Substituent Effects on the pK_a Values and Rates of Hydrolysis of Arylmethylene-erythromycylamines

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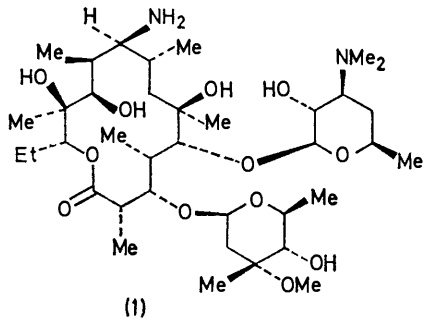
The pK_a values and rates of hydrolysis of a series of *N*-benzylidene derivatives of the macrolide antibiotic, erythromycylamine (I) have been measured. Anomalies in the correlations of the pK_a values with the Hammett equation and of the mechanism of hydrolysis with that of an analogous series of *N*-benzylidene-1,1-dimethylethylamines have been explained in terms of steric and electronic interactions involving the macrolide ring and the unusual effect of *ortho*-methoxy-substituents.

THE mechanism of hydrolysis of Schiff's bases has been well established and reviewed by Jencks.[1] For substituted *N*-benzylidene-1,1-dimethylethylamines,[2] the rate of hydrolysis is independent of pH under basic conditions (pH >8), a consequence of rate-determining attack of hydroxide ion on the protonated Schiff's base [2-5] which is present in low concentration. Under more acidic conditions, in which the Schiff's bases are predominantly protonated, rate-determining attack of water, rather than hydroxide, on the protonated Schiff's base becomes the major reaction path. In strongly acidic solution, a change in the rate-determining step to elimination from the carbinolamine intermediate occurs and the rate decreases with decreasing pH.[2-6] A similar rate profile is found for the hydrolysis of substituted benzylideneanilines,[6-10] but attack of hydroxide on the free imine, competes with the pH-independent reaction at high pH (>12),[6] as is also the case for salicylaldehyde derivatives.[11,12]

The dibasic amine, erythromycylamine (1) (ENH_2) has pK_a values of 8·7 and 9·9.[13] Consequently, the concentration of free base at physiological pH is extremely small. Conversion into a Schiff's base derivative would be expected to decrease the basicity of the primary amine group and to increase the concentration of free amine available for absorption from the gastrointestinal tract. It was therefore of interest to study the effects of substituents on the pK_a and stability of various Schiff's base derivatives of erythromycylamine.



(I)

EXPERIMENTAL

Preparation of Materials.—Erythromycylamine was prepared from erythromycin hydrazone [14] by nitrosation followed by borohydride reduction of the resulting imine.[15] A typical preparation of the Schiff's bases is illustrated for the 2,4,6-trimethylbenzylidene derivative.

Erythromycylamine (15 g, 0·0204 mol) and 2,4,6-trimethylbenzaldehyde (3·03 g, 0·0204 mol) were dissolved, with warming, in isopropyl alcohol (170 ml) and the solution was heated under reflux for 24 h. T.l.c. on silica

[1] W. P. Jencks, *Progr. Phys. Org. Chem.*, 1964, **2**, 63; 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, N.Y., 1969, ch. 10.
[2] E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, 1963, **85**, 2843.
[3] A. V. Willi, *Helv. Chim. Acta*, 1956, **39**, 1193.
[4] K. Koehler, W. Sandstrom, and E. H. Cordes, *J. Amer. Chem. Soc.*, 1964, **86**, 2413.
[5] J. Archila, H. Sull, C. Lagenaur, and E. H. Cordes, *J. Org. Chem.*, 1971, **36**, 1345.
[6] W. P. Jencks, *J. Amer. Chem. Soc.*, 1959, **81**, 475.
[7] R. L. Reeves, *J. Amer. Chem. Soc.*, 1962, **84**, 3332; *J. Org. Chem.*, 1965, **30**, 3129.
[8] E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, 1962, **84**, 832.
[9] L. do Amaral, W. H. Sandstrom, and E. H. Cordes, *J. Amer. Chem. Soc.*, 1966, **88**, 2225.
[10] A. V. Willi and R. E. Robertson, *Canad. J. Chem.*, 1953, **31**, 361.
[11] C. V. McDonnell, jun., M. S. Michailidis, and R. B. Martin, *J. Phys. Chem.*, 1970, **74**, 26.
[12] J. Hoffman, J. Klienor, V. Šterba, and M. Večeřa, *Coll. Czech. Chem. Comm.*, 1970, **35**, 1387.
[13] E. H. Massey, B. Kitchell, L. D. Martin, K. Gerzon, and H. W. Murphy, *Tetrahedron Letters*, 1970, 157.
[14] M. V. Sigal, jun., P. F. Wiley, K. Gerzon, E. H. Flynn, U. C. Quarck, and O. Weaver, *J. Amer. Chem. Soc.*, 1956, **78**, 388.
[15] E. Wildsmith, *Tetrahedron Letters*, 1972, 29.

gel (F254 Merck) with methanol–DMF (3 : 1) as eluant showed virtually complete conversion into the Schiff's base (R_F *ca.* 0·6), with only a trace of slower-running amine remaining. The product crystallised on cooling and was

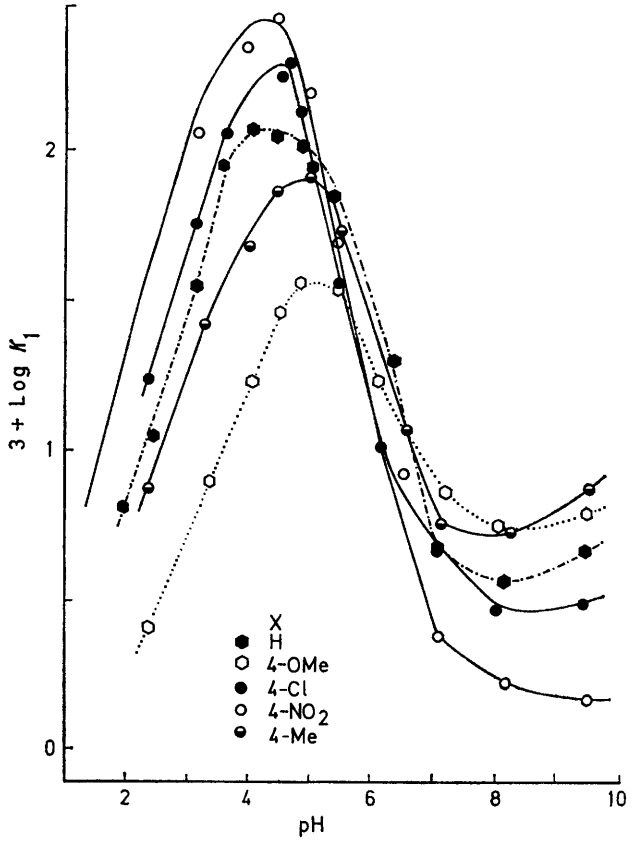


FIGURE 1 Graph of 3 + log k_1 against pH for the hydrolysis of *p*-X-substituted benzylidene-erythromycylamines in methanol–water (47·6%, v/v) at 30 °C; k in min^{-1}. The line drawn for the 4-NO$_2$ derivative is a theoretical curve derived from equation (ii).

filtered off after 2 days to give *N*-(2,4,6-trimethylbenzylidene)erythromycylamine (10·79 g, 61·5%) which was recrystallised from isopropyl alcohol to give material (m.p. 136·5—140°) pure by the above t.l.c. system.

Other compounds were prepared similarly, the reaction time required varying from 1 to 24 h. Since some Schiff's bases failed to crystallise from isopropyl alcohol they were crystallised from anhydrous diethyl ether (see Table 1).

All compounds gave satisfactory microanalyses and n.m.r. spectra and appeared as a single spot on t.l.c.

Kinetic Studies.—(a) *Reaction solutions.* All the hydrolysis studies were carried out in methanol–water (47·6% v/v) to ensure complete homogeneity of solution during the entire reaction. Buffer solutions were prepared, in batches of 50 ml, by mixing 10 ml of Teorell and Stenhagen citrate–borate–phosphate salt mixture [16] with the required volume of hydrochloric acid (0·1M) or sodium hydroxide (0·1M), constant ionic strength being maintained by addition of potassium chloride (0·1M).

(b) *Reaction products.* The u.v. absorption maxima of all the arylidene erythromycylamines (except the *p*-nitrobenzylidene derivative) undergo a pronounced bathochromic shift as the Schiff's bases are protonated. At the conclusion of a kinetic run (10 half-lives) the u.v. spectrum of the reaction solution was always identical to that of a solution of the appropriate aldehyde. The presence of erythromycylamine (1) in the hydrolysate was confirmed by t.l.c.

(c) *Hydrolysis rate and* pK_a *measurements.* All the arylidene-erythromycylamines were stable in methanol for several weeks as indicated by u.v. and t.l.c. analysis. For both pK_a and hydrolysis-rate determinations, a small aliquot (0·03 ml) of a methanol solution of the arylidene-erythromycylamine (*ca.* 20 mg in 5 ml) was rapidly injected into a solution (30 ± 0·1 °C) of the buffer (2·50 ml) contained in a 1-cm silica cell. The solutions were rapidly mixed and the stoppered cell was replaced in the thermostatically controlled cell-compartment of a Unicam SP 700 spectrophotometer; the change in absorbance of the solution with time at a particular wavelength was recorded. Depending on the pH of solution, the selected wavelength was the position of maximum absorption of the Schiff's base, that of the bathochromically shifted peak due to the conjugate acid or the point of maximum overall absorption change. The pK_a values (±0·1 pK unit) were derived from plots of absorbance, extrapolated to zero reaction time,

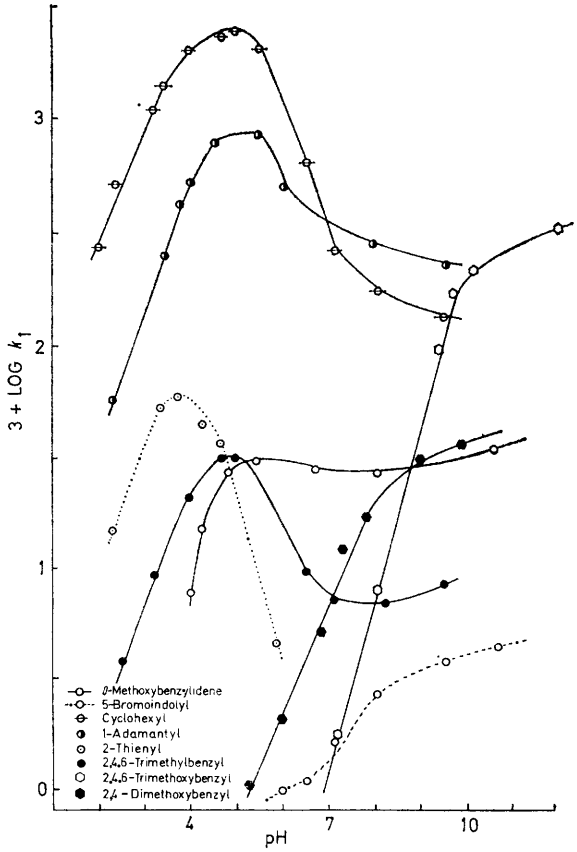


FIGURE 2 Graph of 3 + log k_1 against pH for the hydrolysis of some substituted arylmethylene- and alkylidene-erythromycylamines in methanol–water at 30 °C; k_1 in min^{-1}.

against pH. The pseudo-first-order rate coefficients (±2%) were obtained graphically from a plot of log ($D_t - D_\infty$), where D_t is the absorbance at time t and D_∞ the absorbance at 10 half-lives. The pK_a values are listed in Table 1, and

[16] T. Teorell and E. Stenhagen, *Biochem. Z.*, 1938, **299**, 416.

TABLE 1

Physical characteristics of arylmethylene-erythromycylamines, ArCH=NE

	Ar	M.p. (°C)	Recryst. solvent		Analysis (%) Calc.	Found	pK_a
(1)	Phenyl *	142—146	IPA–water	C	62·9	63·0	4·87
				H	8·9	9·0	
				N	3·4	3·2	
(2)	*p*-Nitrophenyl	151—162	IPA	C	60·9	60·9	4·26
				H	8·5	8·7	
				N	4·8	4·6	
(3)	*p*-Chlorophenyl *	146—155	IPA	C	61·6	61·6	4·75
				H	8·6	8·7	
				N	3·3	3·2	
(4)	*p*-Methylphenyl *	141—143	IPA	C	64·6	64·3	5·12
				H	9·2	9·3	
				N	3·3	3·2	
(5)	*p*-Methoxyphenyl *	135—140	Et_2O	C	63·3	63·1	5·29
				H	9·0	8·9	
				N	3·3	3·0	
(6)	*o*-Methoxyphenyl	134—138	Et_2O	C	63·3	63·5	6·34
				H	9·0	9·0	
				N	3·3	3·1	
(7)	2,4-Dimethoxyphenyl	Amorphous	Et_2O–petrol	C	62·6	62·3	7·57
				H	8·9	9·1	
				N	3·2	3·1	
(8)	2,4,6-Trimethylphenyl	136·5—140	IPA	C	65·2	65·4	4·90
				H	9·3	9·4	
				N	3·2	3·2	
(9)	2,4,6-Trimethoxyphenyl	202·5—204	Et_2O	C	61·8	61·7	9·95
				H	8·8	8·6	
				N	3·1	3·0	
(10)	5-Methyl-2-thienyl	140—145	Et_2O	C	61·2	60·9	4·89
				H	8·9	8·8	
				N	3·3	3·3	
(11)	3-Thienyl	155—160	Et_2O	C	60·8	61·1	5·15
				H	8·7	8·4	
				N	3·4	3·5	
(12)	2-Thienyl	156—162	Et_2O	C	60·8	61·0	4·30
				H	8·7	9·0	
				N	3·4	3·3	
(13)	2-Furyl	145—150	Et_2O	C	62·0	61·8	5·15
				H	8·9	9·2	
				N	3·4	3·2	
(14)	2-Furylidenemethyl	215—217	Et_2O	C	63·0	62·7	6·40
				H	8·9	9·1	
				N	3·3	3·1	
(15)	2-Pyrrolyl	140—146	Et_2O	C	62·1	61·8	6·68
				H	9·1	9·5	
				N	5·2	5·0	
(16)	3-Indolyl	167—170	Et_2O	C	64·1	64·4	7·39
				H	8·8	8·9	
				N	4·9	4·6	
(17)	5-Bromoindolyl	174—180	Pr^i_2O	C	58·7	58·7	6·80
				H	7·9	7·9	
				N	4·5	4·3	
(18)	5-Methylindolyl	155—165	Pr^i_2O	C	64·4	64·5	7·97
				H	8·9	9·1	
				N	4·8	4·5	
(19)	1-Phenyl-2,5-dimethyl-3-pyrrolyl	158—165	Et_2O–petrol	C	65·5	65·6	8·65
				H	8·9	9·1	
				N	4·6	4·4	

* These compounds were first prepared by Dr. E. H. Massey and Mr. L. Martin of Eli Lilly & Co., Indianapolis.

the variations in the rate coefficients with pH are illustrated in Figures 1 and 2.

DISCUSSION

pK_a *Values of Arylmethylene-erythromycylamines.*—(a) p-*Substituted benzylidene-erythromycylamines.* The pK_a values of the conjugate acids of the *p*-substituted benzylidene-erythromycylamines (Table 1, entries 1—5) correlate in a linear manner with the Hammett equation only if the σ^+ substituent constants [17] are used, and give a reaction constant, $\rho^+ = -0{\cdot}68$ (Table 2a). These constants are required to assess the effects of substituents which possess an electron-releasing mesomeric effect, and are capable of participating in a direct resonance interaction with the reaction centre on only one side of an equilibrium. In the present case, conjugative interaction involving the substituent is effectively confined to the conjugate acid [equation (i)].

For the same substituents, the pK_a of the conjugate acids of the substituted benzylidene-1,1-dimethylethylamines [2] give a reaction constant, ρ^+ of $-1{\cdot}54$ (Table

[17] H. C. Brown and Y. Okamoto, *J. Amer. Chem. Soc.*, 1958, **80**, 4979.

2b).* Thus, the substituent effect is much smaller in the erythromycylamine series, despite the decreased medium polarity.[18]

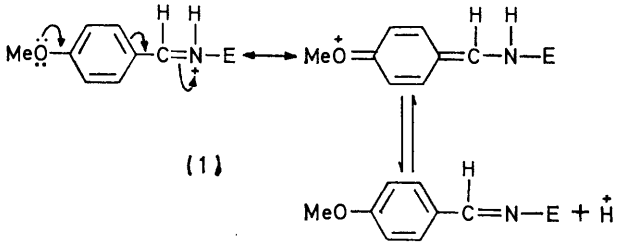
TABLE 2

Hammett reaction constants for the reactivities of arylmethylene-erythromycylamines

Reaction	ρ^+ [a]	r [b]	F-Ratio	Solvent [c]
(a) pK_a Values of *p*-substituted benzylidene-erythromycylamines	−0·68	0·988	126	1
(b) pK_a Values of *p*-substituted 1,1-benzylidenedimethylethylamines [2]	−1·54	0·984	94·4	2
Rate coefficients for reaction between conjugate acid and water in				
(c) *p*-Substituted benzylidene-erythromycylamines	0·58	0·970	47·7	1
(d) *p*-Substituted benzylidene-1,1-dimethylethylamines [2]	2·54	0·979	67·9	2
Rate coefficients for reaction between conjugate acid and hydroxide ion in				
(e) *p*-Substituted benzylidene-erythromycylamines	0·285	0·999	1210	1
(f) *p*-Substituted benzylidene-1,1-dimethylethylamines [2]	1·22	0·984	90·2	2
(g) Hydrolysis rates (at pH 2·0) for *p*-substituted benzylidene-erythromycylamines	0·905	0·978	66·9	1
(h) Hydrolysis rates (at pH 1·0) for *p*-substituted benzylidene-1,1-dimethylethylamines[2]	2·17[2]			2

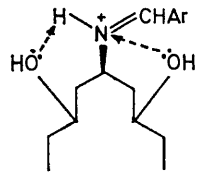
[a] Calculated from the Hammett equation, $\log (k/k_0) = \rho^+\sigma^+$; no significant improvement in the correlation was found using the Yukawa-Tsuno [22] modification of the Hammett equation [*cf.* equation (4)]. [b] Correlation coefficient (5 points). [c] Solvent: 1, 47·6% methanol–water; 2, 3% ethanol–water.

A possible explanation of this reduced ρ^+ value is stabilisation of the conjugate acid by one or more of the suitably placed hydroxy-groups in the macrolide ring [see (2)] which reduces the demand for electron-release from the substituent. Alternatively, the greater steric size of the macrolide ring than of the 1,1-dimethylethyl substituent may reduce the degree of coplanarity of the imine and benzene π-systems and thereby reduce the conjugative interaction between the substituent and the site of protonation.



(1)

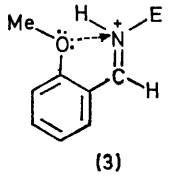
(b) ortho-*and Poly-substituted benzylidene-erythromycylamines*. Our investigations are limited to the methoxy-substituent. For reactions which correlate with the normal Hammett substituent constant, σ, *o*-methoxy is usually less electron releasing than *p*-methoxy [19,20]



(2)

although for the pyrolysis of isopropyl benzoates Jones and Smith [21] quote substituent constants of −0·53 for *o*-methoxy relative to a value of −0·27 for *p*-methoxy. For a number of reactions correlated by σ^+, *o*-methoxy is significantly less electron releasing than *p*-methoxy (Table 3). Values of log $[k_{p\text{-OMe}}/k_H]$ are given as a measure of the sensitivity of the particular reaction to substituent effects

In our series, *o*-methoxybenzylidene-erythromycylamine ($pK_a = 6{\cdot}34$) is considerably more basic than the *p*-isomer ($pK_a = 5{\cdot}29$). Steric deformation of the aromatic and imine π-systems would reduce the transmission of the electron-releasing mesomeric effect of *o*-methoxy. However, the proximity of *o*-methoxy to the site of protonation may account for its apparently greater electron-releasing influence, the conjugate acid being stabilised by a direct interaction between the positive centre and the lone pair on oxygen [(3) or (4)].



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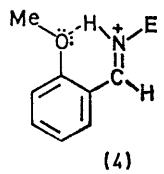


(4)

This type of interaction would lead to a greater degree of co-planarity of the π-systems and this explanation is supported by the increased sensitivity of the pK_a values to further substitution (Table 4). Thus the presence of an *o*-methoxy-group greatly increases the electron-

* The pK_a of benzylidene-1,1-dimethylethylamine is 6·7 in 3% ethanol–water at 25 °C.[2] A referee has commented that this significant difference from our value of 4·87 for benzylidene-erythromycylamine in 47·6 mole % methanol–water may arise because of internal carbinolamine formation in our series of compounds. However, we prefer to attribute the difference to a combination of solvent effect and changing alkyl structure of the parent amine, as the pK_a values of the benzylidene derivatives of cyclohexylamine and 1-adamantylamine are both *ca.* 5·0 in our solvent system (see Table 6, Figure 2). A subsequent determination has revealed that the pK_a of the benzylidene-erythromycylamine is *ca.* 5·5 in 3% ethanol–water.

[18] H. H. Jaffé, *Chem. Rev.*, 1953, **53**, 191.
[19] M. Charton, *J. Amer. Chem. Soc.*, 1969, **91**, 6649.
[20] M. Charton, *J. Org. Chem.*, 1964, **29**, 1222.
[21] D. A. K. Jones and G. G. Smith, *J. Org. Chem.*, 1964, **29**, 3531.

releasing power of an additional *p*-methoxy [compound (7)] and introduction of a further *o*-methoxy [compound (9)] has a much greater electron-releasing effect than that noted for a single *o*-methoxy-group [compound (6)].

For the other types of reaction listed in Table 3 any parameters should differ from those for *p*-substituted derivatives.

It is to be expected that other reactions in which such direct interactions are possible, for example the dehydration of 1-phenyl-2-arylethanols or the solvolysis

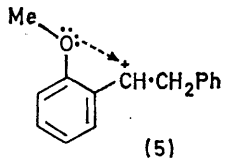
TABLE 3

Comparison of substituent effects of *o*-MeO and *p*-MeO for a number of reactions which involve conjugative interaction between the substituent and the reaction site

		Ref.	log $[k_{p\text{-OMe}}/k_{o\text{-OMe}}]$	log $[k_{p\text{-OMe}}/k_{H}]$
(1)	Rates of dehydration of 1-aryl-2-phenylethanols in 5% ethanol–aqueous sulphuric acid at 25 °C.	*a*	2·76	3·85
(2)	Rates of pyrolysis of 1-arylethyl acetates at 374 °C.	*b*	0·27	0·52
(3)	Rates of pyrolysis of 1-arylethyl methyl carbonates at 346 °C.	*b*	0·24	0·55
(4)	Rates of pyrolysis of 1-arylethyl benzoates at 374 °C.	*b*	0·26	0·62
			$[pK_a^{p\text{-OMe}} - pK_a^{o\text{-OMe}}]$	$[pK_a^{p\text{-OMe}} - pK_a^{H}]$
(5)	pK_a Values of benzylidene-erythromycylamines in 47·6% (v/v) methanol–water at 30 °C.	This work	−1·05	0·42

[a] G. M. Loudon and D. S. Noyce, *J. Amer. Chem. Soc.*, 1969, **91**, 1433. [b] G. G. Smith, K. K. Lum, J. A. Kirby, and J. Posposil, *J. Org. Chem.*, 1969, **34**, 2090.

such interaction would be confined to a geometrically unfavourable four-membered ring [*e.g.* (5)] and it is



(5)

reasonable that these reactions show no enhanced *ortho*-effect. However, a proximity effect of this kind may lend stability to the incipient ion-pair (6) in the pyrolysis of isopropyl *o*-methoxybenzoate and explain

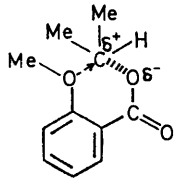
TABLE 4

pK_a Changes induced by incremental methoxy-substitution in benzylidene-erythromycylamines, ArCH=NE

			pK_a Change/methoxy-group	
No.*	Ar	pK_a	*p*-OMe	*o*-OMe
(1)	Phenyl	4·87		
(5)	*p*-Methoxyphenyl	5·29	0·42	
(6)	*o*-Methoxyphenyl	6·34		1·47
(7)	2,4-Dimethoxyphenyl	7·57	1·23	
(9)	2,4,6-Trimethoxyphenyl	9·95		2·38

* These numbers correspond to those in Table 1.

the anomalously high electron-releasing influence of the substituent ($\sigma_{o\text{-MeO}} = -0{\cdot}53$) [21] mentioned above. From models it is clear that, if the transition state resembles



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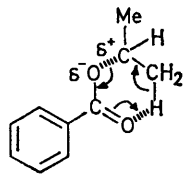


(7)

(6), the normal function of the carboxy carbonyl in acetate pyrolysis is precluded [see (7)] and the activation of the corresponding halides would also show enhanced *ortho*-effects.

(c) *Heteroarylmethylene-erythromycylamines.* It is possible for all the heteroaromatic groups to participate in mesomeric electron-releasing interaction with the imine bond in the conjugate acid, and the appropriate σ^+-constants are listed in Table 5 for the simple five-

TABLE 5

Substituent constants and pK_a values of heteroarylidene-erythromycylamines, ArCH=NE

Ar	$pK_a(ArCO_2H)$ [a]	σ	σ^+_{Ar}	$pK_a(ArCH{=}\overset{+}{N}HE)$
Phenyl	4·21 [b]	0·00	0·00 [i]	4·87
2-Furyl	3·16 [c]	1·05	−0·94 [e]	5·15
2-Thienyl	3·53 [b]	0·68	−0·79 [f]	4·30
3-Thienyl	4·10 [d]	0·11	−0·49 [e]	5·15
2-Pyrrolyl	4·45 [e]	−0·24	−1·53 [h]	6·68

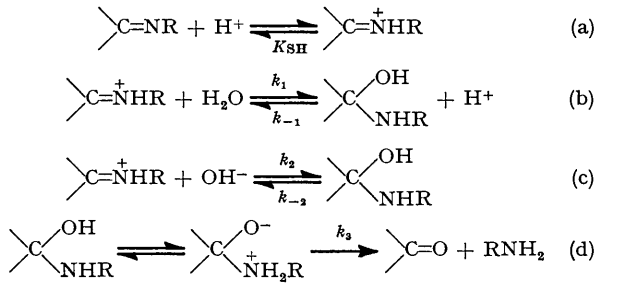
[a] In water at 25 °C. [b] G. Kortum, W. Vogel, and K. Andrussow, 'Dissociation Constants of Organic Acids in Aqueous Solutions,' Butterworth, London, 1961, p. 352. [c] P. O. Lumme, *Suomen Kemist.*, 1960, **33***B*, 87 (*Chem. Abs.*, 1961, **55**, 5014). [d] J. M. Loven, *Z. Physik. Chem.*, 1896, **19**, 456. [e] E. A. Hill, M. L. Gross, M. Stasiewicz, and M. Manion, *J. Amer. Chem. Soc.*, 1969, **91**, 7381. [f] R. Taylor, *J. Chem. Soc.* (*B*), 1968, 1397. [h] P. Linda and G. Marino, *J. Chem. Soc.* (*B*), 1969, 392. [i] Ref. 17.

membered heterocycles. Relative to benzene, the other four groups are all electron releasing, and of these, pyrrole is the most powerful and has the highest imine pK_a value. However, the pK_a value of the 2-thienyl derivative is less than that of the benzylidene compound and this is confirmed by the value of only 4·89 for the 5-methyl-2-thienyl analogue (see Table 1). An explanation for these unexpected results is not obvious from consideration of (i) steric interactions involving the thiophen and macrolide rings (ii) pK_a values of the corresponding heteroaryl carboxylic acids (Table 5), or (iii) Yukawa-Tsuno [22] treatment of the Hammett σ- and σ^+-constants.

[22] Y. Yukawa, Y. Tsuno, and M. Sawada, *Bull. Chem. Soc. Japan*, 1966, **39**, 2274.

Mechanism of Hydrolysis of the Arylmethylene-erythromycylamines.—Cordes and Jencks [2] have clearly elucidated the mechanism of hydrolysis of benzylidene-1,1-dimethylethylamine and their reaction sequence is shown

SCHEME 1



$$k_{obs} = \frac{k_1k_3[H^+] + 10^{-14}k_2k_3}{\{[H^+] + K_{SH}\}\{k_{-1}[H^+] + k_{-2} + k_3\}} \quad \text{(ii)}$$

in Scheme 1, the mathematical expression for this series of reactions being described by equation (ii). Using this expression and the approximations made by Cordes and Jencks, we have calculated the theoretical curve for the hydrolysis of *p*-nitrobenzylidene-erythromycylamine from a knowledge of the pK_a of the protonated imine and the observed rate coefficients. The agreement between the experimental and the calculated results is very good (see Figure 1), suggesting that the hydrolyses

TABLE 6

Rate coefficients for the hydrolysis of substituted benzylidine-erythromycylamines in 47·6% (v/v) methanol-water at an ionic strength of 0·1M at 30 °C

Substituent	k [a] (pH indep.) / min^{-1}	k_1 [b] / min^{-1}	k_2 [b] / l mol^{-1} min^{-1}
4-NO_2	$1·48 \times 10^{-3}$	$4·95 \times 10^{-1}$	$8·14 \times 10^{6}$
	(0·48) [c]	(16)	($1·91 \times 10^{8}$)
4-Cl	$3·06 \times 10^{-3}$	$3·15 \times 10^{-1}$	$5·50 \times 10^{6}$
	(1·09)	(1·1)	($3·45 \times 10^{7}$)
4-H	$3·63 \times 10^{-3}$	$2·13 \times 10^{-1}$	$4·91 \times 10^{6}$
	(1·34)	($4·1 \times 10^{-1}$)	($3·00 \times 10^{7}$)
4-Me	$5·37 \times 10^{-3}$	$1·12 \times 10^{-1}$)	$4·08 \times 10^{6}$
	(1·50)	($1·6 \times 10^{-1}$)	($5·98 \times 10^{6}$)
4-OMe	$5·74 \times 10^{-3}$	$0·706 \times 10^{-1}$	$2·94 \times 10^{6}$
	(1·54)	($0·29 \times 10^{-1}$)	($3·05 \times 10^{6}$)
2-OMe	$3·2 \times 10^{-2}$		$1·4 \times 10^{6}$
2,4-$(OMe)_2$	$4·0 \times 10^{-2}$		$1·3 \times 10^{5}$
2,4,6-$(OMe)_3$	$3·0 \times 10^{-1}$		$3·4 \times 10^{3}$
Benzylidene-cyclohexylamine [d]	$1·26 \times 10^{-1}$	19	$1·3 \times 10^{8}$
Benzylidene-adamantylamine [d]	$2·19 \times 10^{-1}$	3·6	$2·2 \times 10^{8}$

[a] Observed first-order rate coefficient for the pH-independent region. [b] k_1 and k_2 refer to Scheme 1. [c] Values in parentheses are data of Cordes and Jencks [2] for the hydrolysis of the corresponding benzylidene-1,1-dimethylethylamines in 3% (v/v) ethanol–water at 25 °C and ionic strength 0·5M. [d] pK_a of conjugate acid $\simeq$ 5·0 as indicated by the position of the maxima in Figure 2.

of our compounds proceed by the same reaction mechanism. The values of k_1, k_2, and k for the pH-independent reaction are listed in Table 6 and are compared with the data of Cordes and Jencks for the same substituents.

To enable comparison of the influence of the erythromycyl moiety with that of an alkyl substituent, the rates of hydrolysis of the benzylidene derivatives of cyclohexylamine and 1-adamantylamine were measured. These compounds are hydrolysed much more rapidly than benzylidene-erythromycylamine (see Figures), the rate coefficients for attack of hydroxide ion and water on the conjugated acid in both cases being an order of magnitude greater than those observed for benzylidene-erythromycylamine (Table 6). As all three conjugate acids have a similar pK_a, the reduced reactivity of the erythromycyl compound is most probably of steric origin. This substituent is larger than the alkyl groups and if the macrolide hydroxy-groups interact with the conjugate acid, approach of nucleophiles to the protonated imine bond will be further hindered.

The Hammett reaction constants for attack of hydroxide and water on the conjugate acids of the substituted benzylidene-erythromycylamines are both smaller than those for the substituted benzylidene-1,1-dimethylethylamines [2] (Table 2, c—f), despite the fact that the latter data refer to a more polar solvent system (3% ethanol–water). Clearly these results are in agreement with the smaller substituent effect on the pK_a values of the benzylidene-erythromycylamines, and again suggest greater stabilisation of the conjugate acid by the erythromycyl group.

At pH = 2·0, the rate-determining step is the decomposition of the intermediate carbinolamine.[2] The Hammett reaction constant, ρ^+, for the hydrolysis of the benzylidene-erythromycylamines is 0·905, a value which is again smaller than that of 2·17 observed for the benzylidene-1,1-dimethylethylamines, reflecting the expected effects of polar substituents on the pre-equilibrium preceding the rate-determining step (Table 2, g, h).

The rate profiles for the hydrolysis of the heterocyclic and methoxy-substituted arylidene-erythromycylamines are markedly dependent on the basicity of the imine (Figure 2). For the 2,4,6-trimethoxybenzylidene derivative, the rate of hydrolysis increases almost linearly with pH over the region 7—9. Unlike the other imines, this compound is substantially protonated over this pH range and the increase in rate accords with a rise in the concentration of hydroxide ion, which attacks the conjugate acid in the slow step. From the pH-independent rates and the equilibrium constants for dissociation of the conjugate acids, the rate coefficients for hydroxide ion attack on the conjugate acid can be calculated. The figures are listed in Table 6.

A thermodynamic measure of the effect of the methoxy-substituents on the reactivity of the conjugate acid is provided by the pK_a values of the conjugate acids. Using the Hammett reaction constant of −0·68 for the *para*-substituted benzylidene-erythromycylamines (Table 2, a) the following pseudo-σ^+-constants are obtained; 2-OMe, −2·13; 2,4-$(OMe)_2$, −3·94; 2,4,6-$(OMe)_3$, −7·44. If these σ^+ values are used to assess the substituent effect on the reactivity of the conjugate acids towards hydroxide ion, using a reaction constant of

0·285 (Table 2, e); $k_2 = 1{\cdot}2 \times 10^6$ (2-OMe); $= 3{\cdot}7 \times 10^5$ [2,4-$(OMe)_2$]; $= 3{\cdot}7 \times 10^4$ l mol^{-1} min^{-1} [2,4,6-$(OMe)_3$]. The first two of these rate coefficients are close to those found experimentally (Table 6) suggesting that the thermodynamic measure estimates the reactivity towards hydroxide quite well. However, for the 2,4,6-trimethoxy case, the reactivity of the conjugate acid is less than predicted from thermodynamic considerations, probably as a result of increasing steric inhibition of hydroxide attack.

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