Intramolecular General Acid Catalysis of Phosphate Monoester Hydrolysis. The Hydrolysis of Salicyl Phosphate †

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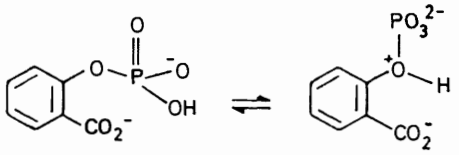
The effects of substituents in the 4- and 5-positions on the rate of hydrolysis of the salicyl phosphate dianion are entirely inconsistent with a mechanism involving a preliminary proton transfer to the leaving group. A mechanism is proposed in which the rate-determining step involves cleavage of the P–O bond concerted with a conformation change, but no significant amount of proton transfer. This mechanism accounts satisfactorily for all the known properties of the reaction, and predicts that intramolecular general acid catalysis by the carboxy-group in this system should be available for S_N2(P) reactions also. It is found that the reactions of substituted pyridines with the salicyl phosphate dianion are indeed subject to catalysis, with rate enhancements as high as 10^8-fold.

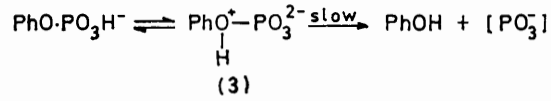
THE hydrolysis of phosphate monoesters is catalysed by a suitably situated carboxy-group. In the best-known example, discovered by Arai [1] in 1934, the hydrolysis of the dianion (1) of salicyl phosphate (2-carboxyphenyl dihydrogen phosphate) is hydrolysed at 39° 1570 times faster than the phenyl hydrogen phosphate monoanion.

The reaction was examined in some detail by Chanley *et al.*,[2] who proposed a mechanism for the catalysis in which the carboxylate group of (1) acts as a nucleophile. Chanley's mechanism requires an acyl phosphate intermediate, and was subsequently ruled out by Bender and Lawlor,[3] who showed that no such intermediate could be involved. The carboxy-group must therefore act as a general acid or base, and Bender and Lawlor suggested an unusual type of general acid catalysis, in which the remaining proton of (1) is transferred completely to the oxygen atom of the leaving group to give the zwitterion (2). The breakdown of (2) to salicylate and the metaphosphate anion, PO_3^-, would then be the rate-determining step of the reaction.



(1) (2)

The hydrolysis of salicyl phosphate is unusual in that the rate enhancement caused by intramolecular catalysis is entirely accounted for by a decrease in the enthalpy of activation: the entropy of activation is close to zero,[2a] as it is for the hydrolysis of the monoanions of phosphate monoesters in general.[4] We have suggested [4] that the hydrolysis of the monoanions of phosphate monoesters, itself a particularly rapid reaction, also involves a pre-equilibrium proton transfer to give a zwitterionic species (3). This mechanism is identical to that proposed by Bender and Lawlor to account for the even faster hydrolysis of salicyl phosphate, which would explain the very similar kinetic behaviour of the two reactions. But this otherwise consistent picture sheds no light on the catalytic role of the carboxy-group in the hydrolysis of salicyl phosphate. Bender and Lawlor [3] suggested that the ionised



(3)

carboxy-group stabilises the zwitterion in some way, perhaps electrostatically. In the light of recent work on the reactivity of phosphate esters this explanation has become difficult to accept.

The breakdown to metaphosphate of a species XPO_3^{2-}, such as (2), is a reaction which is extremely sensitive to the basicity of the leaving group. This is true for the hydrolysis of the dianions of phosphate monoesters, $ArOPO_3^{2-}$, where the leaving group is an oxyanion,[4] and also for the loss of substituted pyridines, in the hydrolysis of the *N*-phosphorylpyridinium ions, $R_3N^+–PO_3^{2-}$.[5] The slope of the logarithmic plot of the rate constant for hydrolysis against the pK_a of the conjugate acid of the leaving group is greater than unity in each case (−1·23 and −1·13, respectively, for the esters and the pyridinium compounds). Consequently, any factor which stabilises the zwitterion form of a monoester monoanion, such as (2), increasing its equilibrium concentration by increasing the effective pK_a of the oxonium group, will stabilise it towards hydrolysis also. Since the slopes of the two linear free-energy relationships that have been measured are both greater than unity, the reduced reactivity towards hydrolysis may actually outweigh the effect of the increase in concentration. Thus the mechanism of Bender and Lawlor,[3] far from explaining the observed rapid rate of hydrolysis, might be expected to lead to a modest *decrease* in reactivity.

For this reason we have re-examined the hydrolysis of salicyl phosphate. We have confirmed many experimental findings of previous investigators, and have provided significant new evidence. In particular we have examined the effects of substituents on the reaction.

† In this paper, salicyl is taken to mean *o*-$HO_2C·C_6H_4–$.

[1] J. Arai, *J. Biochem.* (*Japan*), 1934, **20**, 465.

[2] (*a*) J. D. Chanley, E. M. Gindler, and H. Sobotka, *J. Amer. Chem. Soc.*, 1952, **74**, 4347; (*b*) J. D. Chanley and E. M. Gindler, *ibid.*, 1953, **75**, 4035; (*c*) J. D. Chanley and E. Feageson, *ibid.*, 1955, **77**, 4002.

[3] M. L. Bender and J. M. Lawlor, *J. Amer. Chem. Soc.*, 1963, **85**, 3010.

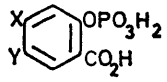
[4] A. J. Kirby and A. G. Varvoglis, *J. Amer. Chem. Soc.*, 1967, **89**, 415.

[5] G. W. Jameson and J. M. Lawlor, *J. Chem. Soc.* (*B*), 1970, 53.

EXPERIMENTAL

Materials.—Inorganic salts were of analytical grade, and were used without further purification. Distilled water was distilled twice more from all-glass apparatus. Salicylic acid and the 5-chloro, 5-iodo, 5-methoxy, and 5-methyl derivatives were obtained commercially, and recrystallised before use. Published procedures were used to prepare the 4-chloro,[6] 4-iodo,[6] 4-methoxy,[7] 4-nitro,[8] and 5-nitro [9] compounds, and for the synthesis of 3-hydroxyphthalic anhydride.[10] 4-Fluorosalicylic acid was prepared from 4-fluoro-2-nitrotoluene. This was oxidised (alkaline permanganate) to 4-fluoro-2-nitrobenzoic acid, m.p. 142—143° (lit.,[11] 145°). Reduction with stannous chloride in HCl gave 4-fluoroanthranilic acid as a mixture of salts, which was diazotised in 15N-H_2SO_4. The diazonium salt was hydrolysed by boiling the aqueous solution for 2 h. On cooling the solution, orange needles were deposited. Sublimation at 80—90° at 0·1 mmHg followed by recrystallisation from ethanol–water gave colourless needles of 4-fluorosalicylic acid (20%), m.p. 188—189° (lit.,[12] 188·2—189·8°).

The substituted salicyl phosphates (4) were prepared by the method used by Chanley *et al.*[2a] for the synthesis



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of salicyl phosphate itself. Only in the case of the 5-nitro-compound was any modification necessary: here the reaction mixture was heated to 90—95° for 2 h before hydrolysis at 0°. Salicyl phosphate (2-carboxyphenyl dihydrogen phosphate) had m.p. 161—161·5° (lit., 162·5—163°,[2a] 160—160·5° [3]).

5-Chloro-2-carboxyphenyl dihydrogen phosphate [(4; X = Cl, Y = H) from 4-chlorosalicylic acid] was recrystallised from chloroform–dimethylformamide and then from wet acetone–chloroform; it had m.p. 177—178° (dec.) (Found: C, 33·1; H, 2·65; Cl, 14·1; P, 12·7. $C_7H_6ClO_6P$ requires C, 33·2; H, 2·4; Cl, 14·05; P, 12·3%).

4-Chloro-2-carboxyphenyl dihydrogen phosphate [(4; X = H, Y = Cl) from 5-chlorosalicylic acid] was recrystallised from chloroform–dioxan and then from wet acetone–chloroform; it had m.p. 158—159° (lit.,[13] 161—162°) and a satisfactory elemental analysis.

5-Iodo-2-carboxyphenyl dihydrogen phosphate [(4; X = I, Y = H) from 4-iodosalicylic acid] was recrystallised from chloroform–dimethylformamide and then from wet acetone–chloroform; it had m.p. 184·5—185·5° (dec.) (Found: C, 24·6; H, 1·95; P, 9·2. $C_7H_6IO_6P$ requires C, 24·4; H, 1·75; P, 9·0%).

4-Iodo-2-carboxyphenyl dihydrogen phosphate [(4; X = H, Y = I) from 5-iodosalicylic acid] was recrystallised from chloroform–dioxan, and had m.p. 165—166° (Found: C, 24·5; H, 1·8; P, 8·85. $C_7H_6IO_6P$ requires C, 24·4; H, 1·75; P, 9·0%).

5-Methoxy-2-carboxyphenyl dihydrogen phosphate [(4; X = MeO, Y = H) from 4-methoxysalicylic acid] was recrystallised from wet acetone–chloroform and had m.p. 146—147° (dec.) (Found: C, 38·7; H, 3·7; P, 12·2. $C_8H_9O_7P$ requires C, 38·7; H, 3·65; P, 12·5%).

4-Methoxy-2-carboxyphenyl dihydrogen phosphate [(4; X = H, Y = MeO) from 5-methoxysalicylic acid] was recrystallised from dioxan–chloroform and had m.p. 151·5—152·5° (Found: C, 38·45; H, 3·6; P, 12·2. $C_8H_9O_7P$ requires C, 38·7; H, 3·65; P, 12·5%).

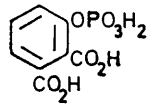
5-Nitro-2-carboxyphenyl dihydrogen phosphate [(4; X = NO_2, Y = H) from 4-nitrosalicylic acid] was recrystallised from acetone–chloroform and had m.p. 167—168° (dec.) (Found: C, 32·25; H, 2·5; N, 5·6; P, 11·9. $C_7H_6NO_8P$ requires C, 31·9; H, 2·3; N, 5·35; P, 11·8%).

4-Nitro-2-carboxyphenyl dihydrogen phosphate [(4; X = H, Y = NO_2) from 5-nitrosalicylic acid] was recrystallised from dry acetone–benzene. It did not melt sharply, but sintered above 145° (Found: C, 31·8; H, 2·4; N, 5·4; P, 11·5. $C_7H_6NO_8P$ requires C, 31·9; H, 2·3; N, 5·35; P, 11·8%).

5-Fluoro-2-carboxyphenyl dihydrogen phosphate [4; X = F, Y = H) from 4-fluorosalicylic acid] was recrystallised from wet acetone–chloroform and had m.p. 161—162° (Found: C, 35·4; H, 2·8; P, 13·2. $C_7H_6FO_6P$ requires C, 35·6; H, 2·55; P, 13·1%).

4-Methyl-2-carboxyphenyl dihydrogen phosphate [(4; X = H, Y = Me) from 5-methylsalicylic acid] was recrystallised from acetone–chloroform and had m.p. 158—159° (lit.,[13] 139·5—140·4°) (Found: C, 41·4; H, 3·95; P, 13·2. $C_8H_9O_6P$ requires C, 41·4; H, 3·9; P, 13·4%).

3-Methyl-2-carboxyphenyl dihydrogen phosphate (from 6-methylsalicyclic acid [14]) was recrystallised from acetone–benzene and had m.p. 142·5—143·5° (Found: C, 41·55; H, 3·95; P, 13·1. $C_8H_9O_6P$ requires C, 41·4; H, 3·9; P, 13·35%).



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2,3-Dicarboxyphenyl dihydrogen phosphate (5). 3-Hydroxyphthalic anhydride (2·0 g, 12·2 mmol) was mixed with PCl_5 (2·5 g, 12·0 mmol) in a flask protected by a drying tube, and the mixture was heated to 80—90° for 1 h; HCl was evolved. The cool liquid product was dissolved in 50% acetone–benzene (5 ml). The solution was cooled in ice, and water (855 μl, 47·5 mmol) was added slowly, with stirring. After 30 min at 0° more benzene was added and the solution was evaporated under reduced pressure to give a brown oil. This crystallised on exposure to the air, and was washed with a little cold ethyl acetate to remove the residual oil. The crude product was then dissolved in boiling ethyl acetate containing a little water; cautious addition of benzene to the solution led to the separation of colourless rhombs (2·0 g, 55%) of 2,3-dicarboxyphenyl dihydrogen phosphate (5). On heating,

[6] H. Ohta, *Nippon Kagaku Zasshi*, 1957, **78**, 1608 (*Chem. Abs.*, 1959, **53**, 21,342*a*).
[7] M. Gomberg and L. C. Johnson, *J. Amer. Chem. Soc.*, 1917, **39**, 1687.
[8] H. Seidel and J. C. Bittner, *Monatsh.*, 1902, **23**, 431.
[9] H. C. Barary and M. Pianka, *J. Chem. Soc.*, 1946, 965.
[10] E. L. Eliel, A. W. Burgstahler, D. E. Rivard, and L. Haefele, *J. Amer. Chem. Soc.*, 1955, **77**, 5092.
[11] B. E. Volkani, S. Sicher, E. D. Bergmann, and H. Bendas, *J. Biol. Chem.*, 1954, **207**, 411.
[12] G. E. Dunn and T. L. Penner, *Canad. J. Chem.*, 1967, **45**, 1699.
[13] R. Anschütz, *Annalen*, 1906, **346**, 286.
[14] W. W. Kaeding and G. R. Collins, *J. Org. Chem.*, 1965, **30**, 3750.

these crystals softened at 108° as they lost water, and finally melted at 160—161° (dec.) (Found: C, 32·4; H, 3·65; P, 10·25. $C_8H_7O_8P,2H_2O$ requires C, 32·3; H, 3·7; P, 10·4%).

Kinetic Methods and Results.—The hydrolysis of the substituted salicyl phosphates was followed spectrophotometrically, by measuring the rate of release of the salicylic acid or anion, at the wavelength shown in Table 1, in the thermostatted cell-compartment of a Zeiss PMQ II spectrophotometer. Reactions were normally measured at 39·00 ± 0·05°, and the ionic strength was maintained constant at 1·0M with added KCl. Where reactions were fast enough (pH *ca.* 3—6) they were followed for 3 half-lives, and end points were taken after at least 10. This procedure gave excellent pseudo-first-order semi-logarithmic plots. Otherwise the initial rate of release of salicylate was followed, for a total change in absorbance of 0·3—0·4, representing 3—5% of reaction. End points were measured using the same solutions after 10 half-lives, by 25-fold dilution with the appropriate buffer–KCl solution. In a few cases both methods were used and the results were found to be identical. The hydrolysis of 6-methylsalicyl phosphate was followed by the initial rate method at all pH values, and end points were obtained by heating the solutions at 100° in sealed tubes. The pH of the reaction mixture was measured at the end of each run at 39°, using an E.I.L. Vibron electrometer fitted with a C-33B pH-measuring attachment and a Pye–Ingold combined glass-reference electrode. Results were reproducible to ±0·03 pH units.

The pH-rate profile was measured for the hydrolysis of each compound over the pH-range 1—8, using HCl, and 0·05M-formate, acetate, phosphate, and TRIS buffers. Buffer catalysis was negligible: in every case a 10-fold increase in buffer concentration had no effect on the rate of hydrolysis. The rate maximum lies close to pH 5 in each case (Table 1), mid-way between the second and third pK_a values of the salicyl phosphate. Since these pK_a values differ by more than 2 pK_a units, and since the dianion is hydrolysed more than 10 times as fast as either the monoanion or the trianion in every case, the rate at the maximum is due almost entirely to the reaction of the dianion. The proportion of starting material actually present as the dianion is in the region of 90% in each case, and was calculated as follows. Kinetic pK_a values for the monoanion and dianion (pK_2 and pK_3) were obtained from the pH-rate profiles using the method of Alberty and Massey.[15] On the basis of the reasonable assumption that the kinetic pK_a values are identical with the true pK_a values in this case, the proportion of dianion present at the pH-rate maximum was calculated; this figure was used to obtain a corrected rate constant for hydrolysis of the dianion. These results are given in Table 1.

TABLE 1

Data for the hydrolysis of substituted salicyl phosphates, at 39° and ionic strength 1·0

Substituent	Followed at (λ in nm)	pH of rate maximum	pK_2, pK_3 [a] (calc)	% of dianion [a] at maximum	k_{hyd} (min.$^{-1}$) for dianion
H	290·5 [c]	4·93	3·76, 6·10	88	$1·02 \times 10^{-2}$
4-Cl	305,[f] 297 [e]	4·57	3·50, 5·64	86	$2·42 \times 10^{-2}$
5-Cl	320,[f] 309 [e]	4·64	3·68, 5·60	82	$1·42 \times 10^{-2}$
4-I	307,[f] 300 [e]	4·66	3·48, 5·74	86	$2·00 \times 10^{-2}$
5-I	320,[d] 313 [e]	4·73	3·43, 6·03	91	$1·43 \times 10^{-2}$
4-NO_2	347,[f] 349 [e]	4·08	2·84, 5·32	90	$1·69 \times 10^{-2}$
5-NO_2	308,[d] 317 [e]	4·56	2·95, 6·17	95	0·10
4-MeO	297,[f] 291·5 [e]	5·10	3·92, 6·28	88	$2·90 \times 10^{-2}$
5-MeO	330,[f] 318 [e]	4·80	3·81, 5·79	83	$4·46 \times 10^{-3}$
5-Me	314,[f] 307 [f]	5·00	3·83, 6·17	88	$5·87 \times 10^{-3}$
4-F	294,[f] 290 [f]	4·66	3·61, 5·71	85	$3·42 \times 10^{-2}$
6-Me	300 [f]	4·75			$4·7 \times 10^{-5}$

[a] Calculated from pH-rate profile, as described in the text. [b] Obtained by dividing observed maximum rate by proportion of dianion from previous column. [c] Isosbestic point for salicylic acid and its anion. [d] Absorption maximum for the substituted acid. [e] Absorption maximum for the anion. [f] Wavelength at which absorption charge on hydrolysis is a maximum.

TABLE 2

Second-order rate constants for the reactions of substituted pyridines with the dianions of salicyl phosphates, at 39° and ionic strength 1·0

Nucleophile	pK_a	Conditions	pH range	Concn. range of free base (M)	No. of runs	k_2 (l mol^{-1} min^{-1})
		Salicyl phosphate				
3-Cyanopyridine	1·45	0·28M-Acetate buffer	4·86—4·91	0·1—0·3	3	$3·4 \times 10^{-3}$
Nicotinamide	3·40	0·3M-Acetate buffer	5·08—5·15	0·1—0·3	3	$8·35 \times 10^{-3}$
Pyridine	5·17	30% Free base	4·97—5·00	0·1—0·3	3	$1·74 \times 10^{-2}$
Pyridine in D_2O		The same, in D_2O		0·1—0·3	3	$1·2 \times 10^{-2}$
		5-Nitrosalicyl phosphate				
Nicotinamide		80% Free base	3·96—4·04	0·05—0·3	6	0·25

Hydrolysis in [^{18}O]*Water.*—Salicyl phosphate and the 5-nitro-compound were hydrolysed completely in water 20·08% enriched with $H_2{}^{18}O$ at the pH-rate maximum for the compound. For example, 2-carboxyphenyl dihydrogen phosphate (60 mg) and anhydrous sodium acetate (90 mg) dissolved in enriched water (0·50 ml) were incubated in a sealed tube at 39° for 10 half-lives at this pH (4·8). The salicylic acid was precipitated at once when a few drops of concentrated hydrochloric acid

[15] R. A. Alberty and V. Massey, *Biochim. Biophys. Acta*, 1954, **13**, 347.

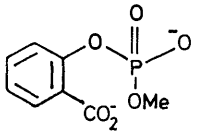
were added to the product solution. Each sample was analysed for incorporation of ^{18}O by mass spectroscopic measurement of the $M + 2/M$ ratio, as described previously.[16] For salicyl phosphate the $M + 2/M$ ratio of the salicylic acid produced was $1{\cdot}97 \pm 0{\cdot}03 \times 10^{-2}$ on hydrolysis in $H_2{}^{18}O$, $1{\cdot}95 \pm 0{\cdot}02 \times 10^{-2}$ for hydrolysis in isotopically normal water. For 5-nitrosalicyl phosphate the ratio was $1{\cdot}62 \pm 0{\cdot}05 \times 10^{-2}$ in each case. Thus no significant incorporation (less than 0·10%) has occurred.

Reactions with Substituted Pyridines.—These reactions were followed as before, at the pH at which the concentration of the salicyl phosphate dianion is a maximum. The pyridine reactions were conveniently self-buffered, while those with nicotinamide and 3-cyanopyridine were carried out in acetate buffers in the range pH 4—5. The catalysed hydrolysis of 5-nitrosalicyl phosphate by nicotinamide accounted for a good proportion of the observed reaction at 0·3M-base, but the catalysis accounted for only up to 10—15% of the observed hydrolysis of salicyl phosphate. There seems no doubt, however, that the rate increase is a genuine catalysis. In about half the reactions the salt composition was unchanged, and relatively low concentrations (up to 0·3M) of the bases were used. Good second-order plots were obtained, after the usual correction [17] for self-association of the pyridines. And the second-order rate constants were correlated accurately by the Brønsted equation (Figure 2), and were of the expected magnitude.

DISCUSSION

The possible mechanisms for intramolecular catalysis in the salicyl phosphate system have been discussed previously.[2,3] Nucleophilic catalysis by the carboxylate group is ruled out by the experimental results of Bender and Lawlor,[3] some of which we have confirmed, and also because this mechanism involves an endocyclic displacement at the phosphorus centre of an anion. Displacements of this type are normally prevented by the highly unfavourable pseudorotation that would be necessary,[18] as is confirmed by the lack of reactivity shown by methyl 2-carboxyphenyl phosphate (6).

Intramolecular general base catalysis, (7), is unlikely on several counts. The reaction shows no significant solvent deuterium isotope effect, and the entropy of activation is close to zero. Mechanism (7) would be expected to show a moderately large negative entropy of activation, and k_H/k_D in the region of 2: although, since intermolecular general base catalysis of the hydrolysis of phosphate diester anions has not been observed,[17] we cannot be absolutely certain what its kinetic behaviour would be. To this extent the kinetic evidence is not entirely conclusive, and we examined the hydrolysis of methyl 2-carboxyphenyl phosphate (6), which we had prepared for an earlier investigation,[19] to provide further evidence. The ester (6) should not be significantly less reactive than salicyl phosphate towards intramolecular general base catalysis of the attack of water, (7), or indeed towards any form of catalysis which involves the carboxylate group as such.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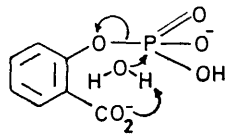
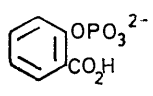

(7)

In fact methyl salicyl phosphate shows none of the reactivity associated with the dianion of salicyl phosphate. The dianion (6) is hydrolysed some 10^5—10^6 times more slowly and the hydrolysis of the monoanion, which is some 10^3 times slower, gives salicyl phosphate as the major product.

From this result it is clear that the key to the mechanism of intramolecular catalysis in the hydrolysis of the salicyl phosphate dianion lies in the role of the remaining proton. This must be associated in some way with the carboxylate group, since both are necessary for catalysis, and the conclusion seems inescapable that some form of general acid catalysis must be involved. This would implicate the less favourable tautomeric species (8), with an undissociated carboxy-group. And the simplest general acid catalysis mechanism would then be one (9) in which the transfer of the proton to the leaving group is concerted with the cleavage of the P–O bond. But a mechanism of this sort, with the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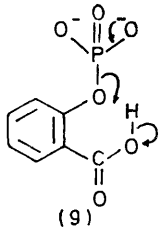
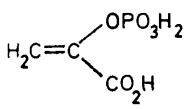

(9)

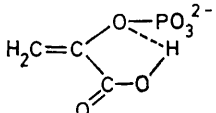
proton partially transferred in the transition state, would normally be expected to be associated with a significant solvent deuterium isotope effect, with $k_H/k_D > 1$. It was for this reason that Bender and Lawlor [3] concluded that the proton transfer must be complete in the transition state, and the breakdown of the zwitterion (2) must be rate determining.

We have discussed above one reason why this conclusion is difficult to accept. A second reason is that although catalysis is highly efficient in the hydrolysis of the dianion of salicyl phosphate, it is not observed in the hydrolysis of the dianion of phosphoenol pyruvate (10).[20] This might be explained if the proton transfer were rate determining, because of the unfavourable cyclic transition state (11) in this case, but not if the proton transfer were already complete: electrostatic

[16] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1967, **89**, 4857.
[17] A. J. Kirby and M. Younas, *J. Chem. Soc.* (*B*), 1970, 1162.
[18] S. A. Khan, A. J. Kirby, M. Wakselman, D. P. Horning, and J. M. Lawlor, *J. Chem. Soc.* (*B*), 1970, 1182.
[19] R. H. Bromilow, S. A. Khan, and A. J. Kirby, *J. Chem. Soc.* (*B*), 1971, 1091.
[20] S. J. Benkovic and K. J. Schray, *Biochemistry*, 1968, **7**, 4090; and unpublished work by A. J. Kirby and M. Wakselman.

stabilisation by the carboxylate group would presumably be more effective if the groups were closer together.


(10)


(11)

In an attempt to resolve this apparent impasse we examined the effects of substituents in the 4- and 5-positions on the rate of hydrolysis of salicyl phosphate dianion (see Table 1). The effects on the carboxy and leaving groups were assumed to be independent, so that the modified Hammett equation of Jaffe [21] applies.

$$\log k/k_0 = \sigma_1\rho_1 + \sigma_2\rho_2$$

The two ρ values are readily obtained as the slope and intercept of a plot of $1/\sigma_1 \log k/k_0$ against σ_2/σ_1: we have used this treatment successfully to separate the effects of substitution on the same two groups in the hydrolysis of substituted aspirins.[22] In the present case also this plot gave an excellent straight line correlating data for both 4- and 5-substituted compounds (see Figure 1), although a simple Hammett plot gave separate lines.

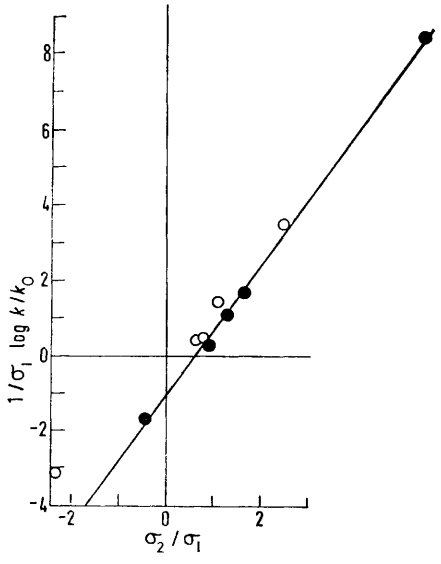
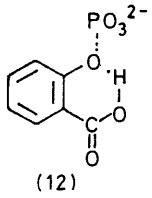


FIGURE 1 Modified Hammett plot (see text) for the hydrolysis of the dianions of 4- (closed circles) and 5-substituted salicyl phosphates, at 39° and ionic strength 1·0

From the modified Hammett plot we find (calculated by the method of least squares) $\rho_{phenol} = 1{\cdot}74 \pm 0{\cdot}06$ and $\rho_{carboxy} = -0{\cdot}99 \pm 0{\cdot}18$. These figures are impossible to reconcile with the mechanism suggested by Bender and Lawlor.[3] The sensitivity to the leaving group is much greater than that found [4] for the hydrolysis of the monoanions of simple monoaryl phosphates, which has ρ = 0·6, and is actually closer to the ρ value of 2·6 found [4] for the hydrolysis of the corresponding dianions. This is evidence that the transfer of the proton to the leaving group is much less far advanced in the transition state for salicyl phosphate hydrolysis, and is certainly not complete.

The ρ value for catalysis by the carboxy-group provides even stronger evidence for this conclusion. The value obtained, $-0{\cdot}99 \pm 0{\cdot}18$, can be taken, within the experimental error, as equal to Brønsted's coefficient for general base catalysis by the carboxy-group. (This is because the pK_a values of substituted benzoic acids generally depend on σ with ρ in the region of 1·0. For example, the calculated values of pK_2 shown in Table 1 for the substituted salicyl phosphates used in this work are correlated by the simple Hammett equation, with $\rho = 1{\cdot}01 \pm 0{\cdot}16$.) A Brønsted coefficient close to unity shows that the electronic requirements of the carboxylate group of the salicyl phosphate dianion change in the same way, and to almost precisely the same extent, on going to the transition state for hydrolysis, as they do on going to the fully protonated form. Since it is certain that the carboxylate group acts as a base, rather than a nucleophile, this means that it is completely, or almost completely, protonated in the transition state.

The picture of the transition state which emerges from this analysis can be represented approximately as (12). P–O bond cleavage, which is well-advanced,


(12)

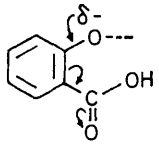
is assisted by general acid catalysis by the neighbouring carboxy-group, although the proton transfer has scarcely begun (Brønsted's coefficient for the CO_2H form is close to zero). This picture is required by the evidence from substituent effects, and is consistent with all the other evidence. For example, the absence of a significant solvent deuterium isotope effect is explained, since the zero-point energy of the O–H bond is conserved almost completely in the transition state. It is, however, surprising that it should be possible for a relatively large amount of negative charge to build up on the leaving group oxygen without attracting a more substantial degree of proton transfer from the adjacent carboxy-group. Consideration of this point has led us to a significant further clarification of the mechanism.

The ionised carboxy-group of the salicyl phosphate dianion is most likely rotated out of the plane of the benzene ring, to minimise non-bonded interactions with the adjacent phosphate group. (The most directly

[21] H. H. Jaffe, *J. Amer. Chem. Soc.*, 1954, **76**, 4261; *Chem. Rev.*, 1953, **53**, 191.

[22] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1967, **89**, 4853.

relevant structural information is an *X*-ray diffraction study of potassium hydrogen 2-acetylsalicylate,[23] in which the plane of the carboxy-group is rotated through 34·5° relative to the plane of the benzene ring). The transfer of the proton from the carboxy to the leaving group, on the other hand, almost certainly takes place with the carboxy-group in the plane of the ring: since this is the conformation most favourable for the formation of a hydrogen-bond between the two oxygen atoms concerned. Thus the carboxy-group has to do two things on going from the ground state to the transition state: it has to accept a proton (formally, and probably also actually, from the phosphate group) and it must rotate about the C–C bond to become coplanar with the benzene ring. Thus it seems highly significant that the cleavage of the P–O bond—which dominates the reaction up to the transition state—also provides an important driving force towards coplanarity: this is because negative charge building up on the oxygen atom *ortho* to the carboxy-group can be efficiently delocalised if this group is in the plane of the ring, as in (13).

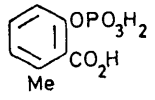


(13)

We thus see the hydrolysis as a reaction of the less-favoured tautomer (8) of the starting material, formed in a rapid pre-equilibrium with the carboxy-group rotated out of the plane of the ring. In states in which P–O bond cleavage has proceeded to a sufficient extent —an extent far short of that necessary to bring about complete cleavage without assistance—the carboxy-group rotates to become coplanar with the ring. This stabilises the developing negative charge on the leaving group oxygen and at the same time brings the proton of the carboxy-group into the correct position to form a hydrogen bond with it. The transition state must occur at, or before, this point, because the ρ value for the carboxy-group shows no evidence that it acts as an electron-sink. This is reasonable because P–O bond cleavage must be very close to complete in the transition state for hydrolysis of a simple monoester dianion; this reflects the need for the highly electrophilic metaphosphate anion PO_3^- to separate from a strongly nucleophilic oxyanion leaving group. Thus, well before this transition state is reached the leaving group oxygen is quite strongly basic, and the system will be stabilised by the start of proton transfer from a sufficiently strong general acid. Thus, the important processes defining the rate-determining step are the cleavage of the phosphorus–oxygen bond and, perhaps concerted with it in a sense, the rotation of the carboxy-group into the plane of the benzene ring.

[23] L. Manojlovic and J. C. Speakman, *J. Chem. Soc.* (*A*), 1967, 971.

If this picture of the reaction is correct several specific predictions can be made. First, steric hindrance to the attainment of coplanarity by the carboxy-group will affect the rate-determining step directly. In fact the introduction of a methyl group *ortho* to the carboxy-group on the side remote from the phosphate, has a much larger (negative) effect on the rate of hydrolysis than any found with the electronically more powerful substituents discussed above. The dianion of the compound concerned, 6-methylsalicyl phosphate (14), is hydrolysed *ca.* 200 times more slowly than salicyl phosphate itself. A second, and more far-reaching prediction is that other reactions involving a large amount

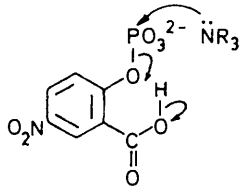


(14)

of P–O cleavage in the transition state ought also to be subject to catalysis. If the hydrolysis of salicyl phosphate is considered as the hydrolysis of a phosphate monoester dianion catalysed by a neighbouring carboxy-group, the efficiency of catalysis is much higher than considered hitherto: since only a small proportion of the reactive form is present, and because the dianion of phenyl phosphate is much less reactive than the monoanion. In fact the rate of hydrolysis of this ester is too slow to measure, though it can readily be estimated from the linear free-energy relationship [4] which correlates the rates of hydrolysis of substituted phenyl phosphate dianions with the pK_a values of the phenols concerned. This calculated rate for phenyl phosphate dianion is *ca.* $2·5 \times 10^{-10}$ min^{-1} at 39°, slower by a factor of 4×10^7 than that of the dianion of salicyl phosphate, so that when the difference in pK_a between the carboxy and phosphate groups is taken into account the rate enhancement due to catalysis by CO_2H is of the order of 10^{10}. Our proposed mechanism implies that the important conditions for this highly efficient catalysis are a large build up of negative charge on the leaving-group oxygen in the transition state for the uncatalysed reaction, and a sufficient concentration of the neutral form of a suitably placed carboxy-group. It is a simple matter to satisfy the second condition using the salicyl phosphate dianion, and a suitable reaction appears to be the S_N2(P) reaction of phosphate monoester dianions. P–O bond cleavage is thought to be well advanced in the transition state for such reactions also: a logarithmic plot of the second-order rate constants for the attack of pyridine on a series of substituted phenyl phosphate dianions, against the pK_a values of the conjugate acids of the leaving groups, had a slope [24] of −1·03, compared with a slope of −1·23 for the hydrolysis reaction.[4]

[24] A. J. Kirby and A. G. Varvoglis, *J. Chem. Soc.* (*B*), 1968, 135.

Catalysis of the $S_N2(P)$ *Reaction with Substituted Pyridines.*—Since the monophenyl phosphate dianion does not react at a detectable rate with amine nucleophiles, we looked first for the reaction between 5-nitrosalicyl phosphate and nicotinamide. (The choice of nucleophiles for these experiments is severely restricted, since the amine has to be present substantially in the free base form at pH 4—5, where the correct ionic form of the salicyl phosphate is present.) Catalysis by nicotinamide was readily apparent. Despite the rapid hydrolysis reaction the rate of appearance of 5-nitrosalicylate is increased by over 50% in the presence of 0·3M-nucleophile. The second-order rate constant obtained for this (presumed) $S_N2(P)$ reaction (15) was 0·25 l mol^{-1} min^{-1}. This is of the same order of magni-



(15)

tude as the first-order constant for hydrolysis (0·10 min^{-1}), which encouraged us to expect that catalysis of the hydrolysis of salicyl phosphate itself would be detectable.

We have observed similar catalysis of the hydrolysis of the dianion of salicyl phosphate, and have measured second-order rate constants for the reactions with 3-cyanopyridine, nicotinamide, and pyridine itself. The proportion of catalysed reaction is smaller than in the case of the 5-nitro-compound, but readily detectable, as described in the Experimental section. The rate enhancement of the $S_N2(P)$ reaction with pyridine due to intramolecular catalysis by the carboxy-group is *ca.* 10^8-fold, and the reactivity of the three pyridines is related to the basicity by the Brønsted equation (Figure 2), with a coefficient $\beta = 0{\cdot}21$.

This low sensitivity to the basicity of the nucleophile is typical of the $S_N2(P)$ reactions of phosphate dianions. For the reactions with substituted pyridines 2,4-dinitrophenyl phosphate dianion had $\beta = 0$,[24] and *p*-nitrophenyl phosphate has $\beta = 0{\cdot}13$,[25] so a value of

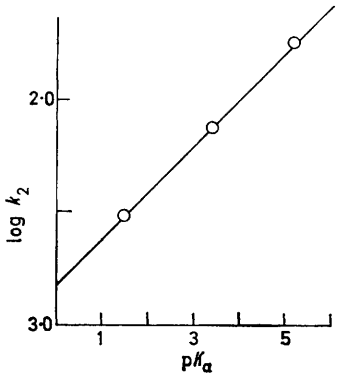


FIGURE 2 Brønsted plot for nucleophilic catalysis by 3-cyanopyridine, nicotinamide, and pyridine, of the hydrolysis of salicyl phosphate dianion, at 39° and ionic strength 1·0

0·2—0·3 would be expected for the reactions with phenyl phosphate, if they could be observed. Catalysis would be expected to reduce this figure, though by only a small factor if P–O bond cleavage is the dominant feature of the transition state. The sensitivity to the basicity of the leaving group is also almost exactly as expected. The sensitivity (ρ value) for the hydrolysis of substituted salicyl phosphate dianions is just over half that for the hydrolysis of aryl phosphate dianions (see above). Nicotinamide reacts with the dianion of 5-nitrosalicyl phosphate 30 ($10^{1.5}$) times faster than with the unsubstituted compound. For the reactions of pyridine with phenyl and *p*-nitrophenyl phosphate dianions the linear free-energy relationship [24] predicts a rate ratio of $10^{2.9}$.

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[1/1666 *Received, September 13th,* 1971]

[25] A. J. Kirby and W. P. Jencks, *J. Amer. Chem. Soc.*, 1965, **87**, 3209.