g.) in water (2 c.c.), and the mixture warmed on a steam-bath for 1 minute. Next day the solid (0·68 g., 100%) was collected and crystallised from *n*-butanol (60 c.c.) giving the *benzylidene* derivative (0·61 g.) as a white, micro-crystalline powder, m. p. 207° (Found: C, 66·6; H, 5·9; N, 12·8. $C_{18}H_{19}O_3N_3$ requires C, 66·5; H, 5·9; N, 12·9%).

Reaction of N-*Phenylacetyl*-DL-*serylazide with* D-*Penicillamine Methyl Ester. Isolation of* N-(N′-*Phenylacetyl*-L-*seryl*)- *and* N-(N′-*Phenylacetyl*-D-*seryl*)-D-*penicillamine Methyl Ester* (II; R = Me).—A solution of sodium nitrite (6·05 g.) in water (80 c.c.) was added with stirring to a solution of *N*-phenylacetyl-DL-serylhydrazide (18·9 g.) in 0·5N-hydrochloric acid (225 c.c.) at 0°. The azide was immediately extracted into ethyl acetate (50-c.c., 25-c.c., and 25-c.c. portions), the combined extracts were washed with 5% aqueous sodium hydrogen carbonate and filtered through anhydrous magnesium sulphate into a solution of D-penicillamine methyl ester (14·1 g.) in ethyl acetate (25 c.c.). All these operations were carried out very rapidly and it was found important to keep the temperature of the solutions close to 0°. After 24 hours at room temperature the ethyl acetate solution was washed with 2N-hydrochloric acid (2 × 25 c.c.), 5% aqueous sodium hydrogen carbonate (25 c.c.), and water (25 c.c.), then dried ($MgSO_4$), and the solvent removed under reduced pressure in an atmosphere of nitrogen, leaving a light yellow oil (24·5 g., 84%), $[\alpha]_D^{25}$ +14·2° (*c*, 5·0 in methanol), which did not crystallise. This oil was dissolved in hot benzene (90 c.c.) and filtered. From a portion (30 c.c.) of the filtrate the solvent was removed under reduced pressure and the residue (*M*) (8·0 g.) was hydrolysed (see below). The remainder of the filtrate was slowly cooled and crystallisation induced by seeding. The crystalline precipitate (7·2 g.), $[\alpha]_D^{20}$ −10·7° (*c*, 3·5 in methanol), m. p. 95—110°, was collected, washed with cold benzene (15 c.c.), and dried (Fraction *A*). Removal of the benzene from the filtrate gave a light yellow glass (Fraction *B*; 10·2 g.), $[\alpha]_D^{20}$ +30·6° (*c*, 3·1 in methanol), $[\alpha]_D^{15}$ +28·7° (*c*, 3·4 in methanol).

Fraction (*A*) was recrystallised twice from benzene (50—100 c.c.) and gave short, colourless needles (6·05 g.), m. p. 122—123°, $[\alpha]_D^{18}$ −12·9° (*c*, 2·6 in methanol). Recrystallisation from ethanol (30 c.c.)–water (60 c.c.) gave N-(N′-*phenylacetyl*-L-*seryl*)-D-*pencillamine methyl ester* (5·5 g.) as long, colourless needles, m. p. 125°, $[\alpha]_D^{18}$ −13·0° (*c*, 2·5 in methanol) (Found: C, 55·4; H, 6·5; N, 7·4; S, 8·5; OMe, 8·0. $C_{17}H_{24}O_5N_2S$ requires C, 55·4; H, 6·5; N, 7·6; S, 8·7; OMe, 8·4%). A mixed m. p. with an authentic specimen of *N*-(N′-phenylacetyl-L-seryl)-D-penicillamine methyl ester (see below) showed no depression and the two substances gave identical *X*-ray powder photographs.

Fraction (*B*) could not be obtained crystalline, and an attempted purification by chromatography of a benzene solution on an alumina column and elution with benzene–ethanol (95 : 5) was not successful. The optical rotation (above) and Raney nickel desulphurisation (see below) showed that it consisted mainly of *N*-(*N*′-phenylacetyl-D-seryl)-D-penicillamine methyl ester.

Hydrolysis of Mixture of Diastereoisomers (*M*).—Mixture (*M*) (8 g.) (above) consisting of *N*-(*N*′-phenyl-L-seryl)- and *N*-(*N*′-phenylacetyl-D-seryl)-D-penicillamine methyl ester was hydrolysed as described for *N*-(*N*′-phenylacetyl-L-seryl)-D-penicillamine methyl ester (see below), giving a non-crystalline mixture of *N*-(*N*′-phenylacetyl-D-seryl)- and *N*-(*N*′-phenylacetyl-L-seryl)-D-penicillamine (6·70 g.). These acids were converted into their sodium salts by dissolution in 2N-sodium carbonate (9·5 c.c.) to give a solution of pH 6, then "freeze-drying" to give the sodium salts as a cream-coloured powder. This was the material used in the laboratories of Boots Pure Drug Company to determine its effect on the yield of penicillin when added to the culture-medium.

N-*Phenylacetyl*-L- *and* N-*Phenylacetyl*-D-*serine* (I; R = OH).—DL-Serine (*Org. Synth.*, 1940, **20**, 81) was resolved as described by Fischer and Jacobs (*Ber.*, 1906, **39**, 2942). Phenylacetyl chloride (11·2 c.c.) and 2N-sodium hydroxide (83 c.c.) were added simultaneously to a stirred solution, at 0°, of L-serine (5 g.) in N-sodium hydroxide (47 c.c.) during half an hour at 0°. After a further 10 minutes, the mixture was filtered, acidified with concentrated hydrochloric acid (20·2 c.c.), and kept in a refrigerator overnight. The precipitate was collected, dried, and washed with a small amount of cold benzene to remove phenylacetic acid. Crystallisation from water gave N-*phenylacetyl*-L-*serine* (1·8 g.) as colourless, rectangular prisms, m. p. 132° (Found: C, 58·9; H, 5·6; N, 6·4. $C_{11}H_{13}O_4N$ requires C, 59·2; H, 5·8; N, 6·3%).

Similarly, D-serine gave *N*-phenylacetyl-D-serine, m. p. 132°.

N-*Phenylacetyl*-L- *and* N-*Phenylacetyl*-D-*serine Methyl Ester* (I; R = OMe).—A mixture of *N*-phenylacetyl-L-serine (1·5 g.), absolute methanol (20 c.c.), anhydrous benzene (5 c.c.), and concentrated sulphuric acid (0·15 c.c.) was refluxed for 2·75 hours in a Soxhlet apparatus, and worked up as in the case of the DL-derivative, to give *N*-phenylacetyl-L-serine methyl ester (1·35 g., 85%) as a light yellow oil.

N-Phenylacetyl-D-serine methyl ester was prepared similarly.

N-*Phenylacetyl*-L-*serylhydrazide* (I; R = $NH{\cdot}NH_2$).—A mixture of *N*-phenylacetyl-L-serine methyl ester (1·35 g.) and 96% hydrazine hydrate (0·65 c.c.) in ethanol (2 c.c.) was left at room temperature for 18 hours. The precipitate (1·0 g., 75%) was collected and crystallised from ethanol (25 c.c.) and water (1·0 c.c.), giving N-*phenylacetyl*-L-*serylhydrazide* (0·76 g.) as colourless needles, m. p. 199°, $[\alpha]_D^{16}$ −39·3° (*c*, 1·1 in water) (Found: C, 56·0; H, 6·4; N, 17·7. $C_{11}H_{15}O_3N_3$ requires C, 55·7; H, 6·3; N, 17·7%). The *benzylidene* derivative (80% yield), prepared as in the previous case, formed colourless platelets (from benzene–ethanol), m. p. 196° (Found: C, 66·8; H, 5·9; N, 13·1. $C_{18}H_{19}O_3N_3$ requires C, 66·5; H, 5·9; N, 12·9%).

N-*Phenylacetyl*-D-*serylhydrazide*.—This *compound* was prepared similarly from *N*-phenylacetyl-D-serine methyl ester and 96% hydrazine hydrate, and crystallised from slightly diluted ethanol as needles, m. p. 199°, $[\alpha]_D^{16}$ +39·1° (*c*, 1·2 in water) (Found: C, 55·9; H, 6·3; N, 18·0%). The *benzylidene* derivative formed colourless platelets from benzene–ethanol, m. p. 196° (Found: C, 67·0; H, 6·4; N, 13·2%).

N-(N′-*Phenylacetyl*-L-*seryl*)-D-*penicillamine Methyl Ester* (II; R = Me).—The azide was prepared as before from *N*-phenylacetyl-L-serylhydrazide (500 mg.) in 0·5N-hydrochloric acid (5·9 c.c.) and sodium nitrite (160 mg.) in water (2 c.c.). A solution of this azide in ethyl acetate (20 c.c.) was added to D-penicillamine methyl ester (850 mg.) in ethyl acetate (4 c.c.). Next day the solution was worked up as in the reaction of *N*-phenylacetyl-DL-serylazide and gave a gummy residue (620 mg., 80%). Crystallisation from benzene–light petroleum (b. p. 60—80°) gave a solid (470 mg.) which was

recrystallised five times from benzene (1 c.c.) and twice from aqueous ethanol, giving N-(N'-*phenylacetyl*-L-*seryl*)-D-*penicillamine methyl ester* as colourless needles, m. p. 123° (Found: C, 55·7; H, 6·6; N, 7·8; S, 8·3; OMe, 9·1. $C_{17}H_{24}O_5N_2S$ requires C, 55·4; H, 6·5; N, 7·6; S, 8·7; OMe, 8·4%).

N-(N'-*Phenylacetyl*-L-*seryl*)-D-*penicillamine* (II; R = H).—*N*-(*N'*-Phenylacetyl-L-seryl)-D-penicillamine methyl ester (0·3 g.) was dissolved in pure dioxan (3 c.c.) and after the addition of thymol-violet (pH range 9·0—13·0) the solution was rapidly titrated with N-sodium hydroxide until the solution was faintly blue (this required almost exactly one equivalent: calc., 0·81 c.c.; found, 0·82 c.c.). Two more equivalents of N-sodium hydroxide (1·64 c.c.) were added and after the solution had been kept at room temperature for 30 hours it was neutralised with N-hydrochloric acid, freed from dioxan under diminished pressure, and extracted with ethyl acetate (one 6-c.c. and two 3-c.c. portions). The combined ethyl acetate extracts were extracted with 5% sodium hydrogen carbonate solution (two 3-c.c. portions) which, after being washed with ether, was acidified with 2N-hydrochloric acid and the liberated acid was extracted into ethyl acetate. After drying ($MgSO_4$), the final ethyl acetate extract gave the hygroscopic, non-crystalline N-(N'-*phenylacetyl*-L-*seryl*)-D-*penicillamine* (213 mg., 74%) (Found: C, 53·0; H, 6·1; N, 7·4; S, 8·1%; equiv., 316. $C_{16}H_{22}O_5N_2S$ requires C, 54·25; H, 6·2; N, 7·9; S, 9·0%; equiv., 354). This material gave a strong, transient blue ferric chloride reaction. It could not be crystallised, nor could crystalline salts be obtained.

N-(N'-*Phenylacetyl*-D-*seryl*)-D-*penicillamine Methyl Ester*.—Conversion of *N*-phenylacetyl-D-seryl-hydrazide (0·5 g.) into its azide, and reaction of this with D-penicillamine methyl ester as in the previous case, gave a gummy residue (583 mg., 75%). This was dissolved in benzene (2 c.c.), and light petroleum (b. p. 60—80°) was added until the solution was slightly cloudy. After being kept in a refrigerator overnight, the solution was filtered and the small amount of solid was discarded. Removal of the solvent gave N-(N'-*phenylacetyl*-D-*seryl*)-D-*penicillamine methyl ester* as a colourless gum, $[\alpha]_D^{14}$ +26·4° (*c*, 2·8 in methanol), which could not be crystallised (Found: C, 55·5; H, 6·6; N, 7·5; S, 9·0; OMe, 8·6%).

Raney Nickel Desulphurisation of N-(N'-*Phenylacetyl*-L-*seryl*)-D-*penicillamine Methyl Ester. Formation of* N-(N'-*Phenylacetyl*-L-*seryl*)-D-*valine Methyl Ester* (VI).—A mixture of *N*-(*N'*-phenylacetyl-L-seryl)-D-penicillamine methyl ester (1 g.), Raney nickel (*ca.* 12 g.), and methanol (75 c.c.) was heated under reflux for 2·5 hours, cooled, and filtered, and the nickel well washed with methanol. The filtrate and washings were combined, the methanol was removed under diminished pressure, the crystalline residue dissolved in methanol (15 c.c.), and water (15 c.c.) added. After being kept in the refrigerator overnight, the precipitate (679 mg.), m. p. 164°, was collected; addition of water to the mother-liquors gave a further precipitate (56 mg.), m. p. 164°. The total yield (735 mg., 81%) was recrystallised from aqueous methanol, giving N-(N'-*phenylacetyl*-L-*seryl*)-D-*valine methyl ester* as colourless needles, m. p. 164·5°, $[\alpha]_D^{24}$ −6·7° (*c*, 1·0 in methanol) (Found: C, 61·1; H, 7·0; N, 8·7; OMe, 9·2. $C_{17}H_{24}O_5N_2$ requires C, 60·7; H, 7·2; N, 8·3; OMe, 9·2%). The identity of this dipeptide was confirmed by hydrolysing a small amount with concentrated hydrochloric acid for 2 hours at 100°. The acid was removed by heating the solution under reduced pressure, adding water to the residue, and removing it by distillation. This was repeated several times. The residue was analysed by paper partition chromatography, using Whatman No. 1 filter paper and as solvent mixture *n*-butanol (480 c.c.), water (195 c.c.), acetic acid (95 c.c.), and *n*-butyl acetate (35 c.c.). Two spots due to serine (R_F = 0·11) and valine (R_F = 0·42) were detected and this behaviour was exactly paralleled by a mixture of these two amino-acids.

Raney Nickel Desulphurisation of N-(N'-*Phenylacetyl*-D-*seryl*)-D-*penicillamine Methyl Ester. Formation of* N-(N'-*Phenylacetyl*-D-*seryl*)-D-*valine Methyl Ester* (VI).—Desulphurisation of *N*-(*N'*-phenylacetyl-D-seryl)-D-penicillamine methyl ester (263 mg.) was carried out with Raney nickel (*ca.* 5 g.) and methanol (20 c.c.) as in the previous case. Removal of the methanol gave a non-crystalline residue (226 mg.) that was dissolved in ethyl acetate (20 c.c.), washed with 2N-hydrochloric acid and 2N-sodium carbonate, then dried ($MgSO_4$), and the solvent removed, giving a partly crystalline residue (151 mg.). This was triturated with light petroleum (b. p. 60—80°), and the microcrystalline solid (115 mg., m. p. 75—80°) collected. Recrystallisation from benzene (4 c.c.)–light petroleum (b. p. 60—80°; 4 c.c.) gave N-(N'-*phenylacetyl*-D-*seryl*)-D-*valine methyl ester* as fine, colourless needles, m. p. 95°, $[\alpha]_D^{25}$ +46° (*c*, 0·35 in methanol) (Found: C, 61·2; H, 7·4; N, 8·5; OMe, 9·0%). The identity of this dipeptide ester was confirmed by paper partition chromatography (see above) by comparison of its acid hydrolysate with an artificial mixture of serine and valine.

Raney Nickel Desulphurisation of Fraction B. *Formation of* N-(N'-*Phenylacetyl*-L-*seryl*)- *and* N-(N'-*Phenylacetyl*-D-*seryl*)-D-*valine Methyl Ester* (VI).—Desulphurisation of fraction *B* (1·3 g.) was carried out with Raney nickel (*ca.* 12 g.) in methanol (80 c.c.) as in the previous cases. After removal of the Raney nickel and the methanol, the remaining oil (1·1 g.) was dissolved in methanol (15 c.c.), and water (40 c.c.) added. After being kept in the refrigerator overnight the precipitate (fraction *X*; m. p. 133—139°; 259 mg.) was collected and dried. Water was added to the filtrate, which was then extracted with ethyl acetate (2 × 20 c.c.), then dried ($MgSO_4$), and the solvent removed, leaving an oil which crystallised on trituration with light petroleum; the solid was collected (fraction *Y*; m. p. 82—84°; 550 mg.). Fraction *X*, after three recrystallisations from aqueous methanol, gave *N*-(*N'*-phenylacetyl-L-seryl)-D-valine methyl ester (10 mg.), m. p. 162° (mixed m. p. with an authentic specimen 163·5°). Fraction *Y* was recrystallised six times from benzene–light petroleum (b. p. 60—80°) and eventually gave *N*-(*N'*-phenylacetyl-D-seryl)-D-valine methyl ester (190 mg.) as colourless needles, m. p. and mixed m. p. 95°.

Racemic 4-*Phenylacetamido-oxazolid*-2-*one* (VII).—Sodium nitrite (0·735 g.) in water (15 c.c.) was added to a solution of *N*-phenylacetyl-DL-serylhydrazide (2·3 g.) in 0·5N-hydrochloric acid (26·2 c.c.) with stirring at 0°. The azide was extracted into ethyl acetate (2 × 15 c.c.) and the combined extracts were quickly washed with water (5 c.c.) and filtered through anhydrous magnesium sulphate. The filtrate was kept at 40° for 20 minutes, then for a further 10 minutes with the temperature rising to 60°.

Nitrogen was evolved and the crystalline precipitate (1·67 g., 78%) which separated was collected and crystallised from water, giving racemic 4-*phenylacetamido-oxazolid*-2-*one* as colourless needles, m. p. 184° (Found : C, 59·8; H, 5·4. $C_{11}H_{12}O_3N_2$ requires C, 60·0; H, 5·4%).

dextro- *and* lævo-4-*Phenylacetamido-oxazolid*-2-*one* (VII).—The *dextro*-compound was prepared as in the previous case by heating the ethyl acetate (4 c.c.) solution of the azide obtained by adding sodium nitrite (33 mg.) in water (0·4 c.c.) to *N*-phenylacetyl-D-serylhydrazide (100 mg.) in 0·5N-hydrochloric acid (1·2 c.c.). The crystalline precipitate (71 mg., 77%) obtained by adding light petroleum (b. p. 60—80°) to the ethyl acetate solution and cooling was collected. Crystallisation from water (2 c.c.) gave dextro-4-*phenylacetamido-oxazolid*-2-*one* as colourless needles, m. p. 157°, $[\alpha]_D^{20}$ +127° (*c*, 0·1 in water) (Found : C, 60·1; H, 5·4; N, 12·6%). Similarly *N*-phenylacetyl-L-serylhydrazide (100 mg.) gave lævo-4-*phenylacetamido-oxazolid*-2-*one* (68 mg., 73%) as colourless needles, m. p. 157° (Found : C, 60·2; H, 5·5; N, 13·0%).

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