813. *The Kinetics and Mechanism of the Removal of the N-Benzyloxycarbonyl Group from N-Benzyloxycarbonylglycine Ethyl Ester and Related Compounds in Acetic Acid containing Hydrobromic Acid or Sulphuric Acid*

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The kinetics of the removal of the *N*-benzyloxycarbonyl group from *N*-benzyloxycarbonylglycine ethyl ester and related compounds by means of sulphuric acid or hydrobromic acid in anhydrous acetic acid has been investigated. Acidity-dependence, salt effects, activation parameters, and substituent effects show that two mechanisms, labelled *A*-1 and *A*-2, may be operative, depending on the reaction conditions, and that they both involve rate-determining decomposition of the ion-pair between the protonated substrate and the anion of the added acid. Some concurrent ester fission, which was also observed, is discussed. Suitable conditions for the removal of the *N*-benzyloxycarbonyl group in preparative work are suggested.

Treatment with solutions of hydrogen bromide in acetic acid is widely used [1] for the removal of the *N*-benzyloxycarbonyl group in peptide syntheses. The mechanism of this important reaction has been discused,[2,3] and a kinetic investigation of substituent effects in this reaction has been reported.[4] There is good evidence [2,5] that the stoicheiometry of the reaction is given by equation (1).

$$R{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CHR'CO_2Et + HBr \longrightarrow R{\cdot}CH_2Br + CO_2 + NH_2{\cdot}CHR'{\cdot}CO_2Et \quad (1)$$

We have studied acidity-dependence, salt effects, substituent effects, and activation parameters in the kinetics of the removal of the benzyloxycarbonyl group from benzyloxycarbonylglycine ethyl ester, using both hydrobromic acid and sulphuric acid as catalysing acids.

Experimental

Materials.—The preparations of anhydrous acetic, sulphuric, and hydrobromic acids and of the tetraethylammonium salts were described previously.[6] 1,3,5-Trimethoxybenzene,[7] benzyloxycarbonylglycine ethyl ester,[8] benzyloxycarbonyl-DL-alanine,[9] and benzyloxycarbonyl-DL-phenylalanine [9] were prepared by standard methods. We are indebted to Dr. B. Ridge for a sample of benzyloxycarbonylglycylglycine, to Dr. F. Serrao for a sample of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine, and to Mr. D. Marlborough for a sample of benzyloxycarbonyl-L-glutamic acid.

p-*Nitrobenzyloxycarbonylglycine ethyl ester* was prepared in a manner identical to that used for preparing the benzyloxycarbonyl derivative [8] and, after recrystallisation from ethanol, had m. p. 107° (Found: C, 51·7; H, 5·25; N, 9·75. $C_{12}H_{14}N_2O_6$ requires C, 51·05; H, 5·0; N, 9·9%).

Kinetic Procedure.—A stock solution of 0·0125M-substrate in anhydrous acetic acid was prepared. A sample (2 ml.) was transferred to one limb of the U-shaped reaction vessel, and the appropriate acetic acid solution (20 ml.) placed in the other limb. After 15 minutes' thermal equilibration at the run temperature, the vessel was inverted and shaken. Samples (2 ml.) were removed at appropriate intervals and quenched by running into the correct amount of aqueous sodium hydroxide to neutralise the sulphuric acid or hydrobromic acid and half the

[1] G. W. Anderson, J. Blodinger, and A. D. Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5309.
[2] D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564; 1954, **19**, 62.
[3] N. F. Albertson and F. C. Mackay, *J. Amer. Chem. Soc.*, 1953, **75**, 5323.
[4] K. Blaha and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1965, **30**, 583.
[5] A. Patchornik and S. Erlich-Ragozinzki, *Analyt. Chem.*, 1961, **33**, 803.
[6] R. B. Homer and R. B. Moodie, preceding Paper.
[7] E. Freudenburg, *Ber.*, 1920, **53**, 1425.
[8] S. Simmonds and J. S. Fruton, *J. Biol. Chem.*, 1951, **188**, 251.
[9] M. Bergmann and L. Servas, *Ber.*, 1932, **65**, 1192.

acetic acid. The quenched sample was made up to 10 ml. For runs with half-lives less than 2 min., a sample of the appropriate acid solution (2 ml.) was transferred to a 10-ml. graduated flask, and after thermal equilibration the stock substrate solution (0·2 ml.) was added by means of a fast-flowing pipette. Immediately after the addition the flask was violently agitated. After the appropriate time the solution was quenched with the appropriate quantity of aqueous sodium hydroxide from a fast-flowing burette, and the quenched solution made up to the mark in the reaction vessel. Analysis of the quenched solution was by ninhydrin estimation of the free amino-group.[10] With careful attention to detail this method gave very reproducible results.

In all runs, good first-order plots were obtained up to at least 60% reaction. However, for runs with added sulphuric acid, the observed first-order rate constant decreased with increasing initial concentration of substrate. This effect was independent of acid concentration, as shown in Figure 1. A 44-fold increase in the initial substrate concentration decreased the rate constant by a factor of 1·7 (Table 1). The possibility of a small contribution from a path of

TABLE 1

Variation of the observed first-order rate constant, k, with substrate concentration in acetic acid. Substrate: *N*-benzyloxycarbonylglycine ethyl ester

[Substrate] (10^3M)	[H_2SO_4] (M)	[HBr] (M)	Temp.	10^5k (sec.$^{-1}$)
1·14	0·556		50°	4·07
49·8	0·556		50	2·38
1·14		0·308	20	23·9
11·4		0·308	20	22·9
1·14		4·98	20	525
11·4		4·98	20	524

order zero, in which the rate-determining step was formation of acetylium ion, suggested the addition of 1,3,5-trimethoxybenzene as a scavenger for these ions, but this had no effect on the rate constant. Similar unexplained departures from first-order kinetics have been observed by Gold and Riley [11] in the boron trifluoride-catalysed acetylation of anisole in acetic acid. Runs in HBr–AcOH were, however, accurately of the first order in substrate; a 10-fold variation in substrate concentration had no effect on the observed first-order rate constant (Table 1).

RESULTS AND DISCUSSION

Mechanism of the Reaction.—The results obtained are consistent with the following reaction scheme:

$$HA \underset{}{\overset{K_i^{HA}}{\rightleftharpoons}} H^+A^- \overset{K_d^{HA}}{\rightleftharpoons} H^+ + A^- \quad (2)$$

$$S + HA \overset{K_i^{SHA}}{\rightleftharpoons} SH^+A^- \overset{K_d^{SHA}}{\rightleftharpoons} SH^+ + A^- \quad (3)$$

$$SH^+A^- \xrightarrow{\text{slow}} \text{Products} \quad (4)$$

(HA is the catalysing acid (HBr or H_2SO_4) and S is the substrate.) It is further shown that two different transition states can be involved in step 4, depending on the nature of A^- and the reaction conditions. The nature of these transition states will be discussed.

Evidence that the ion-pair is the species undergoing further reaction. The mechanisms of ionic reactions in acetic acid are frequently discussed without explicit consideration of ion-association.[12] For brevity we adopted this course in a preliminary communication.[13] A more detailed understanding may be achieved if account is taken of ion-pairing, the importance of which in this medium has been amply demonstrated.[14]

Of the three substrate species present in equilibrium in the solution (neutral substrate, S, protonated substrate, SH^+, and protonated substrate ion-pair, SH^+A^-), further reaction

[10] H. Rosen, *Arch. Biochem. Biophys.*, 1957, **67**, 10.
[11] V. Gold and T. Riley, *J.*, 1961, 1676.
[12] D. Bethell and V. Gold, *J.*, 1958, 1905; R. F. Cieciuch and F. H. Westheimer, *J. Amer. Chem. Soc.*, 1963, **85**, 2591.
[13] R. B. Homer, R. B. Moodie, and H. N. Rydon, *Proc. Chem. Soc.*, 1963, 367.
[14] See, *e.g.*, (*a*) S. Winstein, P. E. Klinedinst, and E. Clippinger, *J. Amer. Chem. Soc.*, 1961, **83**, 4896; (*b*) S. Bruckenstein, *ibid.*, 1960, **82**, 307; (*c*) D. P. N. Satchell, *J.*, 1958, 1916.

through the neutral substrate can be ruled out because no reaction was observed in the absence of added acid, even in the presence of bromide ion. The known low basicity of the substrate,[15] the low value of ion-pair dissociation constants in this medium,[16] and the relatively high buffering concentration of the catalysing acid, shows that the fraction protonated is always small, and suggests that the dominant protonated species is the ion-pair rather than the free ion. Thus, even at the lowest concentration of the weaker of the two acids used, *viz.*, sulphuric acid, using the known overall dissociation constant[17] and assuming a value of 10^{-5} for K_d^{SHA} (a reasonable upper limit[16,18]) we can show that $[SH^+A^-]/[SH^+] \sim 5$; this value will rise with increasing acid concentration.

Taking activity coefficients as unity, and writing $M = [HA] + [H^+A^-]$ for the stoicheiometric concentration of acid, we can show that the proportion of substrate present as SH^+ and SH^+A^- are given, respectively, by equations (5) and (6):

$$[SH^+]/[S] = K' M^{\frac{1}{2}} \quad (5)$$

$$[SH^+A^-]/[S] = K'' M \quad (6)$$

K' and K'' are composites of the various equilibrium constants in equations (2) and (3). Now it is shown that even at the lowest acid concentrations used, $d(\log k)/d(\log M)$ is never less than unity. Extravagant changes in activity coefficients would therefore need to be invoked if further reaction were considered to occur through the protonated-substrate free ion. Thus, further reaction through the ion-pair, SH^+A^-, is indicated by its predominance and demonstrated by the dependence of rate on acidity.

Structure of the Transition States.—The difference in acidity dependence (Figure 1), substituent effects (Tables 2 and 5), salt effects (Table 3), and activation parameters (Table 4) between the reaction in H_2SO_4–AcOH and in HBr–AcOH shows that different transition states for reaction (4) in the two media must be formulated.

Effect of a p-*nitro-substituent.* The introduction of a *p*-nitro-group into the substrate

TABLE 2

Comparison of the observed first-order rate constants, k, for benzyloxycarbonylglycine ethyl ester (I) and *p*-nitrobenzyloxycarbonylglycine ethyl ester (II). Substrate concentration $1{\cdot}14 \times 10^{-3}$M in acetic acid

				10^5k (sec.$^{-1}$)	
$[H_2SO_4]$ (M)	[HBr] (M)	$[Et_4N^+Br^-]$ (M)	Temp.	Substrate (I)	Substrate (II)
0·864			50°	10·3	0·01
4·52			50	341·0	0·141
0·455		0·091	50	40·0	2·0
	0·0308		50	14·7	1·02
	0·0770		50	43·7	3·60
	0·154		50	103·4	8·58
	0·154		20	7·81	0·59
	0·770		20	60·3	3·63
	5·25		20	541·0	28·0

TABLE 3

The effect of common-ion salts on the observed first-order rate constants, k, for the reaction of $1{\cdot}14 \times 10^{-3}$M-benzyloxycarbonylglycine ethyl ester, and on the ionisation ratio, I, of *N*-methyl-2-naphthamide. Temp. 50°

$[H_2SO_4]$ (M)	[HBr] (M)	$[Et_4N^+HSO_4^-]$ (M)	$[Et_4N^+Br^-]$ (M)	log I	10^5k (sec.$^{-1}$)
0·455				1·03	2·46
0·455		0·091		1·10	2·25
	0·077			0·96	43·7
	0·077		0·0455	1·04	47·6

[15] R. B. Homer, R. B. Moodie, and H. N. Rydon, unpublished work.
[16] I. M. Kolthoff and S. Bruckenstein, *J. Amer. Chem. Soc.*, 1956, **78**, 1.
[17] I. M. Kolthoff and S. Bruckenstein, *J. Amer. Chem. Soc.*, 1956, **78**, 2974.
[18] J. T. Denison and J. B. Ramsey, *J. Amer. Chem. Soc.*, 1955, **77**, 2615.

produces a *ca.* 2400-fold decrease in the rate constant for runs in H_2SO_4–AcOH but only a *ca.* 13-fold decrease for runs in HBr–AcOH (Table 2). The substituent will reduce the basicity of the substrate, and hence the rate of reaction in both media. The difference in

TABLE 4

Energies and entropies of activation for the decomposition of benzyloxycarbonylglycine ethyl ester, calculated from observed first-order rate constants at three temperatures

$[H_2SO_4]$ (M)	[HBr] (M)	$[Et_4N^+Br^-]$ (M)	Temp. range	$\Delta H^{\ddagger}$ [a] (kcal. mole^{-1})	$\Delta S^{\ddagger}$ [b] (e.u.)
0·138			50—70°	24·7	−8
0·138		0·091	50—70	19·1	−17
	0·154		20—50	15·5	−23
	0·154 [c]		20—50 [c]	16·4 [c]	−26 [c]

[a] Estimated limits of error ±0·5 kcal. mole^{-1}. [b] Estimated limits of error ±2 e.u. [c] For *p*-nitrobenzyloxycarbonylglycine ethyl ester.

magnitude of the effect in the two media is in accord with other nucleophilic displacements in benzyl compounds [19] if in H_2SO_4–AcOH benzylcarbonium ion separation occurs in step (4) whilst in HBr–AcOH the more nucleophilic bromide ion takes part in an S_N2 displacement at the benzyl group. The two transition states are therefore formulated as:

$$SH^+HSO_4^- \xrightarrow[k_{1a}]{A\text{-}1} C_6H_5CH_2^{\delta+} \cdots O{\cdot}COH{\cdot}NH{\cdot}CH_2CO_2Et\ HSO_4^{\delta+} \qquad (4a)$$

$$SH^+Br^- \xrightarrow[k_{1b}]{A\text{-}2} Br^{\delta-} \cdots C_6H_5CH_2 \cdots O{\cdot}COH{\cdot}NH{\cdot}CH_2CO_2Et^{\delta+} \qquad (4b)$$

Step (4*b*) is described as *A*-2 since both ions are covalently involved in the transition state. (Since only one species, the ion-pair, undergoes further reaction, an alternative description, following Hyne,[20] would be S_Ni.)

Acidity-dependence of the reactions. Equations (2)—(4) lead to the following expression for the observed first-order rate constant, k, in terms of the stoicheiometric concentration of added acid, M.

$$k = \text{Rate}/[S] = k_4K_i^{SHA}[(K_i^{HA}\gamma_{HA}/\gamma_{H^+A}) + 1]^{-1}\gamma_S\gamma_{HA}\gamma_{\ddagger}^{-1}\,M$$

A more tractable expression is obtained if the extent of protonation of an indicator is used as a model for the pre-equilibrium protonation of the substrate. The choice of *N*-methyl-2-naphthamide as the indicator, and the measurement of its ionisation ratio, is discussed in the preceding Paper.[6]

$$B + HA \underset{}{\overset{K_iBHA}{\rightleftharpoons}} BH^+A^- \overset{K_dBHA}{\rightleftharpoons} BH^+ + A^-$$

$$I = [BH^+A^-]/[B] = K_i^{BHA}a_{HA}\gamma_B/\gamma_{BH^+A^-}$$

(The concentration of BH^+ is considered negligible compared with that of BH^+A^-.[14*b*])

$$k = k_4K_i^{SHA}a_{HA}\gamma_S/\gamma_{\ddagger} = \frac{k_4K_i^{SHA}}{K_i^{BHA}}\frac{\gamma_S\gamma_{BH^+A^-}}{\gamma_{\ddagger}\gamma_B}I \qquad (5)$$

B is the indicator with which the ionisation ratio, I, is determined.

A comparison of Figure 1 with Figure 2 shows that part, but not all, of the difference in rate constants for the reaction in the two media is due to the greater acidity of HBr–AcOH, as measured by log I. For reaction in HBr–AcOH, Figure 2 shows that d(log k)/d(log I) is 1·08. This is consistent with the postulated transition state (4*b*) if the

[19] A. Streitwieser, *Chem. Rev.*, 1956, **56**, 571.
[20] J. B. Hyne, *Canad. J. Chem.*, 1961, **39**, 1207.

TABLE 5

The observed first-order rate constants, k, for the removal of the benzyloxycarbonyl group from various substrates

Cpd. to which the *N*-benzyloxycarbonyl group is attached	0·52M-H_2SO_4 at 50° 10^5k (sec.$^{-1}$)	4·98M-HBr at 20° 10^5k (sec.$^{-1}$)	$pK_{NH_3^+}$
Glycine ethyl ester	3·14	5·25	7·75 [22]
Glycylglycine ethyl ester	3·07	5·22	7·75 [23]
DL-Alanine	3·26	7·64	7·80 [23]
DL-Phenylalanine	2·53	8·52	
L-Glutamic acid	2·43	8·75	7·04 [22]
S-Benzyl-L-cysteine	2·22	9·05	6·77 [24]

transition state still resembles the ion-pair, which in turn resembles the indicator–conjugate acid ion-pair, and the activity coefficient term in equation (5) is therefore approximately constant. Where H_2SO_4–AcOH is the medium, d(log k)/d(log I) is much larger, approaching a value of 1·8 at the higher acid concentrations. This is consistent with the transition-state formulation in equation (4*a*) if the benzylcarbonium ion separation in the transition state is near completion, in accord with Hammond's postulate.[21] The transfer of charge from the protonated carbamate group, where the positive charge is mainly located on oxygen and nitrogen atoms bearing hydrogen-bonding protons, to the benzyl group, where the charge is distributed in the ring, will be accompanied by a decrease in solvation, and therefore a decrease in $\gamma_{\ddagger}$ relative to $\gamma_{BH^+A^-}$. A similar loss of solvation in the transition state has been observed for this reaction in aqueous sulphuric acid.[15]

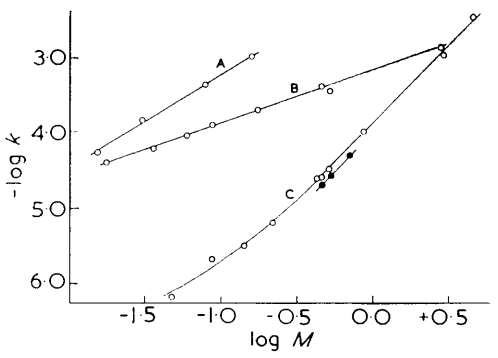

FIGURE 1

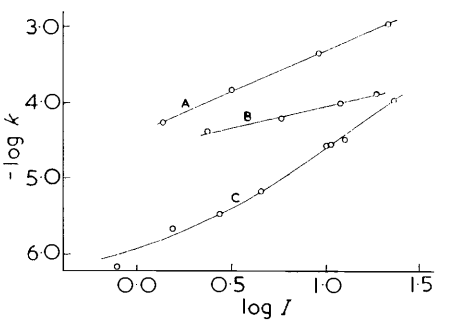

FIGURE 2

FIGURE 1. Variation of the rate constant with the stoicheiometric molar acid concentration in acetic acid at 50°. Substrate: benzyloxycarbonylglycine ethyl ester, $1·14 \times 10^{-3}$M (open circles) or $4·55 \times 10^{-3}$M (full circles)

(A) HBr–AcOH; (B) H_2SO_4–AcOH + 0·091M-$Et_4N^+Br^-$; (C) H_2SO_4–AcOH.

FIGURE 2. Variation of the rate constant with the ionisation ratio, I, of *N*-methyl-2-naphthamide in acetic acid at 50°. Substrate: $1·14 \times 10^{-3}$M-benzyloxycarbonylglycine ethyl ester

(A) HBr–AcOH; (B) H_2SO_4–AcOH + 0·091M-$Et_4N^+Br^-$; (C) H_2SO_4–AcOH.

Salt effects. Added salts can influence the reaction rate by substitution of different ions in the reactive ion-pair, by influencing the extent of protonation of the substrate, and by an ionic-atmosphere effect. The first effect can be avoided by the use of salts of the anion of the catalysing acid, and the second can be allowed for by the supposedly parallel effect on the degree of indicator protonation. The third effect, thus isolated, is shown to be negligible in the case of HBr–AcOH. Thus, the increase in rate when tetraethylammonium bromide is added to HBr–AcOH is accounted for exactly by the increase

[21] G. S. Hammond, *J. Amer. Chem. Soc.*, 1955, **77**, 334.
[22] A. Neuberger, *Biochem. J.*, 1936, **30**, 2085.
[23] J. T. Edsall and M. H. Blanchard, *J. Amer. Chem. Soc.*, 1933, **55**, 2337.
[24] R. E. Benesch and R. Benesch, *J. Amer. Chem. Soc.*, 1955, **77**, 5877.

in the acidity of the medium, as measured by log I. This observation also rules out the possibility of a rate-determining step involving attack by Br^- on SH^+Br^-. Addition of tetraethylammonium hydrogen sulphate to H_2SO_4–AcOH results in an increase in log I and a decrease in k, but both effects are small (Table 3).

The addition of tetraethylammonium bromide to runs in low concentrations of sulphuric acid in acetic acid causes an increase in rate which is too great to be attributed solely to an increase in acidity, as the values of log I show (Figure 2, central line). Substitution of bromide ion for the hydrogen sulphate ion in the reacting ion-pair, which then reacts by the easier A-2 mechanism, accounts well for the observed effects.

$$SH^+HSO_4^- + Br^- = SH^+Br^- + HSO_4^- \quad (6)$$

Increasing the sulphuric acid concentration displaces the equilibrium in equation (6) from right to left and takes the rate constant back from that for a pure A-2 reaction (upper curve) to that for a pure A-1 reaction (lower curve).

Activation parameters. The figures given in Table 4 are derived from the temperature variation of the observed first-order rate constant, and are therefore composite, reflecting the temperature effect on both the pre-equilibria and the rate-determining step. If it is assumed that the thermodynamic parameters for the pre-equilibria for the two acids are similar, then the relative values reflect the difference in the activation parameters for the rate-determining steps. The less negative entropy of activation for the A-1 reaction again suggests that the transition state in this case is less solvated.

The activation parameters for runs in H_2SO_4–AcOH containing tetraethylammonium bromide are of intermediate value, consistent with the mixed mechanism in this case, as discussed in the previous section.

Removal of the benzyloxycarbonyl group from other substrates. If the mechanisms outlined above (eqns. 3 and 4*a* or *b*) are correct, one would not expect the variation of R in the substrate $Ph{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}NHR$ to influence the rate of reaction markedly, since in both mechanisms the polar requirements of the pre-equilibrium and the rate-determining step are opposite, and steric effects at such a distance from the reaction centre should be minimal. The insensitivity of the reactions in either medium to the nature of R is demonstrated in Table 5. Values of $pK_{NH_3^+}$ of the corresponding unprotected compounds where available are included for comparison, but in view of the small differences in rate and the large differences in structure it is thought unwise to discuss the correlation between $pK_{NH_3^+}$ and log k purely in terms of the polar effects of substituents.

Concurrent Ester Fission.—After this work was well under way with benzyloxycarbonylglycine ethyl ester as substrate, investigation by paper chromatography of the reaction mixture in several runs in both HBr–AcOH and H_2SO_4–AcOH showed the presence of glycine as well as glycine ethyl ester. Ester fission was most extensive in the latter medium. No benzyloxycarbonylglycine could be detected in the product, showing that ester fission does not precede the removal of the benzyloxycarbonyl group.

TABLE 6

Comparison of the observed first-order rate constants, k, for benzyloxycarbonylglycine ethyl ester (I) and benzyloxycarbonylglycine (II). Substrate concentration $1{\cdot}14 \times 10^{-3}$M in acetic acid at 50°

		10^5k (sec.$^{-1}$)	
$[H_2SO_4]$ (M)	$[Et_4N^+Br^-]$ (M)	Substrate (I)	Substrate (II)
0·432		2·42	2·27
0·0864	0·091	12·7	12·1

Rate constants for the removal of the benzyloxycarbonyl group from glycine and from glycine ethyl ester were then measured, and are compared in Table 6. The close similarity shows that the concurrent ester fission does not affect the conclusions about the mechanism of the removal of the benzyloxycarbonyl group.

The concurrent ester fission is in apparent contradiction to the widespread use of HBr–AcOH for the selective removal of *N*-benzyloxycarbonyl groups from esters of *N*-benzyloxycarbonyl amino-acids and peptides.[25] However in preparative work substrate concentrations are high, about molar, compared with 10^{-3}M in our kinetic investigations.

Three solutions of benzyloxycarbonylglycine ethyl ester in approximately 5M-HBr in acetic acid, with substrate concentrations of 4×10^{-3}, 10^{-1}, and 1M, respectively, were left for 30 min. at 17°. After dilution of each solution to 4×10^{-3}M, small samples were withdrawn and treated with a little Amberlite IR-45 (OH^-) resin to remove HBr. Paper chromatography revealed the presence of free glycine only in the first solution; significant ester fission had not occurred in the two solutions with the higher substrate concentrations.

A probable explanation is that the reaction

$$R{\cdot}CO_2Et + CH_3{\cdot}CO_2H \rightleftharpoons R{\cdot}CO_2H + CH_3{\cdot}CO_2Et$$

is reversible, and the removal of the ethyl group from the substrate is facilitated by low substrate concentrations, where the back reaction is unimportant. Separation of the solid ester hydrobromide, which also reduces the extent of concurrent ester fission, was observed only in the most concentrated solution.

Conclusions.—Two different mechanisms for the removal of the *N*-benzyloxycarbonyl group have been shown to be consistent with the experimental facts. The first, labelled *A*-1 and described by equations (3) and (4*a*), is operative in H_2SO_4–AcOH, and the second, labelled *A*-2 and described by equations (3) and (4*b*) predominates in HBr–AcOH. The *A*-1 mechanism, which shows the steeper acidity-dependence and should be rather insensitive to the anion of the catalysing acid, may make a significant contribution in HBr–AcOH at the highest HBr concentrations used. In accord with this, the retarding effect of the *p*-nitro-substituent increases slightly with increasing acid concentration (Table 3), suggesting a contribution from the *A*-1 mechanism for the unsubstituted compound of about 30% in 5M-HBr. The probable competition of two mechanisms in this medium has also been demonstrated by Blaha and Rudinger,[4] who studied the rates of decomposition of a range of ring-substituted benzyloxycarbonylglycines, and observed a sharp break in the Hammett $\rho\sigma$ plot in the region of the unsubstituted compound.

The insensitivity of the rate constant to the nature of the amino-acid or peptide group (Table 6 and ref. 4) suggests that the following conditions will in general ensure more than 99% removal of the *N*-benzyloxycarbonyl group, with substrate concentrations up to 1M: (*a*) 1 hr. in 5M-HBr in acetic acid at 20°; (*b*) 1 hr. in 5M-H_2SO_4 in acetic acid at 50°. (These conditions allow 7 half-lives for substrates reacting 3 times more slowly than N-benzyloxycarbonylglycine.) Lower acid concentrations appear to offer no advantages, as complete protonation of the substrate does not occur even at the highest acid concentrations used in this study.

In general, the milder conditions for removal with HBr–AcOH will be preferred. This is particularly true when concurrent ester fission is to be avoided; high substrate concentrations and possibly shorter reaction times will then also be beneficial.

When some part of the substrate is sensitive to nucleophilic attack, high concentrations of bromide ion will be disadvantageous, and H_2SO_4–AcOH may then be preferable. The rate in the latter medium is also more sensitive to ring substituents, and H_2SO_4–AcOH may be useful for the selective removal of one of two differently substituted *N*-benzyloxycarbonyl groups in the same substrate.

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[25] For references see M. Goodman and G. W. Kenner, *Adv. Protein Chem.*, 1957, **12**, 468.