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Amino-acids and Peptides. Part XXVII.[1] Further Studies on Esters of 1-Hydroxypiperidine

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Acylamino-acid 1-piperidyl esters have been obtained in a high state of purity by the use of 'Reagent K' [3-(2-ethyl-5-isoxazolio)benzenesulphonate], and new esters prepared by this and by previously known methods are reported. Standard racemisation tests showed that the new procedure gave an optically pure ester from benzyloxycarbonylglycyl-L-phenylalanine, but some racemate was formed from benzoyl-L-leucine. Other reactions of 1-piperidyl esters are reported. The optimum molar proportion of acetic acid to amine in the condensation of benzyloxycarbonyl-L-leucine 1-piperidyl ester with glycine ethyl ester in chloroform has been found to be between 1 and 2; 1,2,4-triazole catalyses this reaction (with dimethylformamide as solvent) and also that of the ester with benzylamine, but acetic acid is more effective. The preparation of t-butyl 1-piperidyl carbonate, and its use in forming t-butoxycarbonylglycine, are described. With L-lysine, benzyl 1-piperidyl carbonate gives ε-benzyloxycarbonyl-L-lysine in 58% yield. The cleavage of the phthalimido-group of phthalimido-esters by glycine ethyl ester in the presence of acetic acid is reported.

1-Piperidyl esters are selective acylating agents which react readily with unhindered, highly nucleophilic amines such as benzylamine, and condense with less reactive amines such as α-amino-esters in the presence of a weak acid catalyst.[1-3] Their usefulness in peptide synthesis lies particularly in their unusual optical stability, and in the ease with which pure product can be isolated. We report here further experience of their preparation and properties.

For the preparation of small amounts of pure 1-piperidyl esters of acylamino-acids we have found 'Reagent K' [3-(2-ethyl-5-isoxazolio)benzenesulphonate][4] advantageous. For a larger scale we have generally preferred the carbonic mixed anhydride procedure;[2] the dicyclohexylcarbodi-imide method[2] has at times given product contaminated with *N*-acylurea, and when the water-soluble carbodi-imide, 3-(3-dimethylaminopropyl)-1-ethylcarbodi-imide,[5] was used in the preparation of benzyloxycarbonylglycine 1-piperidyl ester the yield was low (53% in a typical experiment). As indicated in a footnote in Part XXIV,[2] we have made benzoyl-L-leucine 1-piperidyl ester by way of the acid azide (prepared by the method of Honzl and Rudinger;[6] the use of nitrous acid gave an impure product); this was particularly valuable for the confirmation of the constants of the ester obtained earlier[2] by repeated crystallisation of the partly racemised product from the dicyclohexylcarbodi-imide procedure. The new α-acylamino-acid 1-piperidyl esters reported here are those of t-butoxycarbonylglycine, benzyloxycarbonyl-L-isoleucine (not crystalline), t-butoxycarbonyl-L-leucine, t-butoxycarbonyl-L-phenylalanine, benzyloxycarbonyl-L-serine, phthaloyl-DL-valine, and α-phthalimidoisobutyric acid, together with γ-methyl α-1-piperidyl benzyloxycarbonyl-L-glutamate. In addition, we report the 1-piperidyl esters of phenylacetic acid and 3-phenylpropionic acid, and the di-ester of phthalic acid. In their reactions with amines, the first two esters showed a selectivity similar to that reported[2] for the benzoic ester. For example, 1-piperidyl 3-phenylpropionate condensed readily with benzylamine but very slowly with diethylamine and with aniline; no reaction with di-isopropylamine, di-n-butylamine, or dicyclohexylamine was detected within 25 days at room temperature. It was shown by Mitin, Stolyarova, and Vlasov[7] that *O*-benzoyl-*NN*-diethylhydroxylamine reacts with aniline in the presence of benzoic acid; the maximum yield, obtained with 1 molar proportion of the acid, was below 50%.

We have sought methods for the direct preparation of 1-piperidyl esters of acyl peptides without risk of racemisation; since these esters are optically stable in the presence of base it seemed possible that, by analogy with methods used for making aryl esters,[8] the action of di-1-piperidyl sulphite or of di-1-piperidyl carbonate with the carboxylic acid and pyridine might be successful. The former ester, prepared by the reaction of thionyl chloride with 1-hydroxypiperidine in ether containing triethylamine, proved to be unstable and difficult to purify, and with benzyloxycarbonylglycine and pyridine in ethyl acetate at room temperature the formation of 1-piperidyl ester (detected chromatographically) was very slow. Di-1-piperidyl carbonate (from phosgene and 1-hydroxypiperidine in ether) is a crystalline stable compound, which with benzyloxycarbonylglycine in pyridine at room temperature gave no detectable 1-piperidyl ester within 3 days. Dichloroacetic esters have been used for the preparation of other active esters,[9] and we therefore prepared 1-piperidyl dichloroacetate by way of the acid chloride; as expected, it is unstable, but did not yield a 1-piperidyl ester with

[1] Part XXVI, J. H. Jones, B. Liberek, and G. T. Young, *J. Chem. Soc.* (*C*), 1967, 2371.
[2] B. O. Handford, J. H. Jones, G. T. Young, and (in part) T. F. N. Johnson, *J. Chem. Soc.*, 1965, 6814.
[3] J. H. Jones, B. Liberek, and G. T. Young, Proc. 8th European Peptide Symposium, Noordwijk, 1966, ed H. C. Beyerman, A. Van de Linde, and W. Maassen Van den Brink, North Holland Publishing Co., Amsterdam, 1967.
[4] R. B. Woodward, R. A. Olofson and H. Mayer, *J. Amer. Chem. Soc.*, 1961, **83**, 1010.
[5] J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, 1961, **26**, 2525.
[6] J. Honzl and J. Rudinger, *Coll. Czech. Chem. Commun.*, 1961, **26**, 2333.
[7] Yu. V. Mitin, T. Yu. Stolyarova, and G. P. Vlasov, *J. Gen. Chem.* (*U.S.S.R.*), 1963, **33**, 3560.
[8] B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373; T. Wieland, B. Heinke, K. Vogler, and H. Morimoto, *Annalen*, 1962, **655**, 189.
[9] S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan*, 1964, **37**, 1231.

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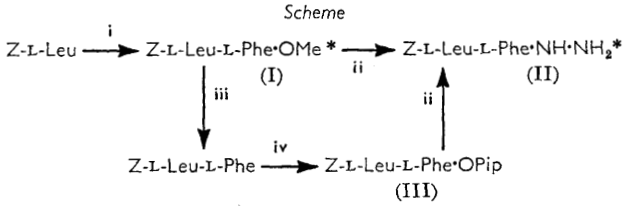
benzyloxycarbonylglycine in pyridine. It seemed possible that the ' Reagent K ' route might yield 1-piperidyl esters of acyl peptides without racemisation, and this was examined in the following way. Benzyloxycarbonylglycyl-L-phenylalanine was converted into the 1-piperidyl ester by means of ' Reagent K,' and the whole product was condensed with glycine ethyl ester; no racemate was found. As a further exercise, the reactions shown in the Scheme were carried through; the conversion of benzyloxycarbonyl-L-leucyl-L-phenylalanine into the 1-piperidyl ester (III) by means of ' Reagent K ' and thence into the hydrazide (II) yielded a product of the same specific rotation as that prepared from the methyl ester (I).

Scheme



* Denotes a new compound. Z = $PhCH_2$·O·CO; Pip = 1-piperidyl.

Reagents: i, L-Phe·OMe and dicyclohexylcarbodi-imide; ii, N_2H_4, H_2O; iii, NaOH; iv, ' Reagent K ' and PipOH.

However, the benzoyl-L-leucine 1-piperidyl ester, prepared by means of ' Reagent K ' had a slightly low optical rotation, and its condensation with glycine ethyl ester (as the hydrochloride with sodium acetate trihydrate) gave a benzoyl-leucylglycine ethyl ester containing (from its rotation) *ca.* 10% of racemate; this was confirmed by saponification, and fractional crystallisation of the resulting acid, from which some racemate was isolated. The preparation of the 1-piperidyl ester of an acyl peptide by means of ' Reagent K ' is not therefore free from all danger of racemisation. Of course, such an ester could be made through the acid azide; this indirect route may prove useful, since the coupling of 1-piperidyl esters not only proceeds without racemisation but also without the side reactions which an acid azide can undergo, and the preparation of benzyloxycarbonyl-L-tyrosyl-L-leucine 1-piperidyl ester in this way has recently been reported.[10] Two points must be made here concerning our racemisation test.[11] Confirmation of the presence of racemate is obtained by saponification of the crude benzoyl-leucylglycine ethyl ester; in the original work, benozyl-L-leucylglycine was slow to crystallise, and benzoyl-DL-leucylglycine was deposited first during crystallisation. However, perhaps partly because of the improvements in coupling methods, it is now common for the L-isomer to crystallise immediately, and it is then necessary to crystallise the product fractionally in order to be certain that small amounts of racemate will be detected.* Secondly, Anderson, Zimmerman, and Callahan[12] have recently reported their inability to raise the optical rotation of authentic benzoyl-L-leucylglycine ethyl ester above $[\alpha]_D^{25}$ $-32{\cdot}5 \pm 0{\cdot}5°$ (*c* 3 in ethanol), whereas we reported $[\alpha]_D^{20}$ $-34{\cdot}0°$ (*c* 3·1 in ethanol); differential thermal analysis of their product gave a single endotherm at 159°. A. W. Williams has repeated the synthesis of this reference compound, and investigated the variation of the specific rotation with concentration and with temperature. His results will be reported separately with related work, but they confirm our earlier constant; the new preparation has $[\alpha]_D^{20}$ $-33{\cdot}9°$ (*c* 3·1 in ethanol), $[\alpha]_D^{20}$ $-33{\cdot}4°$ (*c* 1·0 in ethanol), and $[\alpha]_D^{25}$ $-33{\cdot}7°$ (*c* 3·0 in ethanol).

We have looked further at the effect of the molar proportion of acetic acid catalyst on the rate of condensation of benzyloxycarbonyl-L-leucine 1-piperidyl ester with glycine ethyl ester in chloroform solution; the course of the reaction was followed polarimetrically. The results, which have been presented earlier graphically,[3] show that the optimum molar proportion of acetic acid, relative to the amino-ester, is between 1 and 2. We have obtained some confirmation of the protonation of the piperidyl nitrogen from the changes in the infrared absorption when a solution of hydrogen chloride in chloroform was added to 1-piperidyl 3-phenylpropionate in chloroform; with increasing amounts of hydrogen chloride, the absorption at 1750 $cm.^{-1}$ (ester CO) decreased in intensity and a new peak appeared at 1803 $cm.^{-1}$, which corresponds to that assigned by Huisgen and Kolbeck[13] to the carbonyl absorption (1800 $cm.^{-1}$) of 1-acetoxyquinuclidinium chloride, and so would be consistent with the presence of the ·CO·O·N^+H· grouping.

Beyerman and his co-workers[14] have found that 1,2,4-triazole (a ' bifunctional catalyst '[15]) accelerates the acylation of cyclohexylamine by benzyloxycarbonylglycine 1-piperidyl ester in dimethylformamide, and we have therefore also examined polarimetrically the effect of this catalyst on the condensation of benzyloxycarbonyl-L-leucine 1-piperidyl ester with glycine ethyl ester and with benzylamine in dimethylformamide. The results, presented earlier graphically,[3] show that the triazole does accelerate these reactions, but in both cases acetic acid is a more effective catalyst. These reactions are much slower in dimethylformamide than, *e.g.*, in dioxan.

In Part XXIV [2] we described the use of benzyl and *p*-nitrobenzyl 1-piperidyl carbonates for the preparation of benzyloxycarbonyl- and *p*-nitrobenzyloxycarbonyl-amino-acids, and we have reported[16] the preparation of *p*-methoxybenzyl 1-piperidyl carbonate and its use in the preparation of the *p*-methoxybenzyloxycarbonyl derivatives of glycine (as the free acid), L-alanine, L-leucine,

* Dr. M. Q. Ceprini has informed us that he has had the same experience in this test.

[10] B. O. Handford, T. A. Hylton, J. Preston, and B. Weinstein, *J. Org. Chem.*, 1967, **32**, 1243.

[11] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1963, 881.

[12] G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, 1966, **88**, 1338.

[13] R. Huisgen and W. Kolbeck, *Tetrahedron Letters*, 1965, 783.

[14] W. Maassen Van den Brink, personal communication.

[15] H. C. Beyerman and W. Maassen Van den Brink, *Proc. Chem. Soc.*, 1963, 266.

[16] J. H. Jones and G. T. Young, *Chem. and Ind.*, 1966, 1722.

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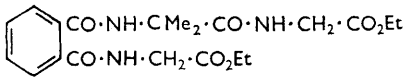
L-phenylalanine, and L-valine (as their dicyclohexylammonium salts); the L-alanine and L-valine derivatives are new and we record their constants here. The reaction of 1-hydroxypiperidine with t-butyl chloroformate[17] gave t-butyl 1-piperidyl carbonate as a liquid (characterised by its infrared and n.m.r. spectra) but it reacted only slowly with glycine ethyl ester (as the hydrochloride, with sodium acetate trihydrate) in dioxan, to give (after saponification of the initial product) t-butoxycarbonylglycine dicyclohexylammonium salt in only 33% yield (overall) after an initial reaction time of 8 days at room temperature. After a reaction time of two hours at reflux temperature, the corresponding yield of salt was 25%; use of free glycine ester and an equivalent of acetic acid, with chloroform as solvent and six and a half hours at reflux temperature, gave a yield of 36%. The reaction of the t-butyl 1-piperidyl carbonate with an excess of benzylamine (no solvent) was still only half complete (judged by n.m.r. spectra) after sixty-four hours at room temperature. We have also (with Miss S. J. Allard) used benzyl 1-piperidyl carbonate in a convenient direct preparation of ε-benzyloxycarbonyl-L-lysine from L-lysine monohydrochloride with an equivalent of triethylamine in aqueous methanol; the yield (58%) is considerably higher than that obtained by the similar reaction with benzyl phenyl carbonate.[18] 1,2,4-Triazole had little effect on the rate of condensation of benzyl 1-piperidyl carbonate with glycine benzyl ester in dioxan, and did not increase the acceleration due to acetic acid.

The reaction of 1-piperidyl α-phthalimidoisobutyrate with glycine ethyl ester in the presence of acetic acid at room temperature was understandably very slow, and when the molar proportion of the latter ester was increased to 4 an unexpected product was isolated, identified as α-(*o*-ethoxycarbonylmethylcarbamoylbenzamido)isobutyrylglycine ethyl ester (IV). Such a



(IV)

cleavage does not appear to have been observed before. Methyl α-phthalimidoisobutyrate reacted analogously with glycine ethyl ester in the presence of acetic acid to give methyl α-(*o*-ethoxycarbonylmethylcarbamoylbenzamido)isobutyrate, and phthaloylglycine ethyl ester gave phthaloyldiglycine diethyl ester. The last experiment was repeated, without the acetic acid; thin-layer chromatography (t.l.c.) indicated the formation of the same by-product, but in amount insufficient for isolation.

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage apparatus, optical rotations with a Perkin-Elmer 141 automatic polarimeter (10 cm. cell), and infrared spectra with a Perkin-Elmer 237 spectrophotometer (0·1 mm. cell); n.m.r. spectra were measured (by Mrs. E. E. Richards) with a Perkin-Elmer R10 spectrometer, with tetramethylsilane as internal standard and deuteriochloroform as solvent. Mass spectra were measured (by Dr. R. T. Aplin) with an A.E.I. MS9 spectrometer, with a direct inlet system operating at 150—220°. Thin-layer chromatography employed Kieselgel-G (unbaked), with ether as solvent and iodine vapour for detection unless otherwise stated. Samples of 1-piperidyl esters were dried at 0·1 mm. and room temperature before analysis. Solutions were evaporated with a rotary evaporator, and solutions in organic solvents were dried over magnesium sulphate. Light petroleum had b. p. 40—60°. 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.

Preparation of 1-Piperidyl Esters by New Methods.—(a) *By use of 'Reagent K' [3-(2-ethyl-5-isoxazolio)benzenesulphonate].*[4] (i) *Benzyloxycarbonylglycine 1-piperidyl ester.* Finely ground 3-(2-ethyl-5-isoxazolio)benzenesulphonate (1·012 g., 4 mmoles) and benzyloxycarbonylglycine (0·836 g., 4 mmoles) were stirred in acetonitrile (10 ml.) at 0°. Triethylamine (0·404 g., 4 mmoles) in acetonitrile (10 ml.) was added, and after 1 hr. 1-hydroxypiperidine (1·00 g., 10 mmoles) was added. After a further 20 min., the mixture was allowed to attain room temperature. Next day, the solvent was removed and the residual yellow oil was distributed between ethyl acetate and water. The organic layer was washed (2N-hydrochloric acid, N-sodium hydrogen carbonate, and water), dried, and evaporated, to leave chromatographically pure benzyloxycarbonylglycine 1-piperidyl ester (0·875 g., 75%), m. p. 109—111° (lit.,[2] 113—114°).

(ii) *Benzyloxycarbonyl-L-alanine 1-piperidyl ester.* An analogous reaction of benzyloxycarbonyl-L-alanine gave the 1-piperidyl ester (71%), m. p. 77—78° (from di-isopropyl ether), $[\alpha]_D^{20}$ −22·0° (*c* 1·0 in dimethylformamide) [lit.,[2] m. p. 77—78°, $[\alpha]_D^{20}$ −23·1° (*c* 1·0 in dimethylformamide)].

(iii) *t-Butoxycarbonylglycine 1-piperidyl ester.* This was prepared from t-butoxycarbonylglycine (0·700 g., 4 mmoles) by the general procedure except that ether was used instead of ethyl acetate, and 10% citric acid instead of 2N-hydrochloric acid for washing. The oil obtained crystallised on trituration with light petroleum, to give the *ester* (0·690 g., 66%), m. p. 60—63°, $\nu_{max.}$ ($CHCl_3$) 1760 and 1715 cm.$^{-1}$, τ 4·7—5·1 (1H, broad triplet, J = 6 c/sec., CO·N*H*·CH_2), 6·06 (2H, doublet, J = 6 c./sec. NH·CH_2·CO), 6·3—7·6 (4H, two broad bands with centres at 6·7 and 7·25, CH_2·N·CH_2), 8·0—8·7 {15H, singlet at 8·53 superimposed on a broad band, (CH_3)$_3$·C and CH_2·[CH_2]$_3$·CH_2} (Found: C, 55·6; H, 8·6; N, 10·6. $C_{12}H_{22}N_2O_4$ requires C, 55·8; H, 8·6; N, 10·8%).

(iv) *Benzyloxycarbonyl-L-serine 1-piperidyl ester.* This was prepared from benzyloxycarbonyl-L-serine (0·956 g., 10 mmoles) by the general procedure except that ether was used instead of ethyl acetate. The residual oil gave needles (1·89 g., 62%), m. p. 75—80° (produced by seeding with crystals obtained in small quantity in an earlier preparation). Recrystallisation from ethyl acetate–light petroleum gave the *ester* (1·48 g., 46%), m. p. 76—80°, $[\alpha]_D^{20}$ −22·6° (*c* 1·0 in dimethylformamide), $\nu_{max.}$ ($CHCl_3$) 1760 and 1720 cm.$^{-1}$, τ 2·66 (5H, singlet, aromatic), 4·05 (1H, doublet, J = 8 c./sec., CO·N*H*·CH), 4·88 (2H, singlet, PhCH_2·O), 5·3—5·8 (1H, complex, NH·C*H*·CO), 6·09 (2H, doublet,

[17] Yu. A. Ovchinnikov, A. A. Kiryushkin, and A. I. Miroshnikov, *Experientia*, 1965, **21**, 418.

[18] H. Zahn and H. R. Falkenburg, *Annalen*, 1960, **636**, 117.

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J = 5 c./sec. ·CH·CH_2OH), 6·3—7·6 (5H, complex, OH and CH_2·N·CH_2), and 7·9—9·0 (6H, complex, CH_2·N·CH_2) (Found: C, 59·3; H, 7·1; N, 8·7. $C_{16}H_{22}N_2O_5$ requires C, 59·6; H, 6·9; N, 8·7%).

(v) *t-Butoxycarbonyl-L-phenylalanine 1-piperidyl ester* (with G. R. Marshall). This was prepared from t-butoxycarbonyl-L-phenylalanine (1·06 g., 4 mmoles) as described for t-butoxycarbonylglycine; the oil obtained was taken up in n-hexane, and evaporation left a solid which gave the *ester* (0·78 g., 56%), m. p. 100—102° (from n-hexane), $[\alpha]_D^{20}$ +15·1° (*c* 1·0 in chloroform), $[\alpha]_D^{20}$ −15·5° (*c* 1·0 in dimethylformamide) (Found: C, 65·5; H, 8·1; N, 8·2. $C_{19}H_{28}N_2O_4$ requires C, 65·6; H, 8·1; N, 8·05%).

(vi) *γ-Methyl α-1-piperidyl benzyloxycarbonyl-L-glutamate.* This was prepared from γ-methyl benzyloxycarbonyl-L-glutamate [19] (1·181 g., 4 mmoles) by the general procedure; evaporation of the ethyl acetate left a sticky solid which gave the *ester* (1·00 g., 75%), as needles, m. p. 95—97° (from di-isopropyl ether and then from ethyl acetate–light petroleum), $[\alpha]_D^{20}$ −5·3° (*c* 1·0 in dioxan), $\nu_{max.}$ (Nujol) 1760, 1735, and 1720 cm.$^{-1}$, τ 2·65 (5H, singlet, aromatic), 4·45 (1H, doublet, J = 8 c./sec. CO·NH·CH), 4·88 (2H, singlet PhCH_2·O), 5·4—5·9 (1H, complex, NH·CH·CO), 6·31 (3H, singlet, OCH_3), and 6·3—9·0 (14H, complex, 7 CH_2-groups) (Found: C, 59·9; H, 6·9; N, 7·6. $C_{19}H_{26}N_2O_6$ requires C, 60·3; H, 6·95; N, 7·4%).

(b) *By the use of 3-(3-dimethylaminopropyl)-1-ethylcarbodi-imide.*[5] *Benzyloxycarbonylglycine 1-piperidyl ester.* A solution of 3-(3-dimethylaminopropyl)-1-ethylcarbodi-imide (1·70 g., 11 mmoles) in ethyl acetate (10 ml.) was added, during 2 min., to a solution of benzyloxycarbonylglycine (2·09 g., 10 mmoles) and 1-hydroxypiperidine (2·0 g., 20 mmoles) in ethyl acetate (20 ml.). After 12 hr., the solution was washed with 2N-hydrochloric acid, N-sodium hydrogen carbonate, and water, and dried. Evaporation left chromatographically pure benzyloxycarbonylglycine 1-piperidyl ester (1·55 g., 53%), m. p. 108—110° (lit.,[2] 113—114°).

A similar experiment but with 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride (12 mmoles) and dichloromethane as solvent gave only an oily product (14%).

(c) *By the acid azide route. Benzoyl-L-leucine 1-piperidyl ester.* The acid azide was formed by the method of Honzl and Rudinger.[6] The gel formed by the addition of hydrogen chloride in dry tetrahydrofuran (3·22N; 6·2 ml.) to benzoyl-L-leucylhydrazide (2·49 g., 10 mmoles) in tetrahydrofuran (11 ml.) was broken up and suspended in dry ether (150 ml.) at −15 to −10°, with stirring. n-Butyl nitrite (1·6 ml., 15 mmoles) was added and the mixture was stirred for 10 min. Not all the solid had by then dissolved, and more n-butyl nitrite (0·35 ml.) was added. After a further 5 min., the solution was quickly washed with cold potassium hydrogen carbonate in brine (0·66N) and then dried for 5 min. at −5°. The solution of acid azide was then filtered into a solution of 1-hydroxypiperidine (2·0 g., 20 mmoles) in anhydrous ether (20 ml.). After 1 hr. at −5° and then 1 hr. at room temperature, the solution was washed with 2N-hydrochloric acid (twice), N-potassium hydrogen carbonate, and water, and dried. Evaporation left an oil which crystallised on the addition of light petroleum, to give chromatographically pure benzoyl-L-leucine 1-piperidyl ester (2·27 g., 78%), m. p. 110—113°, $[\alpha]_D^{20}$ +19·5° (*c* 1·0 in chloroform), $[\alpha]_D^{20}$ +12·3° (*c* 1·0 in ethyl acetate) [lit.[2] (for ester prepared by repeated crystallisation of the partly racemised product of the dicyclohexylcarbodi-imide method), m. p. 112—113·5°, $[\alpha]_D^{25}$ +12·4° (*c* 1·0 in ethyl acetate)].

Preparation of New 1-Piperidyl Esters by Known Methods.—Benzyloxycarbonyl-L-isoleucine 1-piperidyl ester. The mixed carbonic anhydride procedure was used, as in Method (C) in Part XXIV.[2] Benzyloxycarbonyl-L-isoleucine dicyclohexylammonium salt (4·47 g., 10 mmoles) was shaken with 2N-sulphuric acid (30 ml.) for 15 min., and the oil produced was extracted into dichloromethane; the organic layer was washed with brine, dried, and evaporated, to leave benzyloxycarbonyl-L-isoleucine (2·6 g., 10 mmoles). This was dissolved, with triethylamine (1·01 g., 10 mmoles), in dichloromethane (50 ml.) and the solution was cooled to −7°. A solution of ethyl chloroformate (1·08 g., 10 mmoles) in dichloromethane (20 ml.) was added with stirring during 5 min.; after a further 15 min., a solution of 1-hydroxypiperidine (2·0 g., 20 mmoles) in dichloromethane (20 ml.) was added, and after a further 10 min. at −7° the solution was allowed to attain room temperature. After 2 hr. it was evaporated, and the residue was distributed between ether and water. The ethereal layer was washed with 2N-hydrochloric acid, N-sodium hydrogen carbonate, and water, and dried. Evaporation left the chromatographically pure *ester* as an oil (2·47 g., 71%) which solidified when cooled at −5°, but melted when warmed to room temperature; $[\alpha]_D^{20}$ −10·7° (*c* 3·0 in dimethylformamide), $\nu_{max.}$ (film) 1760 and 1720 cm.$^{-1}$, τ 2·62 (5H, singlet, aromatic), 4·4—4·8 (1H, broad, CO·NH·CH), 4·86 (2H, singlet, PhCH_2·O), 5·5—5·9 (1H, broad, NH·CH·CO), 6·3—7·6 (4H, two broad bands with centres at 6·7 and 7·3, CH_2·N·CH_2), 7·8—9·3 {15H, complex, ·CH(CH_3)·CH_2·CH_3 and CH_2·[CH_2]$_3$·CH_2} (Found: N, 8·35. $C_{19}H_{28}N_2O_4$ requires N, 8·0%). An attempted preparation of this compound by the use of dicyclohexylcarbodi-imide [Method (A) of Part XXIV] [2] gave a product with a contaminant which was probably *N*-benzyloxycarbonyl-L-isoleucyl-*NN'*-dicyclohexylurea.

Phthaloyl-DL-valine 1-piperidyl ester (with Miss C. Jackson). Method (B) of Part XXIV [2] gave the *ester*, m. p. 85—87° (from di-isopropyl ether) (Found: C, 65·2; H, 6·7; N, 8·7. $C_{18}H_{22}N_2O_4$ requires C, 65·5; H, 6·7; N, 8·5%).

1-Piperidyl α-phthalimidoisobutyrate. α-Phthalimidoisobutyric acid [20] (3·22 g., 13·8 mmoles) and phosphorus pentachloride (2·88 g., 13·8 mmoles) were mixed intimately and heated at 80—100°. After 0·5 hr., phosphoryl chloride was distilled off at 12 mm./100°, to leave an oil which crystallised when cooled. The crude acid chloride was dissolved in dry ether (80 ml.), and after filtration to remove a small amount of insoluble material the solution was added to 1-hydroxypiperidine (2·5 g., 25 mmoles) in ether (60 ml.) at 0°. The 1-piperidyl ester hydrochloride began to separate after 2 min., and after 30 min. at 0° and 10 min. at room temperature, the suspension was treated with saturated sodium carbonate solution, until effervescence ceased. The ether layer was separated and washed with 2N-hydrochloric acid, N-sodium hydrogen carbonate, and water, and dried. Evaporation left a chromatographically pure solid (3·37 g., 75%) which gave the *ester*, m. p. 103—105° (from di-isopropyl ether), $\nu_{max.}$ ($CHCl_3$) 1780sh, 1760, and 1720 cm.$^{-1}$, τ 2·21 (4H, singlet, aromatic), 6·2—7·6 (4H, two broad bands with centres at 6·7 and 7·3, CH_2·N·CH_2),

[19] W. E. Hanby, S. G. Waley, and J. Watson, *J. Chem. Soc.*, 1950, 3239.

[20] J. H. Billman and W. F. Harting, *J. Amer. Chem. Soc.*, 1948, **70**, 1473.

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8·0—8·7 {12H, comprising (*a*) a singlet at 8·15, $\cdot C(CH_3)_2$, and (*b*) a complex system, $\cdot CH_2\cdot[CH_2]_3\cdot CH_2\cdot$} (Found: C, 64·4; H, 6·4; N, 8·8. $C_{17}H_{20}N_2O_4$ requires C, 64·5; H, 6·4; N, 8·9%).

1-*Piperidyl phenylacetate* (with M. J. Spriggs and Miss C. Jackson). Use of dicyclohexylcarbodi-imide [Method (A) of Part XXIV] [2] gave the *ester*, m. p. 29·5—30·5° after low-temperature recrystallisation from light petroleum (Found: C, 70·9; H, 8·1; N, 6·5. $C_{13}H_{17}NO_2$ requires C, 71·3; H, 7·8; N, 6·4%).

The ester (0·219 g., 1 mmole) reacted with benzylamine (1·284 g., 12 mmoles) in ethyl acetate (5 ml.) within 20 hr. at room temperature (disappearance of ester determined by t.l.c.). The solution was washed as usual, dried, and evaporated, to leave *N*-benzylphenylacetamide (0·198 g., 88%), m. p. 119—121° (lit.,[21] 122°). With dicyclohexylamine no reaction was apparent (no 1-hydroxypiperidine detected chromatographically within 8 days).

1-*Piperidyl 3-phenylpropionate* (with Miss C. Jackson). The acid chloride procedure [Method (B) of Part XXIV] [2] gave the *ester*, m. p. 34·5—35° after low-temperature recrystallisation from n-hexane; $\nu_{max.}$ ($CHCl_3$) 1750 cm.$^{-1}$ (Found: C, 71·9; H, 8·2; N, 6·1. $C_{14}H_{19}NO_2$ requires C, 72·1; H, 8·2; N, 6·0%).

The reaction with benzylamine proceeded as described above for 1-piperidyl phenylacetate, to give *N*-benzyl-3-phenylpropionamide (77%), m. p. 85—86° (lit.,[22] 84—85°). With diethylamine the reaction was incomplete (1-piperidyl ester still detectable by t.l.c.) after 12 days, and with aniline after 3 weeks; with di-isopropylamine, di-n-butylamine, or dicyclohexylamine no reaction was apparent (no 1-hydroxypiperidine detected chromatographically) within 25 days.

Di-1-piperidyl sulphite. Thionyl chloride (11·8 g.) in dry ether (50 ml.) was added druing 5 min. to a stirred solution of 1-hydroxypiperidine (25 g.) and triethylamine (20·2 g.) in ether (100 ml.) at 0°. After a further 5 min. the mixture was washed with water, 2N-hydrochloric acid (3 times), saturated sodium hydrogen carbonate, and water, and then dried. Evaporation left a pale yellow oil which gave one spot only on chromatography (R_F 0·7). The oil was frozen at −60° and triturated with light petroleum to give crystals which were washed with light petroleum at −60° by decantation. At room temperature the crystals melted but after 14 days needles were deposited and were taken up into the same solvent. When a small portion of this solution was warmed with charcoal an explosion occurred. The solvent was evaporated from the main portion, to leave an oil which slowly crystallised, to give the *sulphite* (6·1 g., 24%), m. p. 42°, $\nu_{max.}$ (CCl_4) 1220 and 1210 cm.$^{-1}$ (S=O str.) (Found: C, 48·2; H, 8·1; N, 10·5; S, 11·8. $C_{10}H_{20}N_2O_3$ requires C, 48·2; H, 8·1; N, 11·3; S, 12·9%).

Attempted preparation of benzyloxycarbonylglycine 1-piperidyl ester from di-1-piperidyl sulphite. Di-1-piperidyl sulphite (0·325 g.), benzyloxycarbonylglycine (0·210 g.,) and pyridine (0·16 ml.) were dissolved in ethyl acetate (3 ml.) at room temperature. Thin-layer chromatography showed no reaction until the 16th day, when a faint spot at R_F 0·42 (corresponding to benzyloxycarbonylglycine 1-piperidyl ester) was detected. After 50 days this spot was intense, but sulphite was still present and much brown gum had appeared.

Di-1-piperidyl carbonate. A solution of phosgene (18 g.) in dry ether (40 ml.) was added dropwise to a solution of 1-hydroxypiperidine (31 g.) in ether (20 ml.). After 1·5 hr. the liquid was decanted off and the precipitate was washed once by decantation with ether (150 ml.). It was then suspended in ether (200 ml.) and saturated aqueous sodium carbonate (300 ml.) was added. The mixture was shaken and the ethereal layer was separated, washed with water, and dried. The solution was concentrated to *ca.* 30 ml. and cooled to 0°; needles (11·5 g.) separated, which gave crystals of the *carbonate* (8·2 g., 23%), m. p. 80—81° (from light petroleum), R_F 0·42, $\nu_{max.}$ (CCl_4) 1795 cm.$^{-1}$ (Found: C, 57·4; H, 8·7. $C_{11}H_{20}N_2O_3$ requires C, 57·8; H, 8·8%).

Attempted preparation of benzyloxycarbonylglycine 1-piperidyl ester from di-1-piperidyl carbonate. Di-1-piperidyl carbonate (0·17 g.) and benzyloxycarbonylglycine (0·017 g.) were dissolved in pyridine (1·5 ml.). Thin-layer chromatography detected no reaction within 3 days.

1-*Piperidyl dichloroacetate.* The acid chloride procedure [Method (B) of Part XXIV] [2] gave the *ester* (43%), m. p. 63—64° (from light petroleum), $\nu_{max.}$ (CCl_4) 1795 and 1771 cm.$^{-1}$ [Found (immediately after recrystallisation): C, 39·8; H, 5·4; Cl, 32·8; N, 6·3. $C_7H_{11}Cl_2NO_2$ requires C, 39·6; H, 5·2; Cl, 33·3; N, 6·6%]. The crystals turned yellow after 2 days at room temperature and decomposed to a yellow oil in 1 week.

Attempted preparation of benzyloxycarbonylglycine 1-piperidyl ester from 1-piperidyl dichloroacetate. 1-Piperidyl dichloroacetate (1·0 g.) and benzyloxycarbonylglycine (1·0 g.) were dissolved in pyridine (5 ml.). The solution rapidly turned a deep amber colour. Thin-layer chromatography showed that no dichloroacetate remained after 9 days, but no benzyloxycarbonylglycine 1-piperidyl ester was detected.

Test for racemisation during the conversion of benzyloxycarbonylglycyl-L-phenylalanine into its 1-piperidyl ester by means of 'Reagent K.' Benzyloxycarbonylglycyl-L-phenylalanine (1·421 g., 4 mmoles) was converted into its 1-piperidyl ester by the procedure described above for benzyloxycarbonylglycine 1-piperidyl ester. Evaporation of the solvent left the ester as a glass (1·062 g., 64%). The ester (1·018 g., 2·3 mmoles) was coupled with glycine ethyl ester hydrochloride (0·550 g., 4 mmoles) in the presence of sodium acetate trihydrate (0·550 g., 4 mmoles) in dioxan (10 ml.) with stirring. After 2 days at room temperature the dioxan was evaporated, and the residue was distributed between ethyl acetate and water; the ethyl acetate layer was washed with N-hydrochloric acid, N-sodium hydrogen carbonate, and water, and dried. Evaporation left a glass which crystallised to give benzyloxycarbonylglycyl-L-phenylalanylglycine ethyl ester (0·897 g., 84%), m. p. 110—116°, $[\alpha]_D^{20}$ −12·0° (*c* 2·0 in ethanol). A portion of the product was fractionally crystallised by the standard procedure,[23] except that the storage was at −5°. The first fraction, filtered off after 40 hr., had m. p. 115—118°, $[\alpha]_D^{20}$ −12·7° (*c* 2·0 in ethanol). A week later more crystals (400 mg.) were collected, m. p. 116—118·5°, $[\alpha]_D^{20}$ −12·5° (*c* 2·0 in ethanol). The pure L-isomer has $[\alpha]_D^{25}$ −13·2°.[23] No racemate was found.

Benzyloxycarbonyl-L-leucyl-L-phenylalanine Methyl Ester.—Dicyclohexylcarbodi-imide (10·3 g.) in chloroform (30

[21] R. Weiss, *Sitzungsber. Österr. Akad. Wiss. Wien, Abt. II B.*, 1919, **128**, 139.
[22] E. Möhr, *J. prakt. Chem.*, 1905, [2] **71**, 305.
[23] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1958, **80**, 2902.

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ml.) was added during 20 min. to a stirred mixture of benzyloxycarbonyl-L-leucine (13·2 g.), L-phenylalanine methyl ester hydrochloride (10·7 g.), and triethylamine (5·05 g.) in chloroform (250 ml.) at 12°. Next day the dicyclohexylurea was filtered off and the solution was washed and dried as usual. Evaporation followed by the addition of ether caused the separation of more dicyclohexylurea, which was filtered off; the ether was evaporated off, and trituration of the residue with light petroleum gave a gel which was dried and then reprecipitated from di-isopropyl ether to give the *ester*, m. p. 87—90°, $[\alpha]_D^{20}$ −23·5° (*c* 1·0 in methanol), $\nu_{max.}$ ($CHCl_3$) 1740, 1720, and 1680 cm.$^{-1}$ (Found: C, 67·6; H, 7·0; N, 7·1. $C_{24}H_{30}N_2O_5$ requires C, 67·5; H, 7·1; N, 6·6%).

Benzyloxycarbonyl-L-leucyl-L-phenylalanine.—The above ester (4·26 g.) was saponified with N-sodium hydroxide (13 ml.) in tetrahydrofuran (25 ml.) for 1 hr. at room temperature. The solvents were evaporated, water (60 ml.) was added to the residue, and concentrated hydrochloric acid was added to bring the pH to 2. The sticky solid so formed (4·0 g., 97%) was reprecipitated twice from ethyl acetate by the addition of light petroleum; it was then purified by conversion into the dicyclohexylammonium salt by the addition of an excess of dicyclohexylamine to a solution in ethyl acetate. The *benzyloxycarbonyl-L-leucyl-L-phenylalanine dicyclohexylammonium salt* (82% yield from the acid) had m. p. 165—168° (from chloroform–ether), $[\alpha]_D^{20}$ +37·4° (*c* 1·0 in chloroform), $\nu_{max.}$ ($CHCl_3$) 1720, 1670, and 1640 cm.$^{-1}$ (Found: C, 70·4; H, 8·5; N, 6·9. $C_{35}H_{51}N_3O_5$ requires C, 70·8; H, 8·7; N, 7·1%).

The free acid was liberated by stirring the salt (1·40 g.) with Dowex IR-120 resin (H^+ form) in 80% ethanol–water (50 ml.) for 1 hr. at room temperature. The mixture was filtered and the filtrate was evaporated, to leave an oil which was dissolved in ethyl acetate and dried. Addition of light petroleum precipitated the acid (0·73 g., 76%) which gave one spot only on t.l.c. [in the upper layer of an equilibrium mixture of n-butanol, water, and acetic acid (4 : 5 : 1 v/v); detection by chlorine and starch–iodide], m. p. 120—123° (with earlier softening), $[\alpha]_D^{20}$ +5·8° (*c* 1·0 in pyridine) $\nu_{max.}$ (Nujol) 1720, 1695, and 1680 cm.$^{-1}$ (Found: C, 66·9; H, 7·1; N, 6·8. Calc. for $C_{23}H_{28}N_2O_5$: C, 66·9; H, 6·85; N, 6·8%) (lit.,[24] m. p. 119—121°; no optical rotation given).

Benzyloxycarbonyl-L-leucyl-L-phenylalanylhydrazide.— (a) *From the methyl ester.* Benzyloxycarbonyl-L-leucyl-L-phenylalanine methyl ester (4·26 g.) and hydrazine hydrate (2·0 g.) were dissolved in methanol (30 ml.). Next day the solution was cooled to 0° for 2 hr., and the *hydrazide* was then collected (3·7 g., 87%). It gave needles which melted at 172—174° and then crystallised again as needles, to melt finally at 186—187°; $[\alpha]_D^{20}$ −22·6° (*c* 1·0 in acetic acid), $\nu_{max.}$ (Nujol) 1710 and 1660br cm.$^{-1}$) (Found: C, 64·9; H, 7·1; N, 13·4. $C_{23}H_{39}N_4O_4$ requires C, 64·7; H, 7·1; N, 13·1%).

(b) *By way of the 1-piperidyl ester.* This was prepared from benzyloxycarbonyl-L-leucyl-L-phenylalanine (0·533 g.) by the use of ' Reagent K ' as described for benzyloxycarbonylglycine 1-piperidyl ester, except that ether was used instead of ethyl acetate. Evaporation left the 1-piperidyl ester (0·384 g., 61%) as an oil, $\nu_{max.}$ (film) 1760, 1720, and 1680 cm.$^{-1}$, which was converted directly into the hydrazide by reaction with hydrazine hydrate (1 ml.) in ethanol (2 ml.). Crystals separated immediately, and after 1 hr. the ethanol was evaporated and the residue was triturated with water, to give the hydrazide (0·310 g., 94%), m. p. 175 and 186° (see above), $[\alpha]_D^{20}$ −22·4° (*c* 1·0 in acetic acid). The 1-piperidyl ester has been obtained crystalline by a different route.[25]

Test for Racemisation During the Conversion of Benzoyl-L-leucine into its 1-Piperidyl Ester by means of ' Reagent K.'—Benzoyl-L-leucine (0·941 g., 4 mmoles) was converted into its 1-piperidyl ester by the use of ' Reagent K ' as described for benzyloxycarbonylglycine 1-piperidyl ester. The chromatographically pure product (0·914 g., 78%) had m. p. 100—112°, $[\alpha]_D^{20}$ +17·9° (*c* 1·0 in chloroform), $[\alpha]_D^{20}$ +11·5° (*c* 1·0 in ethyl acetate). The crude product (0·588 g.) was condensed with glycine ethyl ester hydrochloride (0·350 g.) in the presence of sodium acetate trihydrate (0·350 g.) in dioxan (5 ml.). After 15 hr. at room temperature the dioxan was evaporated and the residue was distributed between ethyl acetate and water. The organic layer was washed as usual, dried, and evaporated, to leave benzoyl-leucylglycine ethyl ester (0·472 g., 80%), m. p. 137—157°, $[\alpha]_D^{20}$ −30·5° (*c* 3·0 in ethanol) (Found: C, 63·8; H, 7·5; N, 8·65. Calc. for $C_{17}H_{24}N_2O_4$: C, 63·7; H, 7·6; N, 8·8%). The specific rotation indicated that the crude product contained *ca.* 10% of racemate. The presence of racemate was confirmed by saponification by the following procedure, which is a slight modification of that used originally in this racemisation test.[11] The crude product (0·389 g.) was dissolved in a mixture of dioxan and N-sodium hydroxide (3 : 1; 2 ml.). After 1 hr. at room temperature the solution was diluted with saturated brine (25 ml.), washed with ethyl acetate (2 × 15 ml.), made acid (Congo Red) with concentrated hydrochloric acid, and extracted with ethyl acetate (2 × 20 ml.). The ethyl acetate extracts were combined, washed with saturated brine (2 × 15 ml.), and dried. Evaporation gave benzoyl-leucylglycine as a glass (0·310 g., 87%) which was heated with water (15·8 ml., giving a 2% solution) at 95° until a clear solution was obtained (20 min.) and then allowed to cool to room temperature. Three fractions were obtained: fraction A (120·8 mg.), m. p. 134—138°, $[\alpha]_D^{20}$ −26·8° (*c* 4·0 in ethanol) (Found: C, 61·3; H, 6·7; N, 9·5. Calc. for $C_{15}H_{20}N_2O_4$: C, 61·6; H, 6·9; N, 9·6%); fraction B (7·4 mg.), m. p. 155—162°, $[\alpha]_D^{20}$ −0·9° (*c* 0·35 in ethanol); and fraction C (1 mg.), m. p. 160—162°. Fraction A was essentially L-isomer (lit.,[11] m. p. 134—135°, $[\alpha]_D^{20}$ −26·4° (*c* 4·1 in ethanol), and fractions B and C were mainly racemate (lit.,[11] m. p. 165°). This modified procedure avoids the formation of benzoyl-DL-leucine, which complicated the fractionation in the original experiments.

Polarimetric Investigation of the Effects of Catalysts on the Rate of Condensation of Benzyloxycarbonyl-L-leucine 1-Piperidyl Ester with Glycine Ethyl Ester and with Benzylamine.—(a) *The effect of varying proportions of acetic acid on the condensation with glycine ethyl ester in chloroform.* Solutions of benzyloxycarbonyl-L-leucine 1-piperidyl ester (348 mg., 1·00 mmole), glycine ethyl ester (distilled; 0·120 ml., 1·20 mmoles), and acetic acid [(i) 0, (ii) 0·6, (iii) 1·2, (iv) 2·4, (v) 3·6, and (vi) 4·8 mmoles] in chloroform (5·00 ml.) were prepared. Samples were placed in the cell (1 dm.; jacketed to maintain 20°) of the automatic polarimeter, and the rotations were recorded at suitable intervals. The observed rotations are given after the time (min.). Solution (i): 10 min., −0·562°; 20, −0·565°;

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[25] F. Weygand, W. König, E. Nintz, D. Hoffmann, P. Huber, N. M. Khan, and W. Prinz, *Z. Naturforsch.*, 1966, **21***b*, 325.

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35, −0·569°; 55, −0·578°; 60, −0·581°; 85, −0·589°; 100, −0·599°; 135, −0·617°; 145, −0·623°. Solution (ii): 2 min., −0·677°; 6, −0·770°; 10, −0·834°; 15, −0·889°; 25, −0·959°; 35, −1·006°; 55, −1·063°; 66, −1·100°; 90, −1·128°. Solution (iii): 5 min., −0·880°; 6, −0·906°; 7, −0·923°; 8, −0·947°; 9, −0·965°; 10, −0·982°; 15, −1·063°; 20, −1·129°; 25, −1·184°; 30, −1·231°; 40, −1·302°; 50, −1·356°; 60, −1·399°; 90, −1·478°; 120, −1·559°; 180, −1·583°; 231, −1·610°; 265, −1·621°; ∞ (7 days), −1·651°. Solution (iv): 5 min., −0·860°; 6, −0·876°; 7, −0·894°; 8, −0·909°; 9, −0·925°; 10, −0·939°; 15, −1·011°; 20, −1·068°; 25, −1·118°; 30, −1·163°; 40, −1·235°; 50, −1·292°; 60, −1·338°; 90, −1·435°; 120, −1·498°; 150, −1·540°; 180, −1·572°; ∞ (7 days), −1·654°. Solution (v): 6 min., −0·888°; 7, −0·897°; 8, −0·908°; 9, −0·917°; 10, −0·926°; 15, −0·972°; 20, −1·011°; 25, −1·049°; 30, −1·080°; 40, −1·140°; 50, −1·188°; 60, −1·231°; 80, −1·300°; ∞ (7 days), −1·650°. Soution (vi): 6 min., −0·894°; 7, −0·898°; 8, −0·905°; 9, −0·911°; 10, −0·917°; 15, −0·943°; 20, −0·965°; 25, −0·985°; 30, −1·003°; 40, −1·035°; 50, −1·065°; 60, −1·089°; 80, −1·133°. Solutions (iii), (iv), and (v) gave the same final observed rotation of −1·65°, but solutions (i), (ii), and (vi) became discoloured and the rotations did not reach this value. The reaction proceeds most rapidly in the presence of 1 or 2 molar proportions of acetic acid [solutions (iii) and (iv)]; without acetic acid, the reaction is very slow. The results have been shown graphically in reference **3**. The specific rotation of the l-piperidyl ester is changed by the addition of acetic acid as follows: $[\alpha]_D^{20}$ −7·9° (*c* 7 in chloroform); −9·5° (*c* 7 in chloroform–acetic acid, 99 : 1); −10·4° (*c* 7 in chloroform–acetic acid, 98 : 2).

(b) *The effects of* 1,2,4-*triazole and of acetic acid on the rate of condensation with glycine ethyl ester in dimethylformamide.* Solutions were prepared containing benzyloxycarbonyl-L-leucine l-piperidyl ester (348 mg., 1·00 mmole), distilled glycine ethyl ester (0·120 ml., 1·20 mmoles), and (i) no catalyst, (ii) 1,2,4-triazole (69 mg., 1·00 mmole), and (iii) acetic acid (0·06 ml., 1·00 mmole) in 5·00 ml. of purified dimethylformamide. The change of optical rotation at 20° was observed as before. The observed rotations are given after the time in hr. Solution (i): 0·10 hr., −1·476°; 0·50, −1·475°; 2·5, −1·473°; 7·0, −1·465°; 22·3, −1·404°; ∞ (7 days), −0·844°. Solution (ii): 0·1 hr., −1·490°; 0·2, −1·487°; 0·5, −1·479°; 1·0, −1·466°; 1·5, −1·454°; 2·0, −1·448°; 2·5, −1·439°; 3·0, −1·432°; 7·0, −1·394°; 26·6, −1·175°; ∞ (7 days), −0·836°. Solution (iii): 0·05 hr., −1·455°; 0·1, −1·437°; 0·2, −1·404°; 0·5, −1·321°; 0·75, −1·272°; 1·0, −1·227°; 1·25, −1·192°; 1·5, −1·159°; 6·0, −0·985°; 21·0, −0·862°; ∞ (7 days), −0·844°. Benzyloxycarbonyl-L-leucylglycine ethyl ester has $[\alpha]_D^{20}$ −12·2° (*c* 1·0 in dimethylformamide), from which the calculated final rotation of the above solutions is −0·85°. Acetic acid [solution (iii)] is a much more effective catalyst for this reaction than is triazole. The results have been shown graphically in reference **3**.

(c) *The effect of* 1,2,4-*triazole and of acetic acid on the condensation with benzylamine in dimethylformamide.* Solutions were prepared containing benzyloxycarbonyl-L-leucine l-piperidyl ester (348 mg., 1·0 mmole), benzylamine (128 mg., 1·2 mmoles), and (i) no catalyst, (ii) 1,2,4-triazole (69 mg., 1 mmole), and (iii) acetic acid (0·06 ml., 1 mmole), in dimethylformamide (5 ml.). The change of optical rotation at 20° was observed as before. The observed rotations are given after the time in min. Solution (i): 5 min., −1·464°; 10, −1·462°; 20, −1·457°; 42, −1·451°; 62, −1·446°; 82, −1·441°; 112, −1·438°; ∞ (7 days), −0·286°. Solution (ii): 5 min., −1·452°; 11, −1·422°; 21, −1·377°; 30, −1·336°; 40, −1·294°; 60, −1·217°; 90, −1·130°; 120, −1·049°; ∞ (7 days), −0·284°. Solution (iii): 5 min., −1·405°; 10, −1·249°; 15, −1·190°; 25, −1·094°; 41, −0·925°; 62, −0·857°; 85, −0·747°; 111, −0·660°; ∞ (7 days), −0·282°. Acetic acid is a much more effective catalyst for this reaction than is triazole. The results have been shown graphically in reference **3**.

The Infrared Absorption of Solutions of 1-*Piperidyl* 3-*Phenylpropionate in Chloroform containing Hydrogen Chloride.*—The infrared spectra of solutions of 1-piperidyl 3-phenylpropionate (10·5—10·9 mg.) in chloroform, and in 0·04N-, 0·16N-, and 0·30N-hydrogen chloride in chloroform were examined. The solution in pure chloroform had a single strong carbonyl peak at 1750 cm.$^{-1}$ (ester CO); that in 0·04N-hydrogen chloride in chloroform showed a small peak at 1803 cm.$^{-1}$ and an diminution in the intensity of the 1750 cm.$^{-1}$ peak. With 0·16N-hydrogen chloride the two peaks were approximately equal in intensity, and with 0·30N-hydrogen chloride the intensity of the 1803 cm.$^{-1}$ peak was nearly 3 times that at 1750 cm.$^{-1}$. Huisgen and Kolbeck [13] found the carbonyl absorption in 1-acetoxyquinuclidinium chloride to be at 1800 cm.$^{-1}$.

Preparation of p-*Methoxybenzyloxycarbonylamino-acids by Use of* p-*Methoxybenzyl* 1-*Piperidyl Carbonate.*—The preparation of *p*-methoxybenzyl 1-piperidyl carbonate has been described; [16] the intermediate *p*-methoxybenzyl chloroformate, not previously reported, is stable for a short period in dilute solution at room temperature or as an oil at 0° or below; after 10 min., at room temperature, the oil showed no infrared absorption at 1780 cm.$^{-1}$ (CO·Cl), and a strong peak at 2335 cm.$^{-1}$ (CO_2) appeared. The use of the carbonate in the preparation of the *p*-methoxybenzyloxycarbonyl derivatives of glycine, L-alanine, L-leucine, L-phenylalanine, and L-valine (the last four as their dicyclohexylammonium salts) has also been described [16] but two of these salts are new and their constants are therefore given here:

p-*Methoxybenzyloxycarbonyl-*L-*alanine dicyclohexylammonium salt:* m. p. 151—153°, $[\alpha]_D^{20}$ +2·0°, $[\alpha]_{365}^{20}$ +6·4° (*c* 1·0 in methanol), $\nu_{max.}$ (Nujol) 1710 and 1630 cm.$^{-1}$ (Found: C, 66·8; H, 8·9; N, 6·5. $C_{24}H_{38}N_2O_5$ requires C, 66·3; H, 8·8; N, 6·45%).

p-*Methoxybenzyloxycarbonyl-*L-*valine dicyclohexylammonium salt:* m. p. 162—166°, $[\alpha]_D^{20}$ +2·8°, $[\alpha]_{365}^{20}$ +8·5° (*c* 1·0 in methanol), $\nu_{max.}$ (Nujol) 1710 and 1630 cm.$^{-1}$ (Found: C, 67·2; H, 8·8; N, 5·8. $C_{26}H_{42}N_2O_5$ requires C, 67·5; H, 9·2; N, 6·05%).

t-Butyl 1-*Piperidyl Carbonate.*—t-Butyl chloroformate was prepared from potassium t-butoxide t-butanol solvate (18·6 g., 0·10 mmoles) as described by Ovchinnikov, Kiryushkin, and Miroshnikov.[17] The oil so obtained was dissolved in dry ether (100 ml.) at −25° and a solution of 1-hydroxypiperidine (10 g., 0·10 mmoles) in dry ether (30 ml.) was added during 5 min. After a further 10 min., triethylamine (15 ml.) was added, and the mixture was allowed to warm to room temperature. It was then filtered and the filtrate washed with 10% citric acid (5 times), N-sodium hydrogen carbonate (twice), and brine, and dried. Evaporation left the *carbonate* (5·35 g., 26%) as a chromatographically pure oil, $\nu_{max.}$ (film) 1770 cm.$^{-1}$, τ 6·3—7·6

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(4H, two broad bands with centres at 6·6 and 7·3, $CH_2{\cdot}N{\cdot}CH_2$), 8·0—8·5 (6H, complex, $CH_2{\cdot}[CH_2]_3{\cdot}CH_2$), and 8·48 [9H, singlet, $(CH_3)_3{\cdot}C$].

t-Butoxycarbonylglycine Dicyclohexylammonium Salt.—t-Butyl 1-piperidyl carbonate (2·01 g., 10 mmoles), glycine ethyl ester hydrochloride (0·69 g., 5 mmoles), and sodium acetate trihydrate (0·69 g., 5 mmoles) were stirred in dioxan (4 ml.) at room temperature. After 8 days the solvent was evaporated off, the residue was distributed between ether and water, and the ether layer was washed with 10% citric acid, N-sodium hydrogen carbonate, and brine. Evaporation left an oil which was dissolved in dioxan (7 ml.), and N-sodium hydroxide (7 ml.) was aded; after 1 hr. the solution was evaporated, to leave an oil which was dissolved in water (25 ml.); the solution was cooled to 0° and the pH brought to 2 with N-hydrochloric acid, with the addition of ether (50 ml). The ether layer was washed with brine and dried, and dicyclohexylamine (2 ml.) was added. The *salt* gave needles (0·56 g., 33% overall), m. p. 148—152°, $\nu_{max.}$ (Nujol) 1715 and 1640 cm.$^{-1}$, τ 0·65—1·0 (2H, broad, $=\overset{+}{N}H_2$), 4·5—4·9 (1H, broad, $CO{\cdot}NH{\cdot}CH_2$), 6·31 (2H, doublet, J = 5 c./sec., $NH{\cdot}CH_2{\cdot}CO$), 6·6—7·4 (2H, broad, $CH{\cdot}\overset{+}{N}H_2{\cdot}$), 7·6—9·2 [29H, complex, multiplet from cyclohexane protons and a singlet at 8·52 from $(CH_3)_3{\cdot}C$] (Found: C, 63·7; H, 10·1; N, 8·1. $C_{19}H_{36}N_2O_4$ requires C, 64·0; H, 10·2; N, 7·9%).

When the acylation was effected in refluxing dioxan for 2 hr., the overall yield of dicyclohexylammonium salt was 25%; from free glycine ester and an equivalent of acetic acid in refluxing chloroform for 6·5 hr., the yield was 36%.

ε-Benzyloxycarbonyl-L-lysine (with Miss S. J. Allard).—L-Lysine monohydrochloride (0·91 g., 5 mmoles) and triethylamine (0·50 g., 5 mmoles) were dissolved in the minimum volume of water (2 ml.), and a solution of benzyl 1-piperidyl carbonate (2·1 g., 7·5 mmoles) in methanol (8 ml.) was added. After 2 days, the solid which had separated was collected and washed with a little ice-cold water, methanol, and ether, and dried. Concentration of the filtrate gave a second crop of crystals; total yield 0·83 g. (58%), m. p. 250—252° (from 50% acetic acid) (lit.,[26] 255°).

Reaction of Benzyl 1-Piperidyl Carbonate with Glycine Benzyl Ester in the Presence of 1,2,4-Triazole [15] (with Miss S. J. Allard).—The benzyloxycarbonylation of glycine benzyl ester hydrochloride (12 mmoles) was effected under the conditions described in Part XXIV,[2] except that in experiment (*a*) 1,2,4-triazole (10 mmoles) was added, and in experiment (*b*) 1,2,4-triazole (10 moles) was added and triethylamine (12 mmoles) replaced the sodium acetate trihydrate. In experiment (*a*) the time for reaction (disappearance of benzyl 1-piperidyl carbonate, determined by t.l.c.) was the same as that in the absence of the triazole, 24 hr. In experiment (*b*), in which no acetic acid was present, the time for reaction was 5 days. It is concluded that in this case triazole has little catalytic effect.

Reaction of 1-Piperidyl α-Phthalimidoisobutyrate with Glycine Ethyl Ester.—1-Piperidyl α-phthalimidoisobutyrate (0·316 g., 1 mmole), glycine ethyl ester (0·412 g., 4 mmoles), and acetic acid (0·240 g., 4 mmoles) were dissolved in chloroform (2 ml.) at room temperature. After 22 hr., no 1-piperidyl ester could be detected (by t.l.c.), and the yellow solution was diluted with chloroform and washed with 2N-hydrochloric acid, N-sodium hydrogen carbonate, and water, and dried. Evaporation, and trituration of the residue with light petroleum gave a solid (0·130 g.), which gave needles (60 mg.), m. p. 183·5—185° (from ethyl acetate–light petroleum), of *α-(o-ethoxycarbonylmethylcarbamoylbenzamido)isobutyrylglycine ethyl ester*, $\nu_{max.}$ ($CHCl_3$) 1745 and 1665 cm.$^{-1}$, τ 2·0—2·8 (6H, complex, aromatic protons with 2 $CO{\cdot}NH{\cdot}CH_2$), 3·04 (1H, singlet, $CO{\cdot}NH{\cdot}CMe_2$), 5·5—6·3 [8H from two superimposed systems: (*a*) two identical quartets, J = 7 c./sec., with centres at 5·67 and 5·71, respectively, 2 $O{\cdot}CH_2{\cdot}CH_3$, and (*b*) two identical doublets, J = 8 c./sec., with centres at *ca.* 5·9 and 6·0, respectively, 2 $NH{\cdot}CH_2{\cdot}CO$], 8·42 [6H, singlet, $\cdot C(CH_3)_2$], and 8·5—8·9 (6H, two identical triplets, J = 7 c./sec., with centres at 8·70 and 8·72, respectively, 2 $O{\cdot}CH_2{\cdot}CH_3$), *m/e* 421 (M^+) (Found: C, 57·0; H, 6·5; N, 9·65. $C_{20}H_{27}N_3O_5$ requires C, 57·0; H, 6·5; N, 10·0%).

Methyl α-Phthalimidoisobutyrate.—A solution of diazomethane in ether was added in portions to a solution of α-phthalimidoisobutyric acid (0·932 g., 4 mmoles) in 30% methanol–ether (20 ml.) until the yellow colour persisted; this was discharged with acetic acid, and the solution was evaporated to dryness. The crystalline residue (0·98 g., 99%) gave the *ester*, m. p. 74—76° (from cyclohexane), $\nu_{max.}$ (CCl_4) 1780, 1750, and 1720 cm.$^{-1}$, *m/e* 247 (M^+; the fragmentation pattern was analogous to that already described for the corresponding acid [27]) (Found: C, 63·3; H, 5·35; N, 5·6. $C_{13}H_{13}NO_4$ requires C, 63·1; H, 5·3; N, 5·7%).

The Reaction of Methyl α-Phthalimidoisobutyrate with Glycine Ethyl Ester.—The isobutyrate (0·247 g., 1 mmole), glycine ethyl ester (0·40 ml., 4 mmoles), and acetic acid (0·24 ml., 4 mmoles) were dissolved in chloroform (2 ml.) and left at room temperature for 45 hr. The solution was diluted with ethyl acetate (30 ml.) and washed with water, N-hydrochloric acid, N-sodium hydrogen carbonate, and dried. Evaporation left a sticky solid (0·20 g.), which gave *methyl α-(o-ethoxycarbonylmethylcarbamoylbenzamido)isobutyrate* (0·06 g.), m. p. 162—163° (from ethyl acetate–light petroleum), $\nu_{max.}$ ($CHCl_3$) 1745 and 1665 cm.$^{-1}$, τ 2·2—3·1 (6H, complex, aromatic protons with 2 $CO{\cdot}NH$), 5·6—6·0 [4H comprising (*a*) a quartet, J = 8 c./sec. with centre at 5·8, $O{\cdot}CH_2{\cdot}CH_3$, and (*b*) a doublet, J = 6 c./sec., with centre at 5·9, $NH{\cdot}CH_2{\cdot}CO$], 6·29 (3H, singlet, $O{\cdot}CH_3$), 8·41 [6H, singlet, $\cdot C(CH_3)_2$], and 8·5—8·9 (3H, triplet, J = 8 c./sec., with centre at 8·70, $O{\cdot}CH_2{\cdot}CH_3$), *m/e* 350 (M^+) (Found: C, 58·1; H, 6·7; N, 8·1. $C_{17}H_{22}N_2O_6$ requires C, 58·3; H, 6·3; N, 8·0%).

The Reaction of Phthaloylglycine Ethyl Ester with Glycine Ethyl Ester.—Phthaloylglycine ethyl ester (0·93 g., 4 mmoles), glycine ethyl ester (1·6 ml., 16 mmoles), and acetic acid (0·96 ml., 16 mmoles) were dissolved in chloroform (8·0 ml.) at room temperature. After 47 hr., the solvent was evaporated off and the residue was distributed between ethyl acetate and water. The ethyl acetate layer was washed with N-hydrochloric acid, N-potassium hydrogen carbonate, and water, and dried. Evaporation left a solid (1·2 g.) which gave two spots of similar intensity on t.l.c., one (R_F 0·6) corresponding to unchanged phthaloylglycine ethyl ester, and the other (R_F 0·1) to phthaloyldiglycine diethyl ester. A small quantity of the latter was isolated by fractional crystallisation from ethyl acetate–light petroleum, and was identical with authentic material (see below) (m. p. and mixed m. p., infrared spectrum, and t.l.c.).

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When this experiment was repeated with the omission of the acetic acid, t.l.c. again indicated the presence of the two components in the product, but the amount of phthaloyldiglycine derivative was insufficient for isolation.

Di-1-piperidyl Phthalate.—Phthaloyl chloride (10 g., 0·05 mole) in dry ether (100 ml.) was added during 10 min., to a solution of 1-hydroxypiperidine (13 g., 0·13 mole) in ether (150 ml.) at 0°. After a further 5 min., N-sodium carbonate (200 ml.) was added; the ethereal layer was separated and washed with 2N-hydrochloric acid, N-sodium hydrogen carbonate, and brine, and dried. Evaporation left a crystalline residue (15·4 g., 92%) which gave the *ester*, m. p. 91—92° (from di-isopropyl ether), $\nu_{max.}$ ($CHCl_3$) 1750 cm.$^{-1}$, τ 2·2—2·6 (4H, complex, aromatic), 6·2—7·6 (8H, two broad bands with centres at 6·6 and 7·2, 2 CH_2·N·CH_2), 8·0—8·7 (12H, complex, 2 CH_2·[CH_2]$_3$·CH_2) (Found: C, 64·6; H, 7·4; N, 8·55. $C_{18}H_{24}N_2O_4$ requires C, 65·1; H, 7·3; N, 8·4%).

Phthaloyldiglycine Diethyl Ester.—Glycine ethyl ester (freshly distilled; 1·0 ml., 10 mmoles) and acetic acid (0·60 g., 10 mmoles) were added to a solution of di-1-piperidyl phthalate (3·32 g., 10 mmoles) in the minimum volume of dioxan. After 40 hr., the dioxan was evaporated off, the residue was dissolved in chloroform, and the solution was washed with 2N-hydrochloric acid, N-potassium hydrogen carbonate, and water, and dried. Evaporation left a solid, which was extracted with boiling ether, from which some phthaloylglycine ethyl ester,[28] m. p. 110—111°, was isolated. The ether-insoluble portion gave *phthaloyldiglycine diethyl ester*, m. p. 116—117° (from ethyl acetate), $\nu_{max.}$ ($CHCl_3$) 1740 and 1660 cm.$^{-1}$, τ 2·2—2·8 (6H, complex, aromatic protons and CO·N*H*·CH_2), 5·5—6·0 [8H, consisting of (*a*) a quartet with centre at 5·8, J = 7·5 c./sec., 2 O·C*H*$_2$·CH_3, and (*b*) a doublet with centre at 5·85, J = 6 c./sec., 2 NH·C*H*$_2$·CO], and 8·70 (6H, triplet, J = 7 c./sec., CH_2·C*H*$_3$), *m/e* 336 (M^+) (Found: C, 57·0; H, 5·9; N, 8·7. $C_{16}H_{20}N_2O_6$ requires C, 57·2; H, 6·0; N, 8·3%).

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