

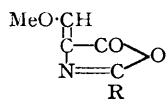
80. Attempted Syntheses of Penicillin Homologues.

By WILSON BAKER AND W. D. OLLIS.

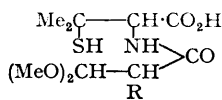
Attempts have been made to synthesise penicillins in which the *C*-formylglycine part of the molecule is replaced by either *C*- β -amino- α -formyl-*n*-butyric or *isovaleric* acid residues. β -Benzamidoisovaleric acid is converted by acetic anhydride into the 1:3-oxazine (IV), a type of compound analogous to the oxazolones derived from the *N*-acyl- α -amino-acids. The lactone group in (IV) is highly reactive, but the methylene group is inert and could not be condensed with ethyl orthoformate to give (III; R = Et), which might yield a penicillin on condensation with D-penicillamine. An alternative route started from methyl β -benzamido-*n*-butyrate which was formylated, converted into the dimethylacetal, and thence *via* the related hydrazide into the azide, which reacted with D-penicillamine giving the acetal (II; R = Ph·CO·NH·CHMe). A similar series of reactions starting from methyl β -phenylacetamido-*n*-butyrate gave the two racemic forms of the related dimethylacetal and of the hydrazide, each of which was converted into an azide and finally into the *N*-acyl-D-penicillamine derivatives

(II; R = Ph·CH₂·CO·NH·CHMe). Attempted thermal ring closure of the acetals (II) gave products devoid of antibacterial activity.

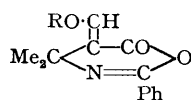
Of the numerous attempts which have been made to synthesise penicillins (see "Monograph on Penicillin"), only the interaction of certain 2-substituted 4-alkoxy (usually methoxy)-methylene-4 : 5-dihydro-oxazole-5-ones (I) with D-penicillamine has consistently given products showing antibiotic activity of the penicillin type (method *a*). By the condensation of 2-benzyl-4-methoxymethylene-4 : 5-dihydro-oxazole-5-one (I; R = CH₂Ph) with D-penicillamine, benzylpenicillin has been synthesised and isolated, though in extremely small yield (see du Vigneaud, Carpenter, Holley, Livermore, and Rachele, *Science*, 1946, **104**, 431, where previous references are given).



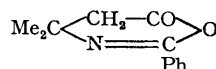
(I.)



(II.)



(III.)

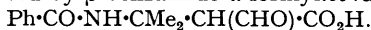


(IV.)

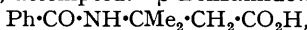
Another reaction which has been claimed to give a product showing weak antibacterial properties is the thermal ring closure of the acetal (II; R = NH·CO·CH₂Ph) (method *b*) (Squibb Institute for Medical Research, Committee for Penicillin Synthesis Reports, CPS, 1945, 414, 452), but the precise conditions necessary to produce biologically-active material have not been defined, and a repetition of the work (Dr. J. F. W. McOmie, Bristol, and Drs. E. P. Abraham and E. B. Chain, Oxford, unpublished experiments) gave only inactive products.

The present work was designed to effect the synthesis of some penicillin homologues, in which the usual penaldic acid portion of the molecule, R·CO·NH·CH(CHO)·CO₂H, which is an α-amino-acid derivative, is replaced by the homologous β-amino-acid grouping R·CO·NH·CR'·Me·CH(CHO)·CO₂H (R' = H or Me). In these cases the penicillins, if produced, would possess the grouping R·CO·NH·CR'·Me attached to the β-lactam ring instead of R·CO·NH, and as it is well known that considerable modifications of this part of the molecule are possible without loss of antibacterial properties it is quite possible that such homologous penicillins would be biologically active.

The first experiments were designed to synthesise by method (*a*) a penicillin in which the penaldic acid grouping is replaced by β-benzamido-α-formylisovaleric acid,



The synthesis of the required 6-keto-2-phenyl-4 : 4-dimethyl-5-alkoxymethylene-5 : 6-dihydro-1 : 3-oxazine (III) was, therefore, attempted. β-Benzamidoisovaleric acid,

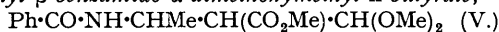


was first prepared by the benzylation of β-aminoisovaleric acid (Slimmer, *Ber.*, 1902, **35**, 408), but a better method is to benzyloxy ethyl β-aminoisovalerate, and hydrolyse the resulting ethyl β-benzamidoisovalerate. By treating β-benzamidoisovaleric acid with acetic anhydride the liquid anhydro-derivative 6-keto-2-phenyl-4 : 4-dimethyl-5 : 6-dihydro-1 : 3-oxazine (IV) is obtained. This 1 : 3-oxazine, when exposed to air or when treated with water, undergoes slow hydration with regeneration of the solid parent acid, but the reaction is catalysed by hydrogen ions, and occurs in a few seconds in presence of dilute hydrochloric acid. The 1 : 3-oxazine (IV) reacts slowly with ethyl alcohol to give ethyl β-benzamidoisovalerate, but it reacts immediately with aniline in ethereal solution to give β-benzamidoisovaleraniide. Unlike the related oxazolones derived from the α-amino-acids, the 1 : 3-oxazine (IV) does not possess a reactive methylene group, and attempts to effect condensation with either benzaldehyde, or with ethyl orthoformate in presence of acetic anhydride to give (III; R = Et) were unsuccessful. Again, no formylation occurred when ethyl β-benzamidoisovalerate was treated with ethyl formate and potassium. The greater degree of reactivity of the methylene group in the oxazolones is to be ascribed to the fact that the methylene group is activated not only by the carbonyl but also by the ·N·C group. Moreover, in the 1 : 3-oxazine (IV) the CMe₂ group exerts a marked steric effect in diminishing reactivity at the adjacent methylene group (see below). In view of the failure to obtain the required alkoxy-methylene-1 : 3-oxazine (III), this approach to the synthesis of a penicillin homologue had to be abandoned.

The 1 : 3-oxazine (IV) appears to represent a new type of anhydro-derivative not previously prepared from an amino-acid. Such 1 : 3-oxazines are only obtainable from β-amino-acids, but might arise by dehydration of, for example, N-acyl derivatives of aspartic acid, which is simultaneously an α- and a β-amino-acid.

Experiments were then directed towards the preparation of acetals of type (II; R =

CHMe·NH·COPh and CHMe·NH·CO·CH₂Ph) which, by thermal ring closure (method *b*), might be converted into homologues of phenylpenicillin and benzylpenicillin respectively. Ethyl β-amino-*n*-butyrate was treated with benzoyl chloride in pyridine, giving ethyl β-benzamido-*n*-butyrate. Reaction of this ester with aniline gave the corresponding anilide, and although when treated with potassium and ethyl formate it yielded a formyl derivative giving a 2 : 4-dinitrophenylhydrazone, it was found desirable, since ethyl β-amino-*n*-butyrate is not a very accessible compound, to devise an alternative route to compounds of this type. A solution of β-amino-*n*-butyric acid was prepared by heating crotonic acid with aqueous ammonia at 150°, and, without isolation, was treated with benzoyl chloride and alkali, yielding β-benzamido-*n*-butyric acid. The methyl ester of this acid was then formylated, and converted into the dimethylacetal, methyl β-benzamido-α-dimethoxymethyl-*n*-butyrate,



characterised as its 2 : 4-dinitrophenylhydrazone. The ester (V) was now converted via the related hydrazide, Ph·CO·NH·CHMe·CH(CO·NH·NH₂)·CH(OMe)₂ (VI), into the corresponding acid azide, and condensation of the latter with D-penicillamine yielded N-(β-benzamido-α-dimethoxymethyl-*n*-butyryl)-D-penicillamine (II; R = Ph·CO·NH·CHMe).

A similar series of reactions was carried out starting from β-phenylacetamido-*n*-butyric acid, the methyl ester of which was formylated and converted into the acetal, methyl β-phenylacetamido-α-dimethoxymethyl-*n*-butyrate, Ph·CH₂·CO·NH·CHMe·CH(CO₂Me)·CH(OMe)₂ (VII). The two possible racemic forms of the acetal (VII), which contains two dissimilar asymmetrical carbon atoms, were isolated and are referred to as the α- and β-forms. Each was converted into an individual hydrazide, giving the α- and β-forms of β-phenylacetamido-α-dimethoxymethyl-*n*-butyrylhydrazide, Ph·CH₂·CO·NH·CHMe·CH(CO·NH·NH₂)·CH(OMe)₂ (VIII), and these were, in turn, separately converted into the acid azides and condensed with D-penicillamine. The α-hydrazide (VIII) thus gave in a crystalline form one of the four possible stereoisomeric forms of N-(β-phenylacetamido-α-dimethoxymethyl-*n*-butyryl)-D-penicillamine (II; R = Ph·CH₂·CO·NH·CMe); the β-hydrazide (VIII), however, gave a crystalline mixture of stereoisomerides of (II; R = Ph·CH₂·CO·NH·CHMe) which could not be separated. These acylpenicillamines (II), including that previously described having R = Ph·CO·NH·CHMe, are not very stable compounds and can seldom be satisfactorily crystallised and obtained in a state of analytical purity. They are all soluble in alkalis, give a blue colouration with ferric chloride showing the presence of the thiol group, and give 2 : 4-dinitrophenylhydrazones.

The three acylpenicillamines (II; R = Ph·CO·NH·CHMe, and both specimens having R = Ph·CH₂·CO·NH·CHMe) were kindly tested as intermediates in the possible production of antibiotics of the penicillin type by Dr. E. P. Abraham of the Sir William Dunn School of Pathology, Oxford. In each case (II) (10 mg.) was heated at (*a*) 110° for 10 minutes, (*b*) 130° for 10 minutes, (*c*) 130° for 10 minutes, followed by a temperature rise to 160° during 10 minutes. The products were stirred with M/5-phosphate buffer (pH 7) (1 c.c.) and the solutions tested for antibacterial activity against *Staphylococcus aureus* by the plate method. In no case was any antibacterial activity observed.

EXPERIMENTAL.

β-Benzamidoisovaleric Acid.—(*a*) β-Aminoisovaleric acid was prepared in excellent yield from ββ-dimethylacrylic acid (*Org. Synth.*, 1943, **23**, 27) by heating under pressure with concentrated aqueous ammonia (Slimmer, *loc. cit.*), but difficulty was encountered in preparing its benzoyl derivative by Slimmer's procedure. The following method gave a 25% yield. To a vigorously-stirred solution of β-aminoisovaleric acid (11.7 g.) in 2*N*-sodium hydroxide (50 c.c.) cooled in ice was slowly added benzoyl chloride (14.5 g.) and 2*N*-sodium hydroxide (50 c.c.) so that the mixture always remained alkaline. After an hour concentrated hydrochloric acid (20 c.c.) was added and the precipitate collected, washed with a little ether, and crystallised from water, giving β-benzamidoisovaleric acid (5.5 g.) as oblong plates, m. p. 141° (cf. Slimmer, *loc. cit.*).

(*b*) A mixture of ethyl β-benzamidoisovalerate (1 g.) (below) and *N*-potassium hydroxide (10 c.c.) was shaken for 1 hour at room temperature, filtered, and acidified, yielding β-benzamidoisovaleric acid (0.95 g.) as oblong plates from water, m. p. and mixed m. p. 141°.

Ethyl β-Benzamidoisovalerate.—β-Aminoisovaleric acid (11.7 g.) was converted by the method of Fischer (*Ber.*, 1901, **34**, 433) into ethyl β-aminoisovalerate (7.5 g.), b. p. 50°/4 mm. A mixture of ethyl β-aminoisovalerate (4.5 g.) and benzoyl chloride (3.7 c.c.) in anhydrous pyridine (20 c.c.) was kept overnight. After heating on a steam-bath for a further 20 minutes the product was poured into water (50 c.c.) and extracted twice with 50 c.c. portions of ether. The combined extracts were washed with 2*N*-hydrochloric acid (10 c.c.), 2*N*-sodium carbonate solution (10 c.c.), and finally with water, and then dried (MgSO₄). The ether was removed and the residue distilled under reduced pressure, giving a colourless oil, b. p. 158°/6 mm., which solidified on cooling (5.7 g.; 74%). Recrystallisation from light petroleum (b. p. 60—80°) gave ethyl β-benzamidoisovalerate as silky needles, m. p. 49° (Found: C, 67.3; H, 7.6; N, 6.0. C₁₄H₁₉O₃N requires C, 67.3; H, 7.6; N, 5.6%).

6-Keto-2-phenyl-4 : 4-dimethyl-5 : 6-dihydro-1 : 3-oxazine (IV).—A mixture of β -benzamidoisovaleric acid (2 g.) and acetic anhydride (6 c.c.) was heated on a steam-bath for 4 hours. After removal of low-boiling material under reduced pressure, the residual liquid (1.7 g.; 92%) distilled as a colourless oil, b. p. 110—111°/3 mm. (Found : C, 70.2; H, 6.2; N, 6.6. $C_{12}H_{13}O_2N$ requires C, 70.9; H, 6.4; N, 6.9%). When this 6-keto-2-phenyl-4 : 4-dimethyl-5 : 6-dihydro-1 : 3-oxazine was treated with dilute mineral acid, it rapidly gave β -benzamidoisovaleric acid, m. p. and mixed m. p. 141°. When the 1 : 3-oxazine (500 mg.) and absolute ethanol (0.7 c.c.) were refluxed for 2 hours, and excess of ethanol removed, a solid (510 mg.; 83%) was left, m. p. 47°; mixed m. p. with ethyl β -benzamidoisovalerate 49°.

β -Benzamidoisovaleranilide.—(a) Aniline (100 mg.) was added to a solution of the 1 : 3-oxazine (200 mg.) in ether (1 c.c.). White needles almost immediately began to separate and after 12 hours were collected (235 mg.; 80%) and crystallised from ethanol; fine needles, m. p. 170° (Found : C, 72.5; H, 6.7; N, 9.5. $C_{18}H_{20}O_2N_2$ requires C, 73.0; H, 6.8; N, 9.5%).

(b) β -Benzamidoisovaleric acid (1 g.) and aniline (2 c.c.) were heated at 170—180° for 1 hour, poured into water, and acidified with hydrochloric acid. The precipitate was washed with sodium carbonate solution and water (yield, 1.3 g.); needles from ethanol, m. p. and mixed m. p. 170°.

Ethyl β -Benzamido-n-butyrate.—A mixture of ethyl β -amino-n-butyrate (9 g.; Décombe, *Ann. Chim. Phys.*, 1932, 18, 133) and benzoyl chloride (9.7 c.c.) in anhydrous pyridine (50 c.c.) was kept overnight, heated on a steam-bath for 1 hour, poured into water (100 c.c.), and extracted twice with ether (100 c.c.). The extracts were washed with 2N-hydrochloric acid, then with 2N-sodium hydroxide, and distilled, giving a colourless oil (9.4 g.; 58%), b. p. 158—160°/2 mm., which solidified on cooling. Recrystallisation from a large volume of light petroleum (b. p. 60—80°) gave ethyl β -benzamido-n-butyrate as a crystalline powder, m. p. 47.5° (Found : C, 66.6; H, 7.7; N, 6.9. $C_{15}H_{17}O_3N$ requires C, 66.4; H, 7.3; N, 6.0%).

This ester was converted into the anilide, which was crystallised from ethyl acetate and then from isopropanol, giving fine needles, m. p. 190° (Found : C, 73.2; H, 6.5; N, 9.6. $C_{17}H_{18}O_2N_2$ requires C, 72.4; H, 6.4; N, 9.9%).

β -Benzamido-n-butyric Acid.—A solution of β -amino-n-butyric acid was prepared by heating crotonic acid (40 g.) with concentrated ammonia (250 c.c.; d 0.880) for 24 hours at 150° (Engle, *Bull. Soc. chim.*, 1888, 50, 102). Air was drawn through the solution to remove most of the free ammonia and the process completed by boiling. The aqueous solution was diluted (to 300 c.c.) and sodium hydroxide (60 g.) added. Benzoyl chloride (165 c.c.) and a solution of sodium hydroxide (70 g.) in water (350 c.c.) were then added simultaneously to the stirred solution at 0° so that it remained alkaline. After 3 hours the mixture was filtered, acidified, and kept overnight in a refrigerator, and the precipitate collected, dried, and extracted with hot light petroleum (b. p. 60—80°) to remove benzoic acid. The insoluble residue was crystallised from hot water (1 l.), giving β -benzamido-n-butyric acid (60 g.; 63%) as colourless needles, m. p. 151.5° (Found : C, 63.9, 64.0; H, 6.6, 6.3; N, 6.3. $C_{11}H_{13}O_3N$ requires C, 63.8; H, 6.3; N, 6.8%).

Methyl β -Benzamido-n-butyrate.— β -Benzamido-n-butyric acid (95 g.) was refluxed with anhydrous methanol (180 c.c.) containing hydrogen chloride (7.5 g.) for 8 hours. Excess of methyl alcohol was removed under reduced pressure, and the residue dissolved in chloroform and extracted with 2N-sodium carbonate solution (acidification gave 5.6 g. of the unchanged acid). The chloroform layer was dried ($MgSO_4$) and distilled, yielding a colourless oil, b. p. 165—166°/3 mm. (81 g.; 80%), which solidified on cooling. Recrystallisation from light petroleum (b. p. 60—80°) gave methyl β -benzamido-n-butyrate as fine needles, m. p. 83° (Found : C, 65.1; H, 6.8; N, 5.5. $C_{12}H_{15}O_3N$ requires C, 65.2; H, 6.8; N, 6.3%).

Methyl β -Benzamido- α -dimethoxymethyl-n-butyrate (V).—Powdered potassium (18 g.) was added to a suspension of methyl β -benzamido-n-butyrate (92 g.) in anhydrous ether (800 c.c.). After addition of anhydrous freshly-distilled methyl formate (125 c.c.) the mixture was stirred mechanically at 0° for 18 hours, when the potassium derivative had separated as a dark brown oil. The bottom layer was poured into a mixture of ice (200 g.), water (125 c.c.), and chloroform (100 c.c.). To this was added the aqueous layer obtained by shaking the ethereal extract with ice (50 g.) and water (50 c.c.); unchanged ester (28 g.) was recovered from this ethereal extract. The mixture was stirred and the chloroform layer discarded. More chloroform (100 c.c.) was added, and a mixture of concentrated hydrochloric acid (50 c.c.) and ice (25 g.) was added slowly with stirring. After several minutes the organic layer was separated and the aqueous layer extracted three times with 50 c.c. portions of chloroform. The chloroform extracts were combined, washed with water (50 c.c.), and dried ($MgSO_4$). Filtering, treatment with charcoal, and removal of the solvent at room temperature under diminished pressure gave methyl β -benzamido- α -formyl-n-butyrate (79 g.; 76%) as a pale yellow oil.

Saturated methanolic hydrogen chloride (35 c.c.) was added to a cooled solution of the formyl compound (79 g.) in absolute methanol (150 c.c.) and the mixture kept at 0° for 48 hours, after which hydrogen chloride and methanol were removed under reduced pressure. The oily residue was dissolved in ether (200 c.c.), the solution shaken with water (250 c.c.) and ice (125 g.), and the ethereal layer washed twice with ice cold 2N-sodium hydroxide and with water, and finally dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure, giving somewhat crude methyl β -benzamido- α -dimethoxymethyl-n-butyrate (31.6 g.; 37%) as a colourless oil (Found : C, 59.3; H, 7.2; N, 4.2; OMe, 26.7. $C_{15}H_{21}O_5N$ requires C, 61.0; H, 7.1; N, 4.7; OMe, 31.1%). This acetal gave a 2 : 4-dinitrophenyl-hydrazone which separated from methanol as fine yellow needles, m. p. 200° (decomp.) (Found : C, 53.3; H, 4.4; N, 15.9. $C_{15}H_{19}O_5N_5$ requires C, 53.1; H, 4.4; N, 16.3%).

β -Benzamido- α -dimethoxymethyl-n-butyryl Hydrazide (VI).—Enough methanol was added to methyl β -benzamido- α -dimethoxymethyl-n-butyrate (13.2 g.) and 96% hydrazine hydrate (4.5 c.c.) to give a homogeneous solution, and the mixture refluxed for 24 hours. On cooling, a crystalline solid separated; this was collected and washed with a little ethanol (yield, 8.6 g.; 66%). Recrystallisation from ethanol gave β -benzamido- α -dimethoxymethyl-n-butyryl hydrazide as fine white needles, m. p. 184.5° (Found : C, 56.4; H, 6.9; N, 14.0. $C_{14}H_{21}O_4N_3$ requires C, 56.9; H, 7.1; N, 14.2%).

N-(β -Benzamido- α -dimethoxymethyl-n-butyryl)-D-penicillamine (II; R = Ph.CO.NH.CHMe).—A solu-

tion of the above hydrazide (1 g.) in *N*/2-hydrochloric acid (10 c.c.) was treated at 0° with a solution of sodium nitrite (260 mg.) in water (8 c.c.). The azide immediately separated as a white microcrystalline solid, which was collected after 20 minutes, washed with water, and added to a solution of *D*-penicillamine hydrochloride (1.890 g.) and anhydrous sodium carbonate (1.350 g.) in water (25 c.c.). The mixture was stirred at 30–40° for 3 hours, and was then filtered from a small amount of solid: this crystallised from ethanol in fine, colourless needles, *m. p.* 148°; it gave a 2:4-dinitrophenylhydrazone and a negative test for sulphur, and analysis suggested it was the *urea* derivative (Ph·CO·NH·CHMe·CH[CH(OMe)₂]·NH)₂CO (Found: C, 61.4; H, 6.5; N, 9.6. C₂₇H₃₈O₇N₄ requires C, 61.2; H, 7.2; N, 10.6%). The filtrate was extracted with ether (25 c.c.), and the aqueous layer covered with ether (25 c.c.) and acidified with 2*N*-hydrochloric acid (15 c.c.). The ethereal layer was separated after shaking, and the aqueous layer extracted twice with ether (25 c.c. portions). The combined extracts were dried (MgSO₄) and evaporated under reduced pressure, leaving a white microcrystalline solid, *m. p.* 105–106° (decomp.) (715 mg.; 51%). This compound could not be recrystallised satisfactorily (Found: C, 53.6; H, 6.9; N, 8.4; S, 6.2; OMe, 12.3. C₁₉H₂₈O₆N₂S requires C, 55.3; H, 6.8; N, 6.8; S, 7.7; OMe, 15.0%). It gave a 2:4-dinitrophenylhydrazone, is soluble in alkali, and gave a strong blue ferric chloride reaction.

β-Phenylacetamido-*n*-butyric Acid.—The phenylacetylation of *β*-amino-*n*-butyric acid was carried out in the same way as the benzylation. A solution of the amino-acid, obtained from crotonic acid (40 g.), was diluted to 300 c.c., and sodium hydroxide (60 g.) was added. To this solution was added phenylacetyl chloride (185 c.c.) and a solution of sodium hydroxide (70 g.) in water (300 c.c.). The precipitate obtained on acidification was washed with cold benzene, giving a crude material (55 g.; 54%) which was crystallised from benzene and then from ethyl acetate. *β*-Phenylacetamido-*n*-butyric acid was obtained as a white microcrystalline powder, *m. p.* 108° (Found: C, 65.3; H, 6.8; N, 6.2. C₁₂H₁₅O₃N requires C, 65.2; H, 6.8; N, 6.3%).

Methyl β-Phenylacetamido-*n*-butyrate.—The preceding acid (28 g.) in absolute methanol (250 c.c.) containing anhydrous hydrogen chloride (12.5 g.) was refluxed for 8 hours. The product was worked up as in the case of the corresponding benzoyl derivative, giving an oil, *b. p.* 171–172°/3 mm. (21.2 g.; 71%), which solidified. Crystallisation from light petroleum (*b. p.* 60–80°) gave *methyl β*-phenylacetamido-*n*-butyrate as fine needles, *m. p.* 50° (Found: C, 66.5; H, 7.3; N, 6.2. C₁₃H₁₇O₃N requires C, 66.4; H, 7.2; N, 6.0%).

α- and *β*-Forms of *Methyl β*-Phenylacetamido-*α*-dimethoxymethyl-*n*-butyrate (VII).—Powdered potassium (7.3 g.) was added to a suspension of *methyl β*-phenylacetamido-*n*-butyrate (40 g.) in anhydrous ether (300 c.c.) and anhydrous redistilled methyl formate (26 c.c.) added. After being stirred at 0° for 18 hours, the potassium derivative was worked up to give *methyl β*-phenylacetamido-*α*-formyl-*n*-butyrate, as a pale yellow oil (38 g.; 85%) which was dissolved in anhydrous methanol (75 c.c.), treated with saturated methanolic hydrogen chloride (15 c.c.), and kept at 0° for 24 hours, after which the excess methanol and hydrogen chloride were removed under reduced pressure at 20°. The oily residue was shaken with ether (100 c.c.) and ice (100 g.), and the ethereal layer washed twice with 2*N*-sodium hydroxide (25 c.c.) and with water. When this ethereal solution was dried (MgSO₄), a solid separated on standing. The drying agent and the solid were collected, the magnesium sulphate dissolved in water, and the residue (3 g.; 7%) crystallised from water, leaving the *α*-form of *methyl β*-phenylacetamido-*α*-dimethoxymethyl-*n*-butyrate as fine, colourless needles, *m. p.* 112° (Found: C, 62.4; H, 7.6; N, 4.3. C₁₆H₂₃O₅N requires C, 62.1; H, 7.4; N, 4.5%).

Removal of the ether from the filtrate at room temperature gave a yellow oil which solidified on standing (26 g.; 58%). Recrystallisation from benzene–light petroleum (*b. p.* 60–80°) gave the *β*-form of (VII) as fine, colourless needles, *m. p.* 76° (Found: C, 62.1, 62.2; H, 7.2, 7.5; N, 4.2%).

α-Form of *β*-Phenylacetamido-*α*-dimethoxymethyl-*n*-butyryl Hydrazide (VIII).—Anhydrous methanol (1.0 c.c.) was added to the *α*-form of *methyl β*-phenylacetamido-*α*-dimethoxymethyl-*n*-butyrate (360 mg.) and 96% hydrazine hydrate (0.19 c.c.) to give a clear solution. Crystals soon separated, and after 48 hours solvent and excess of hydrazine were removed in a vacuum over concentrated sulphuric acid. The *β*-phenylacetamido-*α*-dimethoxymethyl-*n*-butyryl hydrazide crystallised from ethanol in fine, colourless needles, *m. p.* 188° (Found: C, 58.3, 58.5; H, 7.6, 7.5; N, 13.7. C₁₅H₂₃O₄N₃ requires C, 58.3; H, 7.4; N, 13.6%).

α-Form of *N*-(*β*-Phenylacetamido-*α*-dimethoxymethyl-*n*-butyryl)-*D*-penicillamine (II; R = Ph·CH₂·CO·NH·CHMe).—This was prepared from the above hydrazide (1.050 g.) as in the case of the corresponding benzoyl derivative (II; R = Ph·CO·NH·CHMe) (above). The crude *N*-(*β*-phenylacetamido-*α*-dimethoxymethyl-*n*-butyryl)-*D*-penicillamine (1.120 g.; *m. p.* 152–154°) was crystallised from aqueous ethanol, giving needles, *m. p.* 185° (Found: C, 57.1; H, 6.8; N, 5.9; S, 6.2; OMe, 10.1. C₂₀H₃₀O₆N₂S requires C, 56.3; H, 7.0; N, 6.6; S, 7.5; OMe, 11.6%). It gave a 2:4-dinitrophenylhydrazone, was soluble in alkali, and gave a strong blue ferric chloride reaction.

β-Form of *β*-Phenylacetamido-*α*-dimethoxymethyl-*n*-butyryl Hydrazide (VIII).—The *β*-form was prepared as in the case of the *α*-form, and after crystallising from ethanol was obtained as fine needles, *m. p.* 181° (Found: C, 58.2; H, 7.4; N, 13.7. C₁₅H₂₃O₄N₃ requires C, 58.3; H, 7.4; N, 13.6%).

β-Form of *N*-(*β*-Phenylacetamido-*α*-dimethoxymethyl-*n*-butyryl)-*D*-penicillamine (II; R = Ph·CH₂·CO·NH·CHMe).—This was prepared as in the case of the benzoyl derivative (II; R = Ph·CO·NH·CHMe). The crude material, having *m. p. ca.* 80° (decomp.) after shrinking from 60°, could not be crystallised satisfactorily and was analysed directly (Found: N, 7.4; S, 5.9; OMe, 13.6. C₂₀H₃₀O₆N₂S requires N, 6.6; S, 7.5; OMe, 14.6%).

It gave a 2:4-dinitrophenylhydrazone, was soluble in alkali, and gave a strong blue ferric chloride reaction.

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