Amino-acids and Peptides. Part XXV.[1] The Mechanism of the Base-catalysed Racemisation of the *p*-Nitrophenyl Esters of Acylpeptides

By (Miss) **I. Antonovics** and **G. T. Young**, The Dyson Perrins Laboratory, Oxford University

The *p*-nitrophenyl esters of benzoyl- and benzyloxycarbonyl-glycyl-L-phenylalanine are racemised by triethylamine in dichloromethane much more rapidly than are the analogous esters of benzyloxycarbonyl- and phthaloyl-L-phenylalanine. It is shown that the acyldipeptide esters react reversibly with triethylamine to give the corresponding oxazolone, the equilibrium being greatly in favour of the ester. The racemisation of benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester by triethylamine is suppressed by the addition of a large excess of the oxazolone derived from benzyloxycarbonylglycylphenylalanine, which, it is suggested, reacts immediately with the *p*-nitrophenoxide anion and so prevents the back-reaction by which racemic ester is formed. This experiment distinguishes clearly between the " direct exchange " mechanism of racemisation and that through the oxazolone, and it is concluded that such racemisation proceeds through the intermediate formation, racemisation, and coupling of the corresponding oxazolone. Evidence is also given that the conversion of benzyloxycarbonylglycyl-L-phenylalanine into its *p*-nitrophenyl ester by means of diphenyl-keten is accompanied by racemisation.

THE danger of racemisation during the coupling of acylpeptides is now well known, and is a fundamental consideration in planning peptide synthesis.[2] Although we have shown earlier [3] that the base-catalysed racemisation of benzoyl-L-leucine *p*-nitrophenyl ester proceeds chiefly if not exclusively through the intermediate formation, racemisation, and coupling of the corresponding oxazolone, yet this conclusion does not necessarily apply to the more typical acylpeptide esters, and the alternative mechanism of direct hydrogen exchange at the dissymmetric centre of the reactive acid derivative must seriously be considered. Evidence that the base-catalysed racemisation of benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester proceeds chiefly by the oxazolone route has been presented briefly,[4] and the detailed results are now described.

The following new peptide derivatives were prepared by conventional methods for use in this work: benzoylglycyl-L-phenylalanine and its methyl and *p*-nitrophenyl esters; acetylglycyl-L-phenylalanine and its methyl ester; benzoyl- and benzyloxycarbonyl-sarcosyl-L-phenylalanine and their methyl and *p*-nitrophenyl esters. The dimorphism of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester, forming mixtures melting over a wide range, has already been noted,[5,6] and we have found several of these derivatives to be dimorphous. Commonly, crystallisation occurs most readily in a solvated form, resulting again in a melting range, and melting points have proved of little use in establishing purity, for which thin-layer chromatography is a more effective test. A further complication in the case of benzoylglycycl-L-phenylalanine *p*-nitrophenyl ester is that racemisation (possibly through the oxazolone) occurs on melting. The action of dicyclohexylcarbodi-imide [7,8] on benzoylglycyl-L-phenylalanine gave the corresponding crystalline oxazolone, which racemised rapidly in tetrahydrofuran solution without the addition of base.

The addition of one molar proportion of triethylamine to solutions of the *p*-nitrophenyl esters of benzyloxycarbonyl- and benzoyl-glycyl-L-phenylalanine and of benzyloxycarbonyl- and benzoyl-sarcosyl-L-phenylalanine in dichloromethane caused a fall in optical rotation markedly more rapid than with the *p*-nitrophenyl esters of benzyloxycarbonyl-L-phenylalanine or phthaloyl-L-phenylalanine (see Figure). In the last case it is to be expected that the phthaloyl group will increase the acidity of the hydrogen at the dissymmetric centre, and yet racemisation was still slower than for the esters of the acyldipeptides, which would seem to have available

[1] Part XXIV, B. O. Handford. J. H. Jones, G. T. Young, and (in part) T. F. N. Johnson, *J. Chem. Soc.*, 1965, 6814.

[2] Reviews: (*a*) I. Antonovics, A. L. Heard, M. W. Williams, and G. T. Young, Proc. 6th European Peptide Symposium, Athens, 1963, ed. L. Zervas, Pergamon Press, 1966; (*b*) F. Weygand, A. Prox, and W. König, *Chem. Ber.*, 1966, **99**, 1451.

[3] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1964, 3701.

[4] I. Antonovics and G. T. Young, Proc. 7th European Peptide Symposium, Budapest, 1964; *Acta Chim. Hung.*, 1965, **44**, 43; *Chem. Comm.*, 1965, 398.

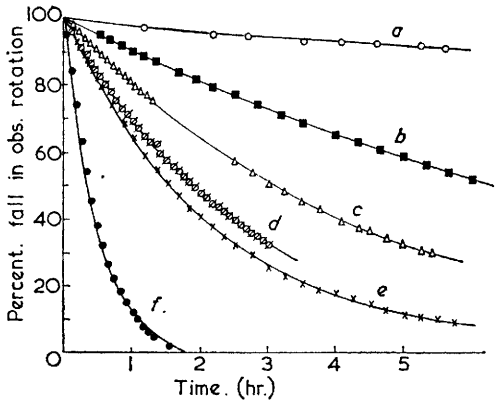
[5] M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 1962, **84**, 1279.

[6] H. C. Beyerman, W. M. van den Brink, F. Weygand, A. Prox, W. König, L. Schmidhammer, and E. Nintz, *Rec. Trav. chim.*, 1965, **84**, 213.

[7] M. Goodman and W. J. McGahren, *J. Amer. Chem. Soc.*, 1965, **87**, 3028.

[8] E. Schnabel, Proc. 6th European Peptide Symposium, Athens, 1963, ed. L. Zervas, Pergamon Press, 1966, p. 71; *Annalen*, 1965, **688**, 238.

a more favourable route. However, the solutions obtained by the action of triethylamine on the two first acyldipeptide *p*-nitrophenyl esters showed at most very weak infrared absorption at 1830 cm.$^{-1}$ (oxazolone

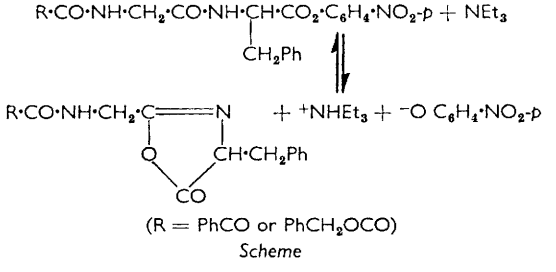


The fall in optical rotation of solutions of *p*-nitrophenyl esters in dichloromethane at 20° after the addition of 1 molar proportion of triethylamine. (*a*) Z-L-Phe·ONp (0·05M); (*b*) Phthaloyl-L-Phe·ONp (0·05M); (*c*) Z·Sar-L-Phe·ONp (0·04M); (*d*) Bz·Sar-L-Phe·ONp (0·04M); (*e*) Z·Gly-L-Phe·ONp (0·05M); (*f*) Bz·Gly-L-Phe·ONp (0·045M); Z = CO·OCH$_2$Ph, ONp = OC$_6$H$_4$·NO$_2$*p*; Sar = MeN·CH$_2$·CO.*

* Abbreviations follow the Rules in "Abbreviated Designation of Amino-acid Derivatives and Polypeptides" (Information Bulletin No. 25, I.U.P.A.C.). Z = PhCH$_2$OCO; Pht = phthaloyl; ONp = OC$_6$H$_4$·NO$_2$*p*; Bz = C$_6$H$_5$·CO; Sar = Sarcosine.

CO), whereas under similar conditions benzoyl-L-leucine *p*-nitrophenyl ester rapidly forms 4-isobutyl-2-phenyloxazolone. The oxazolones prepared from benzyloxycarbonyl- and benzoyl-glycyl-L-phenylalanine show the expected infrared absorption at this frequency, not extinguished by the addition of triethylamine (which greatly modifies the infrared absorption of 4-isobutyl-5-oxazolone [3]).

It seemed possible that the equilibrium shown in the Scheme is indeed established, but that the equilibrium concentration of oxazolone is low:



Scheme

In agreement with this suggestion, the addition of equimolar amounts of *p*-nitrophenol and triethylamine to solutions of each of the oxazolones derived from benzyloxycarbonyl- and benzoyl-glycylphenylalanine immediately resulted in strong absorption at 1770 cm.$^{-1}$ (*p*-nitrophenyl ester CO) with corresponding weakening or near-extinction of the 1830 cm.$^{-1}$ peak. We may mention here that this reaction provides a convenient preparation of (optically inactive) aryl esters of acylpeptides, and we describe its use for benzoylglycyl-DL-phenylalanine *p*-chlorophenyl ester. Goodman and Levine [9] made benzoyl-DL-phenylalanine *p*-nitrophenyl ester similarly in 80% yield, and we have prepared acetyl-DL-leucine *p*-nitrophenyl ester in this way. However, equimolar amounts of 2,4-dinitrophenol and triethylamine failed to react with 2-benzamidomethyl-4-benzyl-5-oxazolone, no ester-carbonyl infrared absorption appearing within 3 hr. at room temperature.

Proof of the equilibrium was obtained by the addition of triethylamine to an equimolar mixture of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester and the oxazolone derived from benzoylglycyl-L-phenylalanine dissolved in dichloromethane; after 1 hr. the reaction was stopped, and thin-layer chromatography of the recovered products showed the presence of the original *p*-nitrophenyl ester together with an approximately equal amount of benzoylglycylphenylalanine *p*-nitrophenyl ester. The former ester was decomposed by means of hydrogen bromide in acetic acid, enabling the latter to be isolated. We conclude that the *p*-nitrophenoxide anion formed as in the Scheme reacts with the added oxazolone and so establishes the equilibrium between the two acyldipeptide esters. No such exchange occurred when benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (which does not form an oxazolone) replaced the benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester in the above reaction, and the ester (still optically active) was recovered in 84% yield.

It does not necessarily follow that the base-catalysed racemisation of such acyldipeptide esters occurs through this equilibrium, but we believe that the following experiment shows that this is so in the case of benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester. To a solution of this ester in dichloromethane was added 10 molar proportions of the oxazolone derived from benzyloxycarbonylglycylphenylalanine and 1 molar proportion of triethylamine. When the optical rotation of the solution had fallen to 51% of the initial value the reaction was stopped; the mixture of esters was recovered and treated with hydrogen bromide in acetic acid (to destroy benzyloxycarbonyl derivatives). Analytically and optically pure benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester was recovered in 46% yield, and no racemic ester could be found. If the racemisation had proceeded by direct exchange, then the recovered ester should be 49% racemic. If, however, the racemisation proceeded through the equilibrium shown in the Scheme, the large excess of oxazolone present should react with the *p*-nitrophenoxide ion formed and so largely prevent the back-reaction by which racemic ester is produced. In that case the original L-isomer should be recoverable in amount corresponding to the residual optical activity of the solution, *i.e.*, 51% of that added. This experiment distinguishes therefore clearly and definitely between the "direct exchange" and the oxazolone routes to racemisation. Control experiments showed that 9% of racemic ester admixed with L-ester could readily be

[9] M. Goodman and L. Levine, *J. Amer. Chem. Soc.*, 1964, **86**, 2918.

detected, and we conclude that in this typical example the base-catalysed racemisation proceeds chiefly if not exclusively through the oxazolone. Important support for this mechanism has recently been presented by Goodman and McGahren,[7] who have shown that when *p*-nitrophenoxide anion or phenylalanine methyl ester reacts with the oxazolone derived from benzyloxycarbonyl-α-aminoisobutyryl-L-phenylalanine racemisation occurs much faster than ring-opening (by factors of 40 and 11, respectively). This is of course consistent with the oxazolone route.

We record also here racemisation tests (carried out with Dr. J. M. Hugo and briefly mentioned earlier)[2a] using diphenylketen and *p*-nitrophenol (with a catalytic amount of triethylamine) in tetrahydrofuran for the preparation of *p*-nitrophenyl esters.[10,11] Benzyloxycarbonylglycyl-L-phenylalanine was converted into the *p*-nitrophenyl ester by this procedure and the whole product, without purification, was condensed with glycine ethyl ester. This sequence constitutes the Anderson test for racemisation.[12] Benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester couples without racemisation under these conditions, and therefore any racemisation observed must have occurred during the preparation of the *p*-nitrophenyl ester. The amount of racemate found varied from 5·8 to 37·5%, depending on the time allowed for anhydride formation (30 sec.—8 min.). In each case the properties of the main product indicated that besides L-peptide it contained some diphenylacetylglycine ethyl ester, which was isolated in one case. Elmore and Smyth [11] have reported finding racemisation when attempting to prepare benzoyl-D- or -L-alanine *p*-nitrophenyl ester by this method. As far as we know, there is still no general procedure by which acylpeptides with optically active residues at the carboxyl-end can be converted into their *p*-nitrophenyl esters without risk of racemisation.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus, and optical rotations on a Perkin-Elmer 141 automatic polarimeter with solutions in a 10 cm. cell. N.m.r. spectra were measured [by (Mrs.) E. Richards] on a Perkin-Elmer R10 spectrometer with tetramethylsilane as internal standard and deuteriochloroform as solvent. Thin-layer chromatography was on Kieselgel G (unbaked), using ether as solvent and iodine vapour for detection, unless otherwise stated; other solvents used were "BWA": n-butanol–water–acetic acid (4 : 1 : 1); "BWP": n-butanol–water–pyridine (2 : 2 : 1). R_f values refer to thin-layer chromatography unless otherwise stated. Evaporation was by rotary evaporator and solutions in organic solvents were dried over magnesium sulphate. Light petroleum was of b. p. 40—60°. Dried and redistilled solvents were used in experiments with active esters, oxazolones, and dicyclohexylcarbodi-imide. Dimethylformamide was dried over potassium hydroxide, redistilled twice at reduced pressure and finally at normal pressure; tetrahydrofuran was dried over sodium, passed down an alumina column, and redistilled in nitrogen. Triethylamine was dried over sodium and redistilled, and pyridine was dried over potassium hydroxide and redistilled. Samples for analysis were dried at 60° below melting point and 0·1 mm., unless otherwise stated.

Benzoylglycyl-L-phenylalanine p-*Nitrophenyl Ester*.—To hippuric acid (2·69 g.; 0·015 mole) in acetonitrile (240 ml.) at 0° was added L-phenylalanine *p*-nitrophenyl ester hydrobromide [13] (5·51 g.; 0·015 mole) and triethylamine (1·51 g.; 0·015 mole) in acetonitrile (10 ml.). After stirring for 1 hr., dicyclohexylcarbodi-imide (3·09 g.; 0·015 mole) was added and the mixture was stirred at 0° for 1 hr. and at room temperature for 5 hr. The dicyclohexylurea was filtered off and washed with ethyl acetate, and the filtrate and washings were evaporated. The residue was taken up in ethyl acetate and the solution was washed with saturated sodium hydrogen carbonate, 2N-hydrochloric acid, and saturated brine, and then dried and evaporated. The yellow residue crystallised on adding methanol, and recrystallisation from methanol acidified with acetic acid gave white needles of ester solvated with methanol, m. p. 61—67° [Found (after drying in a vacuum desiccator): C, 62·4; H, 5·0; N, 8·75. $C_{24}H_{21}N_3O_6$,CH_3OH requires C, 62·6; H, 5·2; N, 8·8%]. The molten product resolidified, melting again at 144—149·5°; the *non-solvated ester* (4·56 g., 68%) was obtained by drying at 75°/0·5 mm. for 5 hr., and had m. p. 144—149·5°, $[\alpha]_D^{22·5}$ −21·8° (*c* 2·0 in $CHCl_3$) $[\alpha]_D^{22·5}$ −29·7° (*c* 2·0 in dimethylformamide); $\nu_{max.}$ ($CHCl_3$) 1775 cm.$^{-1}$ (ester CO); n.m.r. signals at τ 5·00 [multiplet, 1 proton, from NH·C*H*(CH_2–)·CO]; 5·80 (doublet with J = 5 c./sec., 2 protons, from NH·*C*H_2·CO); 6·78 (doublet with J = 6 c./sec. 2 protons, from CH·*C*H_2Ph); R_f 0·15 (Found: C, 64·2; H, 4·6; N, 9·5. $C_{24}H_{21}N_3O_6$ requires C, 64·4; H, 4·7; N, 9·4%). Gradual resolidification often occurred after melting, giving the DL-isomer of m. p. and mixed m. p. 173—176°. The wide melting point range may be due to oxazolone formation during melting. The addition of an equimolar amount of triethylamine to a 0·1M-solution of the ester in dichloromethane caused the appearance in the infrared spectrum of a shoulder at 1830 cm.$^{-1}$, and within 1 hr. crystalline racemic ester separated. With tetrahydrofuran as solvent the shoulder at 1830 cm.$^{-1}$ increased after 45 min. to a weak peak, but no further change was then observed, even when 9 molar equivalents of triethylamine were used. Pyridine caused no change in the infrared absorption of the ester in either solvent up to 6 days.

Benzoylglycyl-DL-phenylalanine p-*Nitrophenyl Ester*.—This was prepared by the action of triethylamine (0·051 g.) on the L-isomer (0·221 g.) in dichloromethane (5 ml.). After 1 hr. the crystals were collected and washed with dichloromethane, leaving colourless needles of the racemate (0·14 g., 64%), m. p. 170—175°. Recrystallisation from methanol gave *racemic ester* of m. p. 174—176° (Found: C, 64·8; H, 4·7; N, 9·25%).

Benzyloxycarbonylglycyl-L-phenylalanine p-*Nitrophenyl Ester*.—This ester was prepared as described by Goodman and Stueben [13] but with a 40% increase in the volume of acetonitrile used as solvent, in 79% yield; after recrystallisation from ethyl acetate–light petroleum it melted partly at 111°, resolidified, and melted finally at 147°;

[10] D. T. Elmore and J. J. Smyth, *Proc. Chem. Soc.*, 1963, 18.
[11] D. T. Elmore and J. J. Smyth, *Biochem. J.*, 1965, **94**, 563.
[12] G. W. Anderson and R. W. Young, *J. Amer. Chem. Soc.*, 1952, **74**, 5307; G. W. Anderson, J. Blodinger, and A. D. Welcher, *ibid.*, p. 5309.
[13] M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.

this behaviour, due to dimorphism, has been observed earlier.[5,6,13,14] The ester had $[\alpha]_D^{20}$ −7·3° (*c* 2·0 in $CHCl_3$), R_f 0·29 [lit., $[\alpha]_D^{25}$ −6·3° (ref. 14) −6·6° (ref. 6); n.m.r. signals at τ 4·26 (triplet with $J = 5$ c./sec., 1 proton, from OCO·N*H*·CH_2); 4·90 (singlet, 2 protons, from PhCH_2O); 4·97 [multiplet partly obscured by the last signal, 1 proton, from NH·C*H*(CH_2–)·CO]; 6·10 (doublet with $J = 5$ c./sec., 2 protons, from NH·CH_2·CO); 6·80 (doublet with $J = 6$ c./sec., 2 protons, from CH·CH_2Ph).

The addition of an equimolar amount of triethylamine to a 0·1M-solution of the ester in dichloromethane or in tetrahydrofuran caused the appearance of a very weak peak at 1830 $cm.^{-1}$, not enhanced by heating at the b. p. for 4·5 hr. in the first case or after 3 months at room temperature in the second case. Pyridine caused no change in the infrared absorption of the ester in either solvent up to 26 hr.

Benzoylglycyl-L-phenylalanine Methyl Ester.—A solution of hippuric acid (7·16 g., 0·04 mole) in tetrahydrofuran (60 ml.) was added to a mixture of L-phenylalanine methyl ester hydrochloride[15] (8·24 g., 0·04 mole) and triethylamine (5·46 ml., 0·04 mole) in tetrahydrofuran (60 ml.). The mixture was stirred and cooled to −10° and dicyclohexylcarbodi-imide (8·24 g., 0·04 mole) in tetrahydrofuran (30 ml.) was added dropwise during 10 min. The cooling bath was removed; next day the solution showed only weak infrared absorption at 2100 $cm.^{-1}$ (N:C:N) and a few drops of acetic acid were added. After 0·5 hr. the solution was filtered and evaporated; the residue was taken up in chloroform and washed as usual; the solution was dried and evaporated, leaving a white solid which was recrystallised from ethyl acetate–light petroleum, giving *ester* (9·24 g., 69%) of m. p. 123—124°, $[\alpha]_D^{19.5}$ +40·4° (*c* 2·0 in $CHCl_3$) (Found: C, 67·4; H, 5·9; N, 8·3. $C_{19}H_{20}N_2O_4$ requires C, 67·05; H, 5·9; N, 8·2%).

Benzoylglycyl-L-phenylalanine.—The above methyl ester (2·51 g., 0·0075 mole) in methanol (11·3 ml.) was saponified by stirring with N-sodium hydroxide (7·9 ml.) at room temperature for 2·5 hr. The solution was acidified (Congo Red) and the methanol was evaporated; the aqueous residue was extracted with ethyl acetate, from which the product was extracted into saturated potassium hydrogen carbonate. The solution was acidified (Congo Red) and the product was extracted into ethyl acetate; the extract was washed with N-hydrochloric acid and saturated brine, and then dried. Evaporation of the solvent left a syrup which slowly crystallised under light petroleum at 0°; it was recrystallised from ethyl acetate–light petroleum to give *acid* (2·15 g., 90%) of m. p. 144·5—148°, $[\alpha]_D^{24}$ +39·3° (*c* 2·0 in EtOH) (Found: C, 66·35; H, 5·6; N, 8·2. $C_{18}H_{18}N_2O_4$ requires C, 66·3; H, 5·5; N, 8·6%).

Acetylglycyl-L-phenylalanine Methyl Ester.—A solution of acetylglycine (2·34 g., 0·02 mole) in a mixture of tetrahydrofuran (60 ml.) and dimethylformamide (8 ml.) containing triethylamine (2·02 g., 0·02 mole) was cooled to −5° with stirring and ethyl chloroformate (1·92 ml., 0·02 mole) was slowly added. 5 Min. after the addition was complete, L-phenylalanine methyl ester hydrochloride[15] (4·12 g., 0·02 mole) and triethylamine (2·02 g.) were added. The cooling bath was removed and stirring was continued overnight. The suspension was filtered, the filtrate was evaporated, and the residual oil was taken up in dichloromethane; on shaking with water the *ester hydrate* (4·66 g., 79%) was precipitated as a white crystalline solid, m. p. 71·5—82°, $[\alpha]_D^{20}$ −0·8° (*c* 2·0 in dimethylformamide) $[\alpha_D]^{20}$ +11·6° (*c* 1·9 in MeOH) [Found (after drying at 20° and 15 mm.): C, 57·0; H, 6·9; N, 9·6. $C_{14}H_{18}N_2O_4,H_2O$ requires C, 56·75; H, 6·8; N, 9·5%]. Recrystallisation from water or aqueous methanol raised the upper limit of the melting point range to 94°; the anhydrous ester could not be solidified. The hydrate gave one spot only on thin-layer chromatography in BWA (R_f 0·74), BWP (R_f 0·72), and in ether (R_f 0·0); detection was by chlorine followed by starch–iodide.

Acetylglycyl-L-phenylalanine.—The above methyl ester (5·92 g.) was saponified for 1·5 hr. as described for the benzoyl analogue. The reaction was followed by paper chromatography in BWP, in which the acid had R_f 0·61; detection was by chlorine followed by starch–iodide. After acidification, the solution was evaporated to dryness, leaving a white solid which was recrystallised from hot water to give product (4·48 g., 85%) of m. p. 157—161·5°, a further crystallisation gave *acid* of m. p. 158—162°, $[\alpha]_D^{20}$ +44° (*c* 2·0 in EtOH), R_f 0·45 (BWA) (Found: C, 59·3; H, 5·9; N, 10·7. $C_{13}H_{16}N_2O_4$ requires C, 59·1; H, 6·05; N, 10·6%). A second recrystallisation from water did not change the melting point; recrystallisation from ethanol–ether gave needles of m. p. 159—162·5° (with indication of an earlier change of form); recrystallisation from ethyl acetate did not change the melting point.

Benzoylsarcosyl-L-phenylalanine Methyl Ester.—This was prepared from benzoylsarcosine[16] as described for the glycyl analogue, except that the residue after evaporation was taken up in ethyl acetate instead of chloroform for washing. Removal of the ethyl acetate left a white solid which was recrystallised from ethyl acetate–light petroleum to give *ester* (80% yield) of m. p. 108·5—112°, raised to 110—112° after a further crystallisation; $[\alpha]_D^{24.5}$ +11·3° (*c* 2·0 in EtOAc) (Found: C, 68·05; H, 6·3; N, 7·5. $C_{20}H_{22}N_2O_4$ requires C, 67·8; H, 6·2; N, 7·9%).

Benzoylsarcosyl-L-phenylalanine.—The above methyl ester was saponified for 1·5 hr. as described for the glycyl analogue. Evaporation of the methanol left a white solid (92%) which was recrystallised from aqueous methanol and dried at 70°/0·1 mm. giving *acid* of m. p. 193—196° (decomp.), $[\alpha]_D^{24}$ +24·8° (*c* 1·7 in MeOH) (Found: C, 66·7; H, 6·25; N, 8·7. $C_{19}H_{20}N_2O_4$ requires C, 67·05; H, 5·9; N, 8·2%).

Benzoylsarcosyl-L-phenylalanine p-*Nitrophenyl Ester.*—This was prepared as described for the glycyl analogue. Evaporation of the ethyl acetate left a yellow gum which crystallised under ethyl acetate–light petroleum; more product was then precipitated by adding ether (yield, 85%). The ester was obtained as a mixture of two crystalline forms not completely separable and melting over a range; benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester behaves somewhat similarly. Recrystallisation from ethanol gave needles of m. p. 135—143°, $[\alpha]_D^{20}$ −13·2° (*c* 2·0 in $CHCl_3$) (Found: C, 64·9; H, 4·9; N, 9·4. $C_{25}H_{23}N_3O_6$ requires C, 65·1; H, 5·0; N, 9·1%). Recrystallisation from ethyl acetate–ether gave *ester* of m. p. 123—130°, $[\alpha]_D^{20}$ −13·0° (*c* 1·1 in $CHCl_3$) (Found: C, 64·7; H, 5·4; N, 9·45%).

[14] M. Goodman and K. C. Stueben, *J. Org. Chem.*, 1962, **27**, 3409.

[15] R. A. Boissonnas, St. Guttmann, P. A. Jaquenoud, and J. P. Waller, *Helv. Chim. Acta*, 1956, **39**, 1421; F. Bergel, J. M. Johnson, and R. Wade, *J. Chem. Soc.*, 1962, 3802.

[16] J. L. O'Brien and C. Niemann, *J. Amer. Chem. Soc.*, 1957, **79**, 80.

Benzyloxycarbonylsarcosyl-L-phenylalanine Methyl Ester.—This was prepared from benzyloxycarbonylsarcosine [17] as described for the benzoyl analogue. Evaporation of the ethyl acetate left a colourless syrup which crystallised under ether at 0°, giving product (65%) of m. p. 73—76·5°. Recrystallisation from ether–light petroleum gave *ester* of the same m. p., $[\alpha]_D^{25}$ +24·0 (*c* 2·0 in EtOAc) (Found: C, 65·8; H, 6·3; N, 7·4. $C_{21}H_{24}N_2O_5$ requires C, 65·6; H, 6·25; N, 7·3%).

Benzyloxycarbonylsarcosyl-L-phenylalanine.—The above ester was saponified for 2 hr. as described in the preparation of benzoylglycyl-L-phenylalanine. Evaporation of the ethyl acetate left product which was recrystallised from ethyl acetate–light petroleum and then from aqueous ethanol, giving *acid* (77%) of m. p. 112—116·5°, unchanged after another crystallisation from aqueous ethanol, $[\alpha]_D^{24}$ +24·8° (*c* 2·0 in MeOH) [Found (after drying at 100°/0·01 mm.): C, 65·1; H, 5·9; N, 7·7. $C_{20}H_{22}N_2O_5$ requires C, 64·9; H, 5·95; N, 7·6%]. The acid crystallised most readily from ethyl acetate–light petroleum as an ethyl acetate *solvate* of m. p. 95° (with previous softening), $[\alpha]_D^{20}$ +22·7° (*c* 2·0 in MeOH); thin-layer chromatography showed one spot only (detection by chlorine and starch–iodide), R_f 0·90 (BWA), R_f 0·70 (BWP) [Found (after drying at 20°/0·1 mm.): C, 63·55; H, 6·5; N, 6·95. $C_{20}H_{22}N_2O_5,\frac{1}{2}(C_4H_8O_2)$ requires C, 63·8; H, 6·3; N, 6·8%].

Benzyloxycarbonylsarcosyl-L-phenylalanine p-*Nitrophenyl Ester.*—This was prepared as described for the glycyl analogue; evaporation of the ethyl acetate left a yellow gel which was crystallised from ethanol. The crystals were washed with ether, leaving a pale yellow powder which was dried at 60°/0·05 mm., giving product (86% yield) of m. p. 134—137·5°. Four recrystallisations from ethanol gave *ester* of m. p. 136—138° (in each case the partial formation of feathery needles was observed at 119·5—121°); $[\alpha]_D^{20}$ −8·3° (*c* 2·0 in $CHCl_3$), R_f 0·26 (Found: C, 63·3; H, 5·2; N, 8·6. $C_{26}H_{25}N_3O_7$ requires C, 63·35; H, 5·1; N, 8·55%). Another preparation gave the lower melting dimorph, m. p. 118—121°, $[\alpha]_D^{20}$ −8·3 (*c* 2·0 in $CHCl_3$) (Found: C, 63·65; H, 5·0; N, 8·4%). Usually this dimorph resolidified after melting, and then had m. p. 132—136°.

4-Benzyl-2-benzyloxycarbonamidomethyl-5-oxazolone.—Benzyloxycarbonylglycyl-L-phenylalanine [18] (7·12 g., 0·02 mole) was shaken with dicyclohexylcarbodi-imide (4·12 g., 0·02 mole) in dichloromethane (50 ml.) for 1·5 hr., after which infrared absorption at 2100 cm.$^{-1}$ (N=C=N) was absent. The dicyclohexylurea was filtered off, the filtrate was evaporated, and the residual gum was taken up in ether (50 ml.). The ether extract was set aside until no more urea separated, and then filtered; from the concentrated filtrate crystals of the oxazolone slowly separated. Recrystallisation from ether gave needles (5·15 g., 76%) of m. p. 75—77°, $[\alpha]_D^{20}$ *ca.* −2° (*c* 0·6 in tetrahydrofuran); $\nu_{max.}$ ($CHCl_3$) 3430, 3010, 1832s, 1725s, 1678s, 1508s, 1190—1230 (broad) cm.$^{-1}$ (Found: C, 67·5; H, 5·3; N, 8·5. Calc. for $C_{19}H_{18}N_2O_4$: C, 67·45; H, 5·3; N, 8·3%). [Lit.,[8] for L-isomer: m. p. 68—76°, $[\alpha]_D$ −15·7° (*c* 0·6 in tetrahydrofuran); lit.,[19] for the DL-isomer, m. p. 70·5—72°]. The crude oxazolone was optically active but the specific rotation fell to zero immediately after the addition of 1 equiv. of triethylamine.

2-Benzamidomethyl-L-4-benzyl-5-oxazolone.—Benzoylglycyl-L-phenylalanine was converted into the *oxazolone* as described above for the benzyloxycarbonyl analogue, yielding white crystals (69%) of m. p. 115—120·5°; concentration of the mother-liquors gave a further crop (16%) of m. p. 114—119°. Recrystallisation from ethyl acetate–ether gave white needles of m. p. 118—121·5°, $[\alpha]_D^{20}$ −12·4° (*c* 2·0 in tetrahydrofuran), $\nu_{max.}$ ($CHCl_3$) 3430, 3050, 1840s, 1670s, 1520s, 1490s, 1391s, 1280, 1130, 1085 cm.$^{-1}$ (Found: C, 70·0; H, 5·6; N, 9·05. $C_{18}H_{16}N_2O_3$ requires C, 70·1; H, 5·2; N, 9·1%). The specific rotation of a 2% solution of the oxazolone in tetrahydrofuran at 20° decreased by 50% in 3 hr.

The Racemisation of Some p-*Nitrophenyl Esters by Triethylamine.*—To a solution of the ester in dichloromethane was added a solution of triethylamine in the same solvent in amount sufficient to provide 1 molar proportion of triethylamine. The time was noted, and the solution was immediately made up to a known volume and the optical rotation was measured in a 10 cm. cell at 20° at 2—10 min. intervals. The results are shown in the Figure. The initial optical rotation was determined by extrapolation from the plot of log α_D obs. against time, which was linear in each case.

Benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester and phthaloyl-L-phenylalanine *p*-nitrophenyl ester were prepared as described by Goodman and Stueben [13] and by Liberek,[20] respectively.

The Reaction of some Oxazolones with Phenols in the Presence of Tertiary Amines.—(*a*) *Infrared spectra.* Solutions of the required oxazolones were prepared as described above by the action of dicyclohexylcarbodi-imide (0·206 g., 0·001 mole) on benzyloxycarbonyl- and benzoylglycyl-L-phenylalanine (0·001 mole) in dichloromethane or tetrahydrofuran (5 ml.) for 1·25 hr.; the urea was filtered off and the infrared spectrum of the filtrate showed the expected oxazolone absorption. *p*-Nitrophenol (0·139 g., 0·001 mole) and tertiary amine (0·001 mole) were added and the infrared absorption was again recorded. With triethylamine, in each case within 6 min. the absorption at 1830 cm.$^{-1}$ had become very weak, and a strong peak had appeared at 1770 cm.$^{-1}$. With pyridine, the same changes occurred in times varying from 5 days to 1 month. When 2,4-dinitrophenol (0·194 g., 0·001 mole) and triethylamine (0·101 g., 0·001 mole) were added to a solution of the oxazolone prepared from benzoylglycycyl-L-phenylalanine (0·001 mole) in tetrahydrofuran (8 ml.), no diminution of the infrared absorption at 1830 cm.$^{-1}$ was observed within 3 hr., and no ester-carbonyl absorption appeared.

(*b*) *Preparation of benzoylglycyl-DL-phenylalanine* p-*chlorophenyl ester.* To a solution of 2-benzamidomethyl-4-benzyl-5-oxazolone (0·616 g., 0·002 mole) in dichloromethane (15 ml.) was added *p*-chlorophenol (0·258 g., 0·002 mole) and triethylamine (0·202 g.). After 1 hr. the infrared absorption at 1830 cm.$^{-1}$ was very weak, and the solution was diluted with dichloromethane (25 ml.) and washed with saturated sodium hydrogen carbonate, 2N-hydrochloric acid, and water, and dried. Evaporation left a pale yellow gum which solidified on the addition of ether and was recrystallised twice from ethyl acetate, giving *ester* (0·70 g., 80%) of m. p. 144—149° (Found: C, 65·9; H, 5·25; N, 6·5; Cl, 8·2. $C_{24}H_{21}ClN_2O_4$ requires C, 66·0; H, 4·8; N,

[17] D. Ben-Ishai and E. Katchalski, *J. Amer. Chem. Soc.*, 1952, **74**, 3688.
[18] K. Hofmann and M. Bergmann, *J. Biol. Chem.*, 1940, **134**, 225.
[19] D. F. De Tar, R. Silverstein, and F. F. Rogers, jun., *J. Amer. Chem. Soc.*, 1966, **88**, 1024.
[20] B. Liberek, *Tetrahedron Letters*, 1963, 1103.

6·4; Cl, 8·1%). Recrystallisation from ethyl acetate with a large volume of ether gave needles of a dimorph of m. p. 104—111°; after melting, resolidification occurred with final melting at 147·5° (Found: C, 65·9; H, 5·0; N, 6·8; Cl, 8·3%).

(*c*) *Preparation of acetyl-*DL*-leucine* p-*nitrophenyl ester.* 4-Isobutyl-2-methyl-5-oxazolone [21] (1·55 g., 0·01 mole) reacted with *p*-nitrophenol (1·39 g., 0·01 mole) and triethylamine (1·01 g., 0·01 mole) in dichloromethane (5 ml.) within 3 hr. The solution was then evaporated to dryness and the solid residue was taken up in ethyl acetate and washed and dried as usual. Evaporation left a white solid which was recrystallised from ethanol–ether to give *ester* (2·17 g., 74%) of m. p. 118—122°. Recrystallisation from ethanol gave product of m. p. 118—122·5° (Found: C, 57·3; H, 6·1; N, 9·65. $C_{14}H_{18}N_2O_5$ requires C, 57·3; H, 6·1; N, 9·6%).

Proof of Equilibrium between Acylpeptide p-*Nitrophenyl Ester and the Corresponding Oxazolone.*—(*a*) *Exchange experiment.* A solution of 2-benzamidomethyl-4-benzyl-5-oxazolone in dichloromethane (5 ml.) was prepared from benzoylglycyl-L-phenylalanine (0·326 g., 0·001 mole) as described above, and then added to a solution of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (0·477 g., 0·001 mole) in dichloromethane (5 ml.), followed by triethylamine (0·101 g., 0·001 mole). After 1 hr. at room temperature, the reaction was stopped by washing the solution with 2N-hydrochloric acid; washing was then continued with sodium hydrogen carbonate and saturated brine. After drying, the solution was evaporated, and the residual gum crystallised on adding acidified methanol. More crystalline product separated after concentration of the filtrate, giving 0·324 g. in all. Thin-layer chromatography of both fractions gave spots of approximately equal intensity at R_f 9·13 and 0·23, parallel with authentic samples of the *p*-nitrophenyl esters of benzoyl- and benzyloxycarbonyl-glycylphenylalanine, respectively. The whole product was dissolved in acetic acid (0·54 ml.) and a solution of hydrogen bromide in acetic acid (4·6N, 0·42 ml.) was added. After 1 hr. the solution was evaporated, finally at 0·5 mm. The residue was extracted with boiling ethyl acetate (10 ml.), the white gelatinous insoluble hydrobromide of glycylphenylalanine *p*-nitrophenyl ester was filtered off, and the filtrate was washed with saturated sodium hydrogen carbonate and saturated brine. After drying, the solvent was evaporated and the residue was recrystallised from the minimum volume of methanol containing a little acetic acid, giving white needles of benzoylglycyl-DL-phenylalanine *p*-nitrophenyl ester (0·081 g.), m. p. 171—175·5°, identical in infrared spectrum (in Nujol) with authentic ester. A similar experiment in which the reaction with triethylamine was continued overnight gave 0·056 g. of benzoylglycyl-DL-phenylalanine *p*-nitrophenyl ester of m. p. 171—175°.

(*b*) *Control experiment.* The above exchange experiment (*a*) was repeated on twice the scale but using benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester [13] (0·84 g., 0·002 mole), 126·5—127·5°, $[\alpha]_D^{20\cdot5}$ −7·5° (*c* 2·0 in $CHCl_3$) instead of the benzyloxycarbonyldipeptide ester. The yellow solid remaining after evaporation of the solvent was triturated with ether and recrystallised twice from ethanol, giving benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (0·662 g.) of m. p. 126—127·5°, $[\alpha]_D^{24\cdot5}$ −8·3° (*c* 2·0 in $CHCl_3$). Concentration of the mother-liquors gave a further 0·047 g. of m. p. 122—127·5°, $[\alpha]_D^{25}$ −7·6° (*c* 2·0 in $CHCl_3$); the total recovery of ester was 84%.

*Recovery of Benzoylglycyl-*L*-phenylalanine* p-*Nitrophenyl Ester after Treatment with Triethylamine in the Presence of a Large Excess of an Oxazolone.*—To a solution of benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester (0·516 g., 0·00115 mole), $[\alpha]_D^{22\cdot5}$ −22·8° (*c* 1·9 in $CHCl_3$) in dichloromethane (26 ml.) was added 4-benzyl-2-benzyloxycarbonamidomethyl-5-oxazolone (3·903 g., 0·00115 mole) and then triethylamine (0·117 g., 0·00117 mole). The optical rotation was followed on a portion of the solution, and when after 54 min. the observed rotation had fallen to 51% of the initial value the solution in the cell was recombined and the reaction was stopped by washing with 2N-hydrochloric acid (2 × 7 ml.). Much benzyloxycarbonylglycyl-DL-phenylalanine separated and was filtered off. The dichloromethane solution was washed with saturated brine (7 ml.), dried, and evaporated. The residual oil was dissolved in ethyl acetate (50 ml.) and washed with saturated sodium hydrogen carbonate (4 × 10 ml.) and saturated brine, and dried. (Washing of the dichloromethane solution with sodium hydrogen carbonate gave emulsions). Evaporation left a gum (1·70 g.) shown by thin-layer chromatography to contain both benzoyl- and benzyloxycarbonyl-glycylphenylalanine *p*-nitrophenyl esters, with unidentified spots at R_f 0·0 and 0·60. The gum was dissolved in acetic acid (5·7 ml.) and a solution of hydrogen bromide in acetic acid (6·8N, 2·73 ml.) was added. After 1 hr. at room temperature the solvent was evaporated, finally at 2 mm. The residue solidified on adding ether; the ether was evaporated and the solid residue was dissolved in ethyl acetate (30 ml.) and water (7 ml.). The organic layer was washed with water (2 × 7 ml.), saturated sodium hydrogen carbonate (3 × 7 ml.), 2N-hydrochloric acid (7 ml.), and saturated brine (7 ml.), and dried. Evaporation left a semi-solid which rapidly crystallised on adding methanol; the crystals were washed with methanol and dried at 70—80° and 0·1 mm. for 5 hr. to give benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester (0·239 g., 46% of the starting material) of m. p. 145—152°, $[\alpha]_D^{23}$ −22·5° (*c* 1·9 in $CHCl_3$) (Found: C, 64·4; H, 4·9; N, 9·7%). The recovery of L-isomer is 90% of that present in the solution when the reaction was stopped, calculated from the optical rotation. In a control experiment using 0·671 g. of benzoylglycyl-L-phenylalanine *p*-nitrophenyl ester, the same procedure was followed except that benzoylglycol-DL-phenylalanine *p*-nitrophenyl ester (0·0671 g.) was added to the product, immediately before the addition of hydrogen bromide in acetic acid. By the same procedure there was obtained fraction 1, 0·310 g. of *p*-nitrophenyl ester of m. p. 145—176·5°, $[\alpha]_D^{20}$ −24·1 (*c* 2·0 in DMF) (Found: C, 64·9; H, 4·9; N, 9·3%); concentration of the mother-liquors and washings gave fraction 2, 0·0168 g., m. p. 135—170°, $[\alpha]_D^{20}$ −27·1° (*c* 0·62 in DMF). The L-ester had $[\alpha]_D^{22\cdot5}$ −29·7° (*c* 2·0 in DMF), and therefore fraction 1 contained 19% and fraction 2 9% of DL-isomer, giving a total recovery of 0·0605 g. (90%) of the DL-isomer added.

Preparation of Benzyloxycarbonylglycylphenylalanine p-*Nitrophenyl Ester by Means of Diphenylketen,*[10,11] *and Application of the Anderson Racemisation Test* [12] *to the Whole Product.* (With J. M. Hugo).—Benzyloxycarbonylglycyl-L-phenylalanine [18] (1·775 g., 0·005 mole) was dissolved in dried tetrahydrofuran (6 ml.) with warming, and the solution was cooled to −15° and stirred during the addition

[21] K. Nowak and I. Z. Siemion, *Roczniki Chem.*, 1961, **35**, 153; A. L. Heard and G. T. Young, *J. Chem. Soc.*, 1963, 5807.

of triethylamine (1 ml. of a 0·001M-solution in tetrahydrofuran) and then diphenylketen (0·86 ml., 0·005 mole). The yellow coloration became faint after 8 min. (anhydride formation), and a solution of *p*-nitrophenol (0·70 g., 0·005 mole) in tetrahydrofuran (6 ml.) was then added. The solution was allowed to rise to room temperature, and next day the solution was evaporated, the residue was taken up in ethyl acetate and washed with saturated sodium hydrogen carbonate, N-hydrochloric acid, water, and saturated brine, and dried. The ethyl acetate was evaporated and the residue was redissolved in ethyl acetate (11 ml.) and glycine ethyl ester (0·5 ml., 0·005 mole) was added. After 2 days at room temperature more ethyl acetate was added with warming to redissolve some crystalline product, and the solution was washed with dilute ammonium hydroxide, saturated sodium hydrogen carbonate, N-hydrochloric acid, water, and saturated brine, and dried. Evaporation left crystalline solid (0·920 g., 39% overall); this was dissolved in dried ethanol to give a 2% solution which on standing at 0° deposited crystals of benzyloxycarbonylglycyl-DL-phenylalanylglycine ethyl ester (0·231 g. 25% of the crude product), m. p. 133—135° (lit.,[12] m. p. 132—133°) (Found: C, 62·3; H, 6·3; N, 9·3. Calc. for $C_{23}H_{27}N_3O_6$: C, 62·6; H, 6·2; N, 9·5%). The mother-liquors were evaporated to give product (0·675 g., 73% yield) of m. p. 82—108°, $[\alpha]_D^{22}$ −5·3° (*c* 1·6 in EtOH) (Found: C, 65·2; H, 6 3; N, 9·1%). Benzyloxycarbonylglycyl-L-phenylalanine ethyl ester has m. p. 120—120·5°, $[\alpha]_D^{25}$ −13·2° [22] and the analysis indicates contamination by diphenylacetylglycine ethyl ester. Reduction of the time allowed for anhydride formation to 5 min., 2 min., 50 sec., and 30 sec. gave racemic benzyloxycarbonylglycylphenylalanylglycine ethyl ester in amounts representing 37·5, 10·6, 5·8, and 7·6% of the crude product, respectively, together with product which by elemental analysis contained much diphenylacetylglycine ethyl ester, which was isolated by ether extraction of the product of the second experiment (2 min. for anhydride formation): m. p. and mixed m. p. 113—117° (lit.,[23] m. p. 118—119°) (Found: C, 72·0; H, 6·6; N, 4·1. Calc. for $C_{18}H_{19}NO_3$: C, 72·7; H, 6·4; N, 4·7%).

Control Experiment: Condensation of Benzyloxycarbonylglycyl-L-phenylalanine p-*Nitrophenyl Ester with Glycine Ethyl Ester.*—To a solution of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (1·89 g., 0·004 mole) in a mixture of dichloromethane (20 ml.) and dimethylformamide (10 ml.) was added glycine ethyl ester (0·44 ml., 0·0044 mole), and stirring was continued for 3 days. The solution was evaporated and the residue was taken up in ethyl acetate; this solution was washed with saturated sodium hydrogen carbonate, N-hydrochloric acid, water, and brine, and dried. Evaporation left a yellow crystalline product (1·69 g., 99%), which was dissolved in ethanol to give a 2% solution. No crystals were deposited after 3 weeks at 0°; concentration gave a first fraction of benzyloxycarbonylglycyl-L-phenylalanine ethyl ester (0·90 g.) of m. p. 116·5—118°, $[\alpha]_D^{25}$ −13·3° (*c* 2·0 in EtOH), and no racemate was found.

We thank the S.R.C. for a Grant for a Special Research and for a Research Studentship (held by I. A.), and Mrs. L. Luckett for technical assistance.

[6/1270 *Received, October 10th,* 1966]

[22] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1958, **80**, 2902.

[23] R. Mozingo and K. Folkers, in "The Chemistry of Penicillin," ed. H. T. Clarke, J. R. Johnson, and R. Robinson, Princeton University Press, 1949, p. 599.