Polypeptides. Part XXIII.[1] The Synthesis of Peptides of α-Benzylphenylalanine by Unconventional Methods, with a Note on Magnetic Non-equivalence in Derivatives of α-Benzylphenylalanine

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α-Benzylphenylalanine is best prepared by use of the Ugi four-component condensation method, followed by hydrolysis and hydrogenolysis of the intermediate *N*-acetyl-*N*,α-dibenzylphenylalanine. The same general procedure gives good yields of α-benzylphenylalanine peptides, but is limited in its application owing to the complete racemisation which attends the conversion of L-amino-acid esters into the isocyanides. The oxazolinone method is useful for the synthesis of peptides of α-benzylphenylalanine by the fragment condensation strategy employing small peptides containing *C*-terminal α-benzylphenylalanine residues, but breaks down with larger peptides.

Many, but not all, derivatives of α-benzylphenylalanine show magnetic non-equivalence of the benzyl methylene protons.

PART XXII [1] described the synthesis of a number of peptides of α-benzylphenylalanine by use of dicyclohexylcarbodi-imide. This method is, however, subject to serious limitations and a better method is desirable. The present paper deals with a study of two unconventional methods, *viz.* the Ugi and oxazolinone procedures, for the synthesis of peptides of α-benzylphenylalanine.

In our earlier work [1] we prepared α-benzylphenylalanine (IV) from ethyl acetoacetate in 32% overall yield by a modification of the method of Felkin.[2] In the present work we used the ' four-component condensation ' procedure devised by Ugi and his colleagues and employed by them for the synthesis of a number of *N*-acylamino-carboxamides.[3] The Schiff's base (I),† prepared from dibenzyl ketone and benzylamine, reacted with phenyl isocyanide and acetic acid in methanol at room temperature to give *N*-acetyl-*N*,α-dibenzylphenylalanine anilide (II). Acid hydrolysis of this anilide gave *N*,α-dibenzylphenylalanine (III), catalytic hydrogenolysis of which gave α-benzylphenylalanine (IV). The overall yield of (IV) was 56% and this method is much more convenient and reproducible than that used before.

$$\underset{\text{(I)}}{AcOH + PhCH_2{\cdot}N{:}C(CH_2Ph)_2} + CNPh \longrightarrow AcN(CH_2Ph){\cdot}C(CH_2Ph)_2{\cdot}CO{\cdot}NHPh \quad \text{(II)}$$

$$PhCH_2{\cdot}NH{\cdot}C(CH_2Ph)_2{\cdot}CO_2H \quad \text{(III)} \longrightarrow H_2N{\cdot}C(CH_2Ph)_2{\cdot}CO_2H \quad \text{(IV)}$$

α-Benzylphenylalanine failed to give any t-butyl ester by acid-catalysed addition to isobutene;[4] a similar observation was made by Kenner and his colleagues [5] in the case of α-methylalanine. The failure of this reaction cannot be due entirely to steric hindrance, however, since *N*,α-dibenzylphenylalanine (III), like α-phenylalanine,[5] gave the t-butyl ester by this method, albeit only in low yield (25%); the reaction is, nevertheless, preparatively acceptable, since unchanged amino-acid is easily recovered and recycled. Catalytic hydrogenolysis of the t-butyl ester of (III) gave an excellent yield of the required α-benzylphenylalanine t-butyl ester.

The successful use of the Ugi ' four-component condensation ' reaction for the preparation of α-benzylphenylalanine prompted us to study its applicability to the synthesis of peptides of α-benzylphenylalanine; Ugi [6] has reported the successful use of the method in the synthesis of a tripeptide of α-methylphenylalanine, but gives no experimental details. The protected α-benzylphenylalanine tripeptide (V) was obtained directly, in high yield, by the reaction between *N*-phthaloylglycine, the Schiff's base (I), and t-butyl isocyanoacetate,[7] thus:

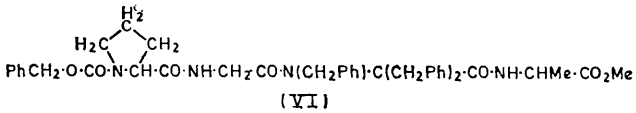
$$C_6H_4(CO)_2N{\cdot}CH_2{\cdot}CO_2H + (I) + CN{\cdot}CH_2{\cdot}CO_2Bu^t \longrightarrow C_6H_4(CO)_2N{\cdot}CH_2{\cdot}CO{\cdot}N(CH_2Ph){\cdot}C(CH_2Ph)_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO_2Bu^t \quad \text{(V)}$$

It seems probable that the method is generally applicable to the synthesis of peptides containing α-benzylphenylalanylglycine at the *C*-terminus. An attempt to apply the reaction to the synthesis of a tripeptide with a *C*-terminal L-alanine residue failed, however, since we were unable to obtain the necessary methyl L-α-isocyanopropionate; treatment of *N*-formyl-L-alanine methyl ester with phosgene in the presence of triethylamine [7] gave only completely racemic methyl α-isocyanopropionate, as also did treatment with toluene-*p*-sulphonyl chloride and pyridine, thionyl chloride and pyridine, and *NN*-dimethylchloromethyleneammonium chloride and tribenzylamine. Reaction of methyl DL-α-isocyanopropionate with the Schiff's base (I) and *N*-benzyloxycarbonyl-L-prolylglycine gave the protected tetrapeptide (VI) in good yield, but the mixture of diastereoisomerides could not be separated into its components by fractional crystallisation. Since our

† The ^{1}H n.m.r. spectrum of this compound in $CDCl_3$ at 33·5 °C was that of the imine (I); the spectrum of a solution in $(CD_3)_2SO$, however, was that of a mixture of (I) (45%) and the *cis*- and *trans*-isomers (25 and 30%, respectively) of the enamine, $PhCH_2{\cdot}NH{\cdot}C(CH_2Ph){:}CHPh$.

[1] Part XXII, G. C. Barrett, P. M. Hardy, T. A. Harrow, and H. N. Rydon, *J.C.S. Perkin I*, 1972, 2634.
[2] H. Felkin, *Bull. Soc. chim. France*, 1959, 20.
[3] G. Gokel, G. Lüdke, and I. Ugi, in ' Isonitrile Chemistry,' ed. I Ugi, Academic Press, 1971, ch. 8.
[4] R Roeske, *J. Org. Chem.*, 1963, **28**, 1251.
[5] D. S. Jones, G. W. Kenner, J. Preston, and R. C. Sheppard, *J. Chem. Soc.*, 1965, 6227.
[6] I. Ugi, *Angew. Chem. Internat. Edn.*, 1962, **1**, 8.
[7] I. Ugi, W. Betz, U. Fetzer, and K. Offermann, *Chem. Ber.*, 1961, **94**, 2814.

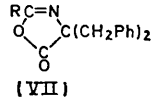
work was completed, it has been reported, without experimental detail,[8] that fully optically active isocyanides can be prepared from *N*-formylamino-acid methyl esters by treatment with phosgene at temperatures below −20° in the presence of pyridine or *N*-methylmorpholine; this claim, when substantiated, will make the Ugi procedure the method of choice for the synthesis of peptides of α-benzylphenylalanine.



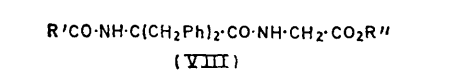
(VI)

The observation of two doublets, each due to one diastereoisomeride, for the methyl protons in the ^{1}H n.m.r. spectra of diastereoisomeric alanine peptides has been suggested [9,10] as a sensitive test for racemisation in such compounds. This test fails completely with our mixture of diastereoisomerides of the protected tetrapeptide (VI) which, at both 60 and at 100 MHz in deuteriochloroform, gives a single sharp doublet (τ 8·83; *J* 7·0 Hz), which collapses to a sharp singlet (τ 8·83) on decoupling from the alanine CH (τ 5·65). A similar mixture of diastereoisomerides of *N*-benzyloxycarbonyl-L-prolylglycylalanine methyl ester, prepared from *N*-benzyloxycarbonyl-L-prolylglycine and DL-alanine methyl ester, however, gave the expected two doublets (τ 8·62; *J* 6·8 Hz and τ 8·83; *J* 6·8 Hz). The failure of the test with (VI) is no doubt due to the overwhelming influence of the three phenyl groups of the neighbouring *N*,α-dibenzylphenylalanine residue on the chemical shift of the alanine methyl protons.

Kenner and his colleagues [5,11] showed that good yields of peptides of α-methyl-, α-ethyl-, and α-phenyl-alanine could be obtained by use of the oxazolin-5-ones derived from the *N*-protected amino-acids. We find that the method is also of value for the preparation of peptides of α-benzylphenylalanine, having used it for the synthesis of glycyl-α-benzylphenylalanylglycine by three routes. The oxazolin-5-ones (VII; R = $ClCH_2$, N_3CH_2, or $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2$) were prepared in high yield by stirring the appropriate *N*-acyl-α-benzylphenylalanine with dicyclohexylcarbodi-imide in ether at room temperature until the di-imide band at 2130 cm^{-1} had disappeared from the i.r. spectrum of the reaction mixture (1·5—2 h); they can also be prepared, but less conveniently, by refluxing the *N*-acylamino-acids with acetic anhydride. These oxazolinones are stable, easily purified crystalline solids and appear to be less reactive than those derived from α-methylalanine.[5,11] The oxazolinones (VII; R = $ClCH_2$) and (VII; R = $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2$) reacted readily with glycine t-butyl ester in acetonitrile at room temperature to give high yields of the peptides (VIII; R′ = $ClCH_2$, R″ = Bu^t) and (VIII; R′ = $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2$, R″ = Bu^t), respectively. The same peptides were prepared by direct dicyclohexylcarbodi-imide



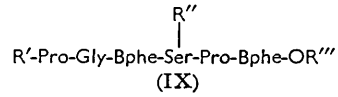
(VII)



(VIII)

coupling of the appropriate *N*-acyl-α-benzylphenylalanines with glycine t-butyl ester, but the yields obtained in these one-stage processes were considerably lower, and the products less easily purified, than those obtained by the two-stage processes involving isolation of the oxazolinones. The stability of the oxazolinones derived from α-benzylphenylalanine enables them to be used for coupling reactions with the sodium salts of amino-acids in aqueous solutions; thus, 80—90% yields of the peptides (VIII; R″ = H) were obtained by reactions between the oxazolinones (VII; R = $ClCH_2$, N_3CH_2, or $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH_2$) and the sodium salt of glycine in 50% aqueous acetone. The protected peptides (VIII), prepared in these various ways, were converted into free glycyl-α-benzylphenylalanylglycine (VIII; R′ = $H_2N{\cdot}CH_2$, R″ = H) by standard methods; the highest overall yield was obtained by the route involving coupling of the oxazolinone from *N*-benzyloxycarbonylglycyl-α-benzylphenylalanine with the sodium salt of glycine.

On the basis of these model experiments we attempted to apply the oxazolinone method to the synthesis of the α-benzylphenylalanine analogue of bradykinin. *N*-Benzyloxycarbonyl-L-prolylglycyl-α-benzylphenylalanine was prepared in satisfactory yield by the dicyclohexylcarbodi-imide coupling of *N*-benzyloxycarbonyl-L-prolylglycine [12] and α-benzylphenylalanine t-butyl ester, followed by removal of the t-butyl group by treatment with trifluoroacetic acid. The oxazolinone (VII; R = Z-Pro-NH·CH_2) * was obtained in good yield by treatment of the *N*-protected tripeptide with dicyclohexylcarbodi-imide in ether. *O*-t-Butyl-L-seryl-L-prolyl-α-benzylphenylalanine t-butyl ester was prepared by the dicyclohexylcarbodi-imide coupling of *N*-benzyloxycarbonyl-*O*-t-butyl-L-seryl-L-proline and α-benzylphenylalanine t-butyl ester, followed by catalytic hydrogenolysis of the product. The reaction between this tripeptide t-butyl ester and the oxazolinone (VII; R = Z-Pro-NH·CH_2) was sluggish and gave only a moderate yield of the fully-protected hexapeptide (IX; R′ = Z,



(IX)

R″ = R‴ = Bu^t), from which the t-butyl groups were removed by treatment with trifluoroacetic acid. The

* The abbreviations are those recommended by I.U.P.A.C.–I.U.B. (*Biochem. J.*, 1972, **126**, 773); Bphe = α-benzylphenylalanine.

[8] P. Hoffmann, D. Marquarding, and I. Ugi, unpublished results, cited in 'Isonitrile Chemistry,' ed. I. Ugi, Academic Press, 1971, pp. 13, 204, 205.

[9] B. Halpern, L. F. Chew, and B. Weinstein, *J. Amer. Chem. Soc.*, 1967, **89**, 5051.

[10] B. Weinstein, *Proc. 1st American Peptide Symp.*, 1970, 371.

[11] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39.

[12] H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 1956, 3642.

resulting *N*-protected hexapeptide (IX; R′ = Z, R″ = R‴ = H), which could not be recrystallised, failed to give the desired oxazolinone on treatment with dicyclohexylcarbodi-imide and the projected synthesis was abandoned at this stage.

We conclude that, although the oxazolinone method is useful for the synthesis of peptides of α-benzylphenylalanine by coupling reactions employing oxazolinones derived from small *N*-protected peptides with *C*-terminal α-benzylphenylalanine residues, it is liable to break down with larger peptides, owing to their lessened reactivity towards dicyclohexylcarbodi-imide and consequent failure to form oxazolinones. It is general experience in peptide synthesis that coupling reactions of all kinds are more difficult to bring about with large peptides than with smaller ones.

Chemical shifts of benzyl methylene protons in some derivatives of α-benzylphenylalanine

(a) R′NH·C(CH_2Ph)$_2$·COR″

R′	R″	τ_A	τ_B	J_{AB}/Hz	Solvent
H_2^+	OH	6·52	6·16	−15	$CF_3·CO_2H$
$ClCH_2·CO$	OH	6·81	6·23	−14	$(CD_3)_2SO$
$N_3CH_2·CO$	OH	6·79	6·20	−14	$(CD_3)_2SO$
Z-Gly	OH	6·85	6·14	−14	$(CD_3)_2SO$
Z-Pro-Gly	OH	6·91	6·21	−14	$(CD_3)_2SO$
Z-Pro-Gly	OH	6·83	6·15	−13·5	$CDCl_3$
$CH_3·CO$	OEt	6·88	6·52	−14	$(CD_3)_2SO$
$CH_3·CO$	OEt	6·79	5·97	−13·5	$CDCl_3$
$Cl^-H_2^+$	OBu^t	6·50	6·44	−14·8	$CDCl_3$ *
H	OBu^t	7·22	6·87	−13·5	$CDCl_3$ *
Z-Pro-Gly	OBu^t	6·84	6·01	−14	$CDCl_3$
Z-Ser(Bu^t)-Pro	OBu^t	6·94	6·10	−13·5	$CDCl_3$
$ClCH_2·CO$	Gly-OH	6·64	6·16	−14	$(CD_3)_2SO$
$N_3CH_2·CO$	Gly-OH	6·60	6·13	−15	$(CD_3)_2SO$
H_2^+-Gly	Gly-OH	6·48	6·30	−15	$CF_3·CO_2H$
Z-Gly	Gly-OH	6·74	6·45	−14	$(CD_3)_2SO$
$ClCH_2·CO$	Gly-OBu^t	6·64	6·17	−14	$(CD_3)_2SO$
$ClCH_2·CO$	Gly-OBu^t	6·72	6·22	−15	$CDCl_3$
Z-Gly	Gly-OBu^t	6·68	6·35	−15	$(CD_3)_2SO$
Z-Gly	Gly-OBu^t	6·66	6·25	−14·5	$CDCl_3$

(b) R′N(CH_2Ph)·C(CH_2Ph)$_2$·COR″

H	OH	6·92 (singlet)			$(CD_3)_2SO$ *,
H_2^+	OH	6·37	6·03	−16	$CF_3·CO_2H$
H	OBu^t	6·97	6·91	−14·9	$CDCl_3$
$CH_3·CO$	NHPh	7·00	6·52	−13	$(CD_3)_2SO$
$CH_3·CO$	NHPh	6·90	6·45	−12·5	$CDCl_3$
$C_6H_4(CO)_2$Gly	Gly-OBu^t	7·14	6·65	−13	$(CD_3)_2SO$
$C_6H_4(CO)_2$Gly	Gly-OBu^t	7·01	6·54	−13·5	$CDCl_3$
Z-Pro-Gly	DL-Ala-OMe	7·01	6·58	−13	$CDCl_3$ *

(c) RC∴N·C(CH_2Ph)$_2$·CO·O

R	τ	Solvent
$ClCH_2$	6·79	$(CD_3)_2SO$
$ClCH_2$	6·78	$CDCl_3$ *
N_3CH_2	6·77	$CDCl_3$
$ZNH·CH_2$	6·85	$CDCl_3$ *
Z-Pro-$NH·CH_2$	6·90	$CDCl_3$

* 100 MHz at 30 °C; all others 60 MHz at 33·5 °C.

The absence of a plane of symmetry relating the two benzyl groups in α-benzylphenylalanine and its derivatives leads to the possibility of observable magnetic non-equivalence [13] of the benzyl methylene protons in such compounds. We have examined the ^1H n.m.r. spectra of 27 such derivatives and the chemical shifts of the *C*-benzyl methylene protons are recorded in the Table. These four protons appear as an AB quartet in the spectra of all the 17 open-chain derivatives of α-benzylphenylalanine and in all but one of those of the six derivatives of *N*,α-dibenzylphenylalanine studied. The coupling constant, J_{AB}, lies between −12·5 and −16 Hz (mean −13·5) for all 22 compounds showing the phenomenon, but $\Delta\tau_{AB}$ varies widely (from 0·06 to 0·84 p.p.m.) without any obvious relationship to chemical structure. All four oxazolinones (VII; R = $ClCH_2$, N_3CH_2, $ZNH·CH_2$, and Z-Pro-$NH·CH_2$) fail to show the phenomenon, the four benzyl methylene protons giving rise to a sharp singlet in the spectra of these compounds. This is, presumably, due to the planarity of the oxazolinone ring and may be general; the absence of any detectable effect of the 2-substituents is surprising. Magnetic non-equivalence is not observable for the benzyl methylene protons of ethyl dibenzylacetoacetate, no doubt owing to the almost identical magnetic shielding effects of the acetyl and ethoxycarbonyl groups.

EXPERIMENTAL

The purity of all peptides and intermediates was confirmed by t.l.c. (Kieselgel GF254); spots were revealed by means of u.v. light, with 0·1% ninhydrin in n-butanol at 100°, by the chlorine–starch–iodide method,[14] by spraying with ammonium sulphate,[15] or by a combination of these methods. Organic solutions were dried over magnesium sulphate and evaporated or concentrated under reduced pressure in a rotary evaporator below 50°. Unless otherwise indicated, light petroleum was the fraction of b.p. 60—80°.

^1H N.m.r. spectra were recorded, usually at *ca.* 30 °C, on either a Perkin-Elmer R10 (60 MHz) or a Jeol JNM-MH-100 (100 MHz) spectrometer. I.r. spectra were recorded on a Perkin-Elmer Infracord spectrometer and mass spectra on a Perkin-Elmer–Hitachi RMU-6 spectrometer. Optical rotations were measured on a Bendix–NPL Polarimeter, model 143A, path-length 1—4 cm.

α-Benzylphenylalanine and Simple Derivatives

Dibenzyl ketone (252 g, 1·2 mol) and benzylamine (220 ml, 2·0 mol), in anhydrous benzene (400 ml) were refluxed, under nitrogen, until the carbonyl stretching peak (1702 cm^{-1}) was no longer observable (72 h); the condensate was continuously returned to the reaction vessel through a column of anhydrous calcium sulphate. Evaporation and distillation of the residue gave N-*benzyl*-1,3-*diphenylpropan*-2-*imine* (I) (337 g, 94%), b.p. 176—182° at 0·02 mmHg (Found: *m/e*, 299. $C_{22}H_{21}N$ requires *M*, 299), n_D^{22} 1·6110, $\nu_{max.}$ (film) 3400 (enamine NH str.), 3020 (CH str.), 2900 (CH str.), 1655 (C∴N str.), 1605 (NH def., C∴C def.), 1495 (C∴C def.), 1455 (C∴C def.), 1075 (CH def.), and 1032 (CH def.) cm^{-1}; τ ($CDCl_3$) 2·65 (5H, s, N·CH_2*Ph*), 2·72 (10H, m, C·CH_2*Ph*), 5·29 (2H, s, N·CH_2Ph), and 6·36 (4H, m, C·CH_2-Ph); τ [$(CD_3)_2SO$] 2·73 (12·25H, imine and enamine CH_2*Ph*), 3·01 (2·75H, m, enamine C∴CH*Ph*), 4·42 (0·55H, t,

[13] Reviews: M. van Gorkom and G. E. Hall, *Quart. Rev.*, 1968, **22**, 14; T. H. Siddall and W. E. Stewart, *Progr. N.M.R. Spectroscopy*, 1969, **5**, 33.

[14] H. N. Rydon and P. W. G. Smith, *Nature*, 1952, **169**, 922.

[15] T. Ziminski and E. Borowski, *J. Chromatog.*, 1966, **23**, 480.

J 5 Hz, NH of enamine), 4·73 (0·55H, s, enamine C:C*H*Ph), 5·38 (0·9H, imine N·CH_2Ph), 5·73 (0·6H, s, *trans*-enamine N·CH_2Ph), 5·82 (0·5H, s, *cis*-enamine N·CH_2Ph), 6·28 (1·8H, s, imine C·CH_2Ph), 6·37 (0·6H, s, *trans*-enamine C·CH_2Ph), and 6·45 (0·5H, s, *cis*-enamine C·CH_2Ph). This product, freshly prepared phenyl isocyanide [7] (b.p. 62—65° at 16 mmHg; 139 g, 1·35 mol) and acetic acid (66·6 g, 1·11 mol) were kept in anhydrous methanol (2·9 l) under nitrogen at room temperature for 6 days; the C:N band at 1655 cm^{-1} was then no longer observable. A small amount of finely divided solid was filtered off under reduced pressure. The crystalline product, which began to separate at once from the filtrate, was collected after 16 h at −10°; a further crop was obtained by concentrating the filtrate to a quarter of its volume and keeping the concentrate at −10° for 24 h. Recrystallisation from ethyl acetate–light petroleum gave N-*acetyl*-N,α-*dibenzylphenylalanine anilide* (II) (352 g, 68%), m.p. 176·5—177·5°; $\nu_{max.}$ (Nujol) 3410 (NH str.), 1690 (anilide C:O str.), 1655 (amide C:O str.), 1600 (C:C def.), 1510 (amide II), and 1498 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·29 (5H, m, NH*Ph*), 2·62 (10H, m, C·CH$_2$*Ph*), 2·81 (5H, s, N·CH$_2$*Ph*), 3·10 (1H, s, NH), 6·26 (2H, s, N·CH_2Ph), 6·68 (4H, ABq, J −12·5 Hz, C·CH_2Ph), and 7·97 (3H, s, CH_3) (Found: C, 80·5; H, 6·7; N, 6·2. $C_{31}H_{30}N_2O_2$ requires C, 80·5; H, 6·5; N, 6·1%). This anilide (116 g, 0·25 mol) was refluxed (bath temp. 140°) with aqueous hydrobromic acid (46—48%; 375 ml) for 3·5 h. The cooled mixture was filtered and the solid product dissolved in M-sodium hydroxide (1 l) and extracted with ether (3 × 250 ml). Acidification of the aqueous phase to pH 3 with 6M-hydrochloric acid, collection of the precipitate, and washing with water (6 × 150 ml), ethanol, and ether, gave N,α-*dibenzylphenylalanine* (III) (84 g, 97%), m.p. 218—220°; a sample recrystallised from ethanol then sublimed (210° at 0·01 mmHg) had m.p. 221—222°; $\nu_{max.}$ (Nujol) 3210 (NH str.), 1620 (C:O str., C:C def.), and 1496 (C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 2·72 (15H, s, C_6H_5), 6·11 (2H, s, PhCH_2·N), 6·5vbr (NH), and 6·92 (4H, s, PhCH_2·C); τ (CF_3·CO_2H) 2·55 (15H, m, C_6H_5), 2·2br (NH_2^+), 5·37 (2H, t, J 5 Hz, PhCH_2·N), and 6·20 (4H, ABq, J −16 Hz, PhCH_2·C) (Found: C, 79·9; H, 6·5; N, 4·2. $C_{23}H_{23}NO_2$ requires C, 80·0; H, 6·7; N, 4·1%). Hydrogen was bubbled through a suspension of N,α-dibenzylphenylalanine (34·5 g) in n-butanol (1 l) containing 5% palladised charcoal (6·9 g) for 12 h at 95°. After cooling and adding M-potassium hydroxide (100 ml), the mixture was filtered through kieselguhr and the residue washed with M-potassium hydroxide (50 ml). The filtrate and washings were evaporated and the residue treated with water (100 ml) and re-evaporated. The oily residue was taken up in water (150 ml), washed with ether (2 × 50 ml), and acidified to pH 5 with M-hydrochloric acid. Filtration and washing with water, ethanol, and ether gave α-benzylphenylalanine (IV) (23·1 g, 91%), m.p. 300° (decomp.), spectroscopically identical with material prepared from ethyl dibenzylacetoacetate.[1] A sample recrystallised from benzyl alcohol then sublimed (280° at 0·001 mmHg) had m.p. 307—308° (lit.,[1,16] 307—308°); $\nu_{max.}$ (Nujol) 3220 (NH str.), 1615 (NH def.), 1598 (C:O str., C:C def.), and 1505 (C:C def.) cm^{-1}; τ (CF_3·CO_2H) 2·56 (10H, s, C_6H_5), 2·97br (3H, s, NH_3^+), and 6·34 (4H, ABq J −15 Hz, CH_2) (Found: C, 75·5; H, 6·4; N, 5·9. Calc. for $C_{16}H_{17}NO_2$: C, 75·3; H, 6·7; N, 5·5%).

Liquid isobutene (200 ml) was added at −10° to a mixture of N,α-dibenzylphenylalanine (31·0 g, 0·895 mol), anhydrous dioxan (200 ml), and conc. sulphuric acid (20 ml) in a pressure bottle. After shaking for 3 days at room temperature, the bottle was cooled to −10°, and the contents were poured into a mixture of ether (500 ml) and M-potassium hydroxide (1 l). After shaking, the aqueous layer was separated and extracted with ether (3 × 150 ml). The combined ethereal solutions were washed with water (3 × 150 ml), dried, and evaporated. Re-evaporation with ethanol, followed by recrystallisation from ethanol (30 ml) at −10°, gave N,α-*dibenzylphenylalanine t-butyl ester* (8·9 g, 25%), m.p. 59—60°; $\nu_{max.}$ (Nujol) 3300 (NH str.), 1735 (C:O str.), 1605 (NH def., C:C def.), and 1498 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·73 (15H, m, C_6H_5), 6·11 (2H, s, N·CH_2Ph), 6·94 (4H, ABq, J −14·9 Hz, C·CH_2Ph), 8·16 (1H, t, J 14 Hz, NH), and 8·69 (9H, s, Bu^t) (Found: C, 80·7; H, 7·9; N, 3·9. $C_{27}H_{31}NO_2$ requires C, 80·7; H, 7·8; N, 3·5%). Acidification of the combined aqueous layer and washings from the reaction mixture precipitated N,α-dibenzylphenylalanine (19·2 g, 62%), which was washed, dried, and used for further esterifications. Hydrogen was bubbled for 9 h through a refluxing solution of the above t-butyl ester (48·9 g) in ethanol (920 ml) in the presence of 5% palladised charcoal (10 g). The mixture was filtered and concentrated to 130 ml; addition of water (85 ml), followed by keeping at −5°, gave α-*benzylphenylalanine t-butyl ester* (32·4 g, 89%), m.p. 74·5—75·5°; $\nu_{max.}$ (Nujol) 3310 (NH str.), 1723 (C:O str.), 1605 (NH def., C:C def.), and 1498 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·74 (10H, s, C_6H_5), 7·05 (4H, ABq, J −13·5 Hz, PhCH_2), and 8·67br (11H, s, Bu^t and NH_2) (Found: C, 77·3; H, 8·3; N, 4·6. $C_{20}H_{25}NO_2$ requires C, 77·1; H, 8·1; N, 4·5%). This ester (3·14 g), in ethyl acetate (50 ml), was shaken with M-hydrochloric acid (4 × 10 ml). The combined aqueous layers were saturated with sodium chloride and extracted with ethyl acetate (4 × 10 ml). The combined ethyl acetate solutions were washed with water (10 ml), dried, and concentrated to 20 ml. Addition of light petroleum (b.p. 40—60°) precipitated the hydrochloride (3·0 g, 86%) which, recrystallised from ethyl acetate–light petroleum, had m.p. 171—172° (decomp.), $\nu_{max.}$ (Nujol) 3180 (NH str.), 1740 (C:O str.), 1595 (NH def., C:C def.), 1515 (NH def.), and 1498 (C:C def.) cm^{-1}; τ ($CDCl_3$) 1·30 (3H, s, NH_3^+), 2·68 (10H, m, C_6H_5), 6·47 (4H, ABq J −14·8 Hz, CH_2), and 8·73 (9H, s, Bu^t).

Chloroacetyl chloride (17·0 g, 150 mmol) was added dropwise at 40—60° to a stirred solution of α-benzylphenylalanine (19·2 g, 75 mmol) in M-sodium hydroxide (75 ml), the pH being kept at 10·5—11·0 by dropwise addition of M-sodium hydroxide with an autotitrator. When no more alkali was taken up, the mixture was cooled and neutralised (pH 7) with M-hydrochloric acid. The precipitate was filtered off, washed with water, and twice re-treated with chloroacetyl chloride (8·5 and 5·7 g). The combined neutral filtrates from the three reactions were acidified (pH 2·9), cooled to 5°, and filtered. The product was washed with water, dried, and recrystallised from ethanol; *N-chloroacetyl-α-benzylphenylalanine* (18·4 g, 75%)* so obtained had m.p. 210—211°; $\nu_{max.}$ (Nujol) 3310 (NH str.), 1723 (carboxyl C:O str.), 1640 (amide C:O str.), 1530 (amide II), and 1498 (C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 2·61 (1H, s, NH), 2·80 (10H, m, C_6H_5), 5·81 (2H, s, CH_2Cl), and 6·52 (4H, ABq J −14 Hz, PhCH_2) (Found: C, 65·6; H, 5·5; N, 4·3. $C_{18}H_{18}ClNO_3$ requires C, 65·1; H, 5·5; N,

* A considerably lower yield was obtained when the acylation was carried out at 0°.

[16] L. Goodson, I. L. Honiberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, 1960, **25**, 1920.

4·2%). Dicyclohexylcarbodi-imide (1·04 g, 5·05 mmol) was added to a suspension of this derivative (1·67 g, 5·05 mmol) in anhydrous ether (25 ml). The mixture was stirred at room temperature until the N:C:N band at 2130 cm^{-1} was no longer observed (90 min). Dicyclohexylurea was filtered off and washed with ether. Light petroleum was added to the filtrate and washings until these became cloudy; the mixture was then slowly evaporated, with cooling (rotary evaporator) until crystallisation began. After 18 h at −5°, the crystals were collected and dried in a vacuum desiccator. *4,4-Dibenzyl-2-chloromethyloxazolin-5-one* (VII; R = $ClCH_2$) (1·45 g, 91%) so obtained had m.p. 58—58·5°; $\nu_{max.}$ (Nujol) 1828 (C:O str.), 1805 (C:O str.), 1672 (C:N str.), 1600 (C:C def.), and 1498 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·78 (10H, m, C_6H_5), 6·24 (2H, s, CH_2Cl), and 6·79 (4H, s, $PhCH_2$) (Found: C, 69·1; H, 4·9; N, 4·7%; *m/e* 313 and 315. $C_{18}H_{16}ClNO_2$ requires C, 68·9; H, 5·1; N, 4·5%; *M*, 313 and 315); the same compound (m.p. 56—57°; b.p. 134° at 0·005 mmHg) was obtained, in the same yield, by refluxing the chloroacetyl derivative (6·64 g) for 1·5 h with acetic anhydride (80 ml).

N-Chloroacetyl-α-benzylphenylalanine (24·9 g, 75 mmol) and sodium azide (14·6 g, 225 mmol) were refluxed for 24 h in 60% aqueous ethanol (75 ml). The ethanol was removed under reduced pressure with the addition of water (25 and 75 ml); the residue was acidified with 6M-hydrochloric acid, extracted with ethyl acetate, washed with water, dried, and evaporated. Recrystallisation from aqueous ethanol gave *N-azidoacetyl-α-benzylphenylalanine* (23·2 g, 80%), m.p. 176·5—180° (decomp.); $\nu_{max.}$ (Nujol) 3320 (NH str.), 2100 (N:N:N str.), 1725 (carboxy C:O str.), 1640br (amide C:O str.), 1525 (amide II), and 1496 (C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 2·68 (11H, m, C_6H_5 and NH), 6·05 (2H, s, CH_2N_3), and 6·50 (4H, ABq, *J* −14 Hz, CH_2Ph) (Found: *m/e* 320. $C_{18}H_{16}N_4O_2 - H_2O$ requires *M*, 320). This compound was heat- and light-sensitive and a satisfactory elemental analysis was not obtained. Treatment with dicyclohexylcarbodi-imide, as for the chloroacetyl analogue, gave *2-azidomethyl-4,4-dibenzyloxazolin-5-one* (VII; R = N_3CH_2) (56%), m.p. 50—51° (from ether–light petroleum); $\nu_{max.}$ (Nujol) 2095 (N:N:N str.), 1825 (C:O str.), 1675 (C:N str.), and 1496 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·82 (10H, s, C_6H_5), 6·49 (2H, s, CH_2N_3), and 6·77 (4H, s, CH_2Ph) (Found: N, 17·5%; *m/e* 320. $C_{18}H_{16}N_4O_2$ requires N, 17·5%; *M*, 320).

Synthesis of Peptides of α-Benzylphenylalanine

(i) *By Ugi's Method*

(a) N-*Phthaloylglycyl*-N,α-*dibenzylphenylalanylglycine t-Butyl Ester* (V).—*N*-Benzyl-1,3-diphenylpropan-2-imine (5·98 g, 20 mmol), *N*-phthaloylglycine [17] (4·10 g, 20 mmol), and t-butyl isocyanoacetate [7] (2·82 g, 20 mmol) were kept at room temperature under nitrogen in methanol (500 ml) for 72 h. Recrystallisation of the crystalline precipitate (10·5 g, 81%) from ethanol gave the pure *protected peptide* (8·8 g, 68%), m.p. 199—200°; $\nu_{max.}$ (Nujol) 3410 (NH str.), 1780 (imide C:O str.), 1747 (ester C:O str.), 1728 (imide C:O str.), 1678 (amide C:O str.), 1605 (C:C def.), 1515 (amide II), and 1499 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·19 (4H, m, C_6H_4), 2·56 (15H, m, C_6H_5), 4·11 (1H, t, *J* 4 Hz, NH), 5·66 (2H, s, $N{\cdot}CH_2Ph$), 6·17 (2H, s, $N{\cdot}CH_2{\cdot}CO$), 6·26 (2H, s, $N{\cdot}CH_2{\cdot}CO$), 6·78 (4H, ABq, *J* −13·5 Hz, $C{\cdot}CH_2Ph$), and 8·61 (9H, s, Bu^t) (Found: C, 72·6; H, 6·5; N, 6·8. $C_{39}H_{39}N_3O_6$ requires C, 72·5; H, 6·1; N, 6·5%).

(b) N-*Benzyloxycarbonyl-L-prolylglycyl*-N,α-*dibenzylphenylalanyl-DL-alanine Methyl Ester* (VI).—*N*-Formyl-L-alanine methyl ester,[18] had b.p. 139—141° at 15 mmHg, n_D^{23} 1·4479, $[\alpha]_D^{22}$ −9·8° (*c* 5·0 in $CHCl_3$). To an ice-cooled solution of this ester (7·90 g, 60 mmol) in triethylamine (20 ml, 135 mmol), M-phosgene in dichloromethane (60 ml) was added dropwise over 30 min with vigorous stirring. The mixture was then allowed to attain room temperature and poured into water (60 ml). Extraction with dichloromethane, drying, and distillation gave methyl DL-2-isocyanopropionate (4·76 g, 70%) as an evil-smelling oil, b.p. 69—70° at 10 mmHg, n_D^{20} 1·4128, $[\alpha]_D^{20}$ 0·0 ± 0·5° (*c* 5·0 in $CHCl_3$), $\nu_{max.}$ (film) 2990 (CH str.), 2920 (CH str.), 2140 (N:C str.), 1755 (C:O str.), 1450 (CH def.), 1438 (CH def.), and 1378 (CH def.) cm^{-1}; τ ($CDCl_3$) 5·60 (1H, q, *J* 8 Hz, α-CH), 6·16 (3H, s, OCH_3), and 8·33 (3H, d, *J* 8 Hz, $C{\cdot}CH_3$). This isocyanide (1·70 g, 15 mmol) was kept for 48 h at room temperature in ether (30 ml) containing anhydrous oxalic acid (1·48 g, 16·5 mmol). The mixture was filtered and the filtrate evaporated to dryness. The residue was kept at room temperature for 48 h with 0·5M-hydrogen chloride in 50% aqueous acetone. The solution (shown by t.l.c. to contain alanine and alanine methyl ester) was evaporated to dryness. The residue was dissolved in acetone (15 ml) and kept for 1 h after adding a little M-sodium hydroxide. Water (15 ml) was added and the acetone removed under reduced pressure. Acidification (pH 5) with 2M-hydrochloric acid and cooling to −5° for 2 h, precipitated DL-alanine, m.p. 270° (decomp.), $[\alpha]_D^{20}$ 0·0 ± 0·2° (*c* 2·0 in AcOH).

N-Benzyl-1,3-diphenylpropan-2-imine (9·0 g, 30 mmol), *N*-benzyloxycarbonyl-L-prolylglycine [12] (9·2 g, 30 mmol), and methyl DL-2-isocyanopropionate (3·4 g, 30 mmol) were kept at room temperature under nitrogen in methanol (75 ml) for 5 days. The solution was evaporated to dryness and the residue dissolved in ethyl acetate (120 ml), washed with saturated sodium hydrogen carbonate (4 × 25 ml), and dried. Evaporation left a gum which solidified on trituration with light petroleum. Crystallisation of the crude product (17·8 g, 83%) from aqueous ethanol gave the *protected tetrapeptide* (13·3 g in 3 crops, 62%), m.p. 100—105°, $[\alpha]_D^{22}$ −38·3° (*c* 5·0 in EtOH); a sample obtained by further recrystallisation of the first crop from aqueous ethanol had m.p. 100—105°, $[\alpha]_D^{27}$ −42·3° (*c* 2·0 in EtOH); $\nu_{max.}$ (Nujol) 3310br (NH str.), 1748 (ester C:O str.), 1673br (urethane C:O str.; amide C:O str.), 1515 (amide II), and 1499 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·72 (21H, m, C_6H_5 and Gly-NH), 3·96 (1H, d, *J* 6 Hz, Ala-NH), 4·89 (2H, s, $O{\cdot}CH_2$) 5·65 (2H, m, Pro-α-CH and Ala-α-CH), 6·14 (2H, s, Gly-CH_2), 6·41 (7H, m, $O{\cdot}CH_3$, $N{\cdot}CH_2Ph$, and Pro-$N{\cdot}CH_2$), 6·80 (4H, ABq, *J* −13 Hz, $C{\cdot}CH_2Ph$), 8·0 (4H, m, Pro-$C{\cdot}CH_2{\cdot}C$), and 8·83 (3H, d, *J* 7 Hz, $C{\cdot}CH_3$) (Found: C, 70·1; H, 6·4; N, 7·5. $C_{42}H_{46}N_4O_7$ requires C, 70·2; H, 6·5; N, 7·8%). The once recrystallised ester (10·8 g, 15 mmol) was kept at room temperature for 24 h with 0·5M-hydrazine hydrate in methanol (60 ml). Evaporation and trituration with ether gave the hydrazide (10·5 g, 97%), m.p. 125—150°. Fractional crystallisation from ether gave a first crop (2·1 g), $[\alpha]_D^{22}$ −6·2° (*c* 2·5 in EtOH), and a second crop (1·3 g), $[\alpha]_D^{22}$ −12·4°; the compound is clearly a mixture of diastereoisomerides.

[17] G. H. L. Nefkens, G. I. Tesser, and J. F. Nivard, *Rec. Trav. chim.*, 1960, **79**, 688.

[18] J. C. Sheehan and D. D. Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1154.

(ii) *By Use of Oxazolinones*

(a) *Glycyl-α-benzylphenylalanylglycine.*—(i) A solution of freshly prepared *N*-benzyloxycarbonylglycyl chloride [19] (10·2 g, 45 mmol) in dioxan (18 ml) was added dropwise to a stirred solution of α-benzylphenylalanine (7·65 g, 30 mmol) in M-sodium hydroxide (30 ml) at 50°; the pH was kept at 10·5—11·0 during the addition by adding M-sodium hydroxide with an autotitrator. When no more alkali was consumed, the mixture was cooled, neutralised (pH 7), and filtered. The solid was re-treated with *N*-benzyloxycarbonylglycyl chloride (3·4 g) and alkali. The combined aqueous filtrates from the two reactions were acidified (pH 2·9) with 6M-hydrochloric acid and extracted with ethyl acetate (3 × 100 ml). The extract was dried and evaporated; recrystallisation of the residue from aqueous ethanol gave *N*-benzyloxycarbonylglycyl-α-benzylphenylalanine (5·9 g, 44%), m.p. 171—172° (lit.,[1] 171—172°); $\nu_{max.}$ (Nujol) 3300br (NH str.), 1726 (carboxy C:O str.), 1698 (urethane C:O str.), 1615 (amide C:O str.), and 1500 (amide II; C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 2·35 (1H, s, Gly-NH), 2·69 (5H, s, O·CH_2Ph), 2·75 (10H, s, C·CH_2Ph), 2·95 (1H, s, Bphe-NH), 4·88 (2H, s, O·CH_2Ph), 6·31 (2H, d, *J* 7 Hz, N·CH_2·CO), and 6·50 (4H, ABq, *J* −14 Hz, C·CH_2Ph) (Found: C, 70·4; H, 5·9; N, 6·3. Calc. for $C_{26}H_{26}N_2O_5$: C, 70·0; H, 5·9; N, 6·3%).

This compound (13·4 g, 30 mmol) and dicyclohexylcarbodi-imide (6·2 g, 30 mmol) were stirred in ether (150 ml) at room temperature for 1·75 h. After 16 h at −10°, dicyclohexylurea was filtered off and washed with a little ether. Light petroleum was added to the combined filtrate and washings until the solution became cloudy. The mixture was then slowly evaporated until crystallisation began. The crude product (10·6 g, 83%) was sufficiently pure (m.p. 71—72·5°) for preparative work; a specimen of 4,4-*dibenzyl*-2-*benzyloxycarbonylaminomethyloxazolin*-5-*one* (VII; R = ZNH·CH_2) obtained by further recrystallisation, had m.p. 73—74°; $\nu_{max.}$ (Nujol) 3300br (NH str.), 1726 (carboxy C:O str.), 1698 (urethane C:O str.), 1615 (amide C:O str.), and 1500 (amide II; C:C def.) cm^{-1}; τ ($CDCl_3$) 2·64 (5H, s, O·CH_2Ph), 2·81 (10H, m, C·CH_2Ph), 4·90 (2H, s, O·CH_2Ph), 5·06 (1H, t, *J* 6 Hz, NH), 6·33 (2H, d, *J* 6 Hz, 2-CH_2), and 6·85 (4H, s, C·CH_2Ph) (Found: C, 73·0; H, 6·1; N, 6·4. $C_{26}H_{24}N_2O_4$ requires C, 72·9; H, 5·7; N, 6·5%).

This oxazolinone (988 mg, 3 mmol) in acetone (1·5 ml) was added to a solution of glycine (225 mg, 3 mmol) in 2M-sodium hydroxide (1·5 ml). The mixture was stirred at room temperature for 18 h and then evaporated to dryness. The residue was treated with 6M-hydrochloric acid (7·5 ml) and the resulting suspension extracted with ethyl acetate. Concentration of the dried extract gave N-*benzyloxycarbonylglycyl*-α-*benzylphenylalanylglycine* (1·17 g, 78%), m.p. 161—162° (from ethyl acetate–ether); $\nu_{max.}$ (Nujol) 3300 (NH str.), 1732 (carboxy C:O str.), 1670 (urethane C:O str.), 1643 (amide C:O str.), 1563 (C:C def.), and 1502 (amide II; C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 1·3 (1H, t, *J* 4 Hz, Gly-NH), 2·23 (1H, m, Gly-NH), 2·73 (5H, s, O·CH_2Ph), 2·89 (11H, s, C·CH_2Ph and Bphe-NH), 5·03 (2H, s, O·CH_2Ph), 6·14 (2H, d, *J* 4 Hz, N·CH_2), 6·35 (2H, d, *J* 9 Hz, N·CH_2), and 6·60 (4H, ABq, *J* −14 Hz, C·CH_2Ph) (Found: C, 66·8; H, 6·0; N, 8·2. $C_{28}H_{29}N_3O_6$ requires C, 66·8; H, 5·8; N, 8·3%). Alternatively, the oxazolinone (2·14 g, 5 mmol) and glycine t-butyl ester [20] (655 mg, 5 mmol) were kept at room temperature for 67 h in acetonitrile (3·5 ml). The solution was then evaporated to dryness and the residue re-evaporated with ethyl acetate. The final residue was dissolved in ethyl acetate (15 ml) and the solution washed with M-hydrochloric acid (3 × 15 ml), saturated sodium hydrogen carbonate (3 × 15 ml), and water, dried, and evaporated. Trituration with light petroleum and recrystallisation from carbon tetrachloride gave N-*benzyloxycarbonylglycyl*-α-*benzylphenylalanylglycine t-butyl ester* (1·7 g, 61%), m.p. 97—99°; $\nu_{max.}$ (Nujol) 3300 (NH str.), 1730 (ester C:O str.), 1682 (urethane C:O str.), 1648 (amide C:O str.), and 1550 and 1510 (amide II) cm^{-1}; τ ($CDCl_3$) 2·60 (5H, s, O·CH_2Ph), 2·76 (10H, s, C·CH_2Ph), 3·18br (2H, m, Gly-NH and Bphe-NH), 4·59 (1H, t, *J* 4 Hz, Gly-NH), 4·89 (2H, s, O·CH_2Ph), 6·07 (2H, d, *J* 4 Hz, N·CH_2), 6·27 (2H, d, *J* 9 Hz, N·CH_2), 6·46 (4H, ABq, *J* −14·5 Hz, C·CH_2Ph), and 8·49 (9H, s, Bu^t) (Found: C, 69·0; H, 6·6; N, 7·7. $C_{32}H_{37}N_3O_6$ requires C, 68·7; H, 6·7; N, 7·5%); this compound was also obtained, in 37% yield, by coupling *N*-benzyloxycarbonylglycyl-α-benzylphenylalanine and glycine t-butyl ester with dicyclohexylcarbodi-imide in acetonitrile. This ester (2·51 g) was kept for 1 h at room temperature in trifluoroacetic acid (1·5 ml). Evaporation, re-evaporation with ethyl acetate (1 ml), and recrystallisation from ethyl acetate–ether gave *N*-benzyloxycarbonylglycyl-α-benzylphenylalanylglycine (1·53 g, 68%), m.p. 154—155°.

The *N*-protected tripeptide (1·07 g) was dissolved in 80% aqueous acetic acid (20 ml); 5% palladised charcoal (220 mg) was added and hydrogen bubbled through the mixture until no more carbon dioxide was evolved (8 h). Filtration, evaporation, trituration with ether, and recrystallisation from acetic acid gave *glycyl*-α-*benzylphenylalanylglycine hemiacetate* (0·65 g, 76%), m.p. 214° (decomp.) (Found: C, 62·9; H, 6·2; N, 11·2. $C_{20}H_{23}N_3O_4$,0·5CH_3·CO_2H requires C, 63·1; H, 6·3; N, 10·5%); the presence of 0·5 mol. equiv. of acetic acid, not removed by drying to constant weight (75° at 0·01 mmHg), was confirmed by n.m.r. spectroscopy.

(ii) 4,4-Dibenzyl-2-chloromethyloxazolin-5-one (627 mg, 2 mmol) in acetone (1 ml) was added to a solution of glycine (150 mg, 2 mmol) in 2M-sodium hydroxide (1 ml). The mixture was stirred at room temperature for 18 h and then evaporated to dryness. The residue was treated with 6M-hydrochloric acid (5 ml) and extracted with ethyl acetate (3 × 5 ml). Addition of ether to the dried extract, followed by recrystallisation of the precipitate from aqueous ethanol, gave N-*chloroacetyl*-α-*benzylphenylalanylglycine* (604 mg, 78%), m.p. 181°; $\nu_{max.}$ (Nujol) 3290 (NH str.), 1738 (chloroacetyl C:O str.), 1718 (carboxy C:O str.), 1645 (amide C:O str.), 1523 (amide II), and 1497 (C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 0·78 (1H, t, *J* 7 Hz, Gly-NH), 2·35 (1H, s, Bphe-NH), 2·74 (10H, s, C_6H_5), 5·89 (2H, s, CH_2Cl), 6·08 (2H, d, *J* 7 Hz, N·CH_2), and 6·40 (4H, ABq, *J* −14 Hz, C·CH_2Ph) (Found: C, 62·0; H, 5·4; N, 7·2. $C_{20}H_{21}ClN_2O_4$ requires C, 61·8; H, 5·4; N, 7·2%). This compound (490 mg, 1·25 mmol) was kept at room temperature for 8 days in 30% aqueous ammonia (3·5 ml). The filtered solution was acidified (pH 1) with 6M-hydrochloric acid and extracted with ethyl acetate (4 × 5 ml). The residual aqueous solution was neutralised with M-sodium hydroxide and the precipitated solid filtered off, washed with water, ethanol, and ether, and dried *in vacuo* (70° at 0·01 mmHg); *glycyl*-α-*benzylphenylalanylglycine* so prepared (144 mg, 31%) had m.p. 223—224° (decomp.); $\nu_{max.}$ (Nujol) 3390 (amide NH str.),

[19] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
[20] A. T. Moore and H. N. Rydon, *Org. Synth.*, 1965, **45**, 47.

3200 (ammonium NH str.), 1698 (amide C:O str.), 1675 (amide C:O str.), 1612 (ammonium NH def.), 1580 (carboxylate C:O str.), 1560 (amide II), and 1497 (C:C def.) cm^{-1}; τ (CF_3·CO_2H) 2·70 (15H, m, C_6H_5, Gly-NH, Bphe-NH, NH_3^+), 5·75 (2H, d, *J* 5 Hz, N·CH_2), 5·90 (2H, d, *J* 6 Hz, N·CH_2), and 6·39 (4H, ABq *J* −15 Hz, CH_2Ph) (Found: C, 64·3; H, 6·1; N, 11·3. $C_{20}H_{23}N_3O_4$ requires C, 65·0; H, 6·3; N, 11·4%).

(iii) 4,4-Dibenzyl-2-chloromethyloxazolin-5-one (7·4 g, 23·5 mmol) and glycine t-butyl ester (3·08 g, 23·5 mmol) were kept at room temperature for 48 h in anhydrous acetonitrile (23·5 ml). Evaporation to dryness, re-evaporation with ethyl acetate, and recrystallisation from ethyl acetate–light petroleum and then from benzene gave *N-chloroacetyl-α-benzylphenylalanylglycine t-butyl ester* (8·3 g, 80%), m.p. 173—174°; a specimen further recrystallised from benzene and then from ethyl acetate had m.p. 177°; $\nu_{max.}$ ($CHCl_3$) 3300 (NH str.), 1730 (ester C:O str.), 1660 (amide C:O str.), and 1500 (amide II; C:C def.) cm^{-1}; τ ($CDCl_3$) 2·54 (1H, s, Bphe-NH), 2·78 (10H, s, C_6H_5), 3·39 (1H, t, *J* 5 Hz, Gly-NH), 6·07 (2H, s, CH_2Cl), 6·08 (2H, d, *J* 6 Hz, N·CH_2), 6·47 (4H, ABq, *J* −15 Hz, CH_2Ph), and 8·50 (9H, s, Bu^t) (Found: C, 64·6; H, 7·0; N, 6·0. $C_{24}H_{29}ClN_2O_4$ requires C, 64·8; H, 6·6; N, 6·3%); the same compound was obtained in 50% yield by coupling *N*-chloroacetyl-α-benzylphenylalanine and glycine t-butyl ester with dicyclohexylcarbodi-imide in acetonitrile. This ester (5·6 g) was kept at room temperature in trifluoroacetic acid (25 ml) for 60 min. Toluene (25 ml) was then added and the solution evaporated to dryness; re-evaporation with more toluene (25 ml) and recrystallisation from aqueous ethanol gave *N*-chloroacetyl-α-benzylphenylalanylglycine (3·3 g, 69%).

(iv) 2-Azidomethyl-4,4-dibenzyloxazolin-5-one (640 mg, 2·0 mmol) and glycine (150 mg, 2·0 mmol) were stirred at room temperature for 18 h in M-sodium hydroxide in 50% aqueous acetone (2·0 ml). Work-up as usual, followed by recrystallisation from aqueous ethanol, gave *N-azidoacetyl-α-benzylphenylalanylglycine* (705 mg, 90%), m.p. 192°; $\nu_{max.}$ (Nujol) 3250 (NH str.), 2105 (N:N:N str.), 1740 (carboxy C:O str.) 1672 (amide C:O str.), 1640 (amide C:O str.), and 1557 and 1518 (amide II) cm^{-1}; τ [$(CD_3)_2SO$] 0·74 (1H, t, *J* 5 Hz, Gly-NH), 2·54 (1H, s, Bphe-NH), 2·64 (10 H, s, C_6H_5), 5·97 (2H, d, *J* 5 Hz, Gly-CH_2), 6·00 (2H, s, CH_2N_3), and 6·37 (4H, ABq *J* −15 Hz, CH_2Ph) (Found: C, 60·9; H, 5·5; N, 17·8. $C_{20}H_{21}N_5O_4$ requires C, 60·8; H, 5·4; N, 17·7%). This compound (395 mg) was kept at room temperature for 1 h in 35% hydrogen bromide in acetic acid (2 ml). Evaporation and trituration with ether gave a solid which was recrystallised from acetic acid–ether. Passage in dimethylformamide (5 ml) through a column of Amberlyst 21 resin (20 equiv.), elution with dimethylformamide (20 ml), evaporation, and trituration with ethanol and water gave glycyl-α-benzylphenylalanylglycine (25 mg, 7%), m.p. 224—225°.

(b) *Derivatives of Prolylglycyl-α-benzylphenylalanylseryl-prolyl-α-benzylphenylalanine.*—*N*-Benzyloxycarbonylglycyl-L-proline [12] (8·40 g, 27·5 mmol), dicyclohexylcarbodi-imide (5·67 g, 27·5 mmol), and α-benzylphenylalanine t-butyl ester (7·77 g, 25 mmol) were stirred for 20 h at room temperature in acetonitrile (75 ml). The precipitated dicyclohexylurea was then filtered off and washed with ethyl acetate. The combined filtrate and washings were evaporated to dryness. Acetic acid (1 ml) was added to a solution of the residue in ethyl acetate (125 ml). After 1 h, the solution was washed with M-hydrochloric acid, water, saturated sodium hydrogen carbonate, and water, dried, and evaporated. Recrystallisation from benzene–light petroleum gave *N-benzyloxycarbonyl-L-prolylglycyl-α-benzylphenylalanine t-butyl ester* (12·1 g, 80%), m.p. 75—76°, $[\alpha]_D^{23}$ −43·0° (*c* 5·0 in MeOH); $\nu_{max.}$ (Nujol) 3290 (NH str.), 1725 (ester C:O str.), 1700 (urethane C:O str.), 1670br (amide C:O str.), and 1510 (amide II) cm^{-1}; τ ($CDCl_3$) 2·65 (5H, s, O·CH_2Ph), 2·80 (11H, s, C·CH_2Ph and Bphe-NH), 4·90 (2H, s, O·CH_2), 5·7br (1H, s, Pro-N·CH), 6·1br (2H, s, Gly-N·CH_2), 6·43 (4H, ABq *J* −14 Hz, CH_2Ph), 6·58br (2H, s, Pro-N·CH_2), 8·1br (4H, m, Pro-C·CH_2·C), and 8·65 (9H, s, Bu^t) (Found: C, 70·0; H, 6·8; N, 6·9. $C_{35}H_{41}N_3O_6$ requires C, 70·1; H, 6·9; N, 7·0%). This ester (16·8 g, 28 mmol) was kept for 1 h in trifluoroacetic acid (56 ml). Evaporation, followed by re-evaporation with benzene (25 ml), gave a gum which was dissolved in ethyl acetate (100 ml). The solution was extracted with saturated sodium carbonate solution; the alkaline extract was acidified with M-hydrochloric acid and extracted with ethyl acetate. Evaporation of the dried extract gave a solid which was precipitated from ether with light petroleum (b.p. 40—60°). The solid product (13·0 g) was dissolved in ethyl acetate (100 ml); dicyclohexylamine (5·25 ml) was added, followed by anhydrous ether (700 ml). After 12 h at −10°, the solution was filtered to remove a little impurity and the filtrate evaporated to dryness. Crystallisation of the residue from ethyl acetate gave *N-benzyloxycarbonyl-L-prolylglycyl-α-benzylphenylalanine dicyclohexylammonium salt* (16·1 g, 77%), m.p. 181—183°, $[\alpha]_D^{26}$ −34·1° (*c* 1·0 in MeOH) (Found: C, 70·9; H, 7·8; N, 7·9. $C_{43}H_{56}N_4O_6$ requires C, 71·2; H, 7·8; N, 7·7%). This salt (7·25 g, 10 mmol) was stirred for 4 h with Dowex 50-X8 (21 g), water (50 ml), and ethyl acetate (50 ml). The mixture was filtered and the resin washed with water (3 × 15 ml) and ethyl acetate (3 × 15 ml). The aqueous layer was extracted with ethyl acetate and the combined ethyl acetate solutions were dried and evaporated. Addition of light petroleum (b.p. 40—60°; 250 ml) to a solution of the residue in ether (250 ml) gave the *free acid* (4·9 g, 91%), m.p. 112—115° $[\alpha]_D^{21}$ −46·9 (*c* 1·0 in MeOH), which was dried in a vacuum desiccator; $\nu_{max.}$ (Nujol) 3300 (NH str.), 1683br (C:O str.), and 1515 (amide II; C:C def.) cm^{-1}; τ [$(CD_3)_2SO$] 1·62 (1H, t, *J* 6 Hz, Gly-NH), 2·67 (5H, s, O·CH_2Ph), 2·82 (10H, s, C·CH_2Ph), 3·03 (1H, s, Bphe-NH), 5·00 (2H, s, O·CH_2Ph), 5·8br (1H, m, Pro-N·CH), 6·31br (2H, s, Gly-N·CH_2), 6·56 (4H, ABq, *J* −14 Hz, C·CH_2Ph), 6·63 (2H, t, *J* 7 Hz, Pro-N·CH_2), and 8·32br (4H, m, Pro-C·CH_2·C) (Found: C, 68·6; H, 5·9; N, 7·6. $C_{31}H_{33}N_3O_6$ requires C, 68·5; H, 6·1; N, 7·7%). Dicyclohexylcarbodi-imide (1·9 g) was added to a solution of this compound in ether (50 ml) and the mixture stirred for 2 h and then set aside at −10°. After 2·5 h the dicyclohexylurea was filtered off and washed with ether. Evaporation of the filtrate and washings, followed by trituration with light petroleum gave *4,4-dibenzyl-2-(N-benzyloxycarbonyl-L-prolylaminomethyl)oxazolin-5-one* (VII; R = Z-Pro-NH·CH_2) (4·6 g, 96%), m.p. 113—116°, $[\alpha]_D^{23}$ −49·2° (*c* 1·0 in MeOH); $\nu_{max.}$ (Et_2O) 3290 (NH str.), 1823 (ring C:O str.), 1705 (urethane C:O str.), 1685 (C:N str.), 1525 (amide II), and 1497 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·70 (5H, s, O·CH_2Ph), 2·85 (11H, m, C·CH_2Ph and Gly-NH), 4·85 (2H, s, O·CH_2Ph), 5·69br (1H, m, Pro-N·CH), 6·28 (2H, d, *J* 6 Hz, Gly-N·CH_2), 6·52 (2H, t, *J* 6 Hz, Pro-N·CH_2), 6·90 (4H, s, C·CH_2Ph), and 8·03br (4H, m, Pro-C·CH_2·C) (Found: *m/e* 525. $C_{31}H_{31}N_3O_5$ requires *M*, 525).

To a solution of *N*-benzyloxycarbonyl-*O*-t-butyl-L-

serine hydrazide [21] (9·27 g, 30 mmol) in dimethylformamide (120 ml) at −30°, 4M-hydrogen chloride in dioxan (30 ml) was added, followed by t-butyl nitrite (3·6 ml, 30 mmol). After 8 min at −20°, the temperature was lowered to −40° and triethylamine (17·6 ml, 126 mmol) was added dropwise. The resulting azide solution was added to L-proline (5·16 g, 45 mmol) and triethylamine (4·2 ml, 30 mmol) in dimethylformamide (60 ml). After stirring for 48 h at 3°, water was added and the solution acidified (pH 1) with 6M-hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated brine and then extracted with saturated sodium hydrogen carbonate. This extract was acidified and extracted with ethyl acetate. Evaporation of the dried extract, trituration with light petroleum, and recrystallisation from ethyl acetate–light petroleum (b.p. 40—60°) gave *N-benzyloxycarbonyl-O-t-butyl-L-seryl-L-proline* (10·0 g, 85%), m.p. 115—116°, $[\alpha]_D^{26}$ −51·8° (*c* 1·0 in MeOH) (Found: C, 61·5; H, 7·4; N, 7·5. $C_{20}H_{28}N_2O_6$ requires C, 61·2; H, 7·2; N, 7·1%). Dicyclohexylcarbodiimide (2·58 g, 12·5 mmol) was added to a solution of this protected dipeptide (4·90 g, 12·5 mmol) and α-benzylphenylalanine t-butyl ester (3·55 g, 11·4 mmol) in acetonitrile (35 ml). After 48 h, the reaction was worked up as usual; *N*-benzyloxycarbonyl-*O*-t-butyl-L-seryl-L-prolyl-α-benzylphenylalanine t-butyl ester (7·3 g, 96%) so obtained was an uncrystallisable gum, contaminated (t.l.c.) with a little dicyclohexylurea; $\nu_{max.}$ (Nujol) 1725 (ester C:O str.), 1690 (urethane C:O str.; amide C:O str.), 1600 (amide C:O str.; C:C def.), 1520 (amide II), and 1498 (C:C def.) cm^{-1}; τ ($CDCl_3$) 2·72 (5H, s, O·CH_2*Ph*), 2·86 (10H, m, C·CH_2*Ph*), 3·12br (1H, s, Ser-NH), 4·60 (1H, t, *J* 8 Hz, Bphe-NH), 4·97 (2H, s, O·C*H*$_2$Ph), 5·49br (2H, m, Ser-N·CH and Pro-N·CH), 6·4br (2H, s, Pro-N·CH_2), 6·52 (2H, d, *J* 6 Hz, Ser-O·CH_2), 6·52 (4H, ABq, *J* −13·5 Hz, C·C*H*$_2$Ph), 8·30br (4H, m, Pro-C·CH_2·C), 8·65 (9H, s, ester Bu^t), and 9·03 (9H, s, ether Bu^t). Hydrogen was bubbled for 2 h through a stirred solution of this peptide (3·43 g, 5 mmol) in methanol (25 ml) containing acetic acid (0·29 ml, 5 mmol) and 10% palladised charcoal (680 mg). The filtered mixture was evaporated and the residue dissolved in ethyl acetate (25 ml), washed with saturated sodium hydrogen carbonate and brine, dried, and evaporated. Chromatography in chloroform and 10% ethanol in chloroform on silica gel gave *O*-t-butyl-L-seryl-L-prolyl-α-benzylphenylalanine t-butyl ester (1·59 g, 60%) (Found: *m/e* 551. Calc. for $C_{32}H_{45}N_3O_5$: *M*, 551).

This ester, without further purification (3·32 g, 6·0 mmol), and the oxazolinone (VII; R = Z-Pro-NH·CH_2) (3·15 g, 6·0 mmol) were refluxed for 14 h in acetonitrile (40 ml). The solution was evaporated to dryness and the residue kept overnight under light petroleum (b.p. 40—60°). The deposited solid was filtered off and dissolved in chloroform (250 ml). The solution was shaken with silica gel (60 g); the gel was then filtered off and washed with more chloroform (250 ml). The chloroform was evaporated and the residue treated twice more with silica gel in the same manner. The final solid product was recrystallised from ether–light petroleum (b.p. 40—60°), at −5°; *N-benzyloxycarbonyl-L-prolylglycyl-α-benzylphenylalanyl-O-t-butyl-L-seryl-L-prolyl-α-benzylphenylalanine t-butyl ester* (IX; R′ = Z, R″ = R‴ = Bu^t) (2·62 g, 40%) so obtained had m.p. 93—98°, $[\alpha]_D^{27}$ −57·2° (*c* 1·0 in MeOH), τ ($CDCl_3$) 2·22 (1H, t, *J* 5 Hz, Gly-NH), 2·70 (5H, s, O·CH_2*Ph*), 2·79 (20H, m, C·CH_2*Ph*), 3·04 (1H, s, Bphe-NH), 3·15 (1H, s, Bphe-NH), 3·30 (1H, d, *J* 6 Hz, Ser-NH), 4·92 (2H, s, O·C*H*$_2$Ph), 5·43br (3H, m, Pro-CH and Ser-CH), 5·6—7·1 (16H, m, Gly-N·CH_2, Ser-O·CH_2, Pro-N·CH_2, and C·C*H*$_2$Ph), 8·2br (8H, m, Pro-C·CH_2·C), 8·60 (9H, s, ester Bu^t), and 8·92 (9H, s, ether Bu^t) (Found: C, 70·1; H, 7·0; N, 7·9. $C_{63}H_{76}N_6O_{10}$ requires C, 70·2; H, 7·1; N, 7·8%).

We thank the University of Luanda for study leave and financial support (to H. L. S. M.) and Miss J. L. Thorne and Mr. V. Šik for the n.m.r. spectra.

[2/1976 *Received*, 21*st August*, 1972]

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