4.05 (quartet, J = 7 Hz, 2 H), and 5.06 (d of multiplets, J = 10 Hz, 1 H).

Methyl (*E*)-2,4-Dimethyl-2-hexenoate. The ethyl ester **11** (51 mg, 0.3 mmol) was stirred with 54 mg (1 mmol) of sodium methoxide in 1.5 ml of methanol at room temperature for 8 hr. Acidification with 10% aqueous HCl and product isolation gave 20 mg (42.7%) of desired methyl ester as a colorless oil. This material was chromatographically identical with a synthetic sample prepared by MacConnell.[12,15] Glpc: column B, 130°, 3.5 min; column C, 90°, 3.25 min. Tlc: pentane–ether 4:1, R_f 0.735; ethyl acetate–cyclohexane 3:2, R_f 0.63.

(*E*)-2,4-Dimethyl-2-hexenoic Acid (12). The corresponding ethyl ester **11** (100 mg, 0.59 mmol) was added to a solution of 300 mg (7.5 mmol) of sodium hydroxide in 3 ml of methanol. The solution was refluxed gently for 5 hr, cooled, and acidified with 10% aqueous HCl. Product isolation gave 80 mg (96%) of a malodorous oil. The material was identical with a synthetic sample prepared by MacConnell and previously shown to be identical with the natural acid **12**. Glpc: column A, 120°, 1.9 min; column D, 140°, 1.7 min. Tlc: methanol, R_f 0.53; cyclohexane–ethyl acetate 2:3, R_f 0.56. Bulb-to-bulb distillation gave a purified sample of the acid: nmr (100 MHz) 0.86 (t, J = 7 Hz, 3 H), 1.04 (d, J = 7 Hz, 3 H), 1.4 (quintet, J = 7 Hz, 2 H), 1.8 (d, J = 1.4 Hz, 2 H), 2.4 (m, 1 H), and 6.62 (doublet of quartets, J = 10 Hz, J = 2 Hz, 1 H); ir 3080–2540 (broad), 1685 (s), 1640, and 1285 cm^{-1}; mass spectrum (70 eV) m/e 142 (M^+) and 42 (base peak).

Anal. Calcd for $C_8H_{14}O_2$: C, 67.57; H, 9.92. Found: C, 67.30; H, 9.93.

(*E*)-2,4-Dimethyl-2-hexenoyl Chloride. The procedure of Engel and Just was used.[23] Oxalyl chloride (0.5 ml) in 1 ml of dry benzene was added to a solution of 50 mg (0.35 mmol) of 2,4-dimethyl-2-hexenoic acid in 2 ml of benzene at 0°. The reaction mixture was stirred for 2 hr at room temperature. The solvent and excess reagent were removed under reduced pressure, and the acid chloride was used without further purification.

(*E*)-4,6-Dimethyl-4-octen-3-one (Manicone, 13).[18] Cuprous iodide (95 mg, 0.5 mmol) was slurried in 1.5 ml of ether under nitrogen and cooled to −10°. Ethyllithium (1 mmol, 1.25 ml of a 0.8 *M* solution in benzene) was added slowly. After 5 min at −10°, the vessel was further cooled to −78°, and a precooled solution of the acid chloride prepared above in 0.3 ml of ether was added *via* syringe. After 15 min at −78°, the reaction was quenched by addition of 0.075 ml (60 mmol) of methanol; the flask was warmed to room temperature, and the reaction mixture was mixed with saturated aqueous NH_4Cl. Product isolation gave 50 mg (92%) of a yellow oil, which was further purified by bulb-to-bulb distillation to give colorless material whose spectroscopic properties were consistent with those reported for the natural material[17]: nmr δ 0.9 (t, J = 7 Hz, 3 H), 1.02 (d, J = 7 Hz, 3 H), 1.06 (t, J = 7 Hz, 3 H), 1.4 (m, broad, 2 H), 1.73 (d, J = 1.4 Hz, 3 H), 2.4 (m, 1 H), 2.59 (quartet, J = 7 Hz, 2 H), and 6.24 (d, J = 10 Hz, 1 H); ir 1750 (w), 1670 (s), and 1650 cm^{-1}.

The 2,4-dinitrophenylhydrazone derivative was prepared by the addition of 15 mg of the ketone **13** to 1 ml of the derivatizing reagent (prepared by the cautious addition of 0.5 ml of concentrated H_2SO_4 to 0.25 g of 2,4-dinitrophenylhydrazine suspended in 5 ml of methanol). The precipitate which formed immediately was filtered and twice recrystallized from ethanol to give red leaf-like crystals, mp 130–131° (lit.[18] 129–131°).

Acknowledgment. Support of this work through grants from the National Institutes of Health (GM 17061) and the National Science Foundation (GP 38441X) is gratefully acknowledged.

(23) Ch. R. Engel and G. Just, *Can. J. Chem.*, **33**, 1515 (1955).

Acylal Hydrolysis. The Hydrolysis of 3-(*p*-Nitrophenoxy)phthalide, α-Acetoxybenzyl *p*-Nitrophenyl Ether, and 3-(Para-Substituted Thiophenyl)phthalides

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Abstract: The rates of hydrolysis of the acylals 3-(*p*-nitrophenoxy)phthalide, α-acetoxybenzyl *p*-nitrophenyl ether, and 3-(para-substituted thiophenyl)phthalides have been measured at 30° in H_2O. In hydrolysis of these compounds hydronium ion and hydroxide ion catalysis takes place. There is also a pH-independent spontaneous reaction. The pH-independent hydrolysis of 3-(*p*-nitrophenoxy)phthalide is characterized by a ΔS^* of −47.1 eu, indicating solvent involvement in the reaction. In the hydronium ion catalyzed hydrolysis of α-acetoxybenzyl *p*-nitrophenyl ether ΔS^* is −17.6 eu. There is pronounced catalysis of the hydrolysis of these compounds by the base component of the buffer. In the case of the *p*-nitrophenoxy acylals there is both a first-order and a second-order dependence on base concentration, suggesting base catalysis of nucleophilic attack by a second molecule of base or a kinetic equivalent. With the 3-(para-substituted thiophenyl)phthalides there is strict first-order dependence on the concentration of the catalyzing base. From the D_2O solvent isotope effect for ethanolamine catalysis of the hydrolysis of 3-(*p*-nitrothiophenyl)phthalide (k_B^H/k_B^D = 1.8) it can be inferred that catalysis is by a general base mechanism involving proton transfer in the rate-determining step. The Hammett ρ value for ethanolamine catalysis is 0.55 and that for hydroxide ion is 0.42. It is apparent that these acylals are hydrolyzing as esters not acetals.

X-Ray crystallographic studies[2] of the glycosidic enzyme lysozyme at 2-Å resolution have indicated that carboxyl groups from glutamic acid-35 and aspartic acid-52 are located at the active site. Several mechanisms have been postulated employing these carboxyl groups.[3] A mechanism recently receiving support involves general acid catalysis by glutamic acid-35 and

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(2) C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Nature (London)*, **206**, 757 (1965); L. N. Johnson and D. C. Phillips, *ibid.*, **206**, 761 (1965).

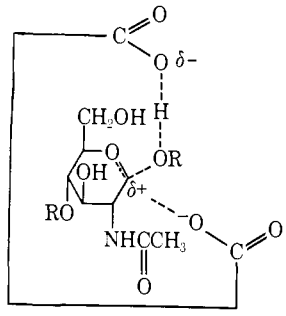
(3) D. C. Phillips, *Sci. Amer.*, **215**, 78 (1969); G. Lowe, G. Sheppard, M. L. Sinnott, and A. Williams, *Biochem. J.*, **104**, 893 (1967); M. A. Raftery and T. Rand-Meir, *Biochemistry*, **7**, 3281 (1968).

Table I. Physical Constants of Thiophthalides

Compd	Para substituent	Mp, °C	Anal., % Calcd C	Anal., % Calcd H	Anal., % Found C	Anal., % Found H
1	NO_2	175–178 (177–178)[a]				
2	Cl	119–120 (121–122)[b]				
3	H	102–103 (102–103)[b]				
4	CH_3	119–120	70.30	4.70	70.31	4.62
5	OCH_3	115–117	66.16	4.42	65.89	4.27

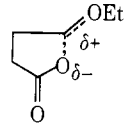
[a] Kh. Feldman and T. I. Gurevich, *J. Gen. Chem. USSR*, **21**, 1544 (1951); *Chem. Abstr.*, **46**, 5016 (1952). [b] D. D. Wheeler, D. C. Young, and D. S. Erley, *J. Org. Chem.*, **22**, 547 (1957).

electrostatic stabilization of a developing carbonium ion by the aspartate carboxylate anion. If covalent

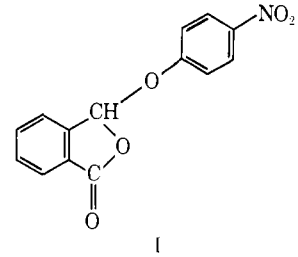


bond formation occurs then an acylal will be produced as an intermediate. A glycosyl enzyme intermediate in which the anomeric carbon is covalently bound to a carboxyl group has also been suggested in reactions of *Escherichia coli* β-galactosidase.[4,5] It has therefore been important to study the hydrolysis reactions of various acylals in order to gain an understanding of the kinetic behavior that would be expected of such an intermediate in the enzymatic reactions.

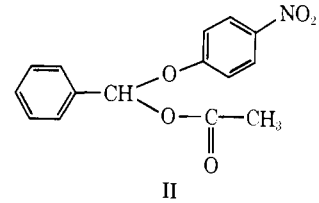
γ-Ethoxy-γ-butyrolactone hydrolyzes as an acetal not an ester at acid or neutral pH values.[6] The mechanism of the acid-catalyzed reaction is A-1 entailing preequilibrium protonation of the substrate by hydronium ion followed by rate-determining unimolecular decomposition of the protonated intermediate to a resonance stabilized intermediate. In the pH range from 5 to 9 a pH-independent reaction occurs. In the reaction unimolecular SN1 decomposition takes place due to the good leaving group and the stabilized carbonium ion intermediate.[7]



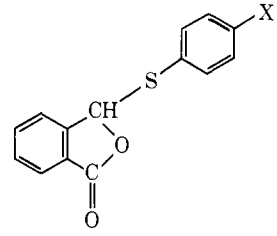
Acylals have generally been observed to undergo acid-catalyzed hydrolysis by an A-1 mechanism,[8] although Salomaa[9] proposed that methoxymethyl formate hydrolyzed by both A-1 and A-2 mechanisms. Also, it was suggested that the mechanism in the case of methyl pseudo-2-benzoylbenzoate[10] was A-2 (rate-determining attack of water on the protonated substrate). It was thought that if the carbonium ion intermediate in acylal hydrolysis is not highly stabilized, then the compound should hydrolyze like a typical ester with the mechanism being AAC2 under acidic conditions, and, in view of the reasonably good leaving group (a hemiacetal oxygen), with catalysis by general bases at neutral or mildly alkaline pH values. We have therefore studied the hydrolysis reactions of the acylals I, II, and III since



I



II



III

little stabilization will result from the nitrophenoxy or thiophenyl groups. We have found that in the hydrolysis of these acylals there is pronounced catalysis by the base component of the buffer in contrast to the lack of such catalysis with acylals that have previously been investigated.

Experimental Section

Materials. Imidazole (K & K Laboratories) was crystallized twice from benzene and then sublimed under reduced pressure. *N*-Methylimidazole, *N*,*N*-dimethylethanolamine, and ethanolamine were obtained from Aldrich and were distilled prior to use. Potassium acetate (Matheson Coleman and Bell) and sodium formate (J. T. Baker) were used without purification. Deuterated oxide (99.75%) and deuterium chloride (38%) in D_2O were purchased from J. T. Baker.

3-(Para-substituted phenyl)thiophthalides were prepared by the following procedure. A mixture of 0.01 mol of *o*-carboxybenzaldehyde and 0.01 mol of para-substituted thiophenol was refluxed in benzene with a trace of *p*-toluenesulfonic acid for 24 hr. The solution was cooled and filtered. The filtrate was extracted with saturated solutions of sodium bicarbonate (2 × 25 ml) and water, dried over $MgSO_4$, and concentrated to give a solid residue. The residue was crystallized from ethanol to give a crystalline solid. Infrared spectra (KBr) of all compounds showed absorption at 1740–1760 cm^{-1}. Physical constants of the thiophthalides are given in Table I.

(4) O. M. Viratelle, J. P. Tenu, J. Garnier, and J. Yon, *Biochem. Biophys. Res. Commun.*, **37**, 1036 (1969); J. P. Tenu, O. M. Viratelle, J. Garnier, and J. Yon, *Eur. J. Biochem.*, **20**, 363 (1971).

(5) For a review of the literature, see B. Capon, *Chem. Rev.*, **69**, 407 (1969).

(6) T. H. Fife, *J. Amer. Chem. Soc.*, **87**, 271 (1965).

(7) For examples of unimolecular decompositions in acetal hydrolysis reactions, see T. H. Fife, *Accounts Chem. Res.*, **5**, 264 (1972).

(8) P. Salomaa, *Acta Chem. Scand.*, **11**, 132 (1957); P. Salomaa and S. Laiho, *Acta Chem. Scand.*, **17**, 103 (1963); P. Salomaa, *Suom. Kemisteleti B*, **37**, 86 (1964).

(9) P. Salomaa, *Acta Chem. Scand.*, **11**, 141, 239 (1957).

(10) D. P. Weeks, A. Grodski, and R. Fanucci, *J. Amer. Chem. Soc.*, **90**, 4958 (1968).

3-(*p*-Nitrophenoxy)phthalide (I). In a 100-ml flask were placed 5.6 g (0.04 mol) of *p*-nitrophenol, 6.0 g (0.04 mol) of *o*-carboxybenzaldehyde, and 1.0 g (0.0053 mol) of *p*-toluenesulfonic acid. The flask was immersed in a preheated oil bath (160–170°) and kept for 30 min. The flask was then cooled, and CH_2Cl_2 (75 ml) was added. The solution was extracted with a saturated solution of sodium bicarbonate (3 × 25 ml) and water, dried with $MgSO_4$, and concentrated to give a solid residue. Crystallization first from ethyl acetate and then with methanol gave I, mp 181–182°. *Anal.* Calcd for $C_{14}H_9NO_5$: C, 62.01; H, 3.32. Found: C, 61.89; H, 3.46.

***p*-Nitrophenyl Benzyl Ether.** In a 500-ml three-necked flask fitted with a condenser, dropping funnel, and a magnetic stirrer was placed 150 ml of absolute ethanol. Sodium (2.3 g, 0.1 g-atom), cut into pieces, was added. When all sodium was in solution, 14.0 g (0.1 mol) of *p*-nitrophenol in 50 ml of absolute ethanol was added slowly. After addition was complete, 12.7 g (0.1 mol) of benzyl chloride was added during a period of 15 min. The flask was heated for 14 hr. Excess ethanol was then removed *in vacuo;* while still hot the mixture was poured into an ice–water mixture. The precipitate thus obtained was crystallized from absolute ethanol to give the desired ether, mp 106° (lit.[11] mp 106°).

α-Acetoxybenzyl *p*-Nitrophenyl Ether (II). In a 250-ml flask were placed 2.2 g (0.01 mol) of *p*-nitrophenyl benzyl ether, 1.78 g (0.01 mol) of *N*-bromosuccinimide, and 50 ml of CCl_4. The mixture was refluxed for 30 min in presence of a light source (150 W) held approximately 8 in. from the flask. Completion of bromination was indicated by the appearance of succinimide at the surface of the reaction mixture.[12] The mixture was cooled and filtered. The filtrate was concentrated *in vacuo* to give a solid residue (bromo ether) which was then used to prepare the acetoxy ether. To the solid residue were added CH_2Cl_2 (40 ml) and 0.98 g (0.01 mol) of potassium acetate which had been dried *in vacuo* for 14 hr. The mixture was stirred for 18 hr at room temperature and was then filtered. The filtrate was concentrated to give an oily liquid which solidified upon standing in the cold. Fractional crystallization with hexane gave the acetoxy ether, mp 91–92.5°. *Anal.* Calcd for $C_{15}H_{13}NO_5$: C, 62.72; H, 4.52. Found: C, 62.69; H, 4.70.

Kinetics. A Gilford Model 2000 and a Zeiss PMQ 11 spectrophotometer were used for collection of rate data. Preliminary scans were taken on a Cary 15 recording spectrophotometer. The reactions were carried out under pseudo-first-order conditions in water and at an ionic strength of 0.5 *M* KCl unless otherwise stated. Stock solutions were prepared in redistilled acetonitrile or methanol. The pH of each solution was determined before and after runs with a Radiometer pHM-22, pH meter to ensure constancy of pH (0.02 unit). Reactions were initiated by addition of substrates to a preequilibrated (30 ± 0.1°) cuvette containing exactly 3 ml of buffer solution. Final concentrations of substrates were 2–7 × 10^{-5} *M*. Ethylenediaminetetraacetic acid (final concentration 2 × 10^{-4} *M*) was added for the reaction of imidazole buffer and **1**. Reactions were monitored at the following wavelengths. Compound I, at pH values greater than 7, 400 nm (appearance of *p*-nitrophenolate), and at pH values less than 7, 290 nm (disappearance of substrate) and 320 nm (appearance of *p*-nitrophenol). Compound II, at pH values greater than 7, 400 nm (appearance of *p*-nitrophenolate), and at pH values less than 7, 255 nm (appearance of benzaldehyde) and 320 nm (appearance of *p*-nitrophenol). Hydrolysis of the thiophthalides III was followed by observing appearance of the corresponding thiophenoxide ion at 410 nm in the case of **1** and at 270 nm for compounds **2–5** or by observing formation of aldehyde at 254 nm.

Pseudo-first-order rate constants were calculated with an IBM 360-40 computer using a least-squares procedure, or from the slopes of plots of log $(OD_\infty - OD_0)/(OD_\infty - OD_t)$ *vs.* time. These plots are linear to several half-lives.

Results

In Tables II and III are given the rate constants for hydrolysis of 3-(*p*-nitrophenoxy)phthalide and α-acetoxybenzyl *p*-nitrophenyl ether. With both compounds there is a hydroxide ion and hydronium ion catalyzed reaction. A pH-independent reaction occurs from 3.0 *M* HCl to pH 8 with I and from pH 3 to 9 in the case of II. The rate constants k_0 for the pH-independent reaction, obtained by extrapolation to zero buffer concentration, are 3.5 × 10^{-5} sec^{-1} for I and 1 × 10^{-4} sec^{-1} for II at 30°. D_2O solvent isotope effects for pH-independent hydrolysis (k_{H_2O}/k_{D_2O}) are 1.84 and 2.50 for I and II, respectively. It will be noted that the rate constants for the hydroxide ion reaction and the pH-independent reaction are quite similar for both compounds. Hydronium ion catalysis, however, is 2500 times more favorable with II. In Table IV rate constants are presented for hydrolysis of 3-(*p*-nitrophenoxy)phthalide in moderately concentrated HCl solutions.

Values of k_{obsd} for hydrolysis of I in 2.0 *M* HCl and II in 0.1 *M* HCl were obtained at various tempera-

Table II. Rate Constants for Hydrolysis of 3-(*p*-Nitrophenoxy)phthalide in H_2O at 30°; μ = 0.5 (with KCl)

Buffer	k_{H^+}, M^{-1} sec^{-1}	k_{OH}, M^{-1} sec^{-1}	k_B, M^{-1} sec^{-1}	k_B', M^{-2} sec^{-1}
HCl	0.00003			
Formic acid			0.00022	
Imidazole			0.0028	0.014
N-Methylimidazole			0.000024	
N,*N*-Dimethylethanolamine			0.028	
Ethanolamine			0.04	0.35
Ethanolamine (D_2O)			0.05	0.27
KOH		7.86[a]		

[a] Determined from a plot of k_{obsd} *vs.* OH^- concentration at five pH values (10.55–11.67).

Table III. Rate Constants for Hydrolysis of α-Acetoxybenzyl *p*-Nitrophenyl Ether in H_2O at 30°; μ = 0.5 (with KCl)

Buffer	k_{H^+}, M^{-1} sec^{-1}	k_{OH}, M^{-1} sec^{-1}	k_B, M^{-1} sec^{-1}	k_B', M^{-2} sec^{-1}
HCl	0.076[a]			
Formic acid			0.000325	
Imidazole			0.00106	
Ethanolamine			0.0079	0.020
Ethanolamine (D_2O)			0.0054	0.015
N,*N*-Dimethylethanolamine			0.0012	
KOH		1.94[b]		

[a] Determined from a plot of k_{obsd} *vs.* HCl concentration at nine pH values (0.35–2.06). [b] Determined from a plot of k_{obsd} *vs.* ^-OH concentration at three pH values (11.30–12.0).

Table IV. Values of k_{obsd} for Hydrolysis of 3-(*p*-Nitrophenoxy)phthalide in Moderately Concentrated HCl at 30°

(HCl), *M*	k_{obsd} × 10^5, sec^{-1}
0.25[a]	4.4[b]
0.185[a,c] (D_2O)	2.4[b]
0.50	4.3[b]
2.0	3.67[b]
3.0	3.4
4.0	5.75
5.5	8.4
5.9	10.1
6.5	12.6
6.9	15.1

[a] μ = 0.5 *M* (with KCl). [b] pH-independent reaction. [c] DCl in D_2O.

(11) E. Vowinkel, *Chem. Ber.*, **95**, 2997 (1962); *Chem. Abstr.*, **58**, 7850[d] (1963).

(12) L. A. Koten and R. J. Sauer, *Org. Syn.*, 42, 26 (1962).

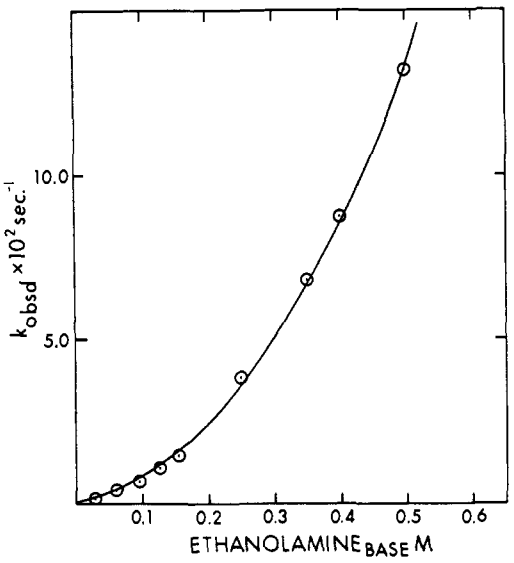


Figure 1. Plot of k_{obsd} for hydrolysis of 3-(*p*-nitrophenoxy)-phthalide at pH 9.50 and 30° (μ = 0.5 with KCl) *vs.* ethanolamine free base concentration (*M*).

Table V. Temperature Dependence of k_{obsd} (sec^{-1}) in Hydrolysis of 3-(*p*-Nitrophenoxy)phthalide and α-Acetoxybenzyl *p*-Nitrophenyl Ether in HCl Solutions

Compd	(HCl), *M*	Temp, °C	k_{obsd}, sec^{-1}
I	2.0	30.0	3.67×10^{-5}
	2.0	38.5	5.86×10^{-5}
	2.0	45.5	7.90×10^{-5}
	2.0	52.0	1.19×10^{-4}
II[a]	0.10	30.0	0.76×10^{-2}
	0.10	38.5	1.51×10^{-2}
	0.10	45.5	2.62×10^{-2}
	0.10	52.0	4.63×10^{-2}

[a] μ = 0.5 *M* with KCl.

tures. These rate constants are given in Table V. The values of ΔH^* and ΔS^* are 9.6 ± 1.0 kcal/mol and −47.1 eu for I and 15.2 ± 1.0 kcal/mol and −17.6 eu for II. The ΔS^* values were calculated at 30° with the rate constants having the units sec^{-1} in the case of I and M^{-1} sec^{-1} in the case of II.

The hydrolysis of I and II is markedly catalyzed by buffer bases. In Figure 1 a plot is shown of k_{obsd} *vs.* ethanolamine free base concentration at pH 9.50 for hydrolysis of I. Marked curvature will be noted. Similar data were also obtained at pH 9.80, 10.05, and 10.50. Plots of k_{obsd} *vs.* the square of the base concentration were linear showing a second-order dependence on the base concentration. In Figure 2 there is presented a plot of $(k_{obsd} - k_{int})$/base *vs.* (base). The plot is linear with a definite intercept. Thus, there is a second-order dependence on base concentration and also a first-order dependence. The minimal equation for k_{obsd} is that given in eq 1.

$$k_{obsd} = k_0 + k_{H^+}(H^+) + k_{OH}(OH^-) + k_B(B) + k_B'(B)^2 \quad (1)$$

Similar behavior was observed with II in ethanolamine buffers, but curvature in plots of k_{obsd} *vs.* base concentration was much less than in the case of I, and in imidazole buffers deviation from linearity is not

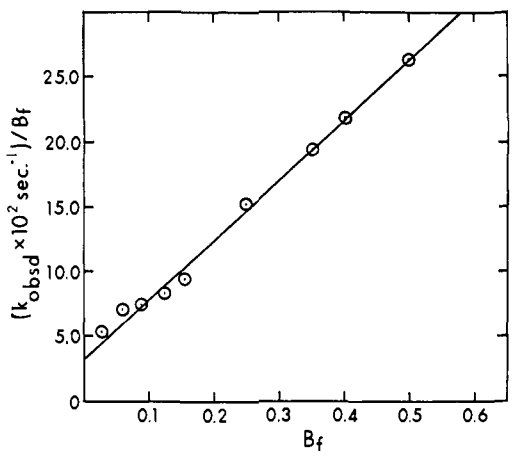


Figure 2. Plot of $(k_{obsd} - k_{int})$/base *vs.* base (*M*) for ethanolamine catalyzed hydrolysis of 3-(*p*-nitrophenoxy)phthalide at 30° and μ = 0.5.

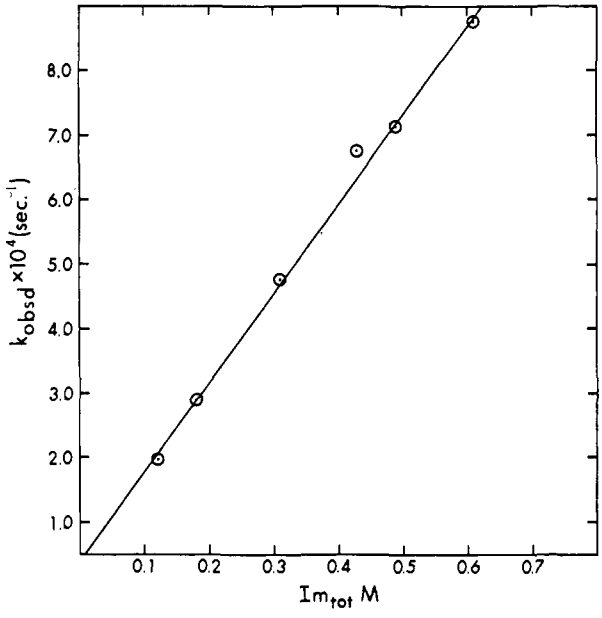


Figure 3. Plot of k_{obsd} for hydrolysis of α-acetoxybenzyl *p*-nitrophenyl ether at 30° and pH 7.0 (μ = 0.5 with KCl) *vs.* imidazole concentration.

statistically significant. A typical plot is shown in Figure 3.

In hydrolysis of the cyclic acylal I in imidazole buffers there is both first-order and second-order dependence on imidazole base concentration with eq 1 being followed. With *N*-methylimidazole as the catalyst, however, only the first-order dependence can be detected (Figure 4), eq 2 being followed. This is also the case with *N,N*-dimethylethanolamine catalysis.

$$k_{obsd} = k_0 + k_{H^+}(H^+) + k_{OH}(OH^-) + k_B(B) \quad (2)$$

The hydrolysis of the thioacylals (III) is subject to catalysis by various bases with a first-order dependence on the base concentration. The rate constant for ethanolamine catalysis with the nitrophenyl derivative is less in D_2O than H_2O (k_B^H/k_B^D = 1.77). Rate constants for thioacylal hydrolysis are given in Table VI. In Figure 5 is shown a plot of log k_B for ethanolamine catalysis of the hydrolysis of the series of thioacylals *vs.*

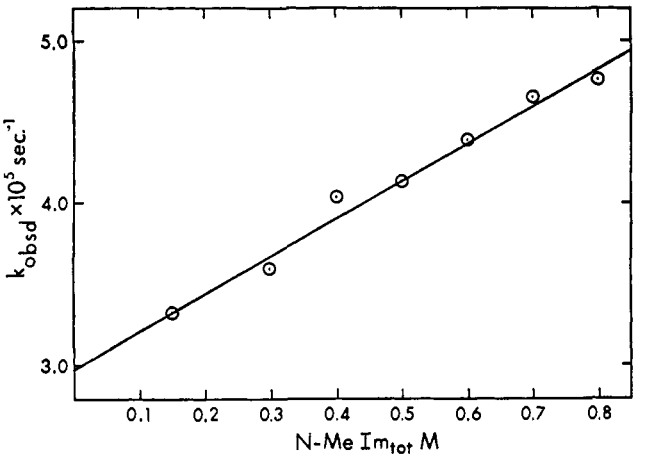


Figure 4. Plot of k_{obsd} for hydrolysis of 3-(*p*-nitrophenoxy)-phthalide at 30° and pH 8.15 (μ = 0.5 with KCl) *vs.* *N*-methylimidazole concentration (*M*).

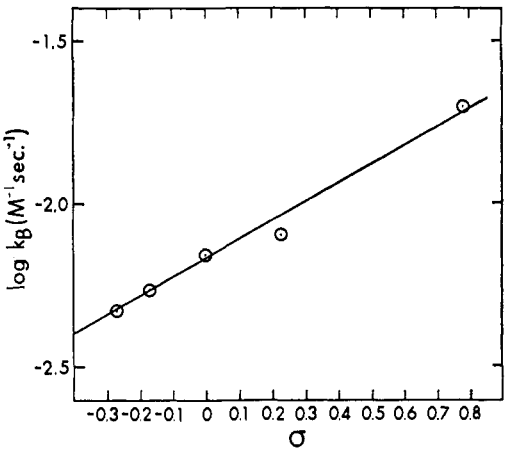


Figure 5. Plot of log k_B for ethanolamine-catalyzed hydrolysis of 3-(para-substituted thiophenyl)phthalides at 30° (μ = 0.5) *vs.* σ, the Hammett substituent constant.

Table VI. Second-Order Rate Constants for Hydrolysis of 3-(Para-Substituted Thiophenyl)phthalides in H_2O at 30°, μ = 0.5

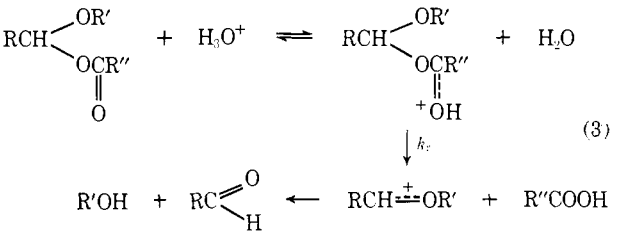
Para substituent	k_{OH}, M^{-1} sec^{-1}	k_{ENH_2},[a] M^{-1} sec^{-1}	k_{Im},[b] M^{-1} sec^{-1}
NO_2	2.84	0.023 (H_2O) 0.013 (D_2O)	0.00022
Cl	1.86	0.008	
H	1.42	0.0069	
CH_3	1.33	0.0055	
OCH_3	0.85	0.0047	

[a] Second-order rate constant for ethanolamine catalysis. [b] Second-order rate constant for imidazole catalysis.

σ, the Hammett substituent constant.[13] The value of ρ is 0.58. The ρ for hydroxide ion catalyzed hydrolysis is 0.42.

Discussion

Acylals generally hydrolyze at acid and neutral pH values like typical acetals, the acid catalyzed reaction occurring by an A-1 mechanism in which preequilibrium protonation by hydronium ion is followed by rate-determining unimolecular decomposition to give a resonance stabilized carbonium ion.[6,8] As with acetals, this mechanism arises because of the stability of the intermediate oxocarbonium ion. In such a case the bond-breaking process is facile.



(3)

The A-1 mechanism is then favored over an A-2 mechanism, in which a water molecule attacks the protonated substrate, because in the unimolecular mechanism ΔS^* will be more positive than in a bimolecular reaction.[14,15]

It has been proposed[16] that for an acid-catalyzed reaction when (log k_{obsd} + H_0) is plotted *vs.* the log of the activity of water, the slope, *w*, will give the difference in number of water molecules in the ground state and transition state of the reaction relative to a standard set by the indicator bases and their conjugate acids used to determine the H_0 values.[17] It is found empirically that negative *w* values, as observed for acetal hydrolysis, are characteristic of A-1 reactions in which a water molecule is not present in the critical transition state.[16] Ester hydrolysis reactions, on the other hand, are characterized by large positive *w* values (4.5–5.5) indicating that a number of water molecules are present as proton transfer agents.[16,18] In the present study of I, a Bunnett plot of log (k_{obsd} − k_0) + H_0 *vs.* $-a_{H_2O}$ (Figure 6) is linear at HCl concentrations greater than 4.0 *M* where the reaction is acid catalyzed, giving a *w* value of 2.3. Such a large positive value is not in accord with an A-1 mechanism but suggests that

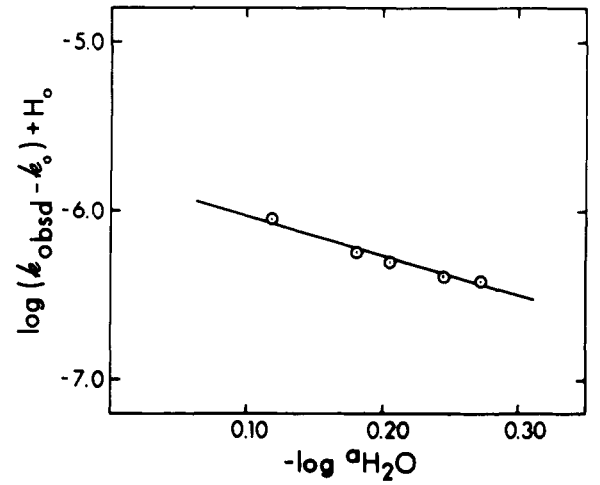


Figure 6. Plot of log (k_{obsd} − k_0) + H_0 *vs.* −log of the activity of water in moderately concentrated HCl solutions at 30° for hydrolysis of I.

(13) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1940.

(14) L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, 1, 1 (1963).

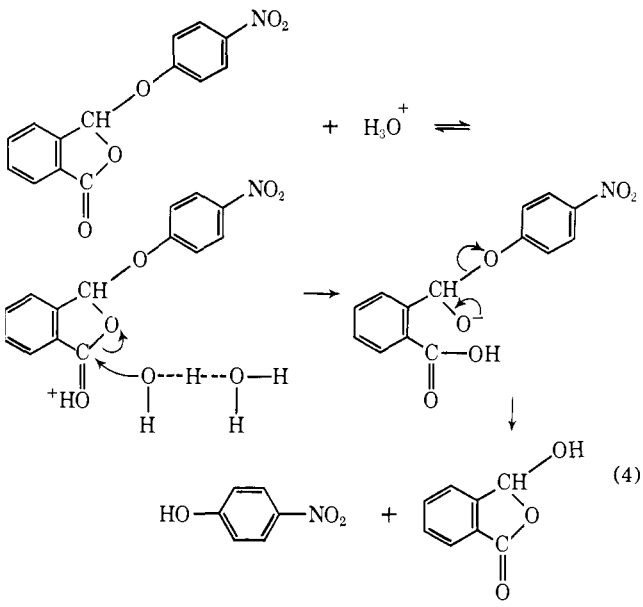
(15) F. A. Long, J. G. Pritchard, and R. E. Stafford, *J. Amer. Chem. Soc.*, 79, 2362 (1957).

(16) J. F. Bunnett, *J. Amer. Chem. Soc.*, 83, 4956, 4968, 4978 (1961).

(17) F. A. Long and M. A. Paul, *Chem. Rev.*, 57, 935 (1960).

(18) T. H. Fife and D. M. McMahon, *J. Amer. Chem. Soc.*, 91, 7481 (1969).

water is involved in the transition state. Thus, although the data are more limited than desired due to the fact that hydronium ion catalysis is only observed at relatively high acid concentration, it is probable that I is hydrolyzing like a typical ester not an acetal (eq 4).



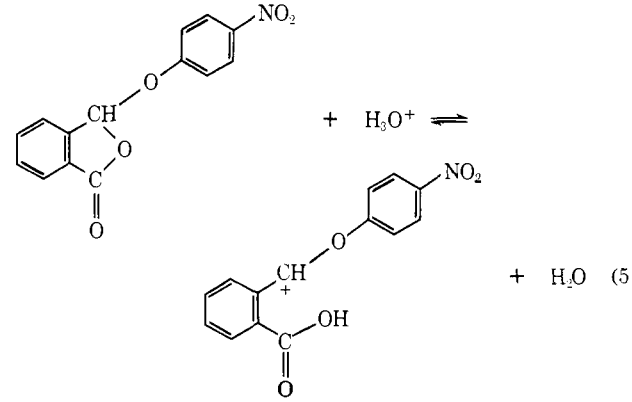
(4)

The pH-independent reaction of I also features participation by water. That reaction is considerably slower in D_2O than in H_2O (k_{H_2O}/k_{D_2O} = 1.84) in contrast to the pH-independent unimolecular hydrolysis of γ-ethoxy-γ-butyrolactone and pH-independent acetal hydrolysis[7] which proceed at nearly the same rate in H_2O and D_2O (k_{H_2O}/k_{D_2O} = 0.9). The ΔS^* for hydrolysis in 2.0 *M* HCl where the reaction is predominantly uncatalyzed is −47.1 eu, a value which strongly points to solvent involvement in the reaction. In view of the similarity in rate constants for pH-independent hydrolysis of I and II it is a reasonable conclusion that the mechanism is the same for both compounds, *i.e.*, attack by H_2O at the carbonyl. Also, for II k_{H_2O}/k_{D_2O} = 2.50, signifying analogous solvent participation.

A nitrophenoxy acylal may hydrolyze like an ester because little stabilization of a developing carbonium ion will be possible by the nitrophenoxy group, making unimolecular bond cleavage difficult. For the reaction to proceed, attack of a water molecule on the neutral or protonated substrate might be required to assist the bond-breaking process even though the entropy of activation would be more negative than if the rate-determining step were unimolecular.

The acid-catalyzed hydrolysis of the open chain acylal II has a ratio k_D/k_H of 2.24, which is greater than expected for an A-2 ester hydrolysis reaction. Likewise, the ΔS^* of −17.6 eu is more negative than expected for an A-1 mechanism but less negative than is usual for A-2 ester hydrolysis. The typical values of ΔS^* and the D_2O solvent isotope effect k_D/k_H point to an A-1 and A-2 mechanism, respectively, for acid-catalyzed acetal and ester hydrolysis.[14-16,19] In acetal hydrolysis ΔS^* is usually close to zero or positive, whereas in ester hydrolysis reactions it is highly negative. The value of k_D/k_H is generally in excess of 2.7 for acetal hydrolysis but is in the range 1.4–1.8 in ester hydrolysis.[20] However, k_D/k_H ratios of 2.2–2.5 have been found in hydrolysis of acyl phosphates where the mechanism is most likely A-2,[21] and in the A-2 hydrolysis of 2-(*p*-methoxyphenyl)-4,4,5,5-tetramethyl-1,3-dioxolane, k_D/k_H is 2.4.[22] While it is probable that the mechanism for acid-catalyzed hydrolysis of the cyclic acylal I is A-2, a conclusive decision cannot be made in the case of II. It is probable, however, that the critical transition state for II is a closer approximation to an A-1 process.

Hydronium ion catalysis is much more favorable with II than I, the second-order rate constants differing by approximately 2500. Considering the structures of the two compounds, it is clear that the cyclic structure of I is responsible for its relatively slow rate of hydrolysis because of either a greater dissociation constant for the conjugate acid or unfavorable steric effects. The ring structure of I could be a contributing factor in giving rise to an A-2 mechanism of hydrolysis. Formation of a carbonium ion intermediate from I in a ring-opening reaction would result in a species with a carboxyl group held rigidly adjacent to the carbonium ion. This might result in rapid reclosure of the ring to regenerate



(5)

starting material. The reaction could then only proceed readily to products if carbonium ion formation were avoided, that is, if the mechanism were A-2. Alternatively, if carbonium ion formation were rapid but reversible, reaction of the carbonium ion with a water molecule could become rate limiting. Similar explanations have been proposed for the A-2 mechanism of hydrolysis of benzaldehyde-4,4,5,5-tetramethyl-1,3-dioxolanes.[7,23]

Weeks and Crane[24] have recently examined acid-catalyzed hydrolysis of 3-methoxyphthalide in aqueous sulfuric acid. Values of ΔS^* (−3.1 eu), k_H/k_D (0.51), and a linear plot of log k_{obsd} *vs.* $-H_0$ with a slope of 0.96 pointed to an A-1 mechanism. A cyclic carbonium ion intermediate was suggested on the basis of substituent effects at the 3 position, the following reactivities being observed: H > CH_3 > Et ~ phenyl. It was considered that A-1 ring opening would not explain this order,[24] but the acid-catalyzed ring opening of 2-substituted-2-phenyl-1,3-dioxolanes proceeding by an A-1 mechanism displays a similar order of reactivity.[25] A mechanism involving a cyclic carbonium

(19) E. H. Cordes, *Progr. Phys. Org. Chem.*, 4, 1 (1967).

(20) F. A. Long, *Ann. N. Y. Acad. Sci.*, 84, 596 (1960).

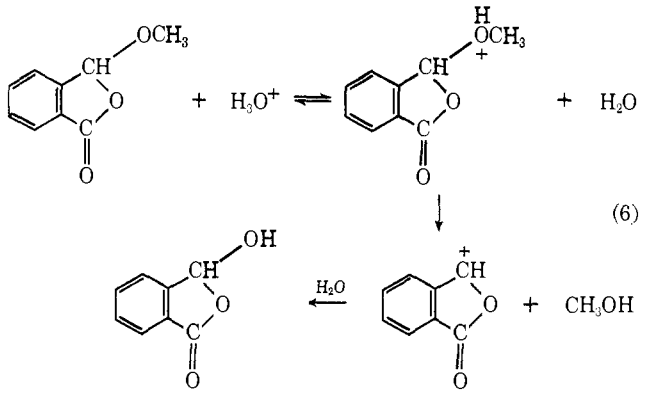
(21) D. R. Phillips and T. H. Fife, *J. Amer. Chem. Soc.*, 90, 6803 (1968).

(22) T. H. Fife, *J. Amer. Chem. Soc.*, 89, 3228 (1967).

(23) T. H. Fife and L. H. Brod, *J. Org. Chem.*, 33, 4136 (1968).

(24) D. P. Weeks and J. P. Crane, *J. Org. Chem.*, 38, 3375 (1973).

(25) T. H. Fife and L. Hagopian, *J. Org. Chem.*, 31, 1772 (1966).



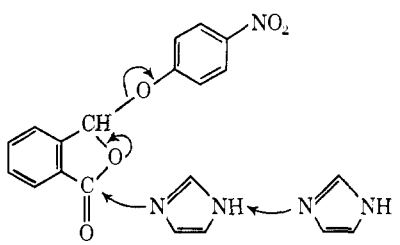
(6)

ion can be criticized on the basis that (1) an open-chain carbonium ion with an adjacent methoxy group would be stabilized to a greater extent by resonance interaction with oxygen; (2) the carboxyl group would be the best leaving group in the reaction; and (3) if formation of a cyclic carbonium ion was energetically favorable it would be formed in the pH-independent reaction of compound I where *p*-nitrophenol is a good leaving group, but that reaction unquestionably utilizes solvent participation. Zucker–Hammett slopes deviate greatly from unity and ΔS^* values become quite negative with increasing size of substituents at the 3 position.[24] With the 3-phenyl derivative the slope of a plot of log k_{obsd} *vs.* $-H_0$ is 0.67 and ΔS^* is -20.6 eu, a value more in accord with an A-2 mechanism. Hence, no conclusive decision should be made in regard to the mechanism of acid-catalyzed hydrolysis of the methoxyphthalide derivatives.

Acid-catalyzed hydrolysis of 3-methoxyphthalide proceeds approximately 1000 times faster than reaction of 3-(*p*-nitrophenoxy)phthalide. This large difference is not consistent with a mechanism in which a cyclic carbonium ion is formed as an intermediate. Electron withdrawal in the leaving group has only a small effect on rate in hydrolysis of glycosides and acetals. The Hammett ρ for substituted phenyl β-D-glucosides[26] is -0.66 and that for hydrolysis of 2-(para-substituted phenoxy)tetrahydropyrans[27] is -0.9. In both cases the *p*-nitrophenoxy and methoxy derivatives hydrolyze at comparable rates. The reactivities of the phthalide acylals are probably reflecting differing stabilities of a ring-opened carbonium ion which may result in a difference in mechanism (A-2 in the case of I). The influence of reversibility would be greater in the case of the less stable carbonium ion intermediate since equilibrium would lie further on the side of protonated acylal.

The pronounced catalysis by formate and imidazole in the hydrolysis of I and II in the pH range where spontaneous hydrolysis is pH independent is in sharp contrast with the lack of such catalysis in hydrolysis of previously studied acylals. For example, the rate of hydrolysis of γ-ethoxy-γ-butyrolactone is unaffected by very high concentrations of imidazole or acetate ion.[6] This was an important piece of evidence leading to the assignment of a unimolecular SN1 type mechanism for the facile pH-independent reaction at pH 5–9. If H_2O was functioning as a nucleophile it might reasonably be expected that more nucleophilic bases would have an effect. The intermediate carbonium ion produced from γ-ethoxy-γ-butyrolactone is well stabilized by the adjoining ethoxy group, making the unimolecular mechanism a favorable process with which bimolecular mechanisms cannot compete, except in the case of the highly basic hydroxide ion. With the acylals I and II this is not the case; the nitrophenoxy group affords little stabilization. Since the leaving group in a bimolecular reaction involving nucleophilic attack at the carbonyl is reasonably good, pH-independent hydrolysis utilizes H_2O participation and catalysis by basic buffer components. In hydrolysis of glucosyl benzoates catalysis by hydrazine is observed,[28] but with such compounds acid catalysis occurs by an A-1 mechanism and the pH-independent reaction (pH 3–5) is unimolecular, proceeding without catalysis by buffer bases.[28]

There is second-order dependence on base concentration in the hydrolysis of I and II as well as first-order dependence. This is most likely due to the nature of the leaving group. The pK_a of the hemiacetal oxygen should be higher than the pK_a of the attacking base. Thus, assistance by a second molecule of base facilitates the reaction, perhaps as in IV. Such behavior has been ob-

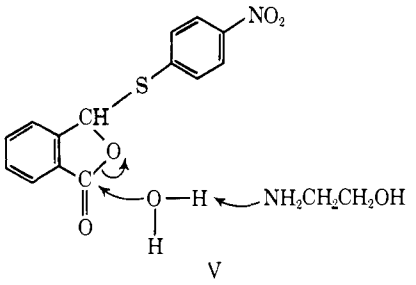


IV

served previously in imidazole reaction with *p*-methoxyphenyl acetate.[29] It will be noted that with *N*-methylimidazole and *N*,*N*-dimethylethanolamine, bases not having a dissociable proton, a second-order dependence on base concentration is not observed. The catalyzed reaction which has first-order dependence on ethanolamine concentration proceeds at nearly the same rate in D_2O as in H_2O for both I and II, $k_B^{H_2O}/k_B^{D_2O}$ being 0.8 and 1.4, respectively, indicating that nucleophile attack is occurring. In the case of the noncyclic acylal II some curvature was detected in plots of k_{obsd} *vs.* base concentration when ethanolamine was the catalyst but not when imidazole is the catalyst.

Imidazole and ethanolamine catalysis is also observed in hydrolysis of the thioacylals III, but in contrast with I and II only first-order dependence on base concentration is detected. The D_2O solvent isotope effect for the ethanolamine catalyzed reaction ($k_B^H/k_B^D = 1.8$) indicates that the reaction involves classical general base catalysis. This would be expected if the pK_a of the leaving group of the thiophenyl derivatives is considerably higher than that of the catalyzing base. To account for the different mechanism, the pK_a of the leaving group would also have to be higher than that of the analogous oxygen acylals. The low ρ values of 0.58 for the ethanolamine reaction and 0.42 for the hydroxide ion catalyzed hydrolysis of the thiophenyl derivatives show there is little sensitivity of the rate to

(26) R. L. Nath and H. N. Rydon, *Biochem. J.*, **57**, 1 (1954).
(27) T. H. Fife and L. K. Jao, *J. Amer. Chem. Soc.*, **90**, 4081 (1968).
(28) A. Brown and T. C. Brulce, *J. Amer. Chem. Soc.*, **95**, 1593 (1973).
(29) J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 833 (1964).



V

the nature of the substituent group in the thiophenyl ring.

In summary, it is clear that the acylals I, II, and III are undergoing hydrolysis with carbonyl attack by nucleophiles in a manner similar to typical esters. This difference in mechanism in comparison with usual types of acylals is most likely due to the ring structure in the case of I and to the fact that hydrolysis in the manner of acetals would demand formation of a rather unstable carbonium ion intermediate. The carbonium ion which would be produced in lysozyme-catalyzed reactions is a species that is not highly stabilized internally relative to simpler acetals. In a reaction sterically constrained by the active site of the enzyme, decomposition of an acylal intermediate to give a carbonium ion and a free carboxyl group (presumably aspartate-52) held in close proximity, should lead to reversibility in the reaction, increasing markedly the normal stability of such an intermediate and necessitating involvement of solvent or other functional groups in its hydrolysis. Thus, it would be expected that if such an intermediate were being formed it would have more than transient existence and should be demonstrable. Therefore since there is no evidence for an acylal intermediate in lysozyme-catalyzed reactions, kinetic or otherwise, it is probable that it is *not* being formed. It will be noted that in hydrolysis of benzaldehyde disalicyl acetal,[30] an acetal having two substituent carboxyl groups properly positioned to participate in the reaction, a bell-shaped pH–rate constant profile is obtained. The enhancement in k_{obsd} in comparison with the dimethyl ester is 3×10^9, an enhancement of the magnitude obtained in enzymatic reactions. In that reaction the carboxylate anion of the monoanionic species contributes little if anything to the large rate facilitation although a stable acylal is the product. Consequently, it is not necessary to postulate involvement of aspartic acid-52 in the transition state to account for the observed kinetic data in lysozyme reactions.

Acknowledgment. This work was supported by a research grant from the National Institutes of Health.

(30) E. Anderson and T. H. Fife, *Chem. Commun.*, 1470 (1971); *J. Amer. Chem. Soc.*, **95**, 6437 (1973).

The Nature of Intramolecular N···C=O Interactions. Crystal Structure of the *Senecio* Alkaloid Senkirkine

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Abstract: The three-dimensional structure of senkirkine, $C_{19}H_{27}NO_6$, was determined by X-ray crystallography. The substance crystallizes in the orthorhombic space group $P2_12_12_1$ with four molecules in a unit cell with dimensions $a = 24.601 \pm 0.002$, $b = 9.133 \pm 0.001$, and $c = 8.708 \pm 0.001$ Å. Intensity data were collected with a diffractometer and the structure was solved by statistical methods. Refinement by least squares, which included hydrogen atoms, converged at R 0.045 for 2275 observed reflections. The transannular N···C distance was found to be 2.292 (4) Å. The extent of the partial bond in this and in several other structures is assessed. A correlation is established between the bond number and the frequency of the carbonyl peak in the infrared spectrum.

During the past years the crystal structures of three alkaloids in which there exists an intramolecular N···C=O interaction were determined in these laboratories, *viz.*, protopine,[1] cryptopine,[2] and clivorine.[3] More recently it was suggested[4] that such an interaction may be pertinent to the physiological activity of methadone. It seemed desirable, therefore, to obtain additional geometrical information and, if possible, to correlate it to the extensive chemical and spectroscopic studies carried out in the past.[5] A transannular N···C=O interaction has been known to exist in the *Senecio* alkaloid senkirkine;[6] this report describes the precise molecular geometry of senkirkine and presents an assessment of the extent of partial bonding in this alkaloid and in several others.

Experimental Section

Senkirkine, $C_{19}H_{27}NO_6$, was isolated from *Senecio vernalis* and crystallized from petroleum ether–chloroform (9:1) by Dr. F. Rulko. Precession photographs showed the colorless prisms (mp 199°) to be orthorhombic; the space group $P2_12_12_1$ was indi-

(1) S. R. Hall and F. R. Ahmed, *Acta Crystallogr., Sect. B*, 24, 337 (1968).

(2) S. R. Hall and F. R. Ahmed, *Acta Crystallogr., Sect. B*, **24**, 346 (1968).

(3) K. B. Birnbaum, *Acta Crystallogr., Sect. B*, **28**, 2825 (1972).

(4) H. B. Bürgi, J. D. Dunitz, and E. Shefter, *Nature (London), New Biol.*, **244**, 186 (1973).

(5) N. J. Leonard, *Rec. Chem. Progr.*, 17, 243 (1956).

(6) L. H. Briggs, R. C. Cambie, B. J. Candy, G. M. O'Donovan, R. H. Russell, and R. N. Seelye, *J. Chem. Soc.*, 2492 (1965).