Tetra-alkylammonium Carboxylate and Imidazole Promoted Esterolysis in Benzene. A Catalytic Model related to the Charge Relay System of Trypsin Enzymes

By **Umberto Tonellato**, Centro ' Meccanismi di Reazioni Organiche ' del C.N.R., Istituto di Chimica Organica, Università di Padova, 35100 Padova, Italy

The esterolysis of *p*-nitrophenyl propionate in benzene in the presence of 3—4 × 10^{-3}M amounts of imidazole is very effectively promoted by addition of tetra-alkylammonium carboxylates. The esterolysis rate in the presence of 3—4 × 10^{-3}M amounts of tetra-alkylammonium carboxylates is enhanced by addition of imidazole up to the point where their molar ratio is *ca.* 1 : 1 and then inhibited by further addition. From the analysis of the kinetic effects observed, including those of water, it is suggested that the main catalytic system is a preformed hydrogen-bonded carboxylate ion–imidazole pair which utilizes the nucleophilic and basic properties of the unsolvated RCO_2^- anion through the relay action of the imidazole.

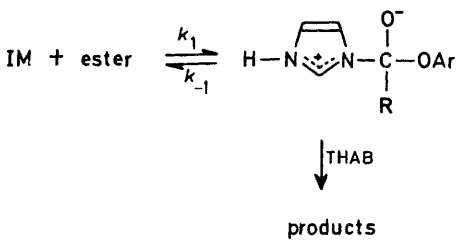
THE unique juxtaposition of the carboxy-, imidazole, and hydroxy-functions of the Asp-102, His-57, and Ser-195 residues in the active site of α-chymotrypsin is regarded [1] as the key to the charge-relay mode of activity of this type of hydrolytic enzyme. In particular, the interaction between the carboxy-group and the imidazolyl residue occurs in a buried depression on the enzyme surface: the environment of the CO_2H function is entirely hydrophobic.[2] The importance of the latter point in revealing possible co-operation between carboxylate ions and imidazole in simple models was first emphasized by Haake and his co-workers.[3] More

[1] (*a*) D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature*, 1969, **221**, 337; (*b*) D. M. Blow, *Accounts Chem. Res.*, 1976, **9**, 145 and references therein.

[2] J. J. Birktoft and D. M. Blow, *J. Mol. Biol.*, 1972, **68**, 187.

[3] G. Wallerberg, J. Boger, and P. Haake, *J. Amer. Chem. Soc.*, 1971, **93**, 4938.

recently, Menger and Vitale [4] reported large catalytic effects due to tetrahexylammonium benzoate (THAB) in the imidazolysis of *p*-nitrophenyl acetate in toluene. Following their mechanistic hypothesis, illustrated in Scheme 1, the carboxylate anion, by removing the proton



SCHEME 1

from the tetrahedral intermediate, strongly favours its partitioning to products.

Our preliminary results on the effect of cetyltrimethylammonium propionate (CTAP) on the imidazolysis of *p*-nitrophenyl propionate (PNPP) indicated that Scheme 1 is not entirely adequate and it was suggested [5] that a major role in the catalytic effects was played by the ion–molecule pair carboxylate–imidazole. Stimulated by this evidence of co-operative interaction and also by the current interest [6,7] in the use of ammonium salts in aprotic solvents, the present study is a kinetic investigation of the esterolysis of PNPP in benzene in the presence of imidazole and/or tetra-alkylammonium carboxylates, $RCO_2^-Q^+$. These were made of different cations, tetrabutylammonium (TBA), tetrahexylammonium (THA), CTA, cetyl(ethyl)(methyl)hydroxyethylammonium (CEMAH),[8] and anions, propionate (P), myristate (M), benzoate (B), and *m*-chlorobenzoate (*m*CB). A kinetic investigation of the reaction between imidazole and PNPP in benzene in the absence of salts has been presented.[9]

RESULTS

The salts used have different physical properties: THAB, THA*m*CB, THAP, and TBAM are liquids of decreasing solubility in benzene whereas TBAP, CEMAHP, and CTAP are solids moderately to slightly soluble in benzene. The liquid salts could only be obtained in the (approximately) hemihydrate form, whereas the solid compounds could be dried after prolonged standing *in vacuo* over P_2O_5.

When excess of $RCO_2^-Q^+$ is added to PNPP in benzene, the solution turns yellow due to the liberation of *p*-nitrophenol (anion; $\lambda_{max.}$ 410—415 nm). The reaction between the salts and PNPP can be reasonably formulated as shown in equation (1). Propionic anhydride was detected by

$$Q^+RCO_2^- + RCO{\cdot}OAr \rightleftharpoons RCO{\cdot}O{\cdot}COR + Q^{+-}OAr \xrightarrow{H_2O} RCO_2H + Q^+(RCO_2 \cdot\cdot H{\cdot}OAr)^- \quad (1)$$

^{1}H n.m.r. in C_6D_6 when carefully dried TBAP (5×10^{-2}M) and PNPP (3×10^{-2}M) were allowed to stand for one day at room temperature. The anhydride could not be detected when THAP hemihydrate was used. Control experiments carried out by mixing equimolar (0.1M) amounts of THAP, PNPP, and propionic anhydride showed that the anhydride hydrolysis occurs faster than PNPP esterolysis. Therefore, it can be safely assumed that, under the kinetic conditions ($[PNPP]_0 = 2—4 \times 10^{-5}$M), the ultimate products are those of hydrolysis.

The reaction between imidazole (IM) and PNPP in benzene was described [9] as the reversible formation of *p*-nitrophenol and propionylimidazole. Addition of RCO_2^--Q^+ greatly increases the rate of formation of *p*-nitrophenol (it appears half-ionized from u.v. spectra, $\lambda_{max.}$ 310 [9] and 410 nm, in the presence of a 20-fold excess of RCO_2^-, with

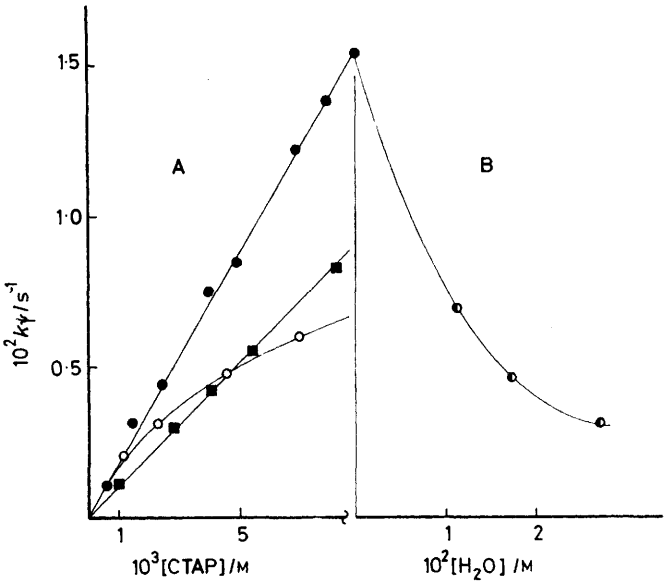


FIGURE 1 The effect of water for solutions containing 1.9×10^{-2}M-imidazole. A, Plot of $k\psi$ against [CTAP]: ● anhydrous in 'dry' benzene; ○ hydrated in 'dry' benzene; ■ anhydrous in moist (8.5×10^{-3}M added water) benzene. B, Plot of $k\psi$ against added [H_2O] for a 8.8×10^{-3}M-CTAP solution in benzene

[IM] 2×10^{-2}M). ^{1}H N.m.r. experiments indicated that propionylimidazole and, perhaps, propionic anhydride were formed by reaction between TBAP (5×10^{-2}M), PNPP, and IM (both 3×10^{-2}M). Analogous experiments carried out with THAP hemihydrate (0.1M) showed the formation of ester hydrolysis products. Therefore, also under the conditions of the kinetic runs where both IM and $RCO_2^-Q^+$ were present, PNPP is irreversibly hydrolysed.

Kinetics.—The observed rate constants k_ψ were measured by following the appearance of *p*-nitrophenol under pseudo-first-order conditions at 25 °C.

The preliminary kinetic runs were aimed at defining the sensitivity of the system toward water. Although benzene was carefully dried,[9] the solutions used for kinetic experi-

[4] F. M. Menger and A. C. Vitale, *J. Amer. Chem. Soc.*, 1973, **95**, 4931.

[5] F. D'Andrea and U. Tonellato, *J.C.S. Chem. Comm.*, 1975, 659.

[6] See, for reversed micelles, J. H. Fendler and E. J. Fendler, 'Catalysis in Micellar and Macromolecular Systems', Academic Press, New York, 1975; J. H. Fendler, *Accounts Chem. Res.*, 1976, **9**, 153.

[7] See, for phase transfer catalysis, E. V. Dehmlow, *Angew. Chem. Internat. Edn.*, 1974, **13**, 170; M. Makosza in 'Modern Synthetic Methods 1976', ed. R. Scheffold, Schweizericher Chemiker Verbund, 1976.

[8] U. Tonellato, *J.C.S. Perkin II*, 1977, 821.

[9] F. Rivetti and U. Tonellato, *J.C.S. Perkin II*, 1977, 1176.

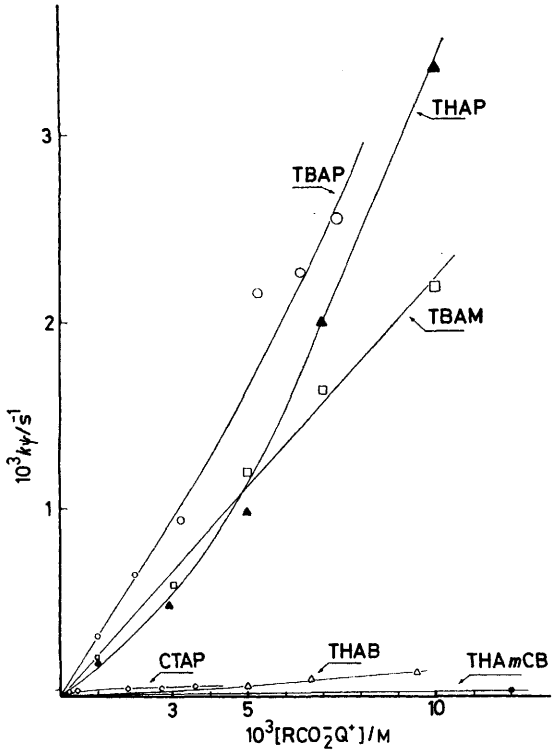


FIGURE 2 Plot of k_ψ against $[RCO_2^-Q^+]$ in the absence of IM. $RCO_2^-Q^+$: □ TBAM; ▲ THAP; ○ TBAP; △ THAB; ■ THA*m*CB; ◇ CTAP

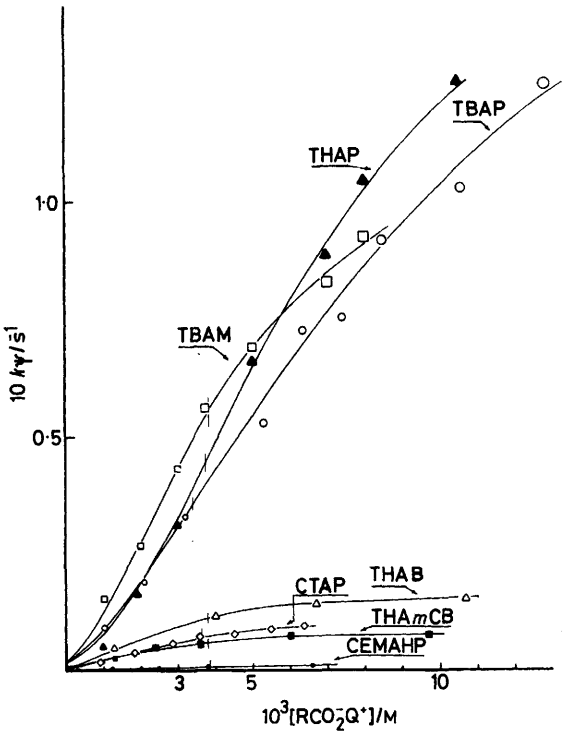


FIGURE 3 Plot of k_ψ against $[RCO_2^-Q^+]$ in the presence of imidazole. $RCO_2^-Q^+$, 10^3M-IM: □ TBAM, 3.83; ▲ THAP, 3.75; ○ TBAP, 3.40; △ THAB, 3.82; ■ THA*m*CB, 3.82; ◇ CTAP, 3.65; ○ CEMAHP, 3.07

ments were estimated to be at least 1—3 × 10^{-3}M in water. The esterolysis rate constants decrease with increasing water concentration: the effect is illustrated in Figure 1 from data obtained for solutions containing IM and either dry CTAP, or hydrated CTAP, or dry CTAP and measured amounts of added water. The k_ψ value decreases approximately two-fold by addition of 0.01M-H_2O: virtually the same results were also obtained for solutions of other salts alone or mixed with IM. We discuss here only much larger rate effects than those observed for addition of water, yet its relevance will not be ignored.

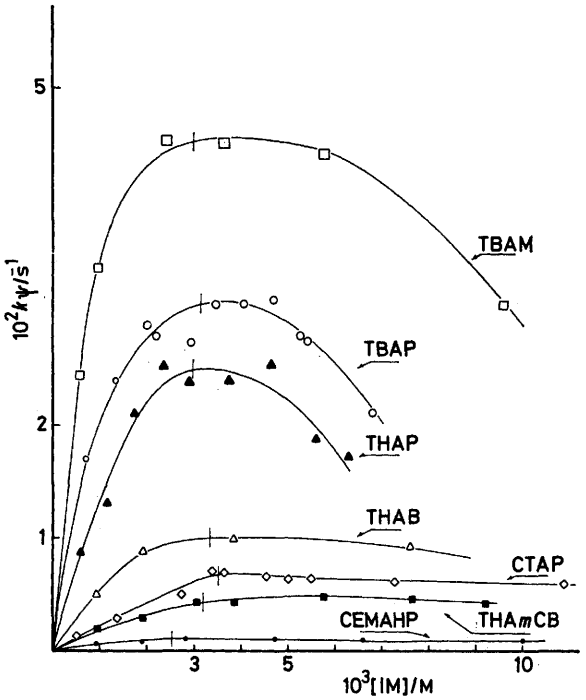


FIGURE 4 Plot of k_ψ against [IM] in the presence of $RCO_2^-Q^+$. 10^3M-$RCO_2^-Q^+$: □ 3.05 TBAM; ▲ 3.03 THAP; ○ 3.18 TBAP; △ 3.35 THAB; ■ 3.05 THA*m*CB; ◇ 3.45 CTAP; ○ 2.65 CEMAHP

The observed rate constants for PNPP esterolysis are shown:[10] in Figure 2 as a function of $[RCO_2^-Q^+]$ in the absence of IM; in Figure 3 as a function of $[RCO_2^-Q^+]$ in the presence of constant [IM]; and in Figure 4 as a function of [IM] in the presence of constant $[RCO_2^-Q^+]$. The lines in the Figures are only intended to connect the points for each salt and make the graphs readable.

DISCUSSION

The major kinetic observations are briefly summarized as follows.

(1) The enhancement of the PNPP esterolysis rate due to addition of $RCO_2^-Q^+$ salts to a solution containing imidazole is very large indeed. Thus, the reaction is 5 × 10^5 times faster for a 3.4 × 10^{-3}M-IM solution in the presence of 1.3 × 10^{-2}M-THAP than in its absence. On the other hand, the rate increase observed by addition of IM to a solution of $RCO_2^-Q^+$ is not so spectacular. Thus the rate is 35-fold faster for a 1 × 10^{-2}M-THAP

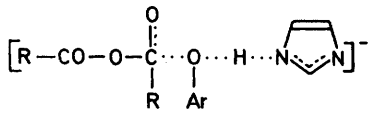
[10] Most of the data are listed in M. Lorenzin, Doctoral Thesis, University of Padova, 1976.

solution when 3.4×10^{-3}M-IM is added. There is a vague similarity between Figures 2 and 3 given the difference of less than two orders of magnitude in the ordinate scale which indicates the amplifying effect of $3—4 \times 10^{-3}$M-IM.

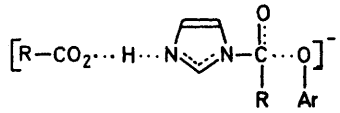
(2) Addition of IM to a $RCO_2^-Q^+$ solution increases the esterolysis rate up to [IM] = *ca.* $[RCO_2^-Q^+]$; further addition (see Figure 4) is virtually ineffective or decreases the observed rate. The plot of k_ψ against $[RCO_2^-Q^+]$ at constant [IM] (see Figures 1 and 3) is virtually linear (CTAP) or slightly convex (THAP, TBAM) up to $[RCO_2^-Q^+]$ = *ca.* [IM] and then displays a more or less pronounced concave curvature. A similar trend was described by Menger and Vitale [4] for the esterolysis of PNPA in toluene in the presence of constant [IM] and increasing amounts of THAP: in this case the point ([THAB] 0.1M) is reached where the corrected (see below) k_ψ value becomes independent of the salt concentration.

(3) The kinetic effects of $RCO_2^-Q^+$ depend on the nature of both cations and anions. Cations can be arranged in the order THA = TBA > CTA > CEMAH, whereas, for anions, the sequence is P = M > B > *m*CB and follows the order of the pK_a value of the conjugate acids (in water).

Point (1) and reports [11,12] on the nucleophilic reactivity of carboxylate ions in aprotic solvents give rise to a preliminary question: which is the nucleophilic reactant and which the catalyst when both imidazole and carboxylate salts are present? The two most reasonable transition states (I) and (II) illustrate the point: (I)



(I)



(II)

would imply nucleophilic attack by a free carboxylate ion while imidazole is the acid catalyst; (II) would involve nucleophilic attack by the imidazole and base catalysis due to RCO_2^-. Experimental evidence and theoretical arguments make structure (I) unlikely. (a) In the esterolysis of substituted phenyl esters in aprotic solvents promoted by imidazole,[4] amines,[13] and azide ion [13] the rate-limiting step is the expulsion of the unprotonated phenoxide ion from a tetrahedral intermediate, as indicated by the very large (4—6) ρ values. (b) Addition of acidic substances inhibit [4,13] the esterolysis rate in the presence of RCO_2^-. (c) Assuming as previously discussed [9,14] that the transition state is not at equilibrium with other components with respect to proton transfer, structure (I) would require an imidazole molecule hydrogen-bonded to a neutral ester molecule, undergoing nucleophilic attack by a ' bare ' carboxylate anion, rather than to the anion itself. Thus, structure (II) is much more reasonable at least when $[RCO_2^-] >$ [IM]. When excess of imidazole is used and acid catalysis is also possible, then (see Figure 4) inhibition is observed.

Therefore, transition state (II) is indicated as the main pathway for the kinetic effects observed when both IM and $RCO_2^-Q^+$ are present and, according to Menger and Vitale,[4] the ' salt + ester ' reaction can be taken as a side-process.

RCO_2^- anions can hardly be assumed to be ' bare ' species when hydrogen-bond donors are present in solution.[15] A simple interpretation of the catalytic effects here observed may be based on the following assumptions. (a) RCO_2^- is involved in equilibria such as (2) and (3). (b) Pair (III) is the most reactive

$$RCO_2^- + HIm \rightleftharpoons \underset{(III)}{[RCO_2 \cdots HIm]^-} \xrightleftharpoons{n\ HIm} [RCO_2 \cdots HIm \cdots (HIm)_n]^- \quad (2)$$

$$RCO_2^- + H_2O \rightleftharpoons [RCO_2 \cdots HOH]^- \quad (3)$$

species in solution. (c) Hydrogen-bonding to hydroxylic compounds (water, alcohols if added) impairs both nucleophilic and basic properties of RCO_2^- or (III).

On this basis, the data in Figure 1 would reflect competition between IM and H_2O for RCO_2^- through equilibria (2) and (3). The data in Figure 4 would be the result of the equilibria (2) between (III) and higher, less reactive aggregates. The data in Figure 3 do not show the ' titration ' pattern of Figure 4 and require further consideration. The main reaction path outlined in Scheme 1 is proposed: the formation of a tetrahedral intermediate, under the condition $[RCO_2^-]$ = *ca.* [IM] (Figure 3), is reversible and further addition of a base catalyst, which would somehow remove the proton from this intermediate, would promote formation of products. Under the conditions explored by Menger and Vitale (large excess of salt) the k_1 step may become rate-limiting and the corrected $[k_\psi - k$ (salt + ester)] rate constant independent of further salt addition.

It is emphasized that the difference between the mechanisms of Schemes 1 and 2, in spite of their formal analogy, is a very substantial one. Following Menger and Vitale's hypothesis [4] (Scheme 1) a ' bare ' imidazole molecule is the nucleophile and the limiting value calculated by the authors ($k_{lim} = k_1$[IM] = 14 l mol^{-1}

[11] C. L. Liotta, E. E. Grisdale, and H. P. Hopkins, jun., *Tetrahedron Letters*, 1975, 4205; C. L. Liotta, H. P. Harris, M. McDermott, T. Gonzales, and K. Smith, *J.C.S. Perkin I*, 1974, 340.

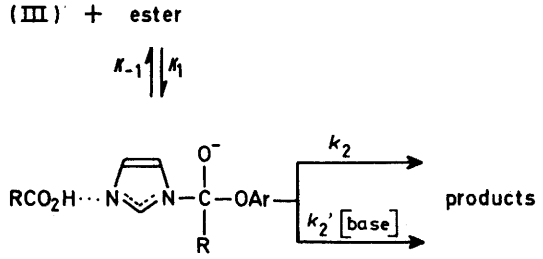
[12] H. D. Durst, *Tetrahedron Letters*, 1974, 2421; S. Akabori and M. Ohtomi, *Bull. Chem. Soc. Japan*, 1975, **48**, 2991.

[13] F. M. Menger and J. H. Smith, *J. Amer. Chem. Soc.*, 1972, **94**, 3824.

[14] W. P. Jencks, *Accounts Chem. Res.*, 1976, **9**. 425.

[15] I. M. Kolthoff and M. K. Chantooni, jun., *Analyt. Chem.*, 1967, **39**, 1080; *J. Amer. Chem. Soc.*, 1963, **85**, 2195; 1969, **91**, 25; G. Frankel and J. P. Kim, *ibid.*, 1966, **88**, 4203.

s^{-1}) should be independent of the type of RCO_2^- anion used. From data in Figure 3, although no limiting value was reached but only approached for the less reactive salts, it is quite evident that it is dependent on the anion. The largest (corrected) k_ψ value of Figure 3 measured for THAP in the presence of 3.8×10^{-3}M-IM would give a limiting value of *ca.* 40 l mol^{-1} s^{-1} which is



SCHEME 2

already higher than that calculated in ref. 4. Under the present hypothesis, the reactivity of (III) is, instead, reasonably anion dependent if the two limiting structures, $RCO_2^- \cdots HIm$ and $RCO_2H \cdots Im^-$, are considered: the more basic the anion the more reactive is (III) as a carbonyl nucleophile. Thus, the question of which (RCO_2^- or IM) catalyses what [see point (1)] seems to be reasonably defined. The flexible imidazole, when hydrogen bonded to the anion, makes use of the basic and nucleophilic properties of its partner.

The role played by the cations also needs to be explained. The 'best' cations are those where the positive charge is better screened as in the case of TBA and THA. This could mean that the less screened cations are more tightly paired to either RCO_2^- or (III), perhaps in ion-pair aggregates, to form less reactive species. CEMAH is a limiting case where anions can hydrogen-bond to the hydroxy-function of the cation. Finally, no evidence whatsoever was found of reversed micellar effects [6] which were particularly sought in the case of CTAP and CEMAHP.

Conclusions.—If the preceding speculations are valid, the observed catalytic effects are due to preformed, hydrogen-bonded carboxylate ion–imidazole pairs. As an amplifier,[16] the system utilizes the basic power of the unsolvated carboxylate anion to obtain an output of greater effectiveness through the relay action of its transducer, *i.e.* the imidazole molecule.

This mode of action is clearly analogous to that suggested for the charge-relay system in the active site of α-chymotrypsin. In the enzyme, the acid–base properties of the unsolvated carboxylic residue are used by imidazole to facilitate proton transfer processes occurring in various steps of the reaction.[1b]

EXPERIMENTAL

Imidazole, *p*-nitrophenyl propionate, propionylimidazole, and benzene were purified and dried as described.[9] Propionic anhydride was fractionally distilled under reduced pressure.

Tetra-alkylammonium carboxylates were obtained from the corresponding bromides, Q^+Br^-, which were either commercial products (THABr, TBABr, CTABr) or prepared as reported [8] (CEMAHBr). Starting from carefully purified products, the following procedure was used. To a solution of Q^+Br^- (0.03 mol) in 2 : 1 methanol–water, freshly prepared silver oxide (0.1 mol) was added and the mixture stirred for 15 min. The filtered solution was immediately neutralized (to pH 6.8—6.6) with a solution of the appropriate acid in methanol or ethanol and the solvent removed under reduced pressure. The residue was repeatedly washed with pentane and then kept under vacuum over P_2O_5 before further purification. The solid samples were very hydroscopic and poor analyses were obtained.

Cetyl(ethyl)(methyl)hydroxyethylammonium propionate (CEMAHP). The residue was lyophilized from benzene and kept under vacuum for five days before use (Found: C, 70.25; H, 13.1; N, 3.15. $C_{24}H_{49}NO_3$ requires C, 71.85; H, 12.7; N, 3.5%), τ(C_6D_6) 5.57 (1 H, s), 6.12 (2 H, m), 6.62 (9 H, m), 7.32 (2 H, q), 8.51br (*ca.* 34 H), and 8.97 (3 H, t).

Cetyltrimethylammonium propionate (CTAP).[17] The residue was twice crystallized from benzene and lyophilized. The amorphous hygroscopic powder, dried under vacuum for 5 h, was probably monohydrated from the elemental analysis (Found: C, 69.85; H, 13.2; N, 3.65. Calc. for $C_{22}H_{47}NO_2 \cdot H_2O$: C, 70.35; H, 13.15; N, 3.75%), τ(D_2O) 6.98 (2 H, m), 7.03 (9 H, s), 7.9 (2 H, q), 8.85br (*ca.* 28 H), 9.1 (3 H, t), and 9.3 (3 H, t). The salt was further dried under vacuum (10^{-2} Torr) over P_2O_5 for ten days prior to preparation of the solution (see Figure 1).

Tetra-n-butylammonium propionate (TBAP).[18] The residue was lyophilized from benzene and dried as described above (Found: C, 70,45; H, 13.15; N, 4.05. Calc. for $C_{19}H_{41}NO_2$: C, 72.4; H, 13.0; N, 4.45%), τ(C_6D_6) 6.65 (8 H, m), 7.36 (2 H, q), 8.5br (*ca.* 19 H), and 8.95 (12 H, t).

Tetra-n-butylammonium myristate (TBAM). The oily residue did not solidify upon attempted crystallization or lyophilization (Found: C, 76.1; H, 13.75; N, 2.75. $C_{30}H_{63}NO_2$ requires C, 76.7; H, 13.5; N, 3.0%), τ(C_6D_6) 6.62 (8 H, m), 7.84 (2 H, q), 8.7br (*ca.* 35 H), and 9.04 (12 H, t).

Tetra-n-hexylammonium benzoate (THAB).[4,19] The hemihydrate had τ(CCl_4) 2.85—2.14 (5 H, m), 5.3br (1 H, H_2O), 6.9 (8 H, m), 8.81br (*ca.* 32 H), and 9.17 (12 H t).

Tetra-n-hexylammonium m-chlorobenzoate (THAmCB). The hemihydrate was a pale yellow oil (Found: C, 71.45; H, 10.85; N, 2.85. $C_{31}H_{56}ClNO_2,0.5H_2O$ requires C, 71.7; H, 11.05; N, 2.7%), τ(CCl_4) 2.85—2.25 (4 H, m), 5.4br (1 H), 6.87 (8 H, m), 8.8br (*ca.* 32 H), and 9.16 (12 H, t).

Tetra-n-hexylammonium propionate (THAP). This was a viscous pale yellow oil, hemihydrate from elemental analysis (Found: C, 73.9; H, 13.55; N, 3.5. $C_{27}H_{57}NO_2 \cdot 0.5H_2O$ requires C, 74.25; H, 13.4; N, 3.2%), τ(C_6D_6), 6.62 (8 H, m), 7.84 (2 H, q), 8.73br (*ca.* 35 H), and 9.13 (12 H, t).

^1H N.m.r. spectra were recorded on Bruker FT-WP 60 MHz or HFX-10 90 MHz instruments. The product

[16] From 'Webster's Seventh New Collegiate Dictionary', Merriam, Springfield, 1965.

[17] J. A. Gautier, J. Renault, and E. Leroi, *Bull. Soc. chim. France*, 1955, 634.

[18] D. L. Fowler, W. V. Loebenstein, D. B. Pall, and C. A. Kraus, *J. Amer. Chem. Soc.*, 1940, **62**, 1140.

[19] C. G. Swain, A. Ohno, D. K. Roe, R. Brown, and T. Maugh, *J. Amer. Chem. Soc.*, 1967, **89**, 2648.

analyses described in the Results section were essentially based on the relative shift and integrals of the CH_2CO_2 signals of the propionate anion (τ 7.8), PNPP (7.95), propionic anhydride (8.03), and propionylimidazole (8.34). These τ values are those observed for solutions of each single substance but vary considerably in mixtures and control experiments with authentic samples were performed where possible.

Kinetics.—Solutions of salts were prepared by transferring under dry nitrogen measured volumes of benzene into the flask where the samples were dried. The procedure was substantially that described.[9] The increase in absorbance at 410 nm was recorded for each run until the reaction was over; the first-order rate law was obeyed up to at least 80% reaction. Rate constants were reproducible to $\pm 3\%$ within a series of measurements involving a single batch of salt solution and to as much as $\pm 15\%$ when different batches were used.

I thank Professor G. Modena for advice and discussions, Drs. F. Rivetti, F. D'Andrea, and M. Lorenzin for their participation, and Mr. E. Castiglione for technical assistance.

[7/960 *Received*, 3*rd June*, 1977]