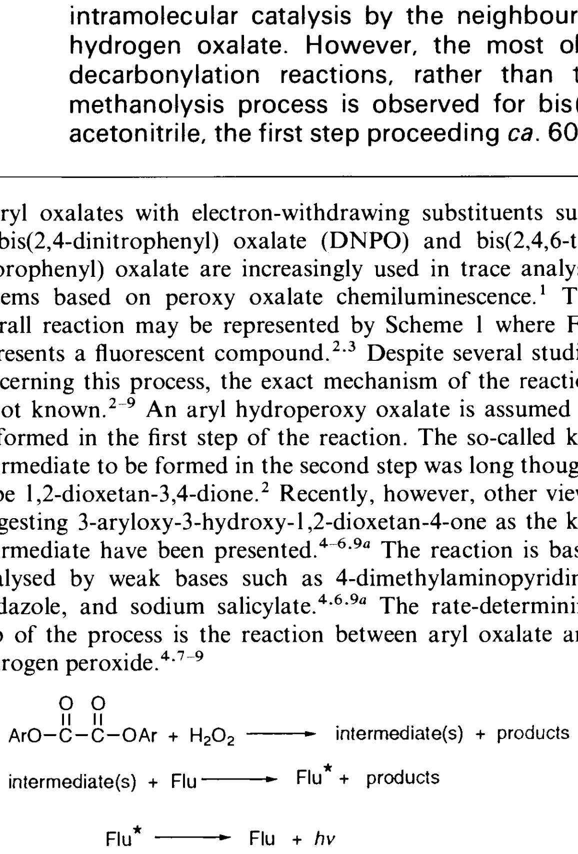
Kinetics of the Decomposition of a Chemiluminescent Reagent Bis(2,4-dinitrophenyl) Oxalate in Aqueous Acetonitrile

Helmi Neuvonen
Department of Chemistry, University of Turku, SF-20500 Turku, Finland

The solution kinetics of a chemiluminescent reagent bis(2,4-dinitrophenyl) oxalate have been studied in acetonitrile–water mixtures in the region $[H_2O] \leq 2.78$ mol dm^{-3}. A stepwise process is suggested for the decomposition of the diaryl ester. The hydrolysis of one of the ester groups is followed by a rapid degradation of the formed intermediate. A $B_{Ac}3$ mechanism is apparent for the first hydrolysis step. The rate acceleration in the second step could be a consequence of intramolecular catalysis by the neighbouring carboxyl group in the hydrolysis of 2,4-dinitrophenyl hydrogen oxalate. However, the most obvious pathway involves successive decarboxylation and decarbonylation reactions, rather than the hydrolysis of the monoaryl oxalate. A consecutive methanolysis process is observed for bis(2,4-dinitrophenyl) oxalate in 1.67 mol dm^{-3} methanol in acetonitrile, the first step proceeding *ca.* 60 times faster than the second step.

Diaryl oxalates with electron-withdrawing substituents such as bis(2,4-dinitrophenyl) oxalate (DNPO) and bis(2,4,6-trichlorophenyl) oxalate are increasingly used in trace analysis systems based on peroxy oxalate chemiluminescence.[1] The overall reaction may be represented by Scheme 1 where Flu represents a fluorescent compound.[2,3] Despite several studies concerning this process, the exact mechanism of the reaction is not known.[2–9] An aryl hydroperoxy oxalate is assumed to be formed in the first step of the reaction. The so-called key intermediate to be formed in the second step was long thought to be 1,2-dioxetan-3,4-dione.[2] Recently, however, other views suggesting 3-aryloxy-3-hydroxy-1,2-dioxetan-4-one as the key intermediate have been presented.[4–6,9a] The reaction is base-catalysed by weak bases such as 4-dimethylaminopyridine, imidazole, and sodium salicylate.[4,6,9a] The rate-determining step of the process is the reaction between aryl oxalate and hydrogen peroxide.[4,7–9]



$$\mathrm{ArO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OAr} + H_2O_2 \longrightarrow \text{intermediate(s)} + \text{products}$$

$$\text{intermediate(s)} + \text{Flu} \longrightarrow \text{Flu}^* + \text{products}$$

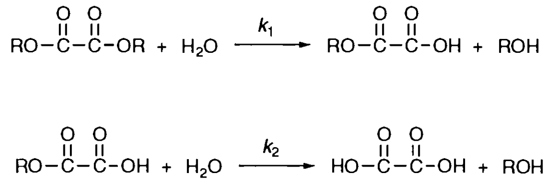
$$\text{Flu}^* \longrightarrow \text{Flu} + h\nu$$

Scheme 1

The knowledge of the hydrolysis kinetics of aryl oxalates is important because in chemiluminescent systems containing water, hydrolysis diminishes chemiluminescence. Further, the understanding of the solvolysis mechanisms of oxalates will help the elucidation of the mechanistic details of the reaction of aryl oxalates with hydrogen peroxide because of obviously similar mechanistic features in these two processes.[3,4]

Although hydrolysis of monocarboxylic esters has been widely studied, there is only limited information in the literature concerning the kinetics of hydrolysis of dicarboxylic acid esters. The neutral hydrolysis of oxalic acid esters is usually believed to proceed in two steps (Scheme 2).[7,10–12] The first step has been proposed to be faster than the second for the hydrolysis of diaryl oxalates,[7,10,11] while the hydrolysis of dimethyl oxalate and methyl hydrogen oxalate has been observed to occur at approximately the same rate.[12]

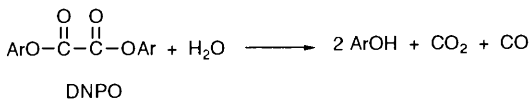
Jennings and Capomacchia[13] studied the hydrolysis of DNPO in acetonitrile–water mixtures ($[H_2O] \leq 2.222$ mol dm^{-3}) by UV measurements. They interpreted the results to indicate the occurrence of a 'concerted mechanism', where both ester groups react simultaneously, liberating two molecules of 2,4-dinitrophenol. 2,4-Dinitrophenyl hydrogen oxalate was not recovered during product analysis and the authors discarded the possibility of k_2 being $> k_1$ in Scheme 2. Later, Orosz and Dudar[14] studied the same reaction in aqueous acetonitrile and aqueous tetrahydrofuran by IR spectroscopy. The changes in the carbonyl region showed neither 2,4-dinitrophenyl hydrogen oxalate nor oxalic acid during the reaction. Instead, the disappearance of DNPO was accompanied by the evolution of carbon dioxide and carbon monoxide. Therefore, 'an unexpected decomposition' was suggested for the reaction between DNPO and water (Scheme 3). It was further suggested in the light of these results that, although generally believed, an aryl hydroperoxy oxalate is perhaps not formed as an intermediate in a chemiluminescence reaction between DNPO and hydrogen peroxide, but a dioxetane derivative is formed directly in the reaction between DNPO and hydrogen peroxide. With less reactive diaryl oxalates, diphenyl oxalate and phenyl 2,4,6-trichlorophenyl oxalate, hydrolysis to phenyl hydrogen oxalate was detected. A stepwise hydrolysis process like that shown in Scheme 2 with $k_1 > k_2$ was suggested for these compounds.[14]



$$\mathrm{RO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OR} + H_2O \xrightarrow{k_1} \mathrm{RO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH} + ROH$$

$$\mathrm{RO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH} + H_2O \xrightarrow{k_2} \mathrm{HO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH} + ROH$$

Scheme 2



$$\underset{\text{DNPO}}{\mathrm{ArO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OAr}} + H_2O \longrightarrow 2\,ArOH + CO_2 + CO$$

Scheme 3

The present study was undertaken to clarify the confusion concerning the mechanism of hydrolysis of DNPO and to get a deeper insight into the details of hydrolysis of diaryl oxalates in general.

Experimental

Materials.—Commercial bis(2,4-dinitrophenyl) oxalate (Sigma) was used as received. 2,4-Dinitrophenyl acetate[15] and

Table 1 First-order rate coefficients (k_{obs}) for the hydrolysis of bis(2,4-dinitrophenyl) oxalate in water–acetonitrile mixtures

$[H_2O]$/mol dm^{-3}	$10^3\ k_{obs}$/s^{-1}
1.11	0.191[a] ± 0.004
	0.183[b] ± 0.009
1.39	0.350[a] ± 0.011
1.67	0.626[a] ± 0.004
	0.587[b] ± 0.003
2.22	1.55[a] ± 0.01
	1.60[b] ± 0.004
2.78	2.89[a] ± 0.12

[a] This study at 298.2 K. The rate coefficients are mean values of five determinations and the error is a standard deviation. [b] Ref. 13 at 296.2 K.

3-nitrophenyl trifluoroacetate[16] were prepared as described earlier. 2,4-Dinitrophenol (Merck) was recrystallized first from ethanol and then from hexane. Acetonitrile (Merck, gradient grade for chromatography) methanol (Rathborn, HPLC grade), trifluoroacetic acid (Fluka, purum), and calcium chloride (Merck) were used as received. Heavy water was from *Norsk Hydro-elektrisk Kvaelstoffaktieselskab* (99.8% D_2O).

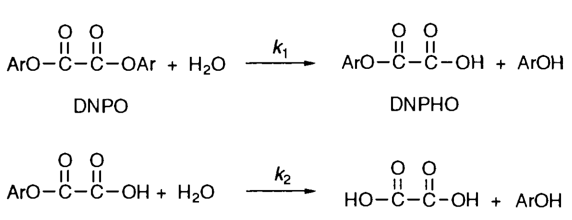
Kinetics.—Reaction rates for the hydrolysis of DNPO were determined by observing the increase in UV absorption due to the 2,4-dinitrophenol formation at 300 nm. It was checked that essentially the same values were obtained by following the ester decay at 240 nm. The rates for methanolysis of DNPO were determined by observing the decrease in UV absorption at 240 nm and the rates for the hydrolysis of 3-nitrophenyl trifluoroacetate by observing the decrease in UV absorption at 250 nm. A Gilford 2600 spectrophotometer equipped with a Gilford Thermoset Temperature Controller was used. The temperatures of the reaction solutions were measured in the optical cell with a calibrated NTC-thermistor. The temperature was accurate to ±0.1 °C. The absorbance changes were recorded with a printer. The reactions were followed for six half-lives and the final value was observed after ten half-lives if needed. In a typical experiment 5 mm^3 of an acetonitrile solution (0.01 mol dm^{-3}) of DNPO was added to an acetonitrile–water mixture (1 cm^3) in a 10 cm^3 flask. The reaction solution was shaken thoroughly, 250 mm^3 of the solution was transferred to a microcell (300 mm^3), and the cell was quickly placed into the thermostatted cell compartment of the spectrophotometer. It was verified with pre-experiments that the reaction solution reached a proper temperature during 2 min. The first-order rate coefficients were determined by the method of Guggenheim.[17] The standard deviations of the individual rate coefficients were 0.4–0.6%. It was checked for all the different reaction mixtures that good linear plots of ln ($A_\infty - A_t$) *versus* time ($r > 0.9999$ over $3t_{\frac{1}{2}}$) were obtained. The rate coefficients k_{obs} are mean values of five determinations and the error given is a standard deviation. The UV spectral scanning drives were performed with the Gilford 2600 spectrophotometer equipped with a HP 7225B plotter.

Results and Discussion

Table 1 summarizes the experimental conditions and the rate coefficients for the hydrolysis of DNPO in acetonitrile–water mixtures. In addition the rate coefficient (1.25 ± 0.02) × 10^{-3} s^{-1} was determined for DNPO in deuterium oxide–acetonitrile containing 2.78 mol dm^{-3} of deuterium oxide. The rate coefficients determined in this study are in agreement with those observed by Jennings and Capomacchia.[13] The latter values are also shown in Table 1. For 3-nitrophenyl trifluoroacetate the following rate coefficients were deteremined at 298.2 K: k_{obs} = (3.70 ± 0.03) × 10^{-3} s^{-1} in 2.78 mol dm^{-3} H_2O in acetonitrile and k_{obs} = (1.462 ± 0.006) × 10^{-3} s^{-1} in 2.78 mol dm^{-3} D_2O in acetonitrile.

As expected, the disappearance of DNPO was accompanied by the formation of 2,4-dinitrophenol (DNP) in the hydrolysis reaction of the oxalate ester in acetonitrile–water mixtures investigated. The rate coefficients determined at 240 nm for the decomposition of DNPO were essentially the same as those determined at 300 nm for the appearance of DNP (*cf.* Table 1). For example, in 2.78 mol dm^{-3} water in acetonitrile and in 1.11 mol dm^{-3} water in acetonitrile the values of 2.82 × 10^{-3} and 2.17 × 10^{-4} s^{-1}, respectively, were obtained for k_{obs} by following the disappearance of DNPO at 240 nm. When the hydrolysis of DNPO was followed by scanning the UV spectrum of the reaction solution (5 × 10^{-5} mol dm^{-3} DNPO in 1.67 mol dm^{-3} H_2O in acetonitrile) repetitively, three constant isosbestic points, at 208, 213, and 256 nm, between the starting compound and the reaction product, were observed. This means that, either there is no absorbing intermediate on the reaction path, or it is a very short lived one. When the first spectrum, taken after $0.15t_{\frac{1}{2}}$ from the mixing of the ester and the reaction solution, was compared with the UV spectrum of 5 × 10^{-5} mol dm^{-3} DNPO in acetonitrile, it could be seen that the measured step of the reaction was not preceded by any fast step including the decomposition of DNPO and a rapid growth of DNP. The UV spectrum of 2,4-dinitrophenyl acetate (DNPAc) shows nearly the same form as that of DNPO but with the molar absorptivity of half of that of the oxalate ester (DNPO), and it obviously satisfactorily mimics the UV spectrum of 2,4-dinitrophenyl hydrogen oxalate (DNPHO).[13] The absorption spectrum of the solution of 5 × 10^{-5} mol dm^{-3} DNPO in 1.67 mol dm^{-3} H_2O in acetonitrile, after ten half-lives of the hydrolysis reaction, was compared both to that of an acetonitrile solution of 1 × 10^{-4} mol dm^{-3} DNP, and to that of a solution containing 5 × 10^{-5} mol dm^{-3} of DNPAc and 5 × 10^{-5} mol dm^{-3} of DNP in acetonitrile. Any indication of the presence of DNPHO was not observed, but the spectrum of the reaction products indicated the formation of 2 mol of 2,4-dinitrophenol from 1 mol of bis(2,4-dinitrophenyl) oxalate.

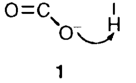
The experiments described above indicate that bis(2,4-dinitrophenyl) oxalate (DNPO) is hydrolysed in acetonitrile–water mixtures with low water content, forming 2 mol of the corresponding phenol (DNP) from 1 mol of DNPO. In accordance with the results of Jennings and Capomacchia[13] and those of Orosz and Dudar,[14] the assumed intermediate, 2,4-dinitrophenyl hydrogen oxalate (DNPHO), is not observed. If the reaction were to be described by Scheme 4 with $k_1 > k_2$ and k_{obs} would refer to the k_1 step, DNPHO should be a detectable reaction intermediate and only 1 mol of DNP should be formed from 1 mol of DNPO in the measured step. If k_1 were $> k_2$ but the detected rate coefficients would refer to the k_2 step, there should be a rapid decrease of DNPO concentration and a burst of DNP before the slow step, which was not observed. Hence, the 'concerted mechanism' suggested by Jennings and Capomacchia,[13] involving simultaneous reaction of both ester groups, or a decomposition process suggested by Orosz and Dudar,[14] seems to be valid.



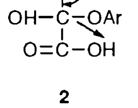
Scheme 4

Alternatively, a consecutive mechanism as shown in Scheme 4 is occurring, k_2 being $> k_1$.

Jennings and Capomacchia[13] calculated a value of 0.15 s^{-1} (9 min^{-1}) for the hydrolysis of DNPHO in water with the aid of the rate coefficients known for 2-nitrophenyl acetate, 2,4-dinitrophenyl acetate, and 2-nitrophenyl hydrogen oxalate. This value they compared with the value of 22.8 s^{-1} (1.37×10^3 min^{-1}) obtained by extrapolation for the hydrolysis of DNPO in water. The latter value was achieved by assuming a constant n for the relationship $k_{obs} = k(H_2O)[H_2O]^n$ all over the region $x(H_2O)$ = 0–1 in acetonitrile–water mixtures. The estimated ratio, *ca.* 150, for $k_{hydr}(DNPO)/k_{hydr}(DNPHO)$ in water was thought to be valid in acetonitrile–water mixtures with low content of water and to disprove the idea of k_2 being $> k_1$ in Scheme 4. This conclusion can, however, be erroneous. DNPHO contains a reactive ester group and a neighbouring carboxyl group, which can participate in the second step of the hydrolysis reaction shown in Scheme 4 and render it faster than the first step under the present reaction conditions. There are several examples of both general acid and general base catalysis by a neighbouring carboxyl group in ester hydrolysis. If a significant amount of 2,4-dinitrophenyl oxalate ion is present in the reaction conditions, the second step of the hydrolysis reaction of DNPO could proceed *via* general base catalysis by the carboxylate anion **1**. A neighbouring-carboxylate catalysis of that type has been verified, for instance, in the hydrolyses of aryl hydrogen malonates[18] and 2-acetyl salicylic acid,[19] and in the hydrolysis of some monohydrogen phthalate esters[20] in aqueous solution. There also are examples of nucleophilic catalysis by neighbouring carboxylate anions both in aqueous solution[21] and in aqueous aprotic solvents[22] in the reactions of carboxylic acid derivatives. However, we can rule out the possibility of a nucleophilc catalysis by $-CO_2^-$ in the present case because of the strain involved in the formation of a three-membered ring. Due to desolvation, anions are known to be more reactive in aprotic solvents than in water.[23] An effective intramolecular general-base catalysis by a desolvated oxalate anion has been detected, for instance, in the reaction of piperidine with 2-nitrophenyl hydrogen oxalate in toluene.[24] Intramolecular general-acid catalysis by $-CO_2H$ has been observed in aqueous solution *e.g.* in the hydrolysis of hydrogen phthalate esters with poor leaving groups such as methyl,[20] ethyl,[25] and 2-chloroethyl[20] hydrogen phthalate. A mechanism shown as **2** has been suggested in acetonitrile, for example, in the aminolysis of phenyl salicylate.[26] This kind of catalysis, which avoids the formation of oxide anion, can be considered to be more meaningful in acetonitrile with low water content than in aqueous solution, because of poor solvation of anions in dipolar aprotic solvents such as acetonitrile.[23]



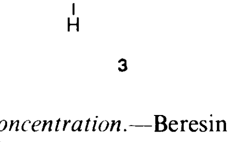
1



2

In any case, the reaction type suggested by Jennings and Capomacchia[13] sounds statistically unattainable and must be entropically extremely unfavourable.[27] Orosz and Dudar[14] detected the formation of CO_2 and CO during the hydrolysis of DNPO. Their original proposal, comprising the straight decomposition of DNPO to DNP, CO_2 and CO (Scheme 3) is also a previously unknown pathway for ester hydrolysis and needs some reconsideration. Oxalic acid is known to decarboxylate easily[28] and the product, formic acid can be decarbonylated. However, these processes usually occur only at elevated temperatures. Therefore, if it can be verified that oxalic acid is stable in the present reaction conditions, some other route than hydrolysis to oxalic acid must be considered for the degradation of DNPO. One possibility is that the reaction goes through successive hydrolysis and decomposition steps, the latter being faster than the former.

First of all, it is necessary to consider the reaction mechanism in the light of the kinetic results. The neutral hydrolysis of both alkyl and aryl esters of monocarboxylic acids has been shown to proceed by water-catalysed attack of water at the carbonyl carbon, *i.e.* by the $B_{AC}3$ mechanism.[29] This mechanism has also been suggested for the pH-independent hydrolyses of 2-nitrophenyl ethyl oxalate and 2-nitrophenyl hydrogen oxalate in aqueous solution.[11] Most studies concerning ester hydrolysis refer to aqueous solution or to solvent mixtures rich in water. Solvent structure of water is known to be destroyed in acetonitrile–water mixtures with water as a minor component, water obviously occurring as monomers when $[H_2O] < 0.5$–1 mol dm^{-3}.[30] A mechanism where a water molecule displaces the leaving group without catalysis, with a transition state shown as **3**, has been suggested by Gopalakrishnan and Hogg[31] for the hydrolysis of 1-acetyl-3-methylimidazolium ion in acetonitrile–water mixtures at water concentrations < 1 mol dm^{-3}. DNPO possesses an activated carbonyl group and a good leaving group, which makes a mechanism through a transition state like **3** conceivable. However, the usual water-catalysed route has been verified for substituted phenyl trifluoroacetates in acetonitrile–water mixtures with low water content ($[H_2O]$ = 3.89–0.56 mol dm^{-3}).[16]



3

Effect of Water Concentration.—Beresin *et al.*[32] have studied the neutral hydrolysis of diphenyl oxalate and diethyl oxalate in binary mixtures of water and several organic solvents on a wide range of solvent compositions with mol fractions of water ≥ 0.23. The influence of solvent composition on the rate in these reactions is very similar to that observed for the neutral hydrolysis of alkyl and halogenoalkyl monocarboxylates shown to be hydrolysed by the $B_{AC}3$ mechanism.[33] In these cases the slopes of the plot of $\log k_{obs}$ *versus* $\log[H_2O]$ vary from 3 to *ca.* 7 depending on the solvent. Fig. 1 shows the dependence of the observed rate coefficient on the water concentration of the solvent mixture for the hydrolysis of DNPO. The slope of the plot has a value of 3.0. The same value was obtained by Jennings and Capomacchia on a somewhat different solvent region.[13] This value is very close to that, 3.4, detected for the hydrolysis of 4-nitrophenyl trifluoroacetate in acetonitrile–water mixtures with $[H_2O]$ = 3.89–0.56 mol dm^{-3} (the plot also shown in Fig. 1).[16] Plots of $\log k$ *versus* $\log[H_2O]$ have been used to determine the order of reaction in water,[34] but these values cannot be interpreted too rigorously.[35] The high value of the slope indicates that several water molecules not involved in the initial state are incorporated in the transition state. Nevertheless, a higher value than 3 is expected if two ester groups would react simultaneously by the $B_{AC}3$ mechanism.

Water Isotope Effect.—A value of 2.3 is obtained in this study for the deuterium oxide isotope effect in the hydrolysis of DNPO in 2.78 mol dm^{-3} water in acetonitrile. The value is just the same as that previously noted for the hydrolysis of both 4-nitrophenyl and 2,4-dinitrophenyl trifluoroacetate in 0.56 mol dm^{-3} water in acetonitrile[16] and close to that, a value of 2.5, determined in the present study for the hydrolysis of 3-nitrophenyl trifluoroacetate in 2.78 mol dm^{-3} water in

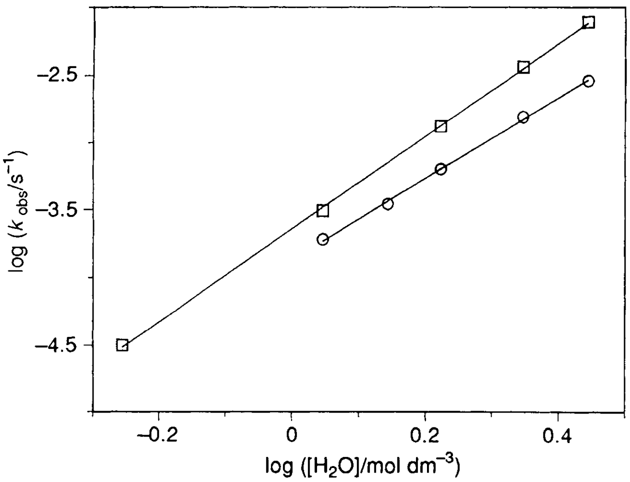
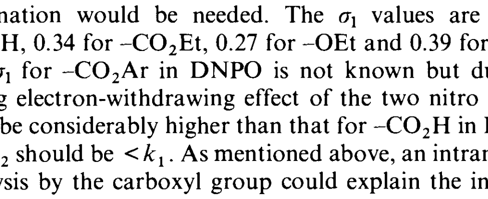


Fig. 1 Plots of log k_{obs} *versus* log[H_2O] for the hydrolysis of bis(2,4-dinitrophenyl) oxalate (○) and 4-nitrophenyl trifluoroacetate (□) [ref. (16)] in acetonitrile–water mixtures at 298.2 K

acetonitrile. As mentioned above, the phenyl esters of trifluoroacetic acid have been shown to be hydrolysed by the $B_{AC}3$ mechanism.[16] Deuterium oxide solvent isotope effects greater than 2 are usually observed for the neutral hydrolysis of alkyl, halogenoalkyl, and aryl carboxylates.[29b–e,36] In accordance with the water-catalysed reaction of water, the value of 3.25 was obtained for $k(H_2O)/k(D_2O)$ in the hydrolysis of 2-nitrophenyl ethyl oxalate in water.[11] In the light of the significant water isotope effect observed in this study, a substitution mechanism where a water molecule displaces the leaving group without catalysis cannot be considered for DNPO. The isotope effect clearly indicates a rate-limiting proton transfer and is consistent with the $B_{AC}3$ mechanism.

Activation Parameters.—Jennings and Capomacchia[13] determined the activation parameters for the hydrolysis of DNPO in 1.11 mol dm^{-3} water in acetonitrile. The values of $\Delta H^{\ddagger} = 22$ kJ mol^{-1} and $\Delta S^{\ddagger} = -242$ J mol^{-1} K^{-1} were obtained. These they compared with values determined, in water or in acetone–water and acetonitrile–water mixtures rich in water, for the neutral hydrolysis of different esters of monocarboxylic acids or for that of dimethyl oxalate. The lower values of $\Delta H^{\ddagger}$ obtained for DNPO was considered to support a 'concerted process' which they suggested for the hydrolysis reaction of this oxalate. In addition, the more negative $\Delta S^{\ddagger}$ for DNPO, in comparison with the monocarboxylic esters or dimethyl oxalate, was expected to reflect the increased structuring of the transition state of a concerted process. Jennings and Capomacchia[13] mentioned that the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are highly solvent dependent, but they had not found literature values concerning hydrolysis reactions in aqueous organic solvents with low content of water. However, for example, for the hydrolysis of phenyl trifluoroacetate, the value of $\Delta H^{\ddagger}$ is known to be 32.0 kJ in 19.4 mol dm^{-3} water in acetonitrile, the mol fraction of water $x(H_2O) = 0.606$, but decreases to 9.5 kJ mol^{-1} in 3.89 mol dm^{-3} water in acetonitrile, $x(H_2O) = 0.181$. For the same reaction $\Delta S^{\ddagger}$ is -172 J mol^{-1} K^{-1} in 19.4 mol dm^{-3} water in acetonitrile, but much more negative, -282 J mol^{-1} K^{-1} in 3.89 mol dm^{-3} water in acetonitrile.[37,38] Gopalakrishnan and Hogg[31] have determined the thermodynamic activation parameters for the water-catalysed hydrolysis reaction of 1-acetyl-3-methylimidazolium ion in the solvent region $x(H_2O) \geqslant 0.1$. The value of $\Delta H^{\ddagger}$ decreases from 42 to 26 kJ mol^{-1} and that of $\Delta S^{\ddagger}$ from -128 to 218 J mol^{-1} K^{-1} as the solvent is changed from water to acetonitrile–water with $x(H_2O) = 0.1$. This remarkable influence of solvent on activation parameters is mainly due to the different response of the thermodynamic functions of transfer of the reactant state and the transition state to the solvent composition.[37,39] The comparison of activation parameters at different solvent compositions must therefore be done carefully. For 4-nitrophenyl and 2,4-dinitrophenyl trifluoroacetates the decrease of the hydrolysis rate, *i.e.* the increase in $\Delta G^{\ddagger}$, with increasing acetonitrile content of the reaction mixture in the region $[H_2O] \leq 2.78$ mol dm^{-3}, the solvent region now under consideration, involves partially compensating behaviour of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ and the values of the latter parameters are highly solvent dependent on solvent composition.[37] For example for 2,4-dinitrophenyl trifluoroacetate $\Delta H^{\ddagger}$ is as small as 4.3 kJ mol^{-1} in 1.11 mol dm^{-3} water in acetonitrile but 13.8 kJ mol^{-1} in 0.56 mol dm^{-3} water in acetonitrile. At the same time the value of $\Delta S^{\ddagger}$ changes from -262 to -248 J mol^{-1} K^{-1}.[37] The value of $\Delta H^{\ddagger}$ obtained for the hydrolysis of DNPO, 22 kJ mol^{-1}, is in fact higher and the value of $\Delta S^{\ddagger}$, -242 J mol^{-1} K^{-1}, less negative than the corresponding values determined for aryl trifluoroacetates under the same reaction conditions. However, keeping in mind their solvent sensitivity, the parameters are alike enough that the same hydrolysis mechanism can be assumed for DNPO and for substituted phenyl trifluoroacetates. The highly negative values of $\Delta S^{\ddagger}$ obviously indicate a preferential solvation of the transition state by water and seem to be typical for water-catalysed hydrolyses in acetonitrile–water mixtures with low water content, and presumably in dipolar aprotic solvent–water mixtures in general.[31,37] If both ester groups of DNPO would react simultaneously, a considerably more negative value of $\Delta S^{\ddagger}$ should be observed because of the increased structuring of the transition state.

Mechanistic Considerations.—In view of the close similarity of the water isotope effect, the activation parameters, and the slopes of the log k *versus* log[H_2O] plots for DNPO and aryl trifluoroacetates, it seems evident that they react by a similar mechanism. Consequently, the hydrolysis of one of the two ester groups by the $B_{AC}3$ mechanism is suggested for the first step of the hydrolysis of DNPO (Scheme 5). However, if it were assumed that the reaction follows Scheme 4 with $k_2 > k_1$, an explanation would be needed. The σ_I values are 0.39 for $-CO_2H$, 0.34 for $-CO_2Et$, 0.27 for $-OEt$ and 0.39 for $-OPh$.[40] The σ_I for $-CO_2Ar$ in DNPO is not known but due to the strong electron-withdrawing effect of the two nitro groups it must be considerably higher than that for $-CO_2H$ in DNPHO, and k_2 should be $< k_1$. As mentioned above, an intramolecular catalysis by the carboxyl group could explain the increase of the rate of the second step.

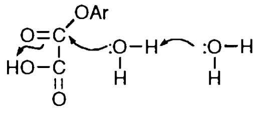


Scheme 5

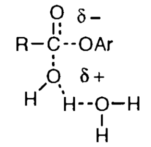
In considering the role of intramolecular general-base catalysis in the hydrolysis of DNPHO by $-CO_2^-$, the reaction of DNPO (5×10^{-5} mol dm^{-3}) was studied in 2.47 mol dm^{-3} water in acetonitrile in the presence of trifluoroacetic acid. The rate coefficients $(1.92 \pm 0.03) \times 10^{-3}$, $(1.92 \pm 0.02) \times 10^{-3}$ and $(1.93 \pm 0.02) \times 10^{-3}$ s^{-1} were observed with 9.39×10^{-4}, 1.88×10^{-3} and 6.68×10^{-3} mol dm^{-3} trifluoroacetic acid, respectively. The reactions nicely followed first-order kinetics.

With the aid of the log k_{obs} *versus* log[H_2O] plot a value of 2.03×10^{-3} s^{-1} can be calculated for the rate coefficient in the absence of trifluoroacetic acid. In scanning drive in the presence of trifluoroacetic acid, the isosbestic points between the reactants and products were similar to those observed in the absence of the acid. The formation of a monoester was not observed. Trifluoroacetic acid does not seem to affect the hydrolysis reaction of DNPO. The pK_a value of trifluoroacetic acid in acetonitrile is 12.65 and those of oxalic acid are 14.5 and 27.7.[41] The pK_a value of DNPHO in acetonitrile or in water is not known. In water the pK_a values of malonic acid are 2.83 and 5.69[42] and that of 4-nitrophenyl hydrogen malonate falls between them, being 3.15.[19] Obviously trifluoroacetic acid is, in the present reaction conditions, more acidic than DNPHO. An estimation of the relative electronic effects of CF_3– and $C_6H_4(NO_2)_2CO_2$– groups may be obtained from the ratio of the rate coefficients for the hydrolysis reactions of 2,4-dinitrophenyl trifluoroacetate (1.937×10^{-2} s^{-1})[16] and that of DNPO (1.91×10^{-4} s^{-1}, Table 1) in 1.11 mol dm^{-3} H_2O in acetonitrile. Trifluoroacetic acid was used in moderate excess (19, 38 and 134 fold) over DNPO. In these conditions the $-CO_2H/-CO_2^-$ ratio of DNPHO should be crucially different to that existing in the absence of trifluoroacetic acid. When the concentration of the dissociated form of DNPHO diminishes, the contribution of catalysis by $-CO_2^-$ is also diminished. Hence, the ratio k_2/k_1 should be changed, and some detectable effect on the reaction is expected if $-CO_2^-$ would be the catalytic species. Although the insignificance of the acidic additive on the measured reaction does not disprove the occurrence of the intramolecular general-base catalysis by $-CO_2^-$ in the second step, faster than the observed step, leaves the catalysis doubtful.

The carboxyl group could catalyse the hydrolysis of DNPHO also in its undissociated form in helping the addition step, as shown in **4** or in helping the departure of the leaving group as shown in **2**. In the hydrolysis of monosubstituted phenyl trifluoroacetates the formation of the addition intermediate obviously is rate-limiting. Alternatively, if the reaction goes through a transition state like **5** only little C–OAr bond breaking has occurred,[16] although for the neutral hydrolysis of 4-nitrophenyl dichloroacetate in aqueous solution a transition state like **5** has been proposed.[39] DNPO and DNPHO contain a better leaving group and a less electron-withdrawing acyl group than monosubstituted phenyl trifluoroacetates. These structural differences should make the departure of the ArO- group easier. Therefore, if the catalysis occurs, it should be in the addition step. Intermolecular acid catalysis by trifluoroacetic acid was, however, not observed.



4



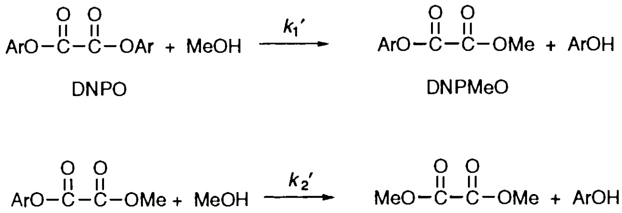
5

The possibilities of intramolecular catalysis are fascinating but they do not satisfactorily explain the formation of carbon dioxide and carbon monoxide, nor the absence of oxalic acid discovered by Orosz and Dudar[14] with the aid of IR analysis. In the present study, we also tried to detect oxalic acid in the reaction solution. From a mixture of 1×10^{-3} mol dm^{-3} oxalic acid and 1×10^{-3} mol dm^{-3} 2,4-dinitrophenol in 2.78 mol dm^{-3} water in acetonitrile a white precipitation, assumed to be calcium oxalate, was obtained by the addition of one drop of a 0.01 mol dm^{-3} solution of calcium chloride in 70% (v/v) acetonitrile–water into 1 cm^3 of the mixture. However, the addition of the calcium chloride solution into the reaction solution after the decomposition of 1×10^{-3} mol dm^{-3} DNPO in 2.78 mol dm^{-3} water in acetonitrile did not reveal the presence of oxalic acid. Again the addition of oxalic acid to reach a concentration of 1×10^{-3} mol dm^{-3} in this mixture gave a white precipitation. Further, it was verified that the added oxalic acid could be detected from the reaction mixture after a prolonged time. In agreement with the results of Orosz and Dudar,[14] these experiments indicate that oxalic acid is not formed at all in the hydrolysis of DNPO.

Orosz and Dudar[14] suggested 'an unexpected decomposition' to DNP, CO_2, CO, and possibly to other gaseous products, rather than hydrolysis, for the reaction of DNPO in aqueous acetonitrile or tetrahydrofuran with low water content, but a normal biphasic hydrolysis reaction (with $k_1 > k_2$ in Scheme 4) for diaryl oxalates of low reactivity. They, however, express that 'the interdependence of these two processes cannot be excluded'. In accordance with this statement, it is now suggested that the second step in the decomposition of DNPO, following the hydrolysis of one ester group, consists of successive decarboxylation and decarbonylation of 2,4-dinitrophenyl hydrogen oxalate, forming carbon dioxide, carbon monoxide and 2,4-dinitrophenol. The strongly electron-withdrawing substituents of the aryloxy group make this process conceivable. The non-ionized 2,4,6-trinitrobenzoic acid is stable toward decarboxylation, resembling the behaviour of aliphatic α-nitro acids and trihaloacetic acids, but the anion decarboxylates easily.[28] The rate coefficient for the decarboxylation of 2,4,6-trinitrobenzoate ion in ethyl alcohol at 35 °C is *ca.* 7×10^{-5} s^{-1}.[43] On the other hand, although it is known that the monoanion of oxalic acid does not lose carbon dioxide as easily as the acid itself, the decarboxylation can take place in both the ionized and non-ionized form. The rate coefficient for the decarboxylation of oxalic acid at 100 °C is 2.24×10^{-7} s^{-1} in quinoline and 4.78×10^{-6} s^{-1} in *m*-cresol.[28] The fact that the added trifluoroacetic acid does not have an effect on the present reaction indicates that the decomposition of DNPHO can proceed through the un-ionized form.

If it is assumed that the decomposition of DNPHO to CO_2, CO, and DNP is faster than the first hydrolysis step of DNPO—and also faster than hydrolysis of DNPHO to oxalic acid and DNP—the simultaneous formation of 2 mol of 2,4-dinitrophenol from 1 mol of DNPO is explained. This reaction route also is in agreement with the observation by Orosz and Dudar[14] that the rate of formation of CO_2 is equal to the rate of disappearance of DNPO. However, even this mechanism leaves unexplained the fact that in the measurements performed by Orosz and Dudar[14] the ratio of carbon dioxide and carbon monoxide was not 1 : 1 but it was between 1 : 1.7 and 1 : 2.2.

In evaluating the proposed reaction mechanism, 2,4-dinitrophenyl methyl oxalate (DNPMeO) was considered as a suitable model, lacking the ability to decompose *via* decarboxylation, decarbonylation or intramolecular catalysis. It was thought to be possible to prepare DNPMeO *in situ* by allowing DNPO to react in methanolic acetonitrile. The methanolysis of DNPO was studied in acetonitrile containing 1.67 mol dm^{-3} of methanol. Due to the methoxide ion reaction, it was necessary to study the neutral methanolysis in an acidified solution. When scanning the UV spectrum of the reaction solution (5×10^{-5} mol dm^{-3} DNPO and 1.58×10^{-3} mol dm^{-3} trifluoroacetic acid in 1.67 mol dm^{-3} methanol in acetonitrile) repetitively, a consecutive process could be identified. A slow step was preceded by a fast step. The difference in the rate of the two steps was large enough for the appearance of a distinct isosbestic point at 254 nm. The reaction was thought to involve the methanolysis of DNPO as given in Scheme 6, k_1' being $> k_2'$. The decrease in UV absorption at around 240 nm due to the disappearance of DNPO was accompanied by an increase of the absorption at around 260 and 290 nm in accordance with the formation of 2,4-dinitrophenol. The UV spectrum of the solution after the fast step showed a maximum at 254–255 nm. The form of the spectra was similar to that obtained for a solution containing 5×10^{-5} mol dm^{-3} of DNPAc and 5×10^{-5} mol dm^{-3} of DNP in acetonitrile, and showing absorption both in the 'ester region' at around 230–245 nm and in the '2,4-dinitrophenol region' at around 260 and 290 nm with a maximum at 255 nm. This is in agreement with the assumption that 2,4-dinitrophenyl methyl oxalate occurs as an intermediate on the reaction path. The absorption spectrum of DNPAc can be expected to be almost identical to that of DNPMeO. After the slow step of the reaction the UV spectrum of the solution showed a maximum at 259–260 nm and fitted for a solution containing 1×10^{-4} mol dm^{-3} of DNP. The contribution of the second expected reaction product, dimethyl oxalate, can be neglected in the UV spectrum due to its low absorptivity.*



$$\mathrm{ArO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OAr} + \mathrm{MeOH} \xrightarrow{k_1'} \mathrm{ArO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OMe} + \mathrm{ArOH}$$

DNPO DNPMeO

$$\mathrm{ArO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OMe} + \mathrm{MeOH} \xrightarrow{k_2'} \mathrm{MeO{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OMe} + \mathrm{ArOH}$$

Scheme 6

The consecutive methanolysis process observed makes the assumption of the biphasic nature of the hydrolysis of DNPO quite reasonable. For the methanolysis of DNPO in 1.67 mol dm^{-3} methanol in acetonitrile, similar rate coefficients (k_1' in Scheme 6) were obtained with 1.58×10^{-3} and 1.58×10^{-2} mol dm^{-3} trifluoroacetic acid $(1.57 \pm 0.07) \times 10^{-3}$ and $(1.60 \pm 0.11) \times 10^{-3}$ s^{-1}, respectively. A value of $(2.56 \pm 0.19) \times 10^{-5}$ s^{-1} was determined for k_2' in Scheme 6 with 1.58×10^{-3} mol dm^{-3} trifluoroacetic acid. In the consecutive methanolysis process k_1' is now *ca.* 60 × k_2', in agreement with the higher inductive effect of $-CO_2Ar$ when compared with $-CO_2Me$. Solvolysis of the first ester group of DNPO is 2.5 times faster in 1.67 mol dm^{-3} methanol than in 1.67 mol dm^{-3} water in acetonitrile, reflecting the higher nucleophilicity of the lower alcohols in comparison with water. If the same ratio is valid for the reactions of DNPMeO, a value of 1.1×10^{-5} s^{-1} can be evaluated for the rate coefficient of the hydrolysis of DNPMeO in 1.67 mol dm^{-3} H_2O in acetonitrile. The value of k_{obs} obtained for the hydrolysis of DNPO in 1.67 mol dm^{-3} H_2O in acetonitrile is 6.26×10^{-4} s^{-1} and it is assumed to represent k_1 in Scheme 4. The rate coefficient for the hydrolysis of DNPHO should be very similar to the rate coefficient for the hydrolysis of DNPMeO in the light of the close similarity of σ_I values of $-CO_2H$ (0.39) and $-CO_2Me$ (0.34)—if intramolecular catalysis does not occur.

Conclusions.—A 'concerted reaction mechanism' where both ester groups react simultaneously[13] or 'an unexpected decomposition'[14] have previously been suggested for the hydrolysis of DNPO in aqueous acetonitrile with low water content. A consecutive hydrolysis mechanism is observed for less reactive oxalate esters in aqueous solution or in aqueous organic solvents.[7,10–12,14] On the basis of the literature data and the present results, a consecutive reaction mechanism is also proposed for the reaction of DNPO. The hydrolysis of one of the ester groups is obviously followed by a fast decomposition of the intermediate, 2,4-dinitrophenyl hydrogen oxalate, through successive decarboxylation and decarbonylation processes rather than by another hydrolysis step. The stepwise reaction mechanism is in agreement with the concept of the occurrence of an aryl hydroperoxy oxalate as an intermediate in a chemiluminescence reaction between DNPO and hydrogen peroxide.

---

* The molar absorptivity of dimethyl oxalate must be very close to that of diethyl oxalate [λ_{max} 225 nm (ε/dm^3 mol^{-1} cm^{-1} 400)].[44]

References

1 See *e.g.* S.-I. Kobayashi and K. Imai, *Anal. Chem.*, 1980, **52**, 424; K. Honda, K. Miyaguchi and K. Imai, *Anal. Chim. Acta*, 1985, **177**, 103; N. Wu, W. J. Horvath, and C. W. Huie, *Anal. Chim. Acta*, 1992, **269**, 99; K. Robards and P. J. Worsfold, *Anal. Chim. Acta*, 1992, **266**, 147; P. J. M. Kwakman and U. A. Th. Brinkman, *Anal. Chim. Acta*, 1992, **266**, 175 and refs. therein.

2 (*a*) M. M. Rauhut, L. J. Bollyky, B. G. Roberts, M. Loy, R. H. Whitman, A. V. Ianotta, A. M. Semsel and R. A. Clarke, *J. Am. Chem. Soc.*, 1967, **89**, 6515; (*b*) M. M. Rauhut, *Acc. Chem. Res.*, 1969, **2**, 80.

3 F. McCapra, *Prog. Org. Chem.*, 1973, **8**, 231.

4 C. L. R. Catherall, T. F. Palmer and R. B. Cundall, *J. Chem. Soc., Faraday Trans. 2*, 1984, **80**, 823, 837.

5 F. J. Alvarez, N. J. Parekh, B. Matuszewski, R. S. Givens, T. Higuchi and R. L. Schowen, *J. Am. Chem. Soc.*, 1986, **108**, 6435.

6 M. Orlović, R. L. Schowen, R. S. Givens, F. Alvarez, B. Matuszewski and N. Parekh, *J. Org. Chem.*, 1989, **54**, 3606.

7 G. Orosz, *Tetrahedron*, 1989, **45**, 3493.

8 R. N. Jennings and A. C. Capomacchia, *Anal. Chim. Acta*, 1989, **227**, 37.

9 (*a*) G. Orosz, K. Torkos and J. Borossay, *Acta Chim. Hung.*, 1991, **128**, 911; (*b*) G. Orosz, R. S. Givens and R. L. Schowen, *Anal. Chim. Acta*, 1992, **266**, 219.

10 M. L. Bender and Y.-L. Chow, *J. Am. Chem. Soc.*, 1959, **81**, 3929.

11 T. C. Bruice and B. Holmquist, *J. Am. Chem. Soc.*, 1967, **89**, 4028.

12 J. Vuori and J. Koskikallio, *Suomen Kemistil. B*, 1969, **42**, 136.

13 R. N. Jennings and A. C. Capomacchia, *Anal. Chim. Acta*, 1988, **205**, 207.

14 G. Orosz and E. Dudar, *Anal. Chim. Acta*, 1991, **247**, 141.

15 H. Neuvonen, *J. Chem. Soc., Perkin Trans. 2*, 1987, 159.

16 H. Neuvonen, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1141.

17 A. A. Frost and R. G. Pearson, *Kinetics and Mechanisms*, Wiley, New York, 1961, 2nd edn., ch. 3, p. 27.

18 A. J. Kirby and G. J. Lloyd, *J. Chem. Soc., Perkin Trans. 2*, 1976, 1753.

19 A. R. Fersht and A. J. Kirby, *J. Am. Chem. Soc.*, 1967, **89**, 4857.

20 J. W. Thanassi and T. C. Bruice, *J. Am. Chem. Soc.*, 1966, **88**, 747.

21 E. Gaetjens and H. Moravetz, *J. Am. Chem. Soc.*, 1960, **82**, 5328.

22 T. C. Bruice and A. Turner, *J. Am. Chem. Soc.*, 1970, **92**, 3422.

23 A. J. Parker, *Chem. Rev.*, 1969, **69**, 1.

24 E. M. Menger, S. Wrenn and H.-S. Rhee, *Bioorg. Chem.*, 1975, **4**, 194.

25 A. Ågren, U. Hedston and B. Jonnson, *Acta Chem. Scand.*, 1961, **15**, 1532.

26 F. M. Menger and J. H. Smith, *J. Am. Chem. Soc.*, 1969, **91**, 5346.

27 A. J. Kirby, in *Comprehensive Chemical Kinetics*, eds. C. H. Bamford and C. F. H. Tipper, Elsevier, Amsterdam, 1972, vol. 10, ch. 2, p. 57.

28 L. W. Clark, in *The Chemistry of Carboxylic Acids and Esters*, ed. S. Patai, Interscience, London, 1969, ch. 12, p. 589.

29 (*a*) S. L. Johnson, *Adv. Phys. Org. Chem.*, 1967, **5**, 237; (*b*) E. K. Euranto, in *The Chemistry of Carboxylic Acids and Esters*, ed. S. Patai, Interscience, London, 1969, ch. 11, p. 505; (*c*) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, 1961, **83**, 1743; E. K. Euranto and N. J. Cleve, *Acta Chem. Scand.*, 1963, **17**, 1584; (*d*) T. H. Fife and D. M. McMahon, *J. Am. Chem. Soc.*, 1969, **91**, 7481; (*e*) J. F. J. Engbersen and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1975, **97**, 1563.

30 W. S. Muney and J. F. Coetzee, *J. Phys. Chem.*, 1962, **66**, 89; M. K. Chantooni, Jr. and I. M. Kolthoff, *J. Am. Chem. Soc.*, 1967, **89**, 1582; I. D. Kuntz, Jr. and C. J. Cheng, *J. Am. Chem. Soc.*, 1975, **97**, 4852; A. J. Easteal, *Aust. J. Chem.*, 1979, **32**, 1379.

31 G. Gopalakrishnan and J. L. Hogg, *J. Org. Chem.*, 1983, **48**, 2038.

32 D. Geheb, N. F. Kasanskaja and I. W. Beresin, *Ber. Bunsenges. Phys. Chem.*, 1972, **76**, 160.

33 N. J. Cleve, *Suom. Kemistil. B*, 1972, **45**, 235; N. J. Cleve, *Ann. Acad. Sci. Fennicae, Ser. A II*, 1972, No. 167; N. J. Cleve, *Suom. Kemistil. B*, 1972, **45**, 385.

34 E. Tommila, *Acta Chem. Scand.*, 1955, **9**, 975; J. W. Henderson and P. Haake, *J. Org. Chem.*, 1977, **42**, 3989; J. Shaskus and P. Haake, *J. Org. Chem.*, 1983, **48**, 2036.

35 C. H. Langford and J. P. K. Tong, *Acc. Chem. Res.*, 1977, **10**, 258; J. R. Haak and J. B. F. N. Engberts, *J. Org. Chem.*, 1988, **49**, 2387.

36 E. K. Euranto and N. J. Cleve, *Suom. Kemistil. B*, 1970, **43**, 147; W. P. Huskey, C. T. Warren and J. L. Hogg, *J. Org. Chem.*, 1981, **46**, 59.

37 H. Neuvonen, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1723.

38 H. Neuvonen, unpublished results as regards the response of $x(H_2O)$ and the concentration of water in acetonitrile–water mixtures.

39 J. B. F. N. Engberts, in *Water. A Comprehensive Treatise*, ed. F. Franks, Plenum, New York, 1979, vol. 6, ch. 4, p. 139.

40 M. Charton, *J. Org. Chem.*, 1964, **29**, 1222.

41 K. Izutsu, *Acid-Base Dissociation Constants in Dipolar Aprotic Solvents*, Blackwell, Oxford, 1990, p. 17.

42 *Handbook of Tables for Organic Compounds Identification*, 3rd edn., comp., Z. Rappoport, Chemical Rubber Co., Cleveland, 1967, p. 430.

43 F. H. Verhoeck, *J. Am. Chem. Soc.*, 1939, **61**, 186.

44 See *Handbook of Chemistry and Physics*, 55th edn., ed. R. C. Weast, CRC Press, Cleveland, 1974, p. C-3.

Paper 3/05086F
Received 23*rd August* 1993
Accepted 21*st September* 1993