Amino-acids and Peptides. Part XXXV.[1] The Effect of Solvent on the Rates of Racemisation and Coupling of Some Acylamino-acid *p*-Nitrophenyl Esters; the Base Strengths of Some Amines in Organic Solvents, and Related Investigations

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The rates of racemisation by triethylamine of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (A) and of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (B) in nine different solvents have been determined. The rates of condensation of the ester (B) with glycine ethyl ester in the same solvents have been measured; the rate for dimethyl sulphoxide is *ca.* 500 times that for chloroform as solvent. The ratios of the rates of racemisation of (A) and (B) to that of the condensation reaction are relatively low for the solvents tetrahydrofuran, dimethylformamide, and dimethyl sulphoxide, but exceptionally high for chloroform, dichloromethane, acetonitrile, and nitromethane, which are unfavourable solvents in this respect. The effect of the nature of the amine on the rate of racemisation of esters (A) and (B) and of benzoyl-L-leucine *p*-nitrophenyl ester in acetonitrile and in dimethyl sulphoxide is reported, and the base strengths of some amines in organic solvents have been measured by use of 2,4-dinitrophenol as spectrophotometric indicator. The change in the base strength of glycine ethyl ester with solvent runs roughly parallel with the change in its nucleophilicity as measured by the rate of its reaction with ester (B). The rates of racemisation of the *p*-chloro-, 2,4-dichloro-, *m*-nitro-, 2,4,5-trichloro-, and *p*-nitro-phenyl esters of benzoyl-L-leucine by base increase in the order given. Benzyltrimethylammonium chloride and iodide racemise benzoyl-L-leucine *p*-nitrophenyl ester in acetonitrile, but the corresponding fluoroborate does not, although it increases the rate of racemisation by bases. It is concluded that the effect of chloride ion in increasing racemisation during coupling is due both to its basicity and to the increase in the ionic strength of the solution.

RACEMISATION at the coupling stage of peptide synthesis is due to carbanion formation at the dissymmetric centre (either directly or as the oxazolone),[2] and this may be caused by the amino-component itself acting as a base, or by the presence of an excess of tertiary amine, added in order to liberate the amino-component from its salt. We report here investigations mainly relevant to the latter case; the compounds studied were mostly *p*-nitrophenyl esters. Our conclusions are not applicable to the new class of active esters, such as those of 1-hydroxypiperidine,[3] 8-hydroxyquinoline,[4] *o*-hydroxyphenol,[5] 2-mercaptopyridine,[1] and 2-hydroxypyridine,[6] for which selective anchimeric assistance of aminolysis is postulated, and for which the ratio of the rate of aminolysis to that of base-catalysed racemisation is abnormally high.

We have measured the rates of racemisation by triethylamine of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (proceeding chiefly through the oxazolone [7]) and of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (assumed to proceed directly through carbanion formation since alkoxycarbonylamino-acids do not form oxazolones) in nine different solvents; the results are shown in Table 1 (columns A and B). The 150-fold increase in rate in changing the solvent from dioxan to nitromethane for the first reaction (column A) emphasises the danger resulting from the presence of base in such highly polar solvents. There is a reasonable correlation of rates in this series with the $E_{T_{30}}$ solvent parameter [8] [the molar transition energy for the charge-transfer absorption of the betaine derived from *N*-(3,5-diphenyl-4-hydroxyphenyl)-2,4,6-triphenylpyridinium perchlorate], given also in Table 1. The sequence for the second reaction (column B) differs significantly for the solvents chloroform and dichloromethane, and the increase in rate from dioxan to nitromethane is 95-fold. As soon as the coupling reagents are present, the significant factor is the relative rate of the racemisation and coupling reactions, and we have determined spectrophotometrically the rates of condensation of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester with glycine ethyl ester in the same solvents; these results are given in column C of Table 1, and the effect of solvent on the ratio of the rate of racemisation to that of coupling is shown by the figures in the fourth and fifth columns. The marked increase in the rate of condensation of the analogous 2,4,5-trichlorophenyl ester with benzylamine, when the solvent was changed from *e.g.* chloroform to dioxan, ethyl acetate, and dimethylformamide, was noted earlier,[9] and our results reveal a still greater range of rates, that for dimethyl sulphoxide being *ca.* 500 times that for chloroform. The ratios of the rate of racemisation to that of coupling are relatively low for tetrahydrofuran, dimethylformamide, and dimethyl sulphoxide; in this typical case at least, advantage could well be taken of the greatly enhanced coupling rate in dimethylformamide and dimethyl sulphoxide. For chloroform, dichloromethane, acetonitrile, and nitromethane the ratios are exceptionally high and these are clearly unfavourable solvents. We

[1] Part XXXIV, K. Lloyd and G. T. Young, *J. Chem. Soc.* (*C*), 1971, 2890.

[2] For a recent review of the mechanism of racemisation during peptide synthesis, see M. Goodman and C. Glaser in 'Peptides: Chemistry and Biochemistry, Proc. 1st Amer. Peptide Symp., 1968,' ed. B. Weinstein and S. Lande, Dekker, New York, 1970, p. 267.

[3] B. O. Handford, J. H. Jones, G. T. Young, and T. F. N. Johnson, *J. Chem. Soc.*, 1965, 6814.

[4] H.-D. Jakubke and A. Voigt, *Chem. Ber.*, 1966, **99**, 2419.

[5] J. H. Jones and G. T. Young, *J. Chem. Soc.* (*C*), 1968, 436.

[6] A. S. Dutta and J. S. Morley, *J. Chem. Soc.* (*C*), 1971, 2896.

[7] I. Antonovics and G. T. Young, *J. Chem. Soc.* (*C*), 1967, 595.

[8] K. Dimroth, C. Reichardt, T. Siepmann, and F. Bohlmann, *Annalen*, 1963, **661**, 1.

[9] J. Pless and R. A. Boissonnas, *Helv. Chim. Acta*, 1963, **46**, 1609.

note that the solvent dependence for these racemisation reactions differs from that observed for the base-catalysed racemisation of the oxazolone derived from benzyloxycarbonyl-α-aminoisobutyryl-L-phenylalanine, which proceeds faster in dioxan than in chloroform,[10] but for those of our reactions which proceed through an oxazolone intermediate the rate-determining step will be oxazolone formation, since tertiary amines cause rapid racemisation of such oxazolones.[7,10] This point is important, because it has been assumed[11] that the optical stability of benzoyl-L-leucine *p*-nitrophenyl ester in the presence of tribenzylamine in chloroform is due to the inability of the amine to racemise the oxazolone; in fact under these conditions no oxazolone is detectable by i.r. spectroscopy, and the optical stability is clearly due to the inability of the amine to cause oxazolone formation. The solvent dependence also differs notably from that found for the aminolysis of *S*-2-pyridyl thioesters[1,12] and *o*-hydroxyphenyl esters;[13] for example, for the condensation of t-butoxycarbonyl-L-leucine *S*-2-pyridyl thioester with *p*-anisidine, the order of decreasing rate was: dioxan ≡ ethyl acetate, dimethylformamide, dimethyl sulphoxide.[1]

We have examined the effect of the nature of the tertiary amine on the rate of racemisation of the *p*-nitrophenyl esters of benzoyl-L-leucine, benzyloxycarbonylglycyl-L-phenylalanine, and benzyloxycarbonyl-L-phenylalanine, in acetonitrile and in dimethyl sulphoxide; the results are given in Table 2. M. and A. Bodanszky[11] found that the racemisation in chloroform of the *p*-nitrophenyl esters of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine and of benzyloxycarbonyl-L-phenylglycine, but not that of benzoyl-L-leucine, was markedly slower with sterically hindered tertiary amines, particularly with ethyldi-isopropylamine. We also find that those esters which racemise through the oxazolone (benzoyl-L-leucine and benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl esters) have similar rates for triethylamine and ethyldi-isopropylamine, whereas the ester which racemises directly through the carbanion (benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester) is racemised more slowly by the hindered amine. Understandably, removal of the amide proton is less susceptible to steric hindrance than is the removal of the proton from the α-carbon atom of an active ester. The anomaly disappears when di-isopropylamine or dicyclohexylamine is used (the rate of condensation of these amines is shown to be too low to affect measurements of the initial rate of racemisation). An extreme case is that of the resin Amberlite IR-45 (containing $ArCH_2{\cdot}NPr^n_2$ groups) equilibrated with dimethyl sulphoxide; with this amine the racemisation even of benzoyl-L-leucine *p*-nitrophenyl ester was very slow.

TABLE 1

Effect of solvent on the rates of racemisation of *p*-nitrophenyl esters by triethylamine and on the rate of coupling of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester with glycine ethyl ester

	Racemisation [a]					
Solvent	A Z-Gly-Phe-ONp	B Z-Phe-ONp	Coupling [b] C	A/C [c]	B/C [c]	$E_{T_{30}}$ [d]
Dioxan	0·7	0·1	0·4	1·8	0·25	36·0
Ethyl acetate	1·3	0·3	0·9	1·4	0·33	38·1
Tetrahydrofuran	1·7	0·1	2·8	0·6	0·04	37·4
Chloroform	6·4	1·3	0·1	64	13	39·1
Dichloromethane	14·7	1·8	0·2	74	9	41·1
Dimethylformamide	37	0·5	20	1·9	0·03	43·8
Dimethyl sulphoxide	47	1·3	48	1·0	0·03	45·0
Acetonitrile	63	1·5	0·8	79	1·9	46·0
Nitromethane	108	9·5	0·4	270	24	46·3

[a] Column A gives 10^5k (pseudo-first-order rate constant) in s^{-1} for the racemisation at 20° of a solution in the stated solvent 0·05M with respect to the ester and triethylamine. Column B gives the analogous figures for the racemisation at 20° of solutions 0·10M with respect to the ester and 0·20M with respect to triethylamine. Abbreviations follow the rules in 'Abbreviated Designation of Amino Acid Derivatives and Polypeptides,' I.U.P.A.C. Information Bulletin No. 26. [b] Column C gives 10^2k (second-order rate constant, l mol^{-1} s^{-1}) for the condensation of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester with glycine ethyl ester at 25°. [c] These columns give the ratios of the figures in columns A and B respectively to those in column C. The ratios are those of a pseudo-first-order rate constant to a second-order rate constant, and they have therefore significance only with respect to figures in the same column. [d] $E_{T_{30}}$ values (in kcal mol^{-1} at 25°) from K. Dimroth, C. Reichardt, T. Siepmann, and F. Bohlmann, *Annalen*, 1963, **661**, 1.

TABLE 2

Effect of the nature of the amine on the rate of racemisation of some *p*-nitrophenyl esters

Amine	Bz-Leu-ONp [a] $t_{\frac{1}{2}}$/h	Z-Gly-Phe-ONp [a] $t_{\frac{1}{2}}$/h	Z-Phe-ONp [b] $t_{\frac{1}{2}}$/h
In acetonitrile			
NEt_3	0·13	0·21	12·7
$EtNPr^i_2$	0·13	0·26	125
$HNPr^i_2$ [c]	0·09		17·8
$(C_6H_{11})_2NH$ [c]	0·07	0·08	12·6
N-Methylmorpholine	10·2	45	210
$(PhCH_2)_3N$	133	Stable [d]	Stable [d]
In dimethyl sulphoxide			
NEt_3	0·21	0·28	15·3
$EtNPr^i_2$	0·29	0·53	78
$HNPr^i_2$ [c]	0·03		1·2
$(C_6H_{11})_2NH$ [c]	0·05	0·10	1·6
N-Methylmorpholine	2·7	7·0	460
$(PhCH_2)_3N$	64	120	Stable [d]
Amberlite IR-45	22 [e]		240 [f]

[a] Except for the reaction with Amberlite IR-45, solutions were 0·10M with respect both to the *p*-nitrophenyl ester and the amine; the times for 50% reaction at 20° are given. [b] Except for the reaction with Amberlite IR-45, solutions were 0·10M with respect to the *p*-nitrophenyl ester and 0·20M with respect to the amine; the times for 50% reaction at 20° are given. [c] For an evaluation of the extent of the competing acylation reaction see the Experimental section. [d] 'Stable' means that no significant change of optical rotation was observed within at least 17 h. [e] The solution was 0·10M with respect to the *p*-nitrophenyl ester, and 3 equiv. of resin were used. [f] The solution was 0·10M with respect to the *p*-nitrophenyl ester, and 6 equiv. of resin were used.

[10] M. Goodman and W. J. McGahren, *Tetrahedron*, 1967, **23**, 2031.

[11] M. Bodanszky and A. Bodanszky, *Chem. Comm.*, 1967, 591.

[12] K. Lloyd, D.Phil. Thesis, Oxford, 1969.

[13] R. D. Cowell and J. H. Jones, personal communication.

TABLE 3

Base strengths of some amines in organic solvents [a]

Solvent	NEt_3	$EtNPr^i_2$	NMM [b]	$(PhCH_2)_3N$	$(C_6H_{11})_2NH$	Gly-OEt	$\frac{\log_{10} 10^4 k_{coupling}}{\log_{10} K_{Gly\text{-}OEt}}$ [c]
Dioxan	3·3 [d]	2·8	1·5	<0	3·3	1·7	0·94
Ethyl acetate	4·1 [d]	3·5	2·5	<0	>4·2	2·4	0·81
Tetrahydrofuran	4·1 [e]		1·9	<0		2·95	0·83
Chloroform	4·2 [d]	4·2	1·75	<0	4·1	0·6 [f]	1·7
Dichloromethane	>4·2	>4·2	2·2	<0		0·7 [f]	1·9
Dimethylformamide	>4·2		>4·2	1·5		>4·2	<0·79
Dimethyl sulphoxide	>4·2	>4·2	>4·2	1·8	>4·2	>4·2	<0·88
Acetonitrile	>4·2	>4·2	3·7	1·5	>4·2	3·1	0·61
Nitromethane	>4·2		3·5	1·2		2·4	0·67

[a] The figures are for $\log_{10} K$, where $K = [\overset{+}{N}HR_3,\overset{-}{O}\cdot C_6H_3(NO_2)_2]/[NR_3][HO\cdot C_6H_3(NO_2)_2]$. [b] *N*-Methylmorpholine. [c] k is the second-order rate constant (from column C, Table 1) for the condensation of benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester with glycine ethyl ester. K is defined in note *a*. [d] R. G. Pearson and D. C. Vogelsong (*J. Amer. Chem. Soc.*, 1958, **80**, 1038) give $K = 1460$ for dioxan, 11,900 for ethyl acetate, and 15,800 for chloroform. [e] G. W. Anderson, J. E. Zimmerman, and F. M. Callahan (*J. Amer. Chem. Soc.*, 1967, **89**, 5012) give $K = 11{,}600$. [f] Calculated assuming an extinction coefficient of 10,000 for the ion-pair.

We have compared the base strengths of some amines in organic solvents, by use of 2,4-dinitrophenol as spectrophotometric indicator.[14] The results, expressed as $\log_{10} K$ where $K = [\overset{+}{N}HR_3,\overset{-}{O}\cdot C_6H_3(NO_2)_2]/[NR_3][HO\cdot C_6H_3(NO_2)_2]$, are given in Table 3. Unfortunately, triethylamine, ethyldi-isopropylamine, and dicyclohexylamine (in the more polar solvents), and tribenzylamine (in the less polar solvents), are beyond the useful range of the indicator. Indeed, it seems unnecessary to invoke steric hindrance to explain the optical stability of *p*-nitrophenyl esters in the presence of tribenzylamine, for it is apparently a much weaker base than is glycine ethyl ester in all the solvents examined, and it is not surprising that its use for the liberation of peptide esters from their salts proved unsatisfactory.[15] The advantages of the use of a base weaker than triethylamine, such as *N*-methylmorpholine, for this and similar purposes have been demonstrated earlier,[16] and the results in Table 2 provide further evidence on this point, but it will be seen from Table 3 that in tetrahydrofuran-*N*-methylmorpholine is apparently weaker than glycine ethyl ester. It must be borne in mind that the use of dissociation constants obtained with an indicator such as dinitrophenol for the evaluation of the relative strengths of bases with respect to a different acid (*e.g.* an amide, for the oxazolone-forming reaction, or the α-CH, for direct exchange) may be misleading, particularly when the acid is an ammonium ion, but it seems likely that in tetrahydrofuran liberation of the free amino-ester by *N*-methylmorpholine may be incomplete. The last column in Table 3 shows that the change in the nucleophilicity of glycine ethyl ester, as measured by the rate of condensation with benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester, with solvent runs roughly parallel with the change in its basicity.

We include (Table 4) some measurements of the rate of racemisation of the *p*-chloro-, 2,4-dichloro-, *m*-nitro-, 2,4,5-trichloro-, and *p*-nitro-phenyl esters of benzoyl-L-leucine by triethylamine and by *N*-methylmorpholine in chloroform and in dioxan. The effects of solvent and of the nature of the amine are again evident. I.r. absorption at 1830 cm^{-1} (oxazolone CO) showed qualitatively that oxazolone formation accompanied racemisation, the absorption being moderately strong after 30 min for the two last esters with triethylamine in chloroform or dioxan, and weak or very weak after the same time for the 2,4-dichlorophenyl and *m*-nitrophenyl esters with triethylamine in chloroform; no such absorption developed within 24 h for the *p*-chlorophenyl ester and triethylamine or for the 2,4-dichlorophenyl ester and *N*-methylmorpholine, in chloroform.

TABLE 4

Racemisation of substituted phenyl esters of benzoyl-L-leucine by tertiary amines [a]

Ester	Amine [b]	Solvent	$t_{\frac{1}{2}}$/h
p-Chlorophenyl	Et_3N	Chloroform	>100
2,4-Dichlorophenyl	Et_3N	Chloroform	16
	Et_3N	Dioxan	50
	NMM	Chloroform	>100
	NMM	Dioxan	>100
m-Nitrophenyl	Et_3N	Chloroform	4·5
	Et_3N	Dioxan	22
	NMM	Chloroform	>100
2,4,5-Trichlorophenyl	Et_3N	Chloroform	1·3
	Et_3N	Dioxan	4·7
	NMM	Chloroform	>100
	NMM	Dioxan	>100
p-Nitrophenyl	Et_3N	Chloroform	0·93
	Et_3N	Dioxan	3·7
	NMM	Chloroform	100
	NMM	Dioxan	140

[a] Solutions for polarimetry were 0·1M with respect to ester and amine. [b] NMM = *N*-Methylmorpholine.

It has been noted that coupling reactions carried out in the presence of tertiary amine hydrochlorides result in many cases in increased racemisation.[17,18] Since quaternary ammonium chlorides caused racemisation of active

[14] R. G. Pearson and D. C. Vogelsong, *J. Amer. Chem. Soc.*, 1958, **80**, 1038.
[15] M. Bodanszky and R. J. Bath, *Chem. Comm.*, 1968, 766.
[16] G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, 1967, **89**, 5012.
[17] G. W. Anderson, J. Blodinger, and A. D. Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5309.
[18] M. A. Smart, G. T. Young, and M. W. Williams, *J. Chem. Soc.*, 1960, 3902.

esters, we attributed this effect to the chloride ion, and since quaternary ammonium perchlorate caused no racemisation we suggested that the basicity of the chloride ion in organic solvents is responsible.[19] On the other hand, Goodman and McGahren [10] found that the racemisation of oxazolones by triethylamine hydrochloride in chloroform is very slow, although the racemisation effected by DL-phenylglycine methyl ester is accelerated by the addition of the salt. They attributed the 'chloride effect' to the increased ionic strength of the solution. Kemp and Chien [20] noted that the addition of triethylammonium fluoroborate could reduce 50-fold the rate of racemisation of *O*-(benzyloxycarbonylglycyl-L-phenylalanyl)-*N*-ethylsalicylamide by triethylamine in dimethylformamide, but such addition will decrease the concentration of the amide anion through which the oxazolone is formed. Unfortunately, the base strength of benzyltrimethylammonium chloride is too low for accurate measurement with dinitrophenol as indicator; on the assumption that the extinction coefficient E for the ion-pair is 10,000, in purified chloroform $\log_{10} K$ would be *ca.* 0·1 (where $K = [HCl,^-O{\cdot}C_6H_3(NO_2)_2]/[Cl^-][HO{\cdot}C_6H_3(NO_2)_2]$), and in dichloromethane and acetonitrile *ca.* 0·3; the addition of one molar proportion of water to the solution of the chloride in chloroform raises $\log_{10} K$ to *ca.* 0·5. Direct comparison has however shown that the order of base strengths of the quaternary ammonium salts is, as expected, $Cl^- > I^- > BF_4^-$; even the addition of 2000 equiv. of the fluoroborate to the dinitrophenol in acetonitrile scarcely changed the absorbance. This difference is paralleled in the rates of racemisation effected by the salts. Benzoyl-L-leucine *p*-nitrophenyl ester (0·10M) in acetonitrile at 20° was 33% racemised by 0·05M-benzyltrimethylammonium chloride after 22 h; at this time i.r. absorption clearly showed the presence of the oxazolone, and after the addition of glycine ethyl ester benzoyl-leucylglycine ethyl ester containing *ca.* 32% of racemate was isolated. The corresponding iodide caused only 5% racemisation under the same conditions; only a weak oxazolone carbonyl absorption developed, and the benzoyl-leucylglycine ethyl ester contained *ca.* 12% of racemate. The corresponding fluoroborate caused no fall in rotation and no oxazolone carbonyl absorption, and the coupling product cannot have contained more than 2% of racemate; even with 1·0M-fluoroborate no change in the optical rotation of the solution occurred within 20 h. It is clear that the racemisation caused by chloride is not due solely to the increase in ionic strength of the solution. On the other hand, we confirm that the addition of salt can accelerate racemisation by bases; the time for 50% racemisation of benzoyl-L-leucine *p*-nitrophenyl ester by *N*-methylmorpholine in acetonitrile was reduced from 10·2 to 5·3 h by the addition of 0·25M-benzyltrimethylammonium fluoroborate. We conclude that the 'chloride effect' is due to both the basicity of the anion and to the increase in the ionic strength of the solution. A tertiary amine hydrochloride should of course be less effective than a quaternary ammonium chloride; accordingly 0·05M-triethylamine hydrochloride in acetonitrile caused 9% racemisation of benzoyl-L-leucine *p*-nitrophenyl ester in 22 h; weak oxazolone carbonyl absorption developed.

We record here our confirmation, briefly mentioned earlier, of the previously reported value for the optical rotation of pure benzoyl-L-leucylglycine ethyl ester, for which a lower value had been reported.[16] We draw attention in the Experimental section to a numerical error in the description of our modified procedure for the saponification of benzoyl-leucylglycine ethyl ester.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus; optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter (1 dm cell). Evaporations were performed with a rotary evaporator and solutions in organic solvents were dried over magnesium sulphate. Tetrahydrofuran was dried over sodium, passed down an alumina column, and distilled in nitrogen. Dioxan was heated under reflux with concentrated hydrochloric acid and then with solid potassium hydroxide, dried over sodium, and distilled in nitrogen. Acetonitrile and nitromethane were dried over phosphoric oxide and distilled. Dimethyl sulphoxide was dried over calcium hydride and fractionally distilled; the fraction of b.p. 40—45° at 0·2 mmHg was retained. Dimethylformamide was dried over anhydrous potassium carbonate, distilled at reduced pressure, and then treated with benzyloxycarbonylglycine *p*-nitrophenyl ester for 10 days; it was then fractionally distilled and the middle fraction of b.p. 24° at 0·2 mmHg was retained. Chloroform and dichloromethane were washed repeatedly with distilled water and dried over calcium chloride; to 500 ml of solvent were then added benzyloxycarbonylglycine *p*-nitrophenyl ester (10 g) and triethylamine (10 g), and after 2 days the solution was washed with 2N-hydrochloric acid, saturated sodium carbonate, and brine. After drying over calcium chloride, the solvent was filtered and distilled; the middle fraction was retained. Triethylamine, ethyldi-isopropylamine, and *N*-methylmorpholine were dried over sodium and distilled; di-isopropylamine and dicyclohexylamine were dried over solid potassium hydroxide and distilled (the latter had b.p. 55—60° at 0·1 mmHg). Tribenzylamine was recrystallised from light petroleum (b.p. 40—60°). *p*-Nitrophenol and 2,4-dinitrophenol were recrystallised from 0·005N-hydrochloric acid. Glycine ethyl ester was redistilled immediately before use.

Measurement of Rates of Racemisation: General.—Solutions in the 1 dm cell were jacketed at 20 ± 0·2° and the optical rotation at 589 nm (exceptionally at 365 nm) was continuously recorded with a Perkin-Elmer 141 automatic polarimeter. The plot of $\log_{10}\alpha_t$ against t, where α_t = the observed rotation at time t, was linear in each case, and the pseudo-first-order rate constant k was calculated from the slope, by use of the equation $kt = \log_e\alpha_0 - \log_e\alpha_t$, where α_0 = the initial rotation and $t_{\frac{1}{2}} = \log_e 2/k$.

(a) *Benzyloxycarbonyl-L-phenylalanine p-nitrophenyl ester.*[21] The ester, prepared by the method using dicyclohexylcarbodi-imide, had m.p. 125—126°, $[\alpha]_D^{20}$ −7·9° (*c* 2 in

[19] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1964, 3701.
[20] D. S. Kemp and S. W. Chien, *J. Amer. Chem. Soc.*, 1967, **89**, 2745.
[21] M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.

$CHCl_3$), $[\alpha]_D^{20}$ −14·2° (*c* 2·0 in EtOAc) (Found: C, 65·5; H, 4·9; N, 6·6. Calc. for $C_{23}H_{20}N_2O_6$: C, 65·7; H, 4·8; N, 6·7%). In each case, the product was recovered from the bulk of the solution (kept at 20 ± 0·2°) by washing (2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, brine), drying, and evaporation; water-miscible solvents were first evaporated off and then the residue was taken up in ethyl acetate for washing as before. The identity of the product was determined by elemental analysis and n.m.r. spectroscopy. The product from the action of triethylamine on this ester in dichloromethane not specially purified (see before) was shown by n.m.r. spectroscopy to contain much of the corresponding methyl ester [τ 6·3 (s, CO_2Me)]. In every other case, excepting those when tribenzylamine (which was not removed by the washing procedure) was used but including that when purified dichloromethane was used, the product was shown to be benzyloxycarbonylphenylalanine *p*-nitrophenyl ester of optical rotation consistent with the kinetic results. The product from the reaction with dicyclohexylamine in acetonitrile for 18 h (86% recovery) had $[\alpha]_D^{20}$ −5·7° (*c* 3·1 in EtOAc) and analytical figures C, 64·9; H, 4·9; N, 6·4% (benzyloxycarbonylphenylalanine *NN*-dicyclohexylamide requires C, 75·3; H, 8·3; N, 6·1%). The n.m.r. spectrum ($CDCl_3$) was as expected except for a weak absorbtion at τ 8·4—9·0 (C_6H_{11}), corresponding to the presence of <10% of the amide. The same amine in dimethyl sulphoxide gave (after 18 h) product (86% recovery) of $[\alpha]_D^{20}$ −0·8° (*c* 2·9 in EtOAc) (Found: C, 65·8; H, 5·0; N, 6·4%). N.m.r. absorbtion at τ 8—9 indicated <15% of amide. The product from the reaction with di-isopropylamine in acetonitrile for 20 h (79% recovery) had $[\alpha]_D^{20}$ −6·6° (*c* 3·2 in EtOAc) (Found: C, 66·7; H, 5·3; N, 6·6%. Benzyloxycarbonylphenylalanine *NN*-di-isopropylamide requires C, 72·3; H, 7·9; N, 7·3%). The n.m.r. spectrum ($CDCl_3$) was as expected except for a weak absorbtion at τ 8—9 (Pr^i) corresponding to the presence of <15% of the amide. The same amine in dimethyl sulphoxide gave (after 3 h; 82% recovery) product of $[\alpha]_D^{20}$ −3·2° (*c* 3·0 in EtOAc) (Found: C, 65·4; H, 5·1; N, 6·9%). The n.m.r. absorbtion at τ 8—9 indicated <10% of the amide.

(b) *Benzyloxycarbonylglycyl-L-phenylalanine* p-*nitrophenyl ester*.[7] The ester had $[\alpha]_D^{20}$ −7·4° (*c* 1 in $CHCl_3$). In order to confirm the low rate of racemisation by triethylamine in dioxan, the product was recovered after 5 h, giving (in duplicate experiments) ester of $[\alpha]_D^{20}$ −6·8° (*c* 1 in $CHCl_3$) (59% recovery) and of $[\alpha]_D^{20}$ −7·6° (67% recovery).

(c) *Benzoyl-L-leucine* p-*nitrophenyl ester*.[22] The ester had m.p. 97—98°, $[\alpha]_D^{20}$ −43° (*c* 3·4 in EtOH). The rates of racemisation of this ester by amines are given in Table 2; the results obtained with quaternary ammonium salts are described here. Solutions were 0·10M with respect to the ester and 0·05M with respect to the salt; observations were made over 22 h. The identity of the product was established by the addition of 2 molar proportions of glycine ethyl ester to the final solution (after 22 h at 20°); benzoylleucylglycine ethyl ester was isolated as usual. 0·16M-Benzoyl-DL-leucine *p*-nitrophenyl ester in chloroform containing one molar proportion of tribenzylamine showed no i.r. absorption at 1830 cm^{-1} within 3 days at room temperature, peaks remaining unchanged at 1670 and 1770 cm^{-1}.

(i) *Benzyltrimethylammonium chloride:* $t_{\frac{1}{2}}$ 36 h. After 22 h the observed rotation had fallen to 67% of the initial value and i.r. absorption at 1830 cm^{-1} indicated the presence of an oxazolone; the isolated benzoyl-leucylglycine ethyl ester (91% yield) had $[\alpha]_D^{20}$ −22·9° (*c* 3·0 in EtOH) (Found: C, 63·4; H, 7·3; N, 9·0%).

(ii) *Benzyltrimethylammonium iodide*. After 22 h, the observed rotation was 95% of the initial value, and very weak i.r. absorption was observed at 1830 cm^{-1}; the benzoyl-leucylglycine ethyl ester (84% yield) had $[\alpha]_D^{20}$ −29·8° (*c* 2·5 in EtOH) (Found: C, 63·3; H, 7·5; N, 8·6%).

(iii) *Benzyltrimethylammonium fluoroborate*. After 22 h the observed rotation was unchanged; no i.r. absorption at 1830 cm^{-1} was detectable; the isolated benzoyl-leucylglycine ethyl ester (89% yield) had $[\alpha]_D^{20}$ −33·4° (*c* 2·9 in EtOH) (Found: C, 63·9; H, 7·6; N, 8·5%). When the concentration of fluoroborate was increased to 1·0M, no change in rotation occurred within 20 h.

(iv) 0·05M-*Triethylamine hydrochloride*. After 22 h the observed rotation was 91% of the initial value; weak absorption at 1830 cm^{-1} was observed.

(v) *Effect of added salt on the racemisation of benzoyl-L-leucine* p-*nitrophenyl ester by* N-*methylmorpholine in acetonitrile*. The solutions were 0·1M with respect to benzoyl-L-leucine *p*-nitrophenyl ester and *N*-methylmorpholine; the experiment recorded in Table 2 gave $t_{\frac{1}{2}}$ 10·2 h (without the addition of salt). With added benzyltrimethylammonium fluoroborate, the $t_{\frac{1}{2}}$ values were: 0·05M fluoroborate, 7·3 h; 0·10M, 6·4 h; 0·25M, 5·3 h; 0·50M, 4·3 h.

(vi) *Benzoyl-L-leucine* p-*chlorophenyl*, 2,4-*dichlorophenyl*, m-*nitrophenyl, and* 2,4,5-*trichlorophenyl esters*. The esters are described later. The results are shown in Table 4. Solutions used for i.r. observations were 0·17M with respect to ester and amine.

(vii) *Experiments with Amberlite* IR-45. The resin (free base) was washed thoroughly with dimethyl sulphoxide and then dried; titration indicated 3·0 mequiv. per g of dried resin. The results are shown in Table 2.

The Rate of Condensation of Benzyloxycarbonyl-L-phenylalanine p-*Nitrophenyl Ester with Glycine Ethyl Ester in Various Solvents*.—Solutions, equimolar with respect to each of the reactants, were made in the specified solvents and maintained at 25 ± 0·2°; the initial concentrations, *a*, given for each reaction, were such as to provide a suitable rate. At intervals samples were withdrawn and (except when otherwise stated) diluted $a \times 10^4$-fold with ethyl acetate, and the absorbance *A* at 320 nm was measured with a Unicam SP 500 spectrophotometer; the same concentration of the solvent in ethyl acetate was used as a blank. The *p*-nitrophenyl ester and *p*-nitrophenol in ethyl acetate have extinction coefficients at 320 nm of 530 and 8850, respectively, and hence the extent of reaction at time $t = x/a = A - 0{\cdot}053/0{\cdot}885 - 0{\cdot}053$, where *a* = initial concentration in mol l^{-1} and *x* = concentration of the products in mol l^{-1} after time *t*. When the dilution conditions were varied these coefficients were redetermined for each case (see later). A plot of $1/(a - x)$ against *t* was linear in each case, and the second-order rate constant *k* was calculated from the slope in the usual way. The initial concentrations of each reactant were: for dioxan and nitromethane, 0·10M; ethyl acetate and acetonitrile, 0·05M; tetrahydrofuran, 0·01M; chloroform and dichloromethane, 0·20M; dimethylformamide, 0·002M; dimethyl sulphoxide, 0·001M. For the solvent dimethylformamide, 0·50 ml of the reaction solution was diluted with 9·5 ml of ethyl acetate; the extinction coefficients at 320 nm of the *p*-nitrophenyl ester and of *p*-nitrophenol in 1 : 19 dimethylformamide–ethyl

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

acetate were 530 and 10,600, respectively, and these values were substituted into the equation given for determining x/a. A control experiment with the purified solvent showed that a 0·002M-solution of the *p*-nitrophenyl ester gave <1% liberation of *p*-nitrophenol during 1 h at 25°. For dimethyl sulphoxide, 1·0 ml of the reaction solution was diluted with 9·0 ml of ethyl acetate; the extinction coefficients at 320 nm of the *p*-nitrophenyl ester and of *p*-nitrophenol in 1 : 9 dimethyl sulphoxide–ethyl acetate were 530 and 11,700, respectively, and these values were substituted into the equation given for determining x/a. A control experiment with the purified solvent showed that a 0·001M-solution of the *p*-nitrophenyl ester gave <1% liberation of *p*-nitrophenol during 1 h at 25°. The rate constants are given in Table 1, column C.

Measurement of the Base Strengths of Some Amines in Organic Solvents.—The procedure is that of Pearson and Vogelsong.[14] To 0·0005M-2,4-dinitrophenol (2 ml) in the required solvent were added varying volumes of amine (usually 0·0020M; see later) in the same solvent and the solution was made up to 10 ml. The absorbance A at 400 nm was then measured. If B is the concentration (mol l^{-1}) of amine, C that of the phenol, E the extinction coefficient of the amine–phenol ion-pair, and $K = [\overset{+}{N}HR_3,\bar{O}{\cdot}C_6H_3(NO_2)_2]/[NR_3][HO{\cdot}C_6H_3(NO_2)_2]$, then $1/A = 1/KEC \,.\, 1/B + 1/EC$. The plot of $1/A$ against $1/B$ was linear in each case. The intercept gives $1/EC$ and the slope gives $1/KEC$; hence E and K are calculated. For triethylamine, ethyldi-isopropylamine, and dicyclohexylamine, the range covered 4—15 equiv. of 0·0020M-amine. For tribenzylamine, 0·25M-amine was used for all solvents, with a range of 250—2000 equiv. of added amine. Values of E were in the range 9000—12,000. When $\log_{10}K > 4{\cdot}2$, 10^{-4}M-dinitrophenol gives over 90% ion-pair formation with only 4 equiv. of amine, and accurate values of K cannot be obtained. For tribenzylamine in dioxan, ethyl acetate, tetrahydrofuran, and chloroform 2000 equiv. of amine gave <10% ion-pair formation and accurate values of E could not be obtained; on the assumption of E 10,000, $\log_{10}K$ would be <0 in these cases. For glycine ethyl ester in chloroform and in dichloromethane the same value of E has been assumed. The results (Table 3) are the means of duplicate experiments.

Substituted Phenyl Esters of Benzyloxycarbonyl-L-leucine. A solution containing equimolar amounts of benzyloxycarbonyl-L-leucine and triethylamine in chloroform at 0° was added dropwise during 4 min to a solution of ethyl chloroformate (1 molar proportion) in chloroform at 0°. After 10 min, equivalent amounts of the phenol and of triethylamine were added. After 2 h at room temperature the solvent was evaporated off; the residue was taken up in ethyl acetate and washed (water, saturated sodium carbonate, 2N-hydrochloric acid, brine), dried, and concentrated. The addition of light petroleum caused crystallisation, and the product was recrystallised from ethyl acetate–light petroleum. The following are new (yields are of recrystallised product with the stated constants and analysis). *Benzyloxycarbonyl-L-leucine* p-*chlorophenyl ester* (38%) had m.p. 80—81°, $[\alpha]_D^{20}$ −19° (*c* 1 in $CHCl_3$) (Found: C, 63·7; H, 6·1; Cl, 9·7; N, 3·8. $C_{20}H_{22}ClNO_4$ requires C, 63·9; H, 5·9; Cl, 9·4; N, 3·7%). *Benzyloxycarbonyl-L-leucine* m-*nitrophenyl ester* (44%) had m.p. 69—71°, $[\alpha]_D^{20}$ −24° (*c* 1 in $CHCl_3$) (Found: C, 62·1; H, 5·8; N, 6·9. $C_{20}H_{22}N_2O_6$ requires C, 62·2; H, 5·7; N, 7·3%). *Benzyloxycarbonyl-L-leucine* 2,4,5-*trichlorophenyl ester* (42%) had m.p. 62—64°, $[\alpha]_D^{20}$ −19° (*c* 1 in $CHCl_3$) (Found: C, 54·0; H, 4·4; Cl, 24·1; N, 3·3. $C_{20}H_{20}Cl_3NO_4$ requires C, 54·0; H, 4·5; Cl, 23·9; N, 3·2%).

Hydrobromides of Substituted Phenyl Esters of L-Leucine.—The benzyloxycarbonyl derivatives were dissolved in hydrogen bromide in acetic acid (45% w/v; 6 molar proportions of hydrogen bromide). Crystalline product separated rapidly; after 1 h dry ether was added, the mixture was cooled to 0°, and the product was collected and washed with dry ether. L-*Leucine* p-*chlorophenyl ester hydrobromide* (74%) had m.p. 202—205°, $[\alpha]_D^{20}$ −6° (*c* 1 in $CHCl_3$) (Found: C, 44·4; H, 5·2; N, 3·7; total halogen, calc. as Cl, 21·7. $C_{12}H_{17}BrClNO_2$ requires C, 44·7; H, 5·3; 'Cl', 22·0; N, 4·3%). L-*Leucine* 2,4-*dichlorophenyl ester hydrobromide* (91%) had m.p. 176—178°, $[\alpha]_D^{20}$ −20° (*c* 1 in $CHCl_3$) (Found: C, 40·6; H, 4·7; Br, 22·1; Cl, 19·8; N, 3·8. $C_{12}H_{16}BrCl_2NO_2$ requires C, 40·4; H, 4·5; Br, 22·4; Cl, 19·9; N, 3·9%). L-*Leucine* m-*nitrophenyl ester hydrobromide* (89%) had m.p. 193—196°, $[\alpha]_D^{20}$ +16° (*c* 1 in EtOH) (Found: C, 43·3; H, 5·3; Br, 23·7; N, 8·5. $C_{12}H_{17}BrN_2O_4$ requires C, 43·3; H, 5·1; Br, 24·0; N, 8·4%). L-*Leucine* 2,4,5-*trichlorophenyl ester hydrobromide* (80%) had m.p. 160—165°, $[\alpha]_D^{20}$ −5° (*c* 1 in $CHCl_3$) (Found: C, 37·0; H, 3·6; Br, 20·8; Cl, 27·1; N, 3·6. $C_{12}H_{15}BrCl_3NO_2$ requires C, 36·8; H, 3·9; Br, 20·4; Cl, 27·2; N, 3·6%).

Substituted Phenyl Esters of Benzoyl-L-leucine.—*N*-Methylmorpholine (2 molar proportions) in dioxan was added in portions during 30 min to a vigorously stirred suspension of equimolar proportions of benzoyl chloride and the L-leucine phenyl ester hydrobromide in dioxan. After a further 1 h, the mixture was filtered, the filtrate was evaporated, and the residue was taken up in ethyl acetate and washed (water, saturated sodium carbonate, 2N-hydrochloric acid, brine) and dried. The solution was concentrated; addition of light petroleum caused crystallisation. *Benzoyl-L-leucine* p-*chlorophenyl ester* (58%) had m.p. 115—117°, $[\alpha]_D^{20}$ −10·5° (*c* 1 in $CHCl_3$) (Found: C, 65·8; H, 6·2; Cl, 10·2; N, 4·2. $C_{19}H_{20}ClNO_3$ requires C, 66·0; H, 5·8; Cl, 10·3; N, 4·1%). *Benzoyl-L-leucine* 2,4-*dichlorophenyl ester* (64%) had m.p. 129—130°, $[\alpha]_D^{20}$ −40° (*c* 1 in $CHCl_3$) (Found: C, 59·9; H, 4·7; Cl, 18·7; N, 3·8. $C_{19}H_{19}Cl_2NO_3$ requires C, 60·0; H, 5·0; Cl, 18·7; N, 3·7%). *Benzoyl-L-leucine* m-*nitrophenyl ester* (63%) had m.p. 120—122°, $[\alpha]_D^{20}$ −29° (*c* 1 in $CHCl_3$) (Found: C, 63·9; H, 5·7; N, 8·0. $C_{19}H_{20}N_2O_5$ requires C, 64·0; H, 5·7; N, 7·9%). *Benzoyl-L-leucine* 2,4,5-*trichlorophenyl ester* (65%) had m.p. 85—86°, $[\alpha]_D^{20}$ −22° (*c* 1 in $CHCl_3$) (Found: C, 55·1; H, 4·6; Cl, 25·4; N, 3·5. $C_{19}H_{18}Cl_3NO_3$ requires C, 55·0; H, 4·4; Cl, 25·7; N, 3·4%).

The rates of racemisation of these esters by amines were determined by the general procedure already described and the results are shown in Table 4. Solutions for i.r. observations were 0·17M with respect to ester and amine.

Benzyltrimethylammonium Salts.—Benzyltrimethylammonium chloride was dried by azeotropic distillation with toluene and then recrystallised from chloroform–ether; this process was repeated (Found: C, 65·0; H, 8·7; Cl, 18·8; N, 7·2. Calc. for $C_{10}H_{16}ClN$: C, 64·7; H, 8·7; Cl, 19·1; N, 7·5%). The iodide was dried similarly and recrystallised from acetonitrile–ether (Found: C, 42·7; H, 5·7; N, 5·3. Calc. for $C_{10}H_{16}IN$: C, 43·3; H, 5·8; N, 5·1%). The fluoroborate was prepared analogously to the iodide (Found: C, 50·3; H, 6·8; N, 6·5. Calc. for $C_{10}H_{16}BF_4N$: C, 50·7; H, 6·8; N, 5·9%).

Relative Base Strengths of Benzyltrimethylammonium

Chloride, Iodide, and Fluoroborate in Some Organic Solvents.—The method is that described for amines. Varying volumes of 0·50M-benzyltrimethylammonium chloride were added to 0·0005M-2,4-dinitrophenol (2 ml), and the solution was made up to 10 ml; for dichloromethane and acetonitrile solubility limited the concentration of salts to 0·125 and 0·05M, respectively. The weakness of the bases made inaccurate the evaluation of E from the graph of A^{-1} against B^{-1}; on the assumption $E = 10{,}000$, the chloride gave $\log_{10}K$ *ca.* 0·1 in chloroform, *ca.* 0·3 in dichloromethane, and *ca.* 0·3 in acetonitrile. The addition of 1 molar proportion of water to the chloride solution in chloroform raised $\log_{10}K$ to *ca.* 0·5. The relative base strengths of the chloride, iodide, and fluoroborate in acetonitrile were estimated by comparing the absorbance (A) at 400 nm for varying concentrations (B) of salts; each solution was 0·0010M with respect to 2,4-dinitrophenol (Table 5). It is

TABLE 5

B	$A(Cl^-)$	$A(I^-)$	$A(BF_4^-)$	$A(Cl^- - I^-)$
0·000M	0·085	0·085	0·085	0·000
0·020M	0·155	0·112	0·078	+0·014
0·025M	0·166	0·116	0·082	+0·025
0·040M	0·182	0·145	0·091	+0·036

seen that the addition of fluoroborate scarcely changes the absorbance. Even with 0·200M-fluoroborate and 0·0001M-dinitrophenol, the absorbance was only 0·037. The last column shows the difference absorbance observed when the iodide solution was placed in the 'solvent' path and the chloride solution in the 'solution' path; the latter is clearly more basic.

Benzoyl-L-leucylglycine Ethyl Ester.—Benzoyloxycarbonyl-L-leucylglycine ethyl ester (7·85 g, 22·5 mmol) was dissolved in a solution of hydrogen bromide in acetic acid (25% w/w; 44 g, 135 mmol). After 1 h, dried ether was added and the precipitate was washed with ether. It was then dissolved in water (20 ml) and sodium hydrogen carbonate (10 g, 120 mmol) and ether (50 ml) were added. Benzoyl chloride (3·16 g, 22·5 mmol) in ethyl acetate (40 ml) was added in portions to the stirred solution. After 30 min, pyridine (5 ml) and water (30 ml) were added. The organic layer was washed (2N-hydrochloric acid and saturated aqueous sodium hydrogen carbonate) and dried. Evaporation left crystalline ester (6·1 g, 85%). Recrystallisation from ethyl acetate–light petroleum gave ester of m.p. 155—156°, $[\alpha]_D^{20}$ −33·9° (*c* 3·0 in EtOH) (Found: C, 63·6; H, 7·7; N, 8·7. Calc. for $C_{17}H_{24}N_2O_4$: C, 63·7; H, 7·6; N, 8·8%). Williams and Young [22] give m.p. 156·5—157°, $[\alpha]_D^{20}$ −34·0° (*c* 3·1 in EtOH); Anderson, Zimmerman, and Callahan [16] found $[\alpha]_D^{25}$ −32·5 ± 0·5° (*c* 3 in EtOH). The specific rotation of our product varied with concentration (*c*) in ethanol as follows: *c* 0·5, $[\alpha]_D^{20}$ −33·2°; 1·0, −33·4°; 2·0, −33·7°; 2·5, −33·9°; 3·5, −34·0°; 4·0, −34·2°; 5·0, −34·3°; 6·0, −34·6°; 8·0, −35·1°; 10·0, −35·5°.

We draw attention here to an error in the experimental description in Part XXVII [23] of the modified procedure for the saponification of benzoyl-leucylglycine ethyl ester; the proportion of dioxan to N-sodium hydroxide should read 1 : 3, not 3 : 1.

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[23] J. H. Jones and G. T. Young, *J. Chem. Soc.* (*C*), 1968, 52.