Kinetics and Mechanism of Hydrolysis of 4-Phenylallophanates

Mohamed Mowafak Al Sabbagh, Michelle Calmon, and Jean-Pierre Calmon *
Laboratoire de Chimie Organique Biologique, Ecole Nationale Supérieure Agronomique, Institut National Polytechnique, 145 avenue de Muret, 31076 Toulouse Cédex, France

The kinetics of hydrolysis of aryl and alkyl 4-phenylallophanates to phenylurea and phenol or alcohol are studied. Acid–base catalysis, deuterium solvent isotope effects, and entropy of activation provide good evidence for a changeover in mechanism from *E*1cB for aryl allophanates to $B_{Ac}2$ for alkyl esters. The parameters of the Hammett and Yukawa–Tsuno relationships for aryl esters and the non-linear Brönsted correlation with the pK_a values of the leaving groups are in good agreement with the proposed mechanisms.

Allophanates $R^3R^4N-CO-NR^2-COOR^1$ show some interest in therapeutics as anticonvulsants,[1-3] antibiotics,[4] antispasmodics,[5] or psychodepressors.[6,7] In agrochemistry, methyl, ethyl, isopropyl, isobutyl, and allyl 4-phenylallophanates show some weed-killing activity.[8] On the other hand, related structures are involved in biological reactions: allophanic acid is an intermediate in the enzymatic cleavage of urea;[9-12] carboxybiotin, which is similar to *NN'*-disubstituted allophanates, reacts in enzymatic carboxylations and transcarboxylations.[13-16]

The quantitative study of the stability of allophanates in aqueous media has not been reported. However, it has been found that ethyl 4-phenylallophanate gives phenylurea quantitatively in alkaline media.[17] By contrast, the hydrolysis of carbamates with a simpler structure has been extensively studied: *NN*-disubstituted carbamates have been shown to hydrolyse by a $B_{Ac}2$ mechanism;[18-20] for *N*-monosubstituted compounds, two mechanisms can occur, $B_{Ac}2$ or *E*lcB, involving the reactivity of the ester or of its anion.[21-23]

This paper is concerned with the mechanism of hydrolysis of a series of aryl or alkyl 4-phenylallophanates. The influence of their structure on their reactivity is discussed.

Results and Discussion

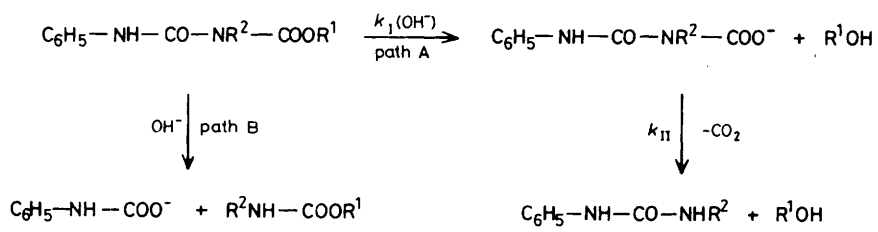
The alkaline cleavage of the esters of phenylallophanic acid can occur either on the ester or on the ureido carbonyl group (Scheme 1). Owing to the well known stability of the urea structure $\rangle N-CO-N\langle$, reaction on the ester function can be predicted (path A). This is confirmed by the following experimental results. (i) Phenylurea has been identified in the reaction products by t.l.c. Furthermore, at the end of the hydrolysis of *p*-acetylphenyl 4-phenylallophanate, the u.v. spectrum is identical to that of a synthetic mixture of phenylurea and *p*-acetylphenol at the same pH and ester concentration. (ii) For *p*-acetylphenyl 4-phenylallophanate, the rate constants k_I and k_{II} of two successive reactions have been determined near neutral pH. The rate constant k_{II} is identical to that measured at the same pH for phenylallophanate anion decarboxylation (Table 1) which confirms the hypothesis of path A. Following path B, the decarboxylation reaction would be that of the carbamate anion, a reaction 10^4 more rapid in the same pH range.[24]

Table 1. Observed rate constants for the hydrolysis of *p*-acetylphenyl 4-phenylallophanate and for the decarboxylation of 4-phenylallophanate anion in water at 25 °C (μ 1.0, KCl)

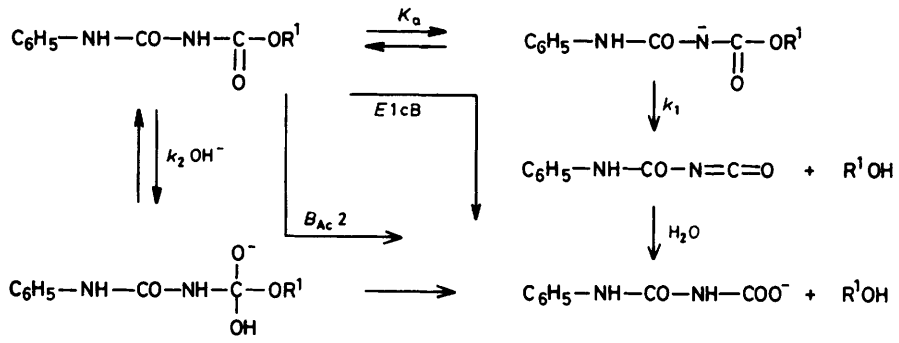
	$C_6H_5-NH-CO-NH-COOC_6H_4COCH_3$-*p*		$C_6H_5-NH-CO-NH-COO^-$
pH	$10^5k_{obs}^{I}/s^{-1}$	$10^5k_{obs}^{II}/s^{-1}$	$10^5k_{obs}/s^{-1}$
5.34	78		263
5.62	84		147
6.00	290	63	65
6.52	780	24.5	26
6.83	1 220	13.0	14.4
7.17	2 350	6.6	7.0
7.59	6 900	3.2	3.0

pH *Dependence of the Hydrolysis Rate Constant for Phenylallophanates.*—The logarithms of the rate constants *versus* pH for the hydrolysis of phenyl 4-phenylallophanate and of the corresponding *N*-methyl material, are plotted in Figure 1. A straight line of slope unity (that is first order in OH^-) is obtained over the whole pH range for the *N*-methyl compound. For the other material, a first-order process is only observed at lower pH and the rate constant levels out near pH 10. The difference between the two compounds is presumably due to the ionisation of the non-methylated substrate on the nitrogen near the ester function. Furthermore, a high difference of reactivity of *ca.* 10^3 is observed between the two compounds.

For ethyl 4-phenylallophanate, the shape of the plot of log k_{obs} *versus* pH is identical to that of phenyl 4-phenylallophanate. Similar results have been observed for other esters, particularly acetoacetates [25] and carbamates.[21,22] Two mechanisms, *E*1cB and $B_{Ac}2$, have been put forward for compounds with a labile proton and are shown in Scheme 2 for phenylallophanates. The corresponding rate equations are (1) for $B_{Ac}2$ and (2) for *E*1cB. These two equations being of the



Scheme 1.



Scheme 2.

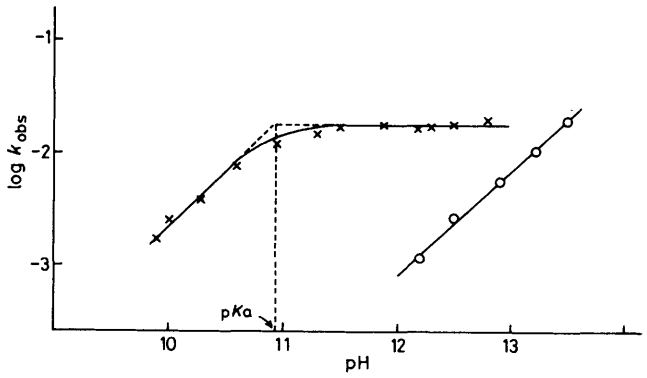


Figure 1. Observed log k_{obs}–pH profiles for the hydrolysis of C_6H_5–NH–CO–NR^2–$COOC_6H_5$ in 25% dioxane: ×, R^2 = H at 10 °C; ○, R^2 = CH_3 at 25 °C (μ 1.0, KCl)

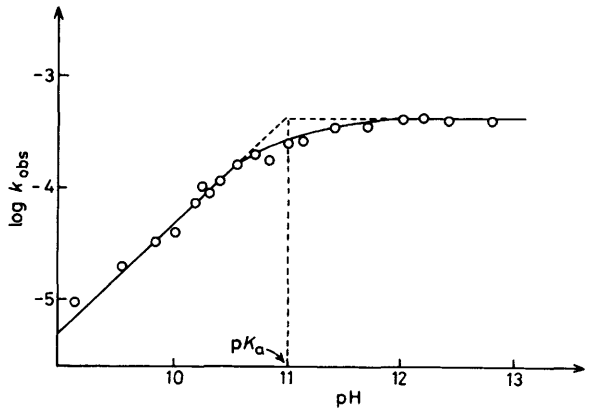


Figure 2. Observed log k_{obs}–pH profile for the hydrolysis of C_6H_5–NH–CO–NH–$COOC_2H_5$ in water at 50 °C (μ 1.0, KCl)

same mathematical form, they do not permit a differentiation

$$k_{obs} = \frac{k_2K_w}{K_a + a_H} \qquad (1)$$

$$k_{obs} = \frac{k_1K_a}{K_a + a_H} \qquad (2)$$

of the two mechanisms. However, the results for the non-methylated derivatives can be correlated in terms of these equations. The limiting expressions are (3) and (4) corresponding to the line of slope unity and (5) and (6) corresponding to the plateau region. The two lines intersect at a pH equal to the pK_a of the substrate.

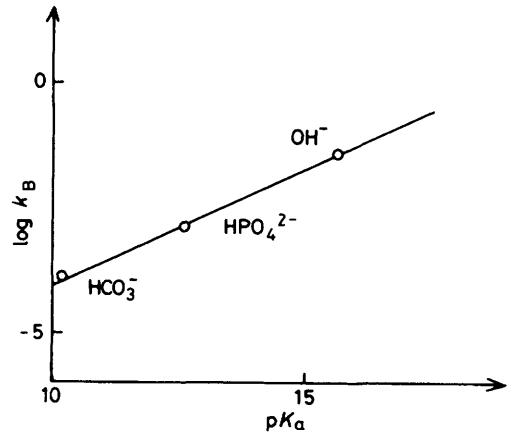


Figure 3. Brönsted plot for the hydrolysis of C_6H_5–NH–CO–NH–$COOC_2H_5$ in water at 50 °C (μ 1.0, KCl)

$$a_H \gg K_a \quad \log k_{obs} = \text{pH} + \log k_2K_w \qquad (B_{Ac}2) \quad (3)$$

$$\log k_{obs} = \text{pH} + \log k_1K_a \qquad (E1cB) \quad (4)$$

$$a_H \ll K_a \quad \log k_{obs} = \log k_2K_w/K_a \qquad (B_{Ac}2) \quad (5)$$

$$\log k_{obs} = \log k_1 \qquad (E1cB) \quad (6)$$

For ethyl 4-phenyllallophanate, the line of Figure 2 is theoretical from the equation $k_{obs} = 0.075\ [OH^-]/(1 + 182\ [OH^-])$ at 50 °C. A pK_a of 11.00 can be read from the graph in good agreement with the value of 11.05 determined by spectrophotometry under the same conditions of temperature and ionic strength (μ 1.0).

For the *N*-methylated compound, the $B_{Ac}2$ scheme alone corresponds to the data. In this case, the rate constant is $k_{obs} = k_2[OH^-]$ in agreement with the line in Figure 1 for phenyl 4-phenyl-2-methylallophanate.

A distinction between *E*1cB and $B_{Ac}2$ mechanisms could be made by isolating the isocyanate involved in the *E*1cB reaction, but this compound is too unstable in aqueous media. Other criteria must be used as in carbamate hydrolysis:[26] investigation of general acid–base catalysis, deuterium solvent isotope effect, entropy of activation, and structural effects.

Table 2. Observed rate constants for the hydrolysis of phenyl 4-phenylallophanate in carbonate buffer and of *p*-nitrophenyl 4-phenylallophanate in acetate buffer at 25 °C in water–dioxane (3 : 1) (μ 1.0, KCl)

C_6H_5–NH–CO–NH–$COOC_6H_5$	Carbonate pH 10.28	$[B_t]$ */M	0.05	0.20	0.35	0.50
		$10^3k_{obs}/s^{-1}$	27.2	29.5	29.2	28.0
C_6H_5–NH–CO–NH–$COOC_6H_4NO_2$-*p*	Acetate pH 5.14	$[B_t]$/M	0.05	0.20	0.35	0.50
		$10^3k_{obs}/s^{-1}$	5.15	5.08	5.22	5.07

* $[B_t]$ = Total buffer concentration ($[B_t] = [B] + [BH^+]$).

Table 3. Observed rate constants of the hydrolysis of ethyl 4-phenylallophanate in buffers at 50 °C (μ 1.0, KCl)

Buffer	pH							
Carbonate	9.16	$[B_t]$ */M	0.60	0.48	0.36	0.24	0.12	0
		$10^3k_{obs}/s^{-1}$	1.11	1.02	0.99	0.99	1.01	0.95
	9.55	$[B_t]$/M	0.50	0.40	0.30	0.20	0.10	0.05 0
		$10^3k_{obs}/s^{-1}$	2.17	2.20	2.18	2.20	1.98	2.00 2.00
	9.85	$[B_t]$/M	0.43	0.34	0.26	0.17	0.085	0
		$10^5k_{obs}/s^{-1}$	5.08	4.70	4.34	3.88	3.68	3.25
Phosphate	10.25	$[B_t]$/M	0.286	0.229	0.172	0.114	0.057	0
		$10^5k_{obs}/s^{-1}$	1.32	1.27	1.22	1.14	1.05	1.00
	10.85	$[B_t]$/M	0.222	0.178	0.133	0.089	0.044	0
		$10^4k_{obs}/s^{-1}$	3.04	2.78	2.47	2.32	2.00	1.76
	11.15	$[B_t]$/M	0.182	0.145	0.109	0.073	0.036	0
		$10^4k_{obs}/s^{-1}$	4.40	3.99	3.79	3.29	2.94	2.60

* $[B_t]$ = total buffer concentration ($[B_t] = [B] + [BH^+]$).

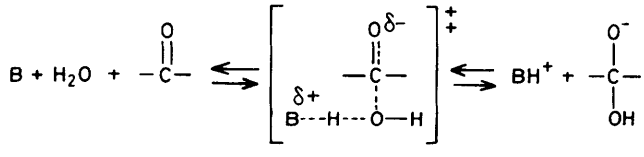
Table 4. Solvent deuterium isotope effect for the hydrolysis of 4-phenylallophanates in water (μ 1.0, KCl)

Allophanates	$[OH^-]$/M or $[OD^-]$/M	$10^4k_{obs}^{H_2O}/s^{-1}$	$10^4k_{obs}^{D_2O}/s^{-1}$	$k_{obs}^{H_2O}/k_{obs}^{D_2O}$
C_6H_5–NH–CO–NH–$COOC_2H_5$	0.5	0.310 *	0.203 *	1.52
	1	0.325 *	0.217 *	
C_6H_5–NH–CO–NH–$COOC_6H_5$	0.005	197 †	204 †	0.96
	0.01	192 †	205 †	
C_6H_5–NH–CO–N(CH_3)–$COOC_6H_5$	0.005	14.0 *	84 *	0.22
	0.01	26.6 *	116 *	

* At 25 °C. † At 10 °C.

Investigation of General Acid–Base Catalysis.—For this study, we have examined the reaction of three esters of 4-phenylallophanic acid with very different reactivities, the *p*-nitrophenyl, phenyl, and ethyl esters. The results for the first two in acetate and phosphate buffers are given in Table 2. No general catalysis is observed, the rate constants being independent of the buffer concentration. On the other hand, in the case of ethyl phenylallophanate, the rate constant increases with phosphate and carbonate buffer concentration (Table 3).

General base catalysis is consistent with the $B_{Ac}2$ mechanism. It has been observed for the chloroacetate hydrolysis and involves the base-catalysed reaction of a water molecule on the ester group.[27]



From the catalytic constants k_B of the buffer species and of the hydroxide ion, a Brönsted β parameter of 0.5 (Figure 3) can be estimated, a value near that obtained for chloroacetate hydrolysis (β 0.47).[28]

For the *E*1cB mechanism, as the rate-determining step is unimolecular anion decomposition, general base catalysis cannot occur. The different behaviour between phenyl and ethyl allophanates is then consistent with a change in mechanism from *E*1cB to $B_{Ac}2$.

Deuterium Solvent Isotope Effect.—The hydrolysis rate constants for phenyl 4-phenylallophanate, its *N*-methyl derivative, and for ethyl phenylallophanate in NaOH and NaOD are in Table 4. For the non-methylated compounds, the isotope effect was measured on the pH-independent plateau.

No significant solvent isotope effect is observed for phenyl 4-phenylallophanate; this lends support to the hypothesis of an *E*1cB pathway since, on the plateau, the rate constant is that of spontaneous anion breakdown which does not involve proton transfer ($k_{obs} = k_1$).

Table 5. Activation parameters for the hydrolysis of C_6H_5–NH–CO–NR^2–$COOR^1$ in water (μ 1.0, KCl)

Compound	pH profile		Rate constants (s^{-1})				$\Delta S^{\ddagger}$/cal mol^{-1} K^{-1}	$\Delta H^{\ddagger}$/kcal mol^{-1}	E_a/kcal mol^{-1}
$R^1 = C_6H_5$, $R^2 = H$	Slope unity	t/°C	25	35	45		36.5	27.4	27.8
		k_{OH}	447	1 910	8 510				
$R^1 = C_6H_5$, $R^2 = CH_3$	Slope unity	t/°C	15	25	35		−39.6	9.0	9.7
		k_{OH}	0.108	0.199	0.322				
$R^1 = C_2H_5$, $R^2 = H$	Slope unity	t/°C	25	50			−13.5	19.2	19.8
		$10^3 k_{OH}$	3.63	48.3					
	Plateau	t/°C	26	30	40	50	−28.5	17.3	18.0
		$10^5 k_{obs}$	4.60	7.29	20.4	43.1			

Table 6. Bimolecular rate constants k_{OH} for the hydrolysis of 4-phenylallophanates C_6H_5–NH–CO–NR^2–$COOR^1$ at 25 °C in water–dioxane (3 : 1) (μ 1.0, KCl)

No.	R^1	R^2	k_{OH} (r)		pK_a of R^1OH
1	p-$NO_2C_6H_4$	H	1.50×10^7	(0.999)	7.15 [a]
2	p-$CH_3COC_6H_4$	H	4.80×10^5	(0.999)	8.05 [a]
3	m-ClC_6H_4	H	1.86×10^4	(0.999)	9.02 [a]
4	m-$CH_3COC_6H_4$	H	1.15×10^4	(0.999)	9.19 [a]
5	p-ClC_6H_4	H	3.89×10^3	(0.999)	9.38 [a]
6	C_6H_5	H	5.62×10^2	(0.999)	10.00 [a]
7	p-$CH_3OC_6H_4$	H	79.4	(0.999)	10.50 [a]
8	$C_2H_2Cl_3$	H	1.17	(0.996)	12.24 [b]
9	CH_3	H	2.88×10^{-3}	(0.995)	15.54 [b]
10	C_2H_5	H	1.32×10^{-3}	(0.996)	16.00 [b]
11	Pr^i	H	5.89×10^{-4}	(0.997)	16.50 [c]
12	p-$NO_2C_6H_4$	CH_3	5.65	(0.995)	7.15 [a]
13	p-$CH_3COC_6H_4$	CH_3	1.15	(0.999)	8.05 [a]
14	m-ClC_6H_4	CH_3	0.562	(0.999)	9.02 [a]
15	m-$CH_3COC_6H_4$	CH_3	0.546	(0.998)	9.19 [a]
16	p-ClC_6H_4	CH_3	0.418	(0.996)	9.38 [a]
17	C_6H_5	CH_3	0.189	(0.999)	10.00 [a]

[a] G. B. Barlin and D. D. Perrin, *Quart. Rev.*, 1966, **20**, 75. [b] P. Balinger and F. A. Long, *J. Am. Chem. Soc.*, 1960, **82**, 795. [c] S. Takahashi L. A. Cohen, H. Y. Miller, and E. G. Peake, *J. Org. Chem.*, 1971, **36**, 1205.

For the *N*-methyl-substituted compound, the *E*1cB pathway is no longer available and the solvent isotope effect k_2^H/k_2^D is clearly less than unity, which is consistent with the greater nucleophilicity of OD^- in the $B_{Ac}2$ mechanism. Similar values have been obtained for the hydrolysis of some aliphatic esters by the same mechanism.[27]

For the ethyl ester, the solvent isotope effect $k_{obs}{}^H/k_{obs}{}^D = 1.52$ is greater than that obtained for the unsubstituted phenyl ester. This value is consistent with the $B_{Ac}2$ pathway for which the rate constant on the plateau is k_2K_w/K_a. If $K_w{}^{H_2O}/K_w{}^{D_2O} = 7.5$ [29] and $K_a{}^{H_2O}/K_a{}^{D_2O} = 4.5$ (calculated for $pK_a{}^{H_2O} = 11.9$ [30]), $k_2{}^{H_2O}/k_2{}^{D_2O} = 0.9$, a value less than unity which again accounts for the greater nucleophilicity of OD^-. Similar results have been obtained for ethyl acetylcarbamate [22] and 5-nitrocoumarone [31] hydrolyses.

Rate as a Function of Temperature.—The rate constants for the hydrolysis of the three preceding esters were determined as a function of temperature for several pH values; the thermodynamic activation parameters were calculated and are given in Table 5.

For phenyl 4-phenylallophanate, the highly positive entropy of activation rules out the $B_{Ac}2$ scheme: measurements were performed on the line of slope unity where $k_{OH} = k_2$, the rate constant for the addition of OH^- to the carbonyl group; for this reaction, a negative entropy should be measured as observed for the hydrolysis of the *N*-methyl-substituted compound ($\Delta S^{\ddagger}$ −40 cal mol^{-1} K^{-1}).

On the other hand, for the *E*1cB scheme, k_{OH} is composite and equal to k_1K_a/K_w. If some compensation operates between the entropies of ionisation of the substrate and water, the positive entropy accounts for unimolecular anion cleavage of rate constant k_1.

The change from a positive to a negative entropy of activation on substituting a methyl group α to an ester group has previously been observed for phenyl *N*-phenylcarbamate [20] and for phenyl *N*-phenylthionocarbamate.[26] In the latter case, the *E*1cB pathway was confirmed for the unsubstituted compound by isolation of the isothiocyanate.

For the hydrolysis of ethyl 4-phenylallophanate, the entropy of activation was determined both on the line of slope unity and on the plateau of a plot of log k_{obs} *versus* pH. The negative values are in good agreement with rate-determining addition of OH^- to the carbonyl group of the carbamate function and accounts for the loss of degrees of freedom of hydroxide ion in the transition state.

To conclude, acid–base catalysis, the deuterium solvent isotope effect, and the entropy of activation provide good evidence for a changeover in mechanism from *E*1cB for phenylallophanate to $B_{Ac}2$ for the ethyl ester.

Substituent Effects.—The bimolecular rate constants $k_{OH} = k_{obs}/[OH^-]$ were determined in the pH range where the rate constant is proportional to hydroxide ion concentration and are given in Table 6. The structural effects were estimated by means of two types of correlations: (i) a Hammett equation for esters with a substituted phenyl group in the R^1 position and (ii) a Brönsted relationship using the pK_a values of the leaving group and extended to all the esters.

Hammet relationship. From the k_{OH} values summarized in Table 6, it is clear that electron-withdrawing R^1 substituents enhance the ester reactivity. A plot of log k_{OH} *versus* σ values for substituents gave a ρ value of 5.01 (*r* 0.966). The correlation is clearly improved by the use for σ^- for *p*-NO_2 (σ^- 1.27) and *p*-$COCH_3$ (σ^- 0.82): ρ 3.41 (*r* 0.998).

For esters with a methyl group on the nitrogen atom near the reacting centre, the Hammett equation, using σ values, gives ρ 1.87 (*r* 0.975). The correlation is not significantly improved by the use of σ^- for *p*-NO_2 and *p*-$COCH_3$ with ρ 1.06 (*r* 0.981). The best correlation was obtained using the Yukawa–Tsuno treatment:[32] $\log k/k_0 = \rho[\sigma + r(\sigma^- - \sigma)]$ with ρ 1.43 and *r* 0.43 (*r* 0.987).

The decrease in ρ value with nitrogen substitution can be explained in terms of a changeover in mechanism from *E*1cB to $B_{Ac}2$. Similar results were reported with phenyl *N*-phenylthionocarbamates (ρ 3.00) and the *N*-methyl-substituted esters (ρ 1.05).[26]

In the *E*1cB scheme, the bimolecular rate constant k_{OH} is composite, $k_{OH} = k_1K_a/K_w$, but electron-withdrawing substituents will tend to increase both the substrate acid strength (K_a) and the leaving group ability (k_1). The correlation is improved by the use of σ^- because the negative charge of the phenolate anion is resonance stabilized by *p*-NO_2 and *p*-$COCH_3$.

As previously noted,[21,33] the *E*1cB pathway involves an important C–O bond cleavage in the transition state in agreement with the large ρ value. The $B_{Ac}2$ pathway involves a degree of resonance interaction (*r* 0.43) larger than that reported for aryl benzoates and acetates hydrolysis (*r* 0.2).[34,35] This value, close to zero for the Yukawa–Tsuno *r* parameter, was explained by rate-limiting hydroxide ion addition with very small cleavage of the C–OAr bond in the transition state. For *N*-methylallophanates, the larger *r* value requires more advanced cleavage of this bond with formation, on the phenolic oxygen, of a negative charge, resonance stabilized by the *para*-substituents. There is however a possible change over in the rate-determining step to the breaking of the tetrahedral intermediate.

Brönsted relationship. In order to include aliphatic and aromatic esters in the same correlation, we established a Brönsted correlation where the pK_a values of the leaving groups are employed: $\log k_{OH} = \beta pK_a + \text{constant}$. With aryl *N*-methyl-4-phenylallophanates, for which the *E*1cB pathway is no longer available, the slope β of the line obtained is −0.48 (*r* 0.984) (Figure 4). A similar value (β −0.47) was reported for aryl *N*-phenyl-*N*-methylthionocarbamates.[26]

With 4-phenylallophanates having a labile proton α to the ester group (R^2 = H), a non-linear plot is obtained (Figure 4). Aromatic esters fit an equation with β 1.56 (*r* 0.999) and for the aliphatic esters β −0.78 (*r* 0.999). The break occurs for a pK_a value of 11.15. These results again provide evidence for the changeover in mechanism that was already put forward from other criteria (entropy, isotope effect, catalysis).

From β parameters previously reported in the literature, the value of −1.56 can be assigned to the *E*1cB pathway: for many aryl esters which are hydrolysed by this mechanism, β values < −1 were obtained: acetoacetates (β −1.29),[25] *N*-phenylcarbamates (β −1.34),[33] acetylcarbamates (β −1.32),[22] *N*-phenylthionocarbamates (β −1.35).[26]

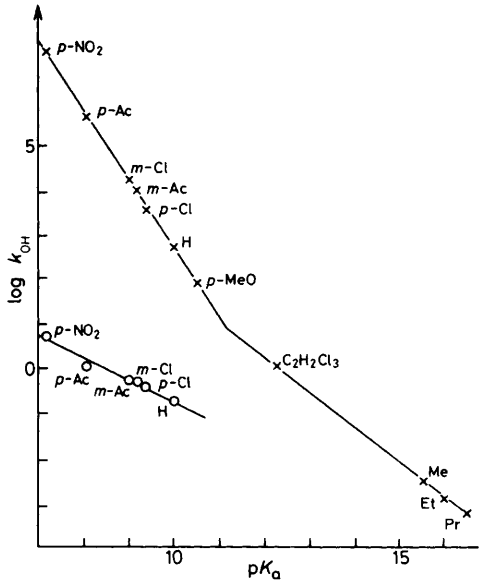


Figure 4. Plots of the bimolecular rate constants k_{OH} for the hydrolysis of 4-phenylallophanates *versus* pK_a of the conjugate acid of leaving group in 25% dioxane at 25 °C (μ 1.0, KCl): ×, C_6H_5–NH–CO–NH–$COOC_6H_4X$; ○, C_6H_5–NH–CO–$N(CH_3)$–$COOC_6H_4X$

For the alternative $B_{Ac}2$ pathway, β values > −1 are generally obtained as for *N*-methylallophanates (β −0.48). A β value of −0.78 is then in good agreement with this mechanism.

Experimental

Materials.—4-Phenylallophanates were prepared by reaction of aryl or alkyl chloroformates on phenylurea as described previously.[36]

Kinetic Procedure.—The rates of hydrolysis were measured spectrophotometrically with a Philips–Pye–Unicam SP 1800 A spectrophotometer equipped with an SP 1805 attachment. A constant temperature was maintained (±0.1 °C) by circulating water in the cell compartment. The pH was measured using a Radiometer model PHM 64 pH-meter equipped with a GK 2301 Radiometer electrode.

For aromatic esters, the rates were measured by following either the decrease in absorbance of the substrate at 260 (R^2 = H) or 240 nm (R^2 = CH_3), or the increase in absorbance of the phenol or of the phenolate ion produced by the reaction.

For aliphatic esters, in alkaline media (sodium hydroxide, carbonate, or phosphate buffers), only small changes in absorbance were obtained. In addition, the rates of ester hydrolysis and of allophanic acid decarboxylation are of the same order of magnitude. For these two reasons, it was not possible to follow the rate by direct absorbance measurements. The optical density was then measured at 255 nm on samples (2 ml) of the reacting solution after addition of concentrated hydrochloric acid (3M, 1 ml) to give phenylurea by fast allophanic acid decarboxylation.

Pseudo-first-order rate constants were calculated from the slopes of the plots of log $(A_\infty - A_t)$ *versus* time or by the Guggenheim method.[37]

The substrate concentration was *ca.* 5×10^{-5}—10^{-4}M.

pK_a *Determination.*—The dissociation constant of ethyl 4-phenylallophanate was determined at 25 °C (μ 1.0, KCl) from

the plot of log $(OD - OD_{AH})/(OD_{A^-} - OD)$ *versus* pH [38] where OD_{AH}, OD_{A^-}, and OD are the optical densities, at the same wavelength, of the non-ionised and ionised forms of the substrate and of their mixture respectively at several pH values. OD_{AH} was determined in a borax buffer and OD_{A^-} in 0.1M-sodium hydroxide. The pK_a value is an average of determinations at several wavelengths.

References

1 M. A. Spielman, J. D. Barnes, and W. J. Close, *J. Am. Chem. Soc.*, 1950, **72**, 2520.
2 S. Umemoto and H. Takamatsu, *Yakugaku Zasshi*, 1963, **83**, 917 (*Chem. Abstr.*, 1964, **60**, 2815cd).
3 K. Nakamura, K. Nakatsuji, K. Ohoshi, Y. Masuda, K. Fujimoto, S. Ose, and K. Tsutsui, *Jpn. J. Pharmacol.*, 1963, **13**, 259 (*Chem. Abstr.*, 1964, **60**, 9790e).
4 Société Industrielle pour la Fabrication des Antibiotiques (S.I.F.A.), F.P. 1962/1,288,153 (*Chem. Abstr.*, 1962, **57**, 15005e).
5 Dainippon Pharmaceutical Co., Ltd., Jpn. P. 1962/10048 (*Chem. Abstr.*, 1965, **63**, 5563f).
6 O. Foussard-Blanpin, G. Uchida-Ernouf, J. Anatol, and J. Berecoecha, *Chim. Ther.*, 1979, **14**, 215.
7 G. Uchida-Ernouf and O. Foussard-Blanpin, *Ann. Pharm. Fr.*, 1979, **17**, 37.
8 Farbenfabriken Bayer Akt.-Ges., B.P. 1959/819 853 (*Chem. Abstr.*, 1960, **54**, 6019d).
9 P. A. Castric, *Anal. Biochem.*, 1976, **72**, 468.
10 P. A. Castric and B. Levenberg, *Biochim. Biophys. Acta*, 1976, **438**, 574.
11 J. W. Leftley and P. J. Syrett, *J. Gen. Microbiol.*, 1973, **77**, 109.
12 E. M. Thomas and P. J. Syrett, *New Phytol.*, 1976, **76**, 409.
13 S. Ochoa and Y. Kaziro, ' Comprehensive Biochemistry,' eds. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1965, vol. 16, pp. 211—249.
14 J. Moss and M. D. Lane, *Adv. Enzymol.*, 1971, **35**, 321.
15 H. G. Wood and R. E. Barden, *Annu. Rev. Biochem.*, 1977, **46**, 385.
16 M. J. Wimmer and I. A. Rose, *Annu. Rev. Biochem.*, 1978, **47**, 1031.
17 A. Helge and O. Backman, *Acta Acad. Aboensis, Ser. B.*, 1944, **14**, 19 (*Chem. Abstr.*, 1948, **42**, 573g).
18 M. L. Bender, *Chem. Rev.*, 1960, **60**, 53.
19 L. W. Dittert and T. J. Higuchi, *J. Pharm. Sci.*, 1963, **52**, 852.
20 I. Christenson, *Acta Chem. Scand.*, 1964, **18**, 904.
21 A. F. Hegarty and L. N. Frost, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1719.
22 M. Bergon and J. P. Calmon, *Bull. Soc. Chim. Fr.*, 1976, 797.
23 A. Williams and K. T. Douglas, *Chem. Rev.*, 1975, **75**, 627.
24 S. L. Johnson and D. L. Morrison, *J. Am. Chem. Soc.*, 1972, **94**, 1323.
25 R. F. Pratt and T. C. Bruice, *J. Am. Chem. Soc.*, 1970, **92**, 5956.
26 G. Sartore, M. Bergon, and J. P. Calmon, *J. Chem. Soc., Perkin Trans. 2*, 1977, 650.
27 T. C. Bruice, H. F. Fife, J. J. Bruno, and P. Benkovic, *J. Am. Chem. Soc.*, 1962, **84**, 3012.
28 W. P. Jencks, in ' Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969, p. 513.
29 P. Salomaa, *Acta Chem. Scand.*, 1971, **25**, 367.
30 R. P. Bell in ' The Proton in Chemistry,' Chapman and Hall, London, 1973, p. 227.
31 P. S. Tobias and F. J. Kezdy, *J. Am. Chem. Soc.*, 1969, **91**, 5171.
32 Y. Tsuno, T. Isabata, and Y. Yukawa, *Bull. Chem. Soc. Jpn.*, 1959, **32**, 960.
33 A. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1972, 808.
34 Z. S. Chaw, A. Fisher, and D. A. R. Happer, *J. Chem. Soc. B*, 1971, 1818.
35 A. A. Humffray and J. J. Ryan, *J. Chem. Soc. B*, 1966, 842.
36 M. M. Al Sabbagh, M. Calmon, and J. P. Calmon, *Bull. Soc. Chim. Fr.*, 1983, *II*, 73.
37 E. A. Guggenheim, *Philos. Mag.*, 1926, **2**, 538.
38 A. Albert and E. P. Serjeant in ' The Determination of Ionization Constants,' Chapman and Hall, London, 1971, p. 44.

Received 31*st October* 1983, *Paper* 3/1938