The Role of the Carboxy-group in Intramolecular Catalysis of Acetal Hydrolysis. The Hydrolysis of Substituted 2-Methoxymethoxybenzoic Acids

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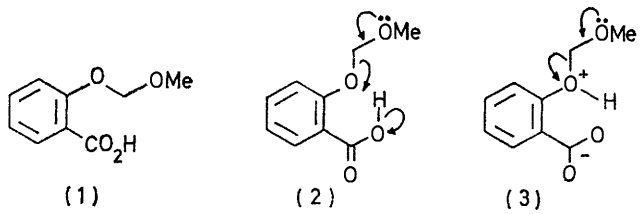
Separating the effects of substituents on the carboxy and leaving groups of 4- and 5-substituted 2-methoxymethoxybenzoic acids, by means of the extended Hammett equation, reveals the expected moderate sensitivity to the leaving group. But the Brönsted coefficient α is zero for intramolecular general acid catalysis. The mechanism proposed to account for this insensitivity to the pK_a of the carboxy-group is analogous to that proposed previously for the hydrolysis of the dianion of salicyl phosphate, and depends on the electronic interaction between the carboxy and leaving groups which occurs in reactions in which the salicylate anion is displaced. The suitability of salicylic acid derivatives as models for enzyme–substrate reactions in which the carboxy and leaving groups are in separate molecules is therefore questionable.

MUCH of the recent interest in the chemistry of acetals is a direct result of the structural work on the enzyme lysozyme. The catalytic groups available at the active site of the enzyme are the side-chain carboxys of aspartic acid-52 and glutamic acid-35; and there have been a number of systematic studies designed to identify the mechanisms by which a carboxy-group or groups can catalyse the hydrolysis of a glycosidic linkage. Several authors have identified and studied catalysis by the carboxy-group in the hydrolysis of simple glycosides and acetals, and this work is lucidly summarised in a recent review by Dunn and Bruice.[1] The important conclusions are that general acid catalysis by carboxylic acids is observed [2] in the hydrolysis of acetals with favourable [3] structural features; that intramolecular general acid catalysis by a suitably positioned carboxy-group can be much more efficient; [1,3,4] and that intramolecular nucleophilic participation by a carboxylate [5] or amide [6] group may occur, though no system has yet been found in which this leads to a large enhancement of efficiency in a reaction already subject to general acid catalysis.[4,7,8]

Intramolecular general acid catalysis of hydrolysis by the carboxy-group is readily observed in acetals derived from salicylic acid, and in fact has been studied in detail only in systems of this sort. Since this reaction is the best available model for at least part of the catalytic action of lysozyme it is important to establish the mechanism of the reaction in simple systems in as much detail as possible.

There is some disagreement over the precise role played by the carboxy-group in such model systems. The simplest model is 2-methoxymethoxybenzoic acid (1), studied independently by Capon and by Bruice. Capon [3] favours a classical general acid catalysis mechanism (2), with proton transfer concerted with C–O bond breaking; while Bruice [1] prefers the kinetically equivalent specific acid catalysis of the hydrolysis of the anion (3). In the latter case (3) the rate enhancement is ascribed to electrostatic stabilisation of the acetal conjugate acid by the carboxylate group. We are not convinced that this factor can account for catalysis in a system of this sort. There is no doubt that the neighbouring carboxylate group could stabilise the conjugate



(1) (2) (3)

acid of the acetal, thus raising its effective pK_a and increasing its concentration. But this effect would stabilise the protonated acetal towards hydrolysis also, and in a reaction which is highly sensitive to the pK_a of the leaving group the result is not likely to be a large increase in the rate of hydrolysis. We have presented this argument in more detail [9] as part of our case for rejection the mechanism represented by (3) for the hydrolysis of salicyl phosphate, which we show below almost certainly involves the same mechanism as the hydrolysis of the 2-methoxymethoxybenzoic acids discussed in this paper. It is equally strong in this case.

There have been two attempts to quantify the role of the carboxy-group in the hydrolysis of the acetal (1). Since the 5-nitro-compound is hydrolysed some 13 times faster, and the pK_a of its carboxy-group is 1·05 lower than that of the unsubstituted compound, Dunn and Bruice [10] estimated the Brönsted coefficient α for general acid catalysis by the carboxy-group to be *ca.* −1·0, consistent with the complete proton transfer in the transition state required by mechanism (3). Capon and his co-workers [3] have done the same calculation for the 4-nitro-compound, and find α −0·79, consistent with their preferred mechanism (2).

These calculations involve the assumption (made explicitly and tentatively by Capon,[3] implicitly by

[1] B. M. Dunn and T. C. Bruice, *Adv. Enzymol.*, 1973, **37**, 1.
[2] T. H. Fife and L. K. Jao, *J. Amer. Chem. Soc.*, 1968, **90**, 4081.
[3] B. Capon, M. C. Smith, E. Anderson, R. H. Dahm, and G. H. Sankey, *J. Chem. Soc.* (*B*), 1969, 1038.
[4] B. Capon, *Tetrahedron Letters*, 1963, 911.
[5] B. Capon and M. I. Page, *J.C.S. Perkin II*, 1972, 2057.
[6] T. C. Bruice and D. Piszkiewicz, *J. Amer. Chem. Soc.*, 1967, **89**, 3568.
[7] T. C. Bruice and D. Piszkiewicz, *J. Amer. Chem. Soc.*, 1968, **90**, 2156.
[8] E. Anderson and T. H. Fife, *Chem. Comm.*, 1971, 1470.
[9] R. H. Bromilow and A. J. Kirby, *J.C.S. Perkin II*, 1972, 149.
[10] B. M. Dunn and T. C. Bruice, *J. Amer. Chem. Soc.*, 1970, **92**, 6589.

Bruice [10]) that substituents act exclusively by their effect on the carboxy-group. This is a highly questionable assumption, since it is known that the general acid catalysed hydrolysis of aryl acetals shows a considerable sensitivity to substitution in the leaving group. The acetic acid catalysed hydrolysis of benzaldehyde aryl methyl acetals, for example, gives a Hammett ρ value [11] of 0·9, as does the formic acid catalysed hydrolysis of 2-aryloxytetrahydropyrans.[12] A ρ value as large as this could be sufficient to account for the substituent effects observed in the hydrolysis of 2-methoxymethoxybenzoic acids in terms of enhanced leaving group capability alone.

The problem is closely similar to that of the mechanism of hydrolysis of salicyl phosphate. In that case,[9] and earlier in the hydrolysis of aspirin,[13] it proved possible to separate the effects of substituents on the carboxy and leaving groups for reactions of salicylic acid derivatives. In this paper we describe a similar detailed examination of substituent effects on the hydrolysis of 4- and 5-substituted 2-methoxymethoxybenzoic acids.

EXPERIMENTAL

Inorganic salts were of analytical grade. Distilled water was distilled twice more from all-glass apparatus. Buffer constituents were also of analytical grade, with the exception of malonic acid, which was recrystallised from aqueous ethanol as the monosodium salt.

Methyl salicylate was obtained commercially. The substituted salicylic acids were those used previously,[9,13] and were converted to the methyl esters by conventional methods. Chloromethyl methyl ether was distilled immediately before use.

Substituted 2-methoxymethoxybenzoic acids were prepared by the method of Reychler,[14] as modified by Dunn and Bruice,[15] using sodium-dried benzene as solvent. The mineral oil in which the NaH had been suspended was separated from the crude acetal as far as possible before distillation, by extraction with low boiling light petroleum where necessary.

2-Methoxymethoxybenzoic acid had m.p. 64—65°, as found previously.[16]

2-Methoxymethoxy-5-nitrobenzoic Acid, Cyclohexylammonium Salt.—Methyl 2-methoxymethoxy-5-nitrobenzoate [10] was carefully hydrolysed to the barium salt by the method described by Fife and Anderson,[7] and the salt generated using cyclohexylammonium sulphate. The centrifuged and filtered aqueous solution was freeze-dried to an off-white solid. When recrystallised from chloroform–ether this gave m.p. 97—105° (Found: C, 55·45; H, 6·95; N, 8·6. $C_{15}H_{22}N_2O$ requires C, 55·2; H, 6·8; N, 8·6%).

2-Methoxymethoxy-2-methylbenzoic Acid.—Methyl 2-methoxymethoxy-5-methylbenzoate [16] was stirred with 2 equiv. of 1M-NaOH at room temperature for 24 h. The solution was extracted with ether, then a further 30 ml of ether was added before the ice-cooled solution was acidified with 3M-HCl. The organic layer was separated and dried to give *2-methoxymethoxy-5-methylbenzoic acid* as an oil (66%) which crystallised from benzene–light petroleum on slow cooling, m.p. 49—50° (Found: C, 61·0; H, 6·15. $C_{10}H_{12}O_4$ requires C, 61·2; H, 6·15%).

4-Methoxy-2-methoxymethoxybenzoic Acid.—Crude methyl 2-methoxymethoxy-4-methoxybenzoate (150 mg) was hydrolysed as described above for the 5-methyl ester. Evaporation of the ether gave *4-methoxy-2-methoxymethoxybenzoic acid* as a solid, which was recrystallised from benzene–light petroleum to m.p. 91—93° (95 mg, 67%) (Found: C, 56·75; H, 5·8. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7%).

5-Methoxy-2-methoxymethoxybenzoic Acid.—Methyl 5-methoxy-2-methoxymethoxybenzoate, b.p. 110—112° at 0·1 mmHg, was hydrolysed with NaOH as above. Acidification with 3M-HCl gave a crystalline precipitate of the desired *acetal acid* (82%) which when recrystallised from benzene–light petroleum had m.p. 66—68° (Found: C, 56·9; H, 5·65%).

5-Chloro-2-methoxymethoxybenzoic Acid.—Methyl 5-chloro-2-methoxymethoxybenzoate (obtained analytically pure by repeated fractional distillation), b.p. 88—90° at 0·1 mmHg, was hydrolysed and the acetal acid isolated by extraction into ether. Evaporation of the solvent gave a *solid* (62%) which when recrystallised from benzene–light petroleum had m.p. 65—67° (Found: C, 49·8; H, 4·15; Cl, 16·5. $C_9H_9ClO_4$ requires C, 49·9; H, 4·2; Cl, 16·4%).

4-Fluoro-2-methoxymethoxybenzoic Acid, Cyclohexylammonium Salt.—Methyl 4-fluoro-2-methoxymethoxybenzoate was hydrolysed by the $Ba(OH)_2$ method [17] used for the 5-nitro-compound (above). After 15 h a sticky solid had appeared and the solution was diluted to dissolve this before conversion to the cyclohexylamine salt. Freeze-drying gave 64% *cyclohexylammonium 4-fluoro-2-methoxymethoxybenzoate* which when recrystallised from chloroform–ether had m.p. 88—109° (Found: C, 60·2; H, 7·35; N, 4·5. $C_{15}H_{22}FNO_4$ requires C, 60·15; H, 7·4; N, 4·7%).

4-Iodo-2-methoxymethoxybenzoic Acid.—Methyl 4-iodo-2-methoxymethoxybenzoate (155 mg of a pale pink solid) was hydrolysed for 24 h in 2 equiv. of 1M-NaOH. At the end of this time a large amount of still undissolved solid remained so dioxan (2 ml) was added. After 4 h more t.l.c. showed that all the starting material had disappeared, and acidification of the ether–aqueous solution mixture followed by evaporation of the organic layer gave a *powder* (125 mg, 84%) which when recrystallised from benzene had m.p. 102—105° (Found: C, 34·9; H, 2·9; I, 41·25. $C_9H_9IO_4$ requires C, 35·05; H, 2·95; I, 41·2%).

5-Iodo-2-methoxymethoxybenzoic Acid.—Crude methyl 5-iodo-2-methoxymethoxybenzoate was hydrolysed as for the 4-iodo-compound. The solid obtained (59%) was recrystallised from benzene to m.p. 96—99° (Found: C, 35·25; H, 2·95; I, 41·45%).

Kinetics.—Reactions were measured at 39·0 ± 0·05° in aqueous buffer solutions maintained at ionic strength 1·0M (KCl) in the thermostatted cell compartment of a Zeiss PMQ II spectrometer. The appearance of the salicylic acid was followed in each case at the wavelength at which the absorbance charge was greatest at the pH concerned (determined by repeated scans on a recording spectrometer). In each case this wavelength was close to the isosbestic

[11] E. Anderson and B. Capon, *J. Chem. Soc.* (*B*), 1969, 1033.
[12] T. H. Fife and L. H. Brod, *J. Amer. Chem. Soc.*, 1970, **92**, 1681.
[13] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1967, **89**, 4853.
[14] A. Reychler, *Bull. Soc. chim. France*, 1907, 1197 (*Chem. Zentr.*, 1908, **79**, 716).
[15] B. M. Dunn and T. C. Bruice, *J. Amer. Chem. Soc.*, 1970, **92**, 2410.
[16] P. Hoering and F. Baum, *Chem. Zentr.*, 1909, **80**, 1681.
[17] T. H. Fife and E. Anderson, *J. Amer. Chem. Soc.*, 1971, **93**, 6610.

point for the acid and its anion. Stock solutions of the 2-methoxymethoxybenzoic acids were made up by dissolving the crystalline acid or cyclohexylammonium salt in 0·03M-NaOH, to give a final pH of *ca.* 11. These solutions were stable over several weeks. Each run was followed for three half-lives and infinity readings taken after at least ten. Then the pH of each reaction mixture was measured at 39° using an E.I.L. Vibron 33B electrometer fitted with a Pye–Ingold combined glass-reference electrode.

RESULTS

The pH-rate profile for hydrolysis was measured for each compound over the range pH 1—5 using HCl and 0·1M-malonic, -formic, and -acetic acid buffers. No catalysis by buffer constituents was observed. In agreement with previous work, the only significant reactions were the spontaneous and acid-catalysed hydrolyses of the undissociated 2-methoxymethoxybenzoic acids. The experimental data were fitted (least squares) to equation (1) (a_H is the hydrogen

$$k_{obs} = k_0 + k_H a_H/(1 + K_{app}/a_H) \quad (1)$$

ion activity measured by the glass electrode, K_{app} is the kinetically apparent dissociation constant of the substrate, k_H is the second-order rate constant for specific acid catalysis of hydrolysis, and k_0 is the first-order constant for the spontaneous hydrolysis). These results are given in full in the Table, and pH rate profiles are illustrated for a representative group of compounds in Figure 1.

Kinetic data for the hydrolysis of substituted 2-methoxymethoxybenzoic acids at 39° and ionic strength 1·0

Substituent	pK_{app}	k_H/l mol^{-1} min^{-1}	k_0/min^{-1}
H	3·77	1·13 ± 0·02	8·60 ± 0·07 [a] × 10^{-2}
5-MeO	3·63	1·23 ± 0·02	6·42 ± 0·07 × 10^{-2}
5-Me	3·83	1·33 ± 0·01	5·82 ± 0·05 × 10^{-2}
5-Cl	3·33	0·61 ± 0·05	1·62 ± 0·02 × 10^{-1}
5-NO_2	3·02	0·6 ± 0·3	1·12 ± 0·01
5-I	3·38	0·71 ± 0·03	1·60 ± 0·02 × 10^{-1}
4-OMe	4·24	1·94 ± 0·03	1·05 ± 0·01 × 10^{-1}
4-F	3·77	0·87 ± 0·04	1·68 ± 0·02 × 10^{-1}
4-I	3·51	0·81 ± 0·03	1·71 ± 0·01 × 10^{-1}
H, in D_2O	4·25 [b]	2·31 ± 0·01	5·33 ± 0·05 × 10^{-2}

[a] Standard deviation. [b] pK_{app} in D_2O.

The dependence on substituent of the various constants given in the Table can be analysed using the Hammett equation or by Jaffé's extended Hammett equation (2),

$$\log k/k_0 = \rho_1\sigma_1 + \rho_2\sigma_2 \quad (2)$$

$$1/\sigma_1 \log k/k_0 = \rho_1 + \sigma_2\rho_2/\sigma_1 \quad (3)$$

(3).[18] The apparent pK_a values of the 5-substituted compounds are well correlated by the simple Hammett equation, and give ρ 1·06 ± 0·03, close to unity as expected for substituted benzoic acids. So $\rho_{carboxy}$ for the effect of substituents on the general acid catalysed reaction can be taken as numerically equal to the Brönsted α value as normally defined.[13]

The rate constants k_H for the specific acid catalysed reaction are not well correlated by the simple Hammett equation, but give an excellent line (correlation coefficient r 0·999) when plotted according to equation (3). (This omits the two least accurate constants, for the 5-chloro- and 5-nitro-compounds, which show only small contributions from the specific acid catalysed reaction at pH 1, and requires the use of σ^+ constants for 4-MeO and 4-I.) The effect of substituents on the two functional groups is comparable, $\rho_{carboxy}$ −0·34 ± 0·01, ρ_{phenol} −0·27 ± 0·01.

The analysis of the k_0 values is clear cut. Two simple Hammett plots can be constructed for a reaction of this sort, using σ values for substituents relative to the leaving group or the carboxy-group. The former plot, which assumes that

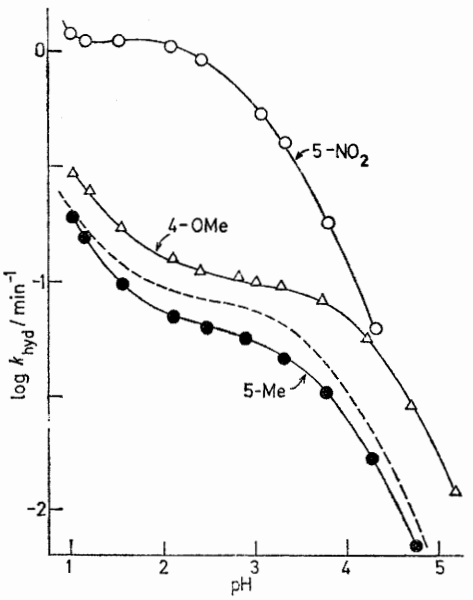


FIGURE 1 pH–Rate profiles for hydrolysis of three substituted 2-methoxymethoxybenzoic acids, and the unsubstituted derivative (broken line), at 39° and ionic strength 1·0

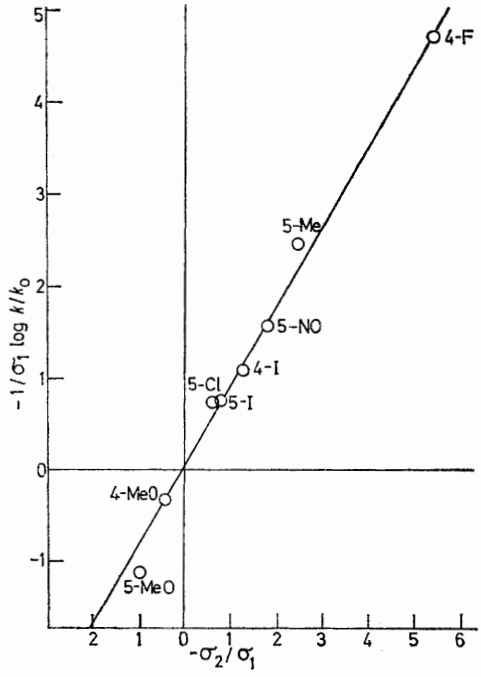


FIGURE 2 Extended Hammett plot of the data (Table) for the hydrolysis of eight substituted 2-methoxymethoxybenzoic acids, at 39° and ionic strength 1·0

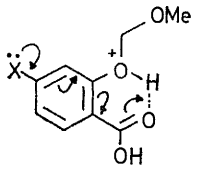
substituents act exclusively by their effect on the leaving group capability of the phenol oxygen atom, gives a good

[18] H. H. Jaffé (*a*) *J. Amer. Chem. Soc.*, 1954, **76**, 4261; (*b*) *Chem. Rev.*, 1953, **53**, 191.

correlation (r 0·997) and ρ 0·89 ± 0·03. The latter plot, for effects through the carboxy-group, gives an apparently random pattern, *e.g.*, both the 4-methoxy- and the 5-nitro-substituents increase the rate of hydrolysis. The extended Hammett equation, on the other hand, correlates all the data acceptably (r 0·995). The least squares values of slope and intercept of this plot (Figure 2) give: $\rho_{phenol} = 0{\cdot}89 \pm 0{\cdot}04$ and $\rho_{carboxy} = 0{\cdot}02 \pm 0{\cdot}08$. Thus the value of ρ_{phenol}, for the leaving group, is normal for the general acid catalysed hydrolysis of a series of phenyl acetals, while $\rho_{carboxy}$ is zero, within experimental error. [This treatment uses normal Hammett σ values [18b] (σ^- for the 5-NO_2 compound), with the exception of the 5-methoxy-substituent. Here we have used van Bekkum's [19] recommended value, σ_p −0·111, for a reaction in which there is no mesomeric interaction between substituent and reaction centre (in this case the leaving group oxygen).]

DISCUSSION

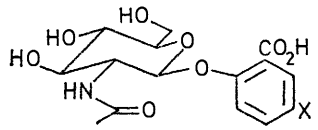
The Specific Acid Catalysed Reaction.—This shows the expected low sensitivity to the leaving group, ρ_{phenol} −0·27. This is identical to that measured by Dunn and Bruice [15] for *para*-substituted aryl methyl acetals of formaldehyde (*ca.* −0·25), and about half that found by Fife and Jao [2] for the specific acid catalysed hydrolysis of 2-aryloxytetrahydropyrans. The comparable effect of substitution through the carboxy-group ($\rho_{carboxy} = -0{\cdot}34$) is unexpected, and suggests that a suitable *ortho*-substituent can stabilise the conjugate acid of the acetal. It is not surprising that a weakly basic group like carboxy should make a contribution to the solvation of an adjacent highly acidic species, as in (4): the geometry of the system is closely similar to that of the salicylate anion, which is known to form a stable hydrogen bond, and the carboxy-group must otherwise hinder the efficient solvation of the hydroxonium cation. The negative value of ρ, and the better fit obtained using σ^+ for the 4-methoxy-group, are both consistent with a



(4)

role of this type: which need not imply that the *ortho*-carboxy (and methoxycarbonyl) groups *catalyse* the reaction, since they probably replace solvent molecules which can do the same job at least as well. The specific acid catalysis rate constant for aryl acetals with an *ortho*-carboxy or -methoxycarbonyl group is in fact usually greater than that for the corresponding *para*-substituted compounds, though not generally by more than an order of magnitude.[15,17]

Intramolecular Catalysis by the Carboxy-group.—The observed effect of substitution specifically on the leaving group, as measured by ρ_{phenol} 0·89 ± 0·04, is identical within experimental error with results in three related systems. ρ is 0·98 for the acetic acid catalysed hydrolysis of aryl methyl acetals of benzaldehyde,[11] and 0·9 for the formic acid catalysed hydrolysis of 2-aryloxytetrahydropyrans.[12] In the intramolecular general acid catalysed hydrolysis of three 1-(2-carboxyaryl)-2-*N*-acetamido-2-deoxy-β-D-glucopyranosides (5) a simple Hammett plot [20] gave ρ 0·86. The simple Hammett plot gives ρ 0·89 ± 0·03 for the hydrolysis of our substituted 2-methoxymethoxybenzoic acids, so if there is concerted catalysis in the hydrolysis of (5), as Piszkiewicz and

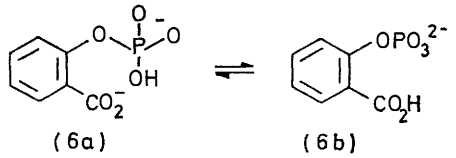


(5)

Bruice [7] suggest, it not only has only a small effect on the rate of the reaction, but also leads to no detectable change in the extent of bond breaking in the transition state. It seems more likely that all the observed acceleration can be accounted for by the catalysis by the carboxy-group.

In contrast to the effect of substitution on the leaving group, the Brönsted α value of zero for carboxy-group catalysis ($\rho_{carboxy}$ 0·02 ± 0·08) is unprecedented in acetal chemistry. Measured values of α for intermolecular general acid catalysed hydrolysis of acetals fall generally [11,21,22] in the range of 0·5—0·7. The value of α observed for this intramolecular reaction is not consistent with either the classical general acid catalysis mechanism (2), with the proton partially transferred in the transition state, or with specific acid catalysis of the hydrolysis of the conjugate anion (3). The simplest interpretation of this evidence is that proton transfer has not proceeded to a significant extent by the time the transition state is reached. This is consistent also with the observed low solvent deuterium isotope effect [k_H/k_D 1·61 at 39° (this work), 1·43 at 45° [3]].

This result is not entirely unexpected because we found very similar behaviour in a recent study [9] of the hydrolysis of salicyl phosphate (6). In that case also



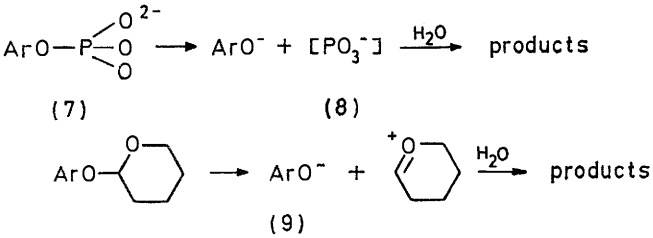
(6a) (6b)

ρ_{phenol} is moderately large, while $\rho_{carboxy}$ indicates that the carboxy-group is essentially fully protonated in the transition state.[9] The catalytic reaction must involve the thermodynamically less favoured tautomer (6b), and

[19] H. van Bekkum, P. E. Verkade, and B. M. Wepster, *Rec. Trav. chim.*, 1959, **78**, 815.
[20] B. M. Dunn and T. C. Bruice, unpublished results quoted in ref. 1, pp. 44—45.
[21] D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, 1967, **89**, 6237.
[22] E. Anderson and T. H. Fife, personal communication quoted in ref. 1.

thus represents intramolecular general acid catalysis by the carboxy-group of the hydrolysis of a phosphate monoester dianion.

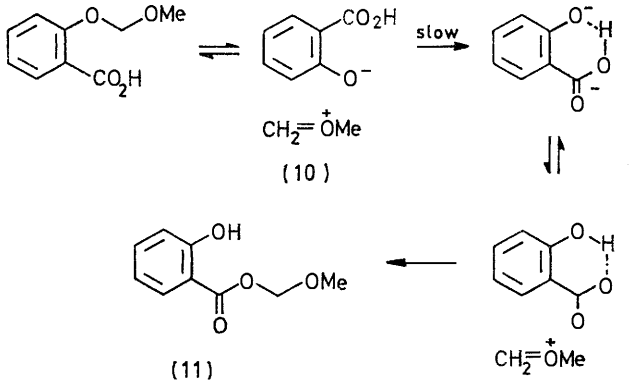
The hydrolysis behaviour of the dianions of phosphate monoesters (7) is closely analogous to that of acetals. The most important mechanism is specific acid catalysis (hydrolysis of the monoanion), a reaction which shows a low sensitivity to the leaving group.[23] This reaction is fastest near pH 4, where the concentration of the monoanion is a maximum, which may account for the failure to observe intermolecular general acid catalysis. Intramolecular catalysis by the carboxy-group of salicyl phosphate is readily observed, and highly efficient. At high pH phosphate monoester dianions are hydrolysed in a pH-independent reaction thought to involve the spontaneous elimination of the leaving group and the formation of the high-energy species metaphosphate (8). This reaction becomes significant when the leaving group is *p*-nitrophenoxide, or the anion of a more strongly acidic phenol; as is the case also for the unimolecular elimination reaction (9) of acetals such as 2-*p*-nitrophenoxytetrahydropyran.[12]



These spontaneous reactions are characterised by a high sensitivity to substitution in the leaving group: ρ^- 2·6 for the phosphate monoester dianions,[23] and at least 2·6 for the acetal reaction. (This latter figure is that found for a series of three aryl 2-acetamido-2-deoxy-β-D-glucopyranosides by Piszkiewicz and Bruice,[7] and insofar as the reaction is assisted in this system by the neighbouring acetamido-group,[21] a higher sensitivity might be expected in a system where such assistance does not occur.)

Since the spontaneous hydrolyses of acetals and phosphate monoester dianions are closely similar, and since the kinetic properties of the intramolecular carboxy-catalysed reactions are almost identical ($\rho_{carboxy} = 0$, $\Delta S^{\ddagger}$ close to 0,[7,24] k_H/k_D close to 1 [3,25]) there seems little doubt that the same mechanism must account for the observed intramolecular catalysis in the two cases. We have proposed a mechanism for the hydrolysis of the salicyl phosphate dianion [9] in which proton transfer from the carboxy to the leaving group does not begin until P–O bond cleavage is well advanced, because the most stable ground state conformation has the carboxy-group rotated out of the plane of the aromatic ring. (This is the case in crystal structures of salicylic acid derivatives which do not have intramolecular hydrogen bonds,[26] and is likely to be true also for 2-methoxymethoxybenzoic acids: these are thought not to be intramolecularly hydrogen bonded in water,[3] and external solvation of the carboxy-group, which is presumably thermodynamically more favourable than internal hydrogen bonding, can be more efficient if the group is rotated out of plane.) In this conformation the molecule will behave much as if the carboxy-group were absent: the high sensitivity to substitution in the leaving group is evidence that the bond to be cleaved is almost completely broken in the transition state for the spontaneous hydrolysis of such substrates, so considerable negative charge can build up on the leaving group oxygen before the transition state is reached. We suggest that this developing negative charge provides the driving force for the conformational change which brings the carboxy-group into plane. This process acts to stabilise the system because the partial negative charge can then be efficiently delocalised onto the *ortho*-carboxy-group, and because the hydrogen bond of the salicylate anion will form. By definition, the transition state is reached at the point along the reaction co-ordinate where stabilisation begins, and if at this point the proton transfer process is thermodynamically favourable it will not have proceeded to a significant extent in the transition state.

We believe that this mechanism, with the reaction co-ordinates for the spontaneous and catalysed reactions diverging only at fairly high degrees of bond-breaking, is the best available description consistent with the evidence for the hydrolysis of both salicyl phosphate dianion and 2-methoxymethoxybenzoic acid. The conformation change and concomitant solvent reorganisation must be concerted with bond breaking, because they are fundamentally slower processes than molecular



vibrations. We have considered, but do not favour, a similar mechanism in which the conformational change occurs when the anion is fully formed in an ion-pair (10). For this mechanism the relatively low sensitivity to the leaving group becomes difficult to explain; and the salicylate–oxocarbonium ion-pair (10) would be expected

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to collapse to form the acetal ester (11), which has been shown [3] not to be an initial product of the reaction.

We have now measured $\rho_{carboxy}$ for three different reactions catalysed by the undissociated carboxy-group of derivatives of salicylic acid, the hydrolyses of 2-methoxymethoxybenzoic acids described in this work, of salicyl phosphate dianions,[9] and of substituted acetylsalicylic acids,[27] and in each case the observed value of α is zero. This raises the strong possibility that the observed value of α is characteristic of the salicylate anion as a leaving group, rather than of the particular reaction concerned; and thus that acetals derived from salicylic acid are not well suited as models for the enzyme catalysed hydrolysis of glycosides, because of the direct electronic interaction between the carboxy and leaving groups. Since the only detailed model system work based on the lysozyme structure has involved derivatives of salicylic acid, it is clear that evidence from an entirely different system is needed to provide a firmer basis for an informed discussion of the enzyme mechanism.

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