Reactive Acyl Dipeptides as Potential Penicillin Analogues. Part I. α-(Acylaminosuccinimido)-carboxylic Acids

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A number of esters of α-(acylaminosuccinimido)-carboxylic acids were prepared from diesters of *N*-acyl-α-aspartyl- or *N*-acyl-β-aspartyl-α-amino-acids; cyclisation occurred either spontaneously or on heating with triethylamine in ethanol. α-(Acylaminosuccinimido)-carboxylic acids were prepared by catalytic hydrogenation of the benzyl esters or, in less pure form, by heating a dry mixture of the appropriate *N*-acyl aspartic anhydride and α-amino-acid *in vacuo*. Like penicillin, the α-(acylaminosuccinimido)-carboxylic acids could be regarded as acyl dipeptides containing a reactive peptide bond, but they displayed little or no antibacterial activity.

PENICILLIN is considered to exert its antibiotic action by interfering with the formation of certain peptide cross-linkages necessary to the completion of the wall structure in the dividing bacterial cell.[1,2] The penicillin molecule (I) may be regarded as an acyl dipeptide in which the peptide bond has acquired unusual chemical reactivity, including the capacity to act as an acylating agent, by reason of its incorporation in the fused β-lactam–thiazolidine structure. It has therefore been proposed[1] that penicillin may act by acylating the active site of a bacterial enzyme. If this is so, then synthetic acyl dipeptides containing peptide bonds which have been 'activated' in other ways might also display antibacterial properties. Such reasoning led Henery-Logan and Limburg[3] to synthesise *N*-phenylacetylglycylaziridine-2-carboxylic acid (II) which, however, showed

[1] F. P. Doyle and J. H. C. Nayler, *Adv. Drug Research*, 1964, **1**, 59.

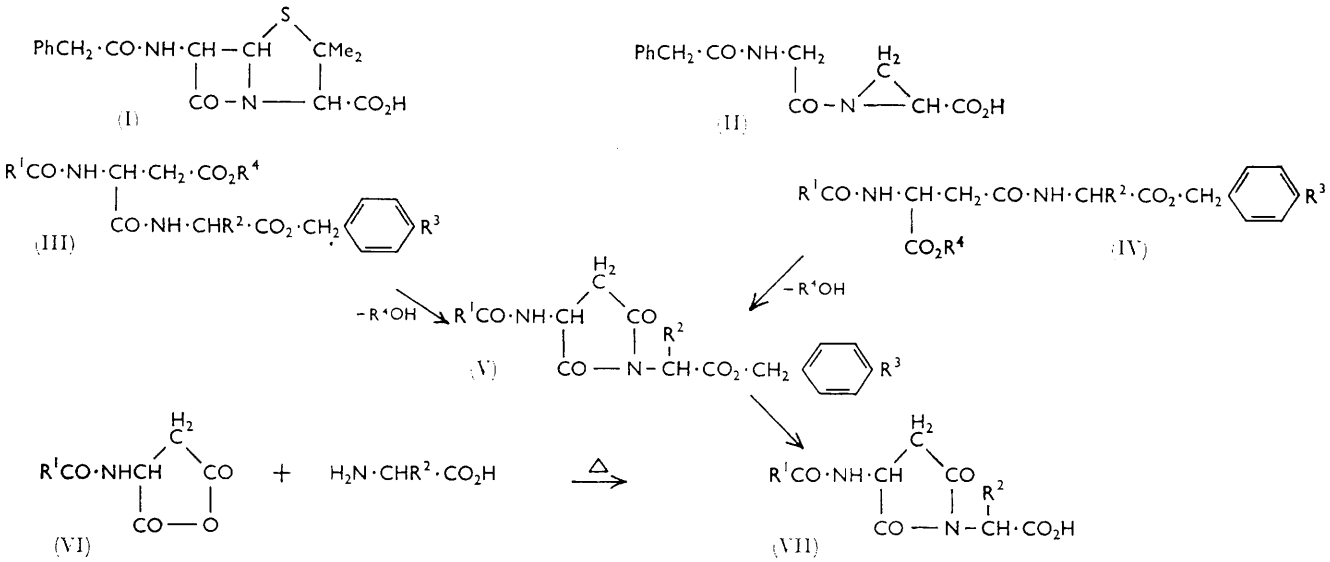
[2] E. Wise and J. Park, *Proc. Nat. Acad. Sci. U.S.A.*, 1965, **54**, 75; D. J. Tipper and J. L. Strominger, *ibid.*, p. 1133; B. F. Erlanger and L. Goode, *Nature*, 1967, **213**, 183.

[3] K. R. Henery-Logan and A. M. Limburg, *Tetrahedron Letters*, 1966, 4915.

only a very low order of antistaphylococcal activity. It occurred to us that the peptide bond could also be 'activated' by incorporation into a diacylimide structure, and in the present Paper we describe the preparation of a number of α-(acylaminosuccinimido)-carboxylic acids (VII). Racemic amino-acids were employed throughout.

Several investigators[4,5] have postulated participation of cyclic intermediates [*e.g.*, (V) or (VII)] in order to account for formation of both α- and β-aspartyl structures during alkaline hydrolysis of various derivatives of aspartyl peptides. According to Iselin and Schwyzer,[6] treatment of *N*-benzyloxycarbonyl-(β-benzyl)-α-L-aspartylglycine *p*-nitrobenzyl ester (III; $R^1 = PhCH_2O$, $R^2 = H$, $R^3 = NO_2$, $R^4 = CH_2Ph$) with dilute aqueous or alcoholic base gave the imide (V; $R^1 = PhCH_2O$, $R^2 = H$, $R^3 = NO_2$), isolated as the ethanolate, m. p. 95—96°. Attempting to repeat their work in the DL-series we condensed β-benzyl *N*-benzyloxycarbonylaspartate and *p*-nitrobenzyl glycinate in the presence of *NN'*-dicyclohexylcarbodi-imide but obtained, in addition to the expected α-aspartyl peptide, m. p. 104—105°, a second product, m. p. 148—149°. Contrary to Iselin and Schwyzer's experience we were unable to cyclise the compound, m. p. 104—105°, by treatment with potassium hydrogen carbonate in aqueous methanol, but elemental analysis of the by-product, m. p. 148—149°, indicated that it was the desired imide.

The corresponding acid (VII; $R^1 = PhCH_2O$, $R^2 = H$) was prepared by a procedure analogous to that of Sheehan and Laubach[7] for succinimidoacetic acid. A dry mixture of glycine and *N*-benzyloxycarbonylaspartic anhydride (VI; $R^1 = PhCH_2O$) was heated under high vacuum to give a hygroscopic acid which could not be obtained in pure crystalline form but which was readily characterised as the *p*-nitrobenzyl ester, m. p. 148—149°, identical with the previous specimen. Treatment with hydrogen bromide in dry acetic acid cleaved the *N*-benzyloxycarbonyl group but not the *p*-nitrobenzyl ester function. The resulting amine was not purified but was treated at once with phenylacetyl chloride to give *p*-nitrobenzyl α-(phenylacetamidosuccinimido)acetate (V; $R^1 = PhCH_2$, $R^2 = H$, $R^3 = NO_2$).



The phenylacetyl derivative was obtained more conveniently by condensing β-benzyl *N*-phenylacetylaspartate with *p*-nitrobenzyl glycinate in the presence of *NN'*-dicyclohexylcarbodi-imide to give the α-aspartyl peptide diester (III; $R^1 = R^4 = PhCH_2$, $R^2 = H$, $R^3 = NO_2$) which, on treatment with triethylamine in boiling ethanol, lost benzyl alcohol to give the imide (V; $R^1 = PhCH_2$, $R^2 = H$, $R^3 = NO_2$). Triethylamine proved to be an excellent general reagent for such cyclisations and was applicable to α- as well as β-esters. Thus the same imide was obtained by elimination of ethanol from *N*-phenylacetyl-(α-ethyl)-β-aspartylglycine *p*-nitrobenzyl ester (IV; $R^1 = PhCH_2$, $R^2 = H$, $R^3 = NO_2$, $R^4 = Et$). In other examples described later in this Paper the hydroxy-compound eliminated was *p*-nitrophenol rather than an alcohol.

In order to obtain the penicillin analogue (VII; $R^1 = PhCH_2$, $R^2 = H$), the benzyl ester (V; $R^1 = PhCH_2$, $R^2 = R^3 = H$) was prepared by a cyclisation procedure similar to that used for the *p*-nitrobenzyl ester. Catalytic hydrogenation removed the benzyl group and, since the resulting acid could not be obtained in pure crystal-

[4] A. R. Battersby and J. C. Robinson, *J. Chem. Soc.*, 1955, 259.

[5] S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Amer. Chem. Soc.*, 1962, **84**, 2421; R. W. Hanson and H. N. Rydon, *J. Chem. Soc.*, 1964, 836; G. Folsch, *Acta Chem. Scand.*, 1966, **20**, 459.

[6] B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, 1962, **45**, 1499.

[7] J. C. Sheehan and G. D. Laubach, *J. Amer. Chem. Soc.*, 1951, **73**, 4376.

line form, it was characterised as the *p*-nitrobenzyl ester described previously. The same esterification procedure was used to characterise a less pure form of the acid (VII; $R^1 = PhCH_2$, $R^2 = H$) obtained by heating a dry mixture of glycine and *N*-phenylacetylaspartic anhydride (VI; $R^1 = PhCH_2$) under high vacuum.

Several of the procedures outlined above were next utilised to prepare α-(benzamidosuccinimido)acetic acid (VII; $R^1 = Ph$, $R^2 = H$). Reaction of *N*-benzoylaspartic anhydride (VI; $R^1 = Ph$) with benzyl glycinate might in theory have given either an α- or a β-aspartyl peptide, but in fact only the former was isolated; esterification of the product with benzyl alcohol gave a peptide ester identical with that prepared from β-benzyl *N*-benzoylaspartate and benzyl glycinate. An example of spontaneous cyclisation of a peptide ester was encountered during an attempt to esterify the free β-carboxy-group of *N*-benzoyl-α-aspartylglycine benzyl ester (III; $R^1 = Ph$, $R^2 = R^3 = R^4 = H$) with *p*-nitrophenol in the presence of *NN'*-dicyclohexylcarbodiimide; the product was benzyl α-(benzamidosuccinimido)acetate (V; $R^1 = Ph$, $R^2 = R^3 = H$). Cyclisation could not be brought about by the carbodi-imide alone, so the initial product must have been the *p*-nitrophenyl ester, which then lost *p*-nitrophenol. α-(Benzamidosuccinimido)acetic acid (VII; $R^1 = Ph$, $R^2 = H$), prepared either by hydrogenation of the benzyl ester or in less pure form by fusion of glycine and *N*-benzoylaspartic anhydride, was conveniently characterised as the *NN'*-dibenzylethylenediamine salt.

An imido-acid (VII) would resemble penicillin (I) even more closely if the α-substituent (R^2) were a suitable alkyl group instead of hydrogen, as in the compounds described hitherto. Reaction of *N*-phenylacetylaspartic anhydride (VI; $R^1 = PhCH_2$) with valine benzyl ester gave a product which may have been either *N*-phenylacetyl-α-aspartylvaline benzyl ester (III; $R^1 = PhCH_2$, $R^2 = CHMe_2$, $R^3 = R^4 = H$) or the β-isomer (IV). The free carboxy-group was then esterified with *p*-nitrophenol and the diester was cyclised with triethylamine to give a product which could only be benzyl α-(phenylacetamidosuccinimido)isovalerate (V; $R^1 = PhCH_2$, $R^2 = CHMe_2$, $R^3 = H$). The free acid (VII; $R^1 = PhCH_2$, $R^2 = CHMe_2$), obtained by catalytic hydrogenolysis, was the only one of its kind obtained in pure crystalline form. A corresponding compound (VII; $R^1 = PhCH_2$, $R^2 = MeS{\cdot}CH_2{\cdot}CH_2$) derived from methionine was characterised as the ethyl ester, and one from glutamine (VII; $R^1 = PhCH_2$, $R^2 = CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2$) as the benzylamine salt.

The various α-(acylaminosuccinimido)-carboxylic acids (VII) 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*. The only active compound was γ-carbamoyl-α-(phenylacetamidosuccinimido)butyric acid which, at a dilution of 1 in 2000, inhibited the growth of *Staphylococcus* Oxford and *Streptococcus pneumoniae*. This represents only about one ten-thousandth of the activity of benzylpenicillin.

[8] A. Berger and E. Katchalski, *J. Amer. Chem. Soc.*, 1951, **73**, 4084.

EXPERIMENTAL

All α-amino-acids were racemic.

N-*Phenylacetylaspartic Acid*.—Phenylacetyl chloride (35 ml.) and 4N-sodium hydroxide (65 ml.) were added simultaneously during 1 hr. to a stirred solution of aspartic acid (35 g.) in 2N-sodium hydroxide (260 ml.). The mixture was stirred at room temperature for 2 hr. more, then acidified with hydrochloric acid. The precipitate was collected and warmed with light petroleum to remove phenylacetic acid, and the residue gave N-*phenylacetylaspartic acid monohydrate* (52 g.), m. p. 117° (from water) (Found: C, 53·4; H, 5·3; N, 4·9. $C_{12}H_{13}NO_5,H_2O$ requires C, 53·5; H, 5·6; N, 5·2%).

Dibenzyl N-*Phenylacetylaspartate*.—A solution of *N*-phenylacetylaspartic acid (9 g.), benzyl alcohol (40 ml.), and toluene-*p*-sulphonic acid (0·5 g.) in toluene (50 ml.) was refluxed for 4 hr. with azeotropic removal of water. The mixture was cooled, stirred with magnesium oxide (1 g.) for 15 min., and filtered. Evaporation of the filtrate left an oil which solidified when triturated with light petroleum and gave the *dibenzyl ester* (93%), m. p. 74—75° (from ether–light petroleum) (Found: C, 72·2; H, 6·2; N, 3·25. $C_{26}H_{25}NO_5$ requires C, 72·4; H, 5·8; N, 3·25%).

β-*Benzyl* N-*Phenylacetylaspartate*.—A solution of dibenzyl *N*-phenylacetylaspartate (4·31 g., 0·01 mole) and sodium hydroxide (0·01 mole) in water (70 ml.) and dioxan (100 ml.) was kept at room temperature for 24 hr. The pH was then adjusted to 5·5 with hydrochloric acid and the solution was concentrated under reduced pressure. The residue was treated with N-potassium hydrogen carbonate solution (10 ml.) and extracted with ether to remove some unchanged dibenzyl ester. Acidification of the aqueous layer gave the monobenzyl ester (81% based on diester consumed) which was formulated as the β-isomer by analogy with the known mode of hydrolysis of the *N*-benzyloxycarbonyl analogue.[8] Crystallisation from aqueous methanol gave β-*benzyl* N-*phenylacetylaspartate*, m. p. 130—131° (Found: C, 66·6; H, 5·9; N, 4·2. $C_{19}H_{19}NO_5$ requires C, 66·8; H, 5·6; N, 4·1%).

N-*Phenylacetylaspartic Anhydride*.—A mixture of *N*-phenylacetylaspartic acid (52 g.) and acetic anhydride (150 ml.) was heated on a steam-bath for 20 min., cooled slightly, and then poured into ether–light petroleum (b. p. 40—60°) (1 : 1; 500 ml.). The precipitate gave the *cyclic anhydride* as needles (41·5 g.), m. p. 162° (from ethyl acetate) (Found: C, 61·9; H, 4·7; N, 5·6. $C_{12}H_{11}NO_4$ requires C, 61·8; H, 4·7; N, 6·0%).

α-*Ethyl* N-*Phenylacetylaspartate*.—A solution of *N*-phenylacetylaspartic anhydride in ethanol was refluxed for 4 hr., then concentrated *in vacuo*. The residual oil was dissolved in ether and extracted with N-sodium carbonate solution. Acidification of the extracts gave an oil which slowly solidified and gave α-*ethyl* N-*phenylacetylaspartate hemihydrate* (60%), m. p. 52° (from water) (Found C, 58·3; H, 6·9; N, 4·9. $C_{14}H_{17}NO_5,0{\cdot}5H_2O$ requires C, 58·3; H, 6·3; N, 4·9%). The product is formulated as the α-ester since this is known to be the major alcoholysis product from *N*-benzoylaspartic anhydride.[4]

p-*Nitrobenzyl* α-(*Benzyloxycarbonylaminosuccinimido*)-*acetate*.—(*a*) β-Benzyl *N*-benzyloxycarbonylaspartate, m. p. 99—101° (from benzene), was prepared from the dibenzyl ester and one equivalent of base as described for the

L-isomer.[8] A solution of the mono-ester (3·6 g.), *p*-nitrobenzyl glycinate (2·1 g.), and *NN'*-dicyclohexylcarbodi-imide (2·1 g.) in methylene dichloride was kept at 0° for 24 hr. and filtered to remove *NN'*-dicyclohexylurea; the filtrate was evaporated under reduced pressure. Treatment of the residue with ether gave a white solid (1·85 g.), m. p. 100—103°, which gave N-*benzyloxycarbonyl*-(β-*benzyl*)-α-*aspartylglycine* p-*nitrobenzyl ester* (1·57 g.), m. p. 104—105° (Found: C, 61·0; H, 5·2; N, 7·7. $C_{28}H_{27}N_3O_9$ requires C, 61·2; H, 5·0; N, 7·7%). Meanwhile the ether filtrate was concentrated to give more solid (1·37 g.), m. p. 142—144°, which gave p-*nitrobenzyl* α-(*benzyloxycarbonylaminosuccinimido*)*acetate*, m. p. 148—149° (from ethyl acetate) (Found: C, 57·3; H, 4·7; N, 9·2. $C_{21}H_{19}N_3O_8$ requires C, 57·1; H, 4·3; N, 9·5%).

(*b*) An intimate mixture of *N*-benzyloxycarbonylaspartic anhydride (5 g.) and glycine (5 g.) was heated in an oil-bath at 170° under high vacuum for 1 hr., then allowed to cool. The resulting viscous oil was extracted with ethanol and the excess of glycine was filtered off. Evaporation of the ethanol filtrate left an amorphous hygroscopic acid (6 g.) which resisted purification. The bulk of this solid (5 g.) was dissolved in a mixture of ethyl acetate (60 ml.) and ethanol (20 ml.) treated with *p*-nitrobenzyl bromide (4 g.) and triethylamine (2·8 ml.), and refluxed for 4 hr. The solvents were removed under reduced pressure and the residue gave *p*-nitrobenzyl α-(benzyloxycarbonylaminosuccinimido)-acetate (2·5 g.), m. p. and mixed m. p. with product of (*a*) 147—149° (from ethyl acetate) (Found: C, 57·2; H, 4·3. N, 9·5%).

p-*Nitrobenzyl* α-(*Phenylacetamidosuccinimido*)*acetate*.—(*a*) *p*-Nitrobenzyl α-(benzyloxycarbonylaminosuccinimido)-acetate (3·19 g.) was stirred with an anhydrous solution of hydrogen bromide in acetic acid (36%; 18 g.) until dissolution was complete, then treated with dry ether until precipitation of crude *p*-nitrobenzyl α-(aminosuccinimido)-acetate hydrobromide ceased. The salt was crystallised once from ethanol (yield 0·8 g.; m. p. 162—164°), then dissolved in dry chloroform (80 ml.) containing triethylamine (0·56 ml.) and treated dropwise with a solution of phenylacetyl chloride (0·28 ml.) in dry chloroform (40 ml.). The mixture was set aside for 4 hr., then washed successively with sodium hydrogen carbonate solution (3%; 40 ml.) and water (2 × 40 ml.). The chloroform layer was dried and evaporated, then the residue was triturated with ether. The resulting p-*nitrobenzyl* α-(*phenylacetamidosuccinimido*)-*acetate* (0·72 g.) had m. p. 123—124° (from ethyl acetate) (Found: C, 59·0; H, 4·8; N, 10·1. $C_{21}H_{19}N_3O_7$ requires C, 59·3; H, 4·5; N, 9·9%).

(*b*) A solution of β-benzyl *N*-phenylacetylaspartate (6·8 g., 0·02 mole) and *p*-nitrobenzyl glycinate (0·02 mole; freshly liberated from the hydrobromide) in methylene dichloride was treated with *NN'*-dicyclohexylcarbodi-imide (4·2 g., 0·02 mole). After 24 hr. *NN'*-dicyclohexylurea was filtered off and the filtrate was evaporated. The residue gave N-*phenylacetyl*-(β-*benzyl*)-α-*aspartylglycine* p-*nitrobenzyl ester* (6·5 g.), m. p. 151—153° (from ethanol) (Found: C, 63·1; H, 5·2; N, 7·8. $C_{28}H_{27}N_3O_8$ requires C, 63·0; H, 5·1; N, 7·9%). This ester (1 g.) was dissolved in ethanol and treated with triethylamine (1 ml.); the mixture was refluxed for 2 hr. and evaporated. Trituration of the residue with ether gave *p*-nitrobenzyl α-(phenylacetamidosuccinimido)acetate (0·45 g.), which, after purification as before, was identical with the product from (*a*).

(*c*) A solution of α-ethyl *N*-phenylacetylaspartate (7 g., 0·025 mole) and *p*-nitrobenzyl glycinate (5 g., 0·025 mole) in dry chloroform (100 ml.) was treated with *NN'*-dicyclohexylcarbodi-imide (5 g., 0·025 mole) and set aside at room temperature. Next morning *NN'*-dicyclohexylurea was filtered off, the filtrate was evaporated, and the residue gave N-*phenylacetyl*-(α-*ethyl*)-β-*aspartylglycine* p-*nitrobenzyl ester* (9·7 g.), m. p. 135—136° (from ethyl acetate) (Found: C, 58·6; H, 5·4. $C_{23}H_{25}N_3O_8$ requires C, 58·6; H, 5·3%). This ester (3·5 g.) and triethylamine (7 ml.) were refluxed in ethanol for 2 hr., the solvent was evaporated off, and the residue was triturated with ether. The resulting solid, crystallised from ethyl acetate, gave *p*-nitrobenzyl α-(phenylacetamidosuccinimido)acetate (2·5 g.) identical with the previous samples.

(*d*) A solution of β-benzyl *N*-phenylacetylaspartate (8·5 g., 0·025 mole) and benzyl glycinate (freshly liberated from 0·03 mole of the benzenesulphonate) in methylene dichloride was treated with *NN'*-dicyclohexylcarbodi-imide (6·25 g.) at 0° for 24 hr., then filtered to remove *NN'*-dicyclohexylurea. The filtrate was evaporated and the residue gave N-*phenylacetyl*-(β-*benzyl*)-α-*aspartylglycine benzyl ester* (6·8 g.), m. p. 119·5—120° (from ethanol) (Found: C, 68·6; H, 5·7; N, 6·0. $C_{28}H_{23}N_2O_6$ requires C, 68·8; H, 5·8; N, 5·7%). This ester (12 g.) and triethylamine (12 ml.) in ethanol were refluxed for 2 hr., the mixture was evaporated under reduced pressure, and the residue was triturated with ether. The resulting solid gave *benzyl* α-(*phenylacetamidosuccinimido*)*acetate* (4·5 g.), m. p. 101—102° (from ethanol) (Found: C, 66·1; H, 5·6; N, 7·6. $C_{21}H_{20}N_2O_5$ requires C, 66·3; H, 5·3; N, 7·4%). A solution of this ester in ethanol was hydrogenated at room temperature and pressure over 10% palladium–charcoal. A quantitative yield of α-(phenylacetamidosuccinimido)acetic acid was obtained as a hygroscopic amorphous solid which could not be induced to crystallise. A portion of this acid (0·58 g.) in ethyl acetate (25 ml.) was refluxed with *p*-nitrobenzylbromide (0·44 g.) and triethylamine (0·2 ml.) for 2 hr. The solution was concentrated to *ca.* 10 ml., cooled, and filtered to remove triethylamine hydrobromide. Addition of light petroleum to the filtrate gave crystals of *p*-nitrobenzyl α-(phenylacetamidosuccinimido)acetate (0·23 g.), m. p. 123—124°, identical with previous samples.

(*e*) Crude α-(phenylacetamidosuccinimido)acetic acid was prepared by fusion of *N*-phenylacetylaspartic anhydride and glycine as described for the *N*-benzyloxycarbonyl series. The hygroscopic product (4·5 g.) in ethanol (100 ml.) was treated with *p*-nitrobenzyl bromide (3·5 g.) and triethylamine (2·2 ml.); the solution was refluxed for 2 hr. and evaporated. Repeated recrystallisation of the residue, first from ethyl acetate–light petroleum and then from ethyl acetate alone, gave *p*-nitrobenzyl α-(phenylacetamidosuccinimido)acetate (0·75 g.) identical with all previous specimens.

p-*Nitrobenzyl* α-(*Benzamidosuccinimido*)*acetate*.— (*a*) β-Benzyl *N*-benzoylaspartate [9] (3·2 g.) and *p*-nitrobenzyl glycinate (2·2 g.) in methylene dichloride (60 ml.) were treated with *NN'*-dicyclohexylcarbodi-imide (2·1 g.) at 0° for 24 hr., then filtered to remove *NN'*-dicyclohexylurea. The filtrate was evaporated under reduced pressure and the residue gave N-*benzoyl*-(β-*benzyl*)-α-*aspartylglycine* p-*nitrobenzyl ester* (4·4 g.), m. p. 128—129° (from ethyl acetate–ethanol) (Found: C, 62·4; H, 4·9; N, 8·1.

[9] L. Benoiton, R. W. Hanson, and H. N. Rydon, *J. Chem. Soc.*, 1964, 824.

$C_{27}H_{25}N_3O_8$ requires C, 62·4; H, 4·9; N, 8·1%). This ester (0·5 g.) in ethanol (10 ml.) was refluxed with triethylamine (1 ml.) for 2 hr., then the mixture was evaporated under reduced pressure and the residue triturated with ether. The resulting solid gave p-*nitrobenzyl* α-(*benzamidosuccinimido*)*acetate* (0·2 g.), m. p. 164—165° (from ethyl acetate–light petroleum) (Found: C, 58·4; H, 4·6; N, 10·2. $C_{20}H_{17}N_3O_7$ requires C, 58·4; H, 4·2; N, 10·2%).

(*b*) α-Ethyl *N*-benzoylaspartate [4] (1·1 g.) and *p*-nitrobenzyl glycinate (1 g.) were coupled by the carbodi-imide procedure as in (*a*) to give *N-benzoyl-*(α-*ethyl*)-β-*aspartylglycine* p-*nitrobenzyl ester* (1·05 g.), m. p. 177—178° (from ethanol) (Found: C, 57·6; H, 5·3; N, 9·2. $C_{22}H_{23}N_3O_8$ requires C, 57·8; H, 5·1; N, 9·2%). This was cyclised as before with triethylamine in boiling ethanol to give *p*-nitrobenzyl α-(benzamidosuccinimido)acetate (40%), m. p. and mixed m. p. with product of (*a*) 164—165° (Found: C, 58·7; H, 4·3; N, 10·2%).

N-Benzoyl-(β-*benzyl*)-α-*aspartylglycine Benzyl Ester.*—(*a*) β-Benzyl *N*-benzoylaspartate [9] (13 g.) was condensed with benzyl glycinate by the usual carbodi-imide procedure to give *N-benzoyl-*(β-*benzyl*)-α-*aspartylglycine benzyl ester* (4 g.), m. p. 95—96° (from ethanol) (Found: C, 68·8; H, 5·7; N, 5·9. $C_{27}H_{26}N_2O_6$ requires C, 68·3; H, 5·5; N, 5·9%).

(*b*) *N*-Benzoylaspartic anhydride (10·5 g., 0·05 mole) was suspended in dry ethyl acetate (100 ml.) and stirred while benzyl glycinate (freshly liberated from 40 g. of the benzene sulphonate) in ethyl acetate (50 ml.) was added rapidly. Dissolution of the anhydride was followed by slow precipitation of a heavy white solid, which gave *N-benzoyl-*α-*aspartylglycine benzyl ester* (15 g.), m. p. 165—166° (from methanol) (Found: C, 62·4; H, 5·2; N, 7·4. $C_{20}H_{20}N_2O_6$ requires C, 62·5; H, 5·2; N, 7·3%). This ester (4·26 g.) and toluene-*p*-sulphonic acid (0·2 g.) in benzyl alcohol (15 ml.) and toluene (20 ml.) were refluxed for 3 hr. with azeotropic removal of water. The solution was cooled, stirred with magnesium oxide (0·4 g.) for 30 min., and filtered, and the filtrate was evaporated *in vacuo*. The residue gave *N*-benzoyl-(β-benzyl)-α-aspartylglycine benzyl ester (4·65 g., 91%), m. p. and mixed m. p. with product of (*a*) 95—96° (from ethanol) (Found: C, 68·5; H, 5·8; N, 5·9%).

Benzyl α-(*Benzamidosuccinimido*)*acetate.*—(*a*) *N*-Benzoyl-(β-benzyl)-α-aspartylglycine benzyl ester (1·6 g.) and triethylamine (1·6 ml.) in ethanol (25 ml.) were refluxed for 3 hr.; the solution was then concentrated under reduced pressure. The residue gave *benzyl* α-(*benzamidosuccinimido*)*acetate* (0·6 g.), m. p. 148—149° (from aqueous ethanol) (Found: C, 65·6; H, 5·2; N, 7·8. $C_{20}H_{18}N_2O_5$ requires C, 65·6; H, 5·0; N, 7·7%).

(*b*) *N*-Benzoyl-α-aspartylglycine benzyl ester (8·3 g.) and *p*-nitrophenol (3 g.) in acetone (250 ml.) were treated with *NN'*-dicyclohexylcarbodi-imide (4·45 g.) and set aside overnight. *NN'*-Dicyclohexylurea was filtered off, the filtrate was evaporated under reduced pressure, and the residual oil was triturated with ether. The resulting solid (from aqueous methanol) gave, instead of the expected *p*-nitrophenyl ester, benzyl α-(benzamidosuccinimido)-acetate (3·5 g.) identical with the product from (*a*) (Found: C, 65·5; H, 5·1; N, 7·5%).

α-(*Benzamidosuccinimido*)*acetic Acid.*—(*a*) Benzyl α-(benzamidosuccinimido)acetate (3 g.) in acetone was hydrogenated at room temperature and pressure over 10% palladium–charcoal (0·5 g.) for 8 hr. The product was α-(benzamidosuccinimido)acetic acid (2 g.), an amorphous hygroscopic solid which could not be induced to crystallise. A portion of the acid (0·26 g.) was dissolved in sodium hydrogen carbonate solution (3%; 3 ml.) and treated with *NN'*-dibenzylethylenediamine diacetate (0·18 g.) in water (1 ml.). The *NN'*-dibenzylethylenediamine *salt* (1·85 g.) crystallised when scratched; m. p. 239—240° (Found: C, 63·7; H, 5·3; N, 10·7. $C_{42}H_{44}N_6O_{10}$ requires C, 63·6; H, 5·6; N, 10·6%).

(*b*) An intimate mixture of *N*-benzoylaspartic anhydride (5 g.) and glycine (5 g.) was heated at 170° under high vacuum for 1 hr. The cooled mixture was extracted with ethanol and filtered to remove residual glycine, and the filtrate was evaporated to leave a crude hygroscopic acid (90%). A portion was characterised as in (*a*) as the *NN'*-dibenzylethylenediamine salt (43%), m. p. 239—240° (from ethanol).

Benzyl α-(*Phenylacetamidosuccinimido*)*isovalerate.*—A suspension of *N*-phenylacetylaspartic anhydride (0·078 mole) in dry ethyl acetate was stirred with benzyl valinate (freshly liberated from 0·1 mole of the benzene sulphonate). Next morning some undissolved anhydride was removed by filtration, then the filtrate was extracted with 10% sodium carbonate solution. Acidification of the extracts gave a solid, which gave *N-phenylacetyl-*α(β)-*aspartylvaline benzyl ester* (54%), m. p. 138—139° (Found: C, 65·4; H, 6·5; N, 6·3. $C_{24}H_{28}N_2O_6$ requires C, 65·5; H, 6·4; N, 6·4%). Equimolecular quantities of this peptide, *p*-nitrophenol, and *NN'*-dicyclohexylcarbodi-imide in dry acetone were set aside overnight, then the mixture was filtered to remove *NN'*-dicyclohexylurea. Evaporation of the filtrate left a yellow oil which solidified on trituration with ether and gave *N-phenylacetyl-*[β(α)-p-*nitrophenyl*]-α(β)-*aspartylvaline benzyl ester* (84%), m. p. 124—125° (from ethanol) (Found: C, 63·8; H, 5·5; N, 7·6. $C_{30}H_{31}N_3O_8$ requires C, 64·2; H, 5·6; N, 7·5%). This ester in ethanol was refluxed with triethylamine for 2 hr., then the mixture was concentrated under reduced pressure. The residual oil was triturated with ether and the resulting solid gave *benzyl* α-(*phenylacetamidosuccinimido*)*isovalerate* (33%), m. p. 157—159° (Found: C, 68·6; H, 6·2; N, 6·6. $C_{24}H_{26}N_2O_5$ requires C, 68·2; H, 6·2; N, 6·6%).

α-(*Phenylacetamidosuccinimido*)*isovaleric Acid.*—A solution of benzyl α-(phenylacetamidosuccinimido)isovalerate (1·63 g.) in ethanol was hydrogenated at room temperature and pressure over 10% palladium–charcoal (0·2 g.). The solid product was washed with light petroleum and gave α-(*phenylacetamidosuccinimido*)*isovaleric acid* (82%), m. p. 162—163° (from water) (Found: C, 61·0; H, 6·1; N, 8·2. $C_{17}H_{20}N_2O_5$ requires C, 61·4; H, 6·1; N, 8·4%).

*Ethyl γ-Methylthio-*α-(*phenylacetamidosuccinimido*)-*butyrate.*—(*a*) Equimolecular quantities of α-ethyl *N*-phenylacetylaspartate and methionine ethyl ester in acetone were condensed by the usual *NN'*-dicyclohexylcarbodi-imide procedure to give *N-phenylacetyl-*(α-*ethyl*)-β-*aspartylmethionine ethyl ester* (73%), m. p. 93—94° (from ethyl acetate–light petroleum) (Found: C, 57·8; H, 6·6; N, 6·2; S, 7·3. $C_{21}H_{30}N_2O_6S$ requires C, 57·5; H, 6·9; N, 6·4; S, 7·3%). This ester (3 g.) and triethylamine (9 ml.) in ethanol were refluxed for 2 hr., then the solution was evaporated *in vacuo* and the residual oil was triturated with ether. The resulting solid gave *ethyl γ-methylthio-*α-(*phenylacetamidosuccinimido*)*butyrate* (27%), m. p. 129—130° (from ethyl acetate–light petroleum) (Found: C, 58·4; H, 6·2; N, 7·0; S, 7·9. $C_{19}H_{24}N_2O_5S$ requires C, 58·2; H, 6·2; N, 7·1; S, 8·2%).

(*b*) An intimate mixture of *N*-phenylacetylaspartic anhydride (10 g.) and methionine (10 g.) was heated under high vacuum at 175° for 1 hr., cooled, and extracted with ethanol. Unreacted methionine was filtered off, then the filtrate was evaporated under reduced pressure to leave γ-methylthio-α-(phenylacetamidosuccinimido)butyric acid as an amorphous hygroscopic solid which could not be recrystallised. The crude acid (6 g.) was esterified with saturated ethanolic hydrogen chloride (1 hr. under reflux). The solution was evaporated under reduced pressure and the residual oil was set aside under dry ether. A solid (0·5 g.) gradually separated which gave ethyl γ-methylthio-α-(phenylacetamidosuccinimido)butyrate (0·35 g.), m. p. and mixed m. p. with product of (*a*) 130° (from ethyl acetate–light petroleum) (Found: C, 58·1; H, 6·2; N, 7·1; S, 8·3%).

N-*Phenylacetyl*-(β-*benzyl*)-α-*aspartylglutamine*.— Ethyl chloroformate (0·03 mole) was added dropwise to an ice-cold solution of β-benzyl *N*-phenylacetylaspartate (0·02 mole) and triethylamine (0·03 mole) in dry acetone (50 ml.). The mixture was stirred at 0° for 15 min. until formation of the mixed anhydride was complete, then an aqueous solution of glutamine (0·02 mole) and sodium hydrogen carbonate (0·02 mole) was added during 1 hr. and the mixture was stirred at room temperature for 3 hr. Acetone was removed under reduced pressure and the aqueous concentrate was washed with ether and then acidified to precipitate an oil which solidified when scratched. The product (86%) gave N-*phenylacetyl*-(β-*benzyl*)-α-*aspartylglutamine*, m. p. 177—178° (from ethanol) (Found: C, 61·8; H, 5·6; N, 9·2. $C_{24}H_{27}N_3O_7$ requires C, 61·4; H, 5·8; N, 9·0%).

N-*Phenylacetyl*-(β-*benzyl*)-α-*aspartylglutamine Benzyl Ester*.— *N*-Phenylacetyl-(β-benzyl)-α-aspartylglutamine (0·05 mole) in dioxan was treated dropwise at room temperature with a solution of α-diazotoluene (0·1 mole) in ether. The solution was set aside for 1 hr., cooled, clarified by filtration, and evaporated under reduced pressure. Trituration of the residual oil with ether gave N-*phenylacetyl*-(β-*benzyl*)-α-*aspartylglutamine benzyl ester* (41%), m. p. 157—158° (from ethanol) (Found: C, 66·8; H, 6·0; N, 7·4. $C_{31}H_{33}N_3O_7$ requires C, 66·5; H, 5·9; N, 7·5%).

Benzyl γ-Carbamoyl-α-(phenylacetamidosuccinimido)-butyrate.— *N*-Phenylacetyl-(β-benzyl)-α-aspartylglutamine benzyl ester (0·02 mole) in ethanol was refluxed with triethylamine (0·2 mole) for 2 hr., then evaporated to an oil which crystallised when triturated with ether and gave *benzyl γ-carbamoyl-α-(phenylacetamidosuccinimido)butyrate* (20%), m. p. 147° (from ethyl acetate) (Found: C, 63·2; H, 5·6; N, 9·6. $C_{24}H_{25}N_3O_6$ requires C, 63·8; H, 5·6; N, 9·3%).

γ-Carbamoyl-α-(phenylacetamidosuccinimido)butyric Acid.—A solution of benzyl γ-carbamoyl-α-(phenylacetamidosuccinimido)butyrate (1·3 g.) in acetone was hydrogenated at room temperature and pressure over 10% palladium–charcoal catalyst (0·5 g.). The product was the amorphous hygroscopic acid (80%), which could not itself be crystallised, so a solution of it in tetrahydrofuran was treated with benzylamine in ether to precipitate the *benzylamine salt*. This crystallised from ethyl acetate as the monohydrate, m. p. 124° (Found: C, 59·6; H, 6·1; N, 11·7. $C_{24}H_{28}N_4O_6,H_2O$ requires C, 59·3; H, 6·2; N, 11·5%).

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