Chemical Reactions of Omeprazole and Omeprazole Analogues. II. Kinetics of the Reaction of Omeprazole in the Presence of 2-Mercaptoethanol

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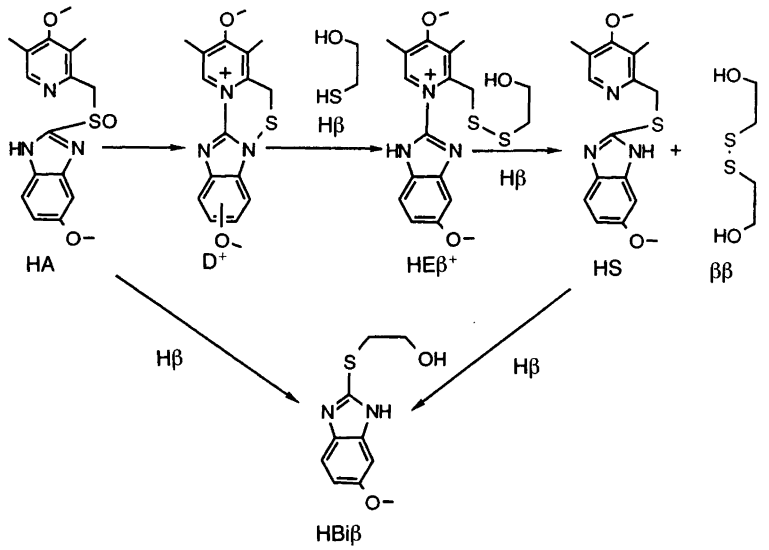
The rate at which 5-methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinylmethylsulfinyl)-1*H*-benzimidazole, omeprazole, reacts with 2-mercaptoethanol is equal to the rate at which it is converted into a sulfenamide. This reaction proceeds via two intermediates, a spiro compound and a sulfenic acid. A kinetic study of the reaction at different pH values has revealed that the conversion into the spiro compound is the slowest step. It can be an uncatalyzed reaction, but can also be acid catalyzed. Even the conversion of the spiro compound into the sulfenic acid can occur by an uncatalyzed reaction as well as by an acid-catalyzed one. The conversion of the sulfenic acid to the sulfenamide is very rapid. The rate constants for the different steps can usually be predicted.

The reaction of omeprazole, HA, in the presence of an excess of 2-mercaptoethanol, Hβ, was studied in buffer solutions. At intervals, samples were withdrawn and analyzed by means of HPLC. Depending on the conditions, the chromatograms contained two or three main peaks corresponding to the starting material HA, the disulfide HEβ$^+$ and the sulfide HS related to the sulfoxide HA. In addition there were sometimes a few minor peaks.

The following observations were made:

(1) The concentration of HA, decreases according to first-order kinetics.
(2) The pseudo first-order rate constant k_{obs} is almost totally independent of the concentration of Hβ. Only at high values of [Hβ] is a slight increase in k_{obs} observed.
(3) The sulfide HS is formed by a second-order reaction, first order in HEβ$^+$ and first order in Hβ.



Scheme 1.

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(4) The pseudo first-order rate constant k_{obs} for the disappearance of omeprazole is equal to the rate constant k_{AD} for the forward reaction in the reversible conversion of HA into the sulfenamide D^+ in the absence of Hβ.

(5) When the reaction is performed at different pH values, the pseudo first-order rate constant k_{obs} usually decreases with an increase in the pH of the solution.

Since both D^+ and $HE\beta^+$ have been isolated in a pure state, it has been possible to study their reactions with Hβ separately (see part IV of this series[1]). Based on all of the facts available we propose the reaction sequence shown in Scheme 1.

Derivation of rate equations

The overall reaction (HA → $HE\beta^+$ → HS). Under most conditions the reactions leading to HBiβ can be disregarded.[1] Eqns. (1a)–(1d) then give the rate equations for the reactions, where [A] is the total concentration of all protolytic forms of HA and so on.

$$\frac{d[A]}{dt} = -k_{AD}[A] + k_{DA}[D] \quad (1a)$$

$$\frac{d[D]}{dt} = k_{AD}[A] - k_{DA}[D] - k_{DE\beta}[\beta][D] \quad (1b)$$

$$\frac{d[E\beta]}{dt} = k_{DE\beta}[D][\beta] - k_{E\beta S}[\beta][E\beta] \quad (1c)$$

$$\frac{d[S]}{dt} = k_{E\beta S}[\beta][E\beta] \quad (1d)$$

Since D^+ is very rapidly consumed by its reaction with Hβ, [D] is very small and the steady-state approximation can be applied to D^+. Only when [β] is low ($\leq 10^{-4}$ M) and D^+ has its lowest reactivity towards Hβ (in 0.01 M HCl; see part IV[1]) is a detectable concentration of D^+ observed. An example is given in Appendix 2.

Setting $d[D]/dt = 0$ and remembering that [β] can be regarded as being constant during the experiment and that $k_{DA} \ll k_{DE\beta}$ [β], gives eqn. (2). This lead to eqns. (3a)–(3c), which are readily integrated to give eqns. (4a)–(4c) where $[E\beta]_{max}$ and $[S]_{max}$ means the total concentration of $HE\beta^+$ and HS obtained if all HA is converted into $HE\beta^+$ or HS.

$$[D] = k_{AD}[A]/(k_{DE\beta}[\beta]) \quad (2)$$

$$\frac{d[A]}{dt} = -k_{AD}[A] \quad (3a)$$

$$\frac{d[E\beta]}{dt} = k_{AD}[A] - k_{E\beta S}[\beta]\,[E\beta] \quad (3b)$$

$$\frac{d[S]}{dt} = k_{E\beta S}[\beta]\,[E\beta] \quad (3c)$$

$$[A] = [A]_0 e^{-k_{AD}t} \quad (4a)$$

$$[E\beta] = [E\beta]_{max}\frac{k_{AD}}{k_{E\beta S}[\beta] - k_{AD}}(e^{-k_{AD}t} - e^{-k_{E\beta S}[\beta]t}) \quad (4b)$$

$$[S] = [S]_{max}\left(1 - \frac{k_{E\beta S}[\beta]e^{-k_{AD}t} - k_{AD}e^{-k_{E\beta S}[\beta]t}}{k_{E\beta S}[\beta] - k_{AD}}\right) \quad (4c)$$

In the HPLC analyses (see the Experimental) the peaks corresponding to HA, $HE\beta^+$ and HS are expressed in integration units, which are proportional to the concentrations. For convenience, in the calculations we have used the integration units. If one or more of the peaks are followed as a function of time, a non-linear least-squares regression gives k_{AD} and $k_{E\beta S}$, together with one or more of the values $[A]_0$, $[E\beta]_{max}$ or $[S]_{max}$ (in integration units). A comparison of these values with the corresponding values for reference peaks for HA, $HE\beta^+$ and HS reveals whether or not side reactions have occurred. Our usage of measurements of more than one of the compounds HA, $HE\beta^+$ and HS gives k_{AD} and $k_{E\beta S}$ values of very good accuracy, see Appendix 1.

A plot of log k_{AD} against pH is given in Fig. 1. From the shape of the curve we can see that the pH dependence of the reaction is quite complicated. It is, nevertheless, possible to explain all of the observations in the way given below.

Possible intermediates in the reaction HA ⇌ D^+. From theoretical considerations and various experiments reported in this and other parts of this series, we have arrived at the reaction sequence in Scheme 2 for the transformation of omeprazole to the sulfenamide D^+.

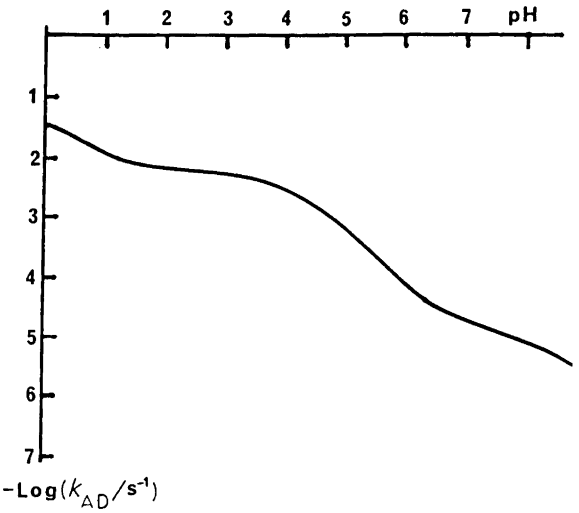
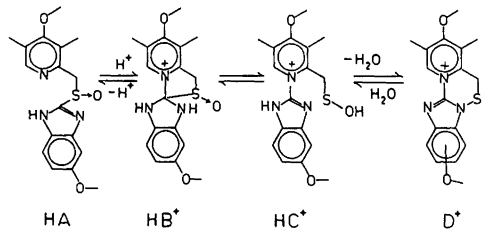


Fig. 1. The pH-dependence of log (k_{AD}/s^{-1}) for the reaction of omeprazole in the presence of β-mercaptoethanol. T = 37 °C.



Scheme 2.

We have good indications that HB^+ and HC^+ are present in very low concentrations. The reactions are reversible, since some HA is formed when a pure salt of D^+ is dissolved in dilute acid (see the Experimental). In order to understand the total reaction we must consider each individual step in this reaction sequence. We have the rate expressions of eqns. (5)–(7) for HA, HB^+, and HC^+.

$$\frac{d[A]}{dt} = -k_{AB}[A] + k_{BA}[B] \tag{5}$$

$$\frac{d[B]}{dt} = k_{AB}[A] + k_{CB}[C] - (k_{BA} + k_{BC})[B] \tag{6}$$

$$\frac{d[C]}{dt} = k_{BC}[B] + k_{DC}[D] - (k_{CB} + k_{CD})[C] \tag{7}$$

The constants are defined on the basis of 'total concentrations' and are pH dependent.

If HB^+ is present in very low concentrations compared with HA or HC^+, the 'steady-state approximation' can be applied. $d[B]/dt = 0$ gives eqn. (8). If this expression is introduced into eqn. (5) we obtain eqn. (9). The rate expression for a direct reversible conversion $HA \rightleftharpoons HC^+$ is given by eqn. (10). If eqns. (9) and (10) are compared we obtain eqn. (11).

$$[B] = \frac{k_{AB}[A] + k_{CB}[C]}{k_{BA} + k_{BC}} \tag{8}$$

$$\frac{d[A]}{dt} = -\frac{k_{AB}k_{BC}}{k_{BA} + k_{BC}}[A] + \frac{k_{CB}k_{BA}}{k_{BA} + k_{BC}}[C] \tag{9}$$

$$\frac{d[A]}{dt} = -k_{AC}[A] + k_{CA}[C] \tag{10}$$

$$k_{AC} = \frac{k_{AB}k_{BC}}{k_{BA} + k_{BC}}\ ;\ k_{CA} = \frac{k_{CB}k_{BA}}{k_{BA} + k_{BC}} \tag{11}$$

The rate expression for the reversible conversion $HA \rightleftharpoons D^+$ is given by eqn. (12). If the steady-state approximation is applied to the intermediates HB^+ and HC^+, we obtain eqn. (13). A comparison between eqns. (12) and (13) gives eqns. (14) and (15). These expressions are considerably simplified if eqn. (11) is introduced. We thus obtain eqn. (16).

$$\frac{d[A]}{dt} = -k_{AD}[A] + k_{DA}[D] \tag{12}$$

$$\frac{d[A]}{dt} = \frac{-k_{AB}k_{BC}k_{CD}[A] + k_{DC}k_{CB}k_{BA}[D]}{k_{BA}k_{CB} + k_{CD}(k_{BA} + k_{BC})} \tag{13}$$

$$k_{AD} = \frac{k_{AB}k_{BC}k_{CD}}{k_{CB}k_{BA} + k_{CD}(k_{BA} + k_{BC})} \tag{14}$$

$$k_{DA} = \frac{k_{DC}k_{CB}k_{BA}}{k_{CB}k_{BA} + k_{CD}(k_{BA} + k_{BC})} \tag{15}$$

$$k_{AD} = \frac{k_{AC}k_{CD}}{k_{CA} + k_{CD}}\ ;\ k_{DA} = \frac{k_{DC}k_{CA}}{k_{CA} + k_{CD}} \tag{16}$$

From NMR measurements at different temperatures[2,3] we know that the rate of the interconversion $HC^+ \rightleftharpoons D^+$ is very rapid. The constants k_{CD} and k_{DC} are thus very large. From kinetic measurements we know both k_{AD} and k_{DA}, both of which are much smaller than k_{CD} and k_{DC}.

If k_{DA} is written as in eqn. (17), we can see that the two observations above are understandable only if $k_{CA} \ll k_{CD}$. In the present case, eqn. (16) is thus simplified to eqns. (18) and (19).

$$k_{DA} = \frac{k_{DC}}{1 + \dfrac{k_{CD}}{k_{CA}}} \tag{17}$$

$$k_{AD} = k_{AC} = \frac{k_{AB}k_{BC}}{k_{BA} + k_{BC}} \tag{18}$$

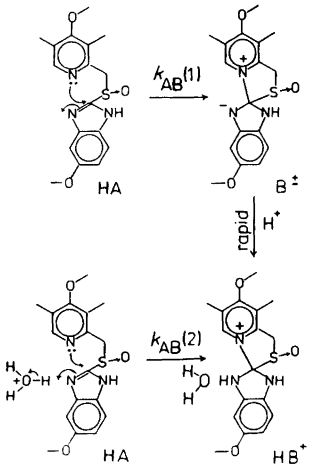
$$k_{DA} = k_{CA} \cdot \frac{k_{DC}}{k_{CD}} = \frac{k_{DC}k_{CB}k_{BA}}{k_{CD}(k_{BA} + k_{BC})} \tag{19}$$

pH *Dependence of the rate constants.* The observed variation in k_{AD} with pH is thus to be found in the variations of k_{AB}, k_{BA} and k_{BC} with pH. We must therefore consider each step in detail.

The reaction $HA \rightarrow HB^+$ means that the electron pair on the nitrogen atom of the pyridine ring attacks the electron-deficient 2-carbon atom of the benzimidazole ring. Protonation of the pyridine ring blocks the crucial electron pair and thus the form H_2A^+ should be unreactive. The same should be valid for the form H_3A^{2+} in which HA is protonated on both the pyridine ring and on the benzimidazole ring. Since the electron-deficient carbon on the benzimidazole ring loses this property in forming the anion A^-, we can expect that the form A^-, is also unreactive in the conversion into $B^\pm$. The conversion of $HA \rightarrow HB^+$ therefore takes place with the form HA.

An intramolecular reaction of HA results in the zwitterion $B^\pm$. This zwitterion is a very strong base,[4] and in the aqueous reaction medium it should very rapidly be converted into the cation HB^+. Energetically, the con-

version of HA into HB^+ might be favored if HA and a proton were to react in a second-order reaction directly to HB^+. Kinetically, this is almost equivalent with a protonation on the nitrogen atom of the benzimidazole ring followed by a first-order conversion into HB^+. This possibility will be discussed later. We thus have to consider the two reactions in Scheme 3.



Scheme 3.

At any pH value, we can now find the relationship expressed in eqn. (20) where HA is the fraction of A present as the form HA and $K^*_{H_3A^{2+}}$, and K^*_{HA} are concentration ionization constants ($K^*_{H_2A^+} = K_{H_2A^+}$).

$$k_{AB} = X_{HA}\{k_{AB}(2)[H_3O^+] + k_{AB}(1)\} \qquad (20)$$

$$X_{HA} = \frac{1}{1 + \frac{[H_3O^+]}{K_{H_2A^+}}\,1 + \frac{[H_3O^+]}{K^*_{H_3A^{2+}}} + \frac{K^*_{HA}}{[H_3O^+]}}$$

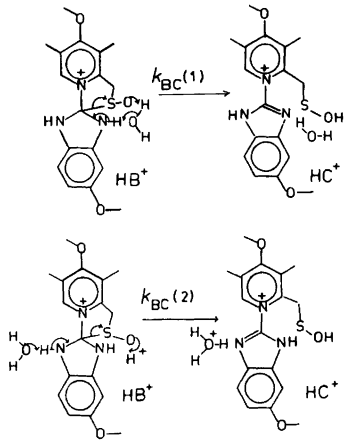
In contrast with the parameter k_{AB}, the parameters $k_{AB}(2)$ and $k_{AB}(1)$ should be constant independent of the pH of the solution.

Eqn. (20) contains the constant $K^*_{H_3A^{2+}}$ which is the stoichiometric constant for the protolysis of H_3A^{2+} which is HA protonated on both the pyridine and the benzimidazole ring. This constant is not available by direct measurement. An approximate value for $K^*_{H_3A^{2+}}$ can be estimated directly from the structure and more reliable values are available by a combination of calculation and measurement as described below and in part III.[4]

For the reaction $HB^+ \rightarrow HA$ we should recall that B is entirely present in the form HB^+. Here we do not have the possibility of a second-order reaction involving a proton, and the reaction rate constant k_{BA} should thus be independent of the pH of the solution.

In neutral and alkaline solutions we have the possibility that HB^+ reacts with OH^- in a second-order reaction to HA. Since our interest has been concentrated on the acidic solutions, we have not considered this possibility in the following discussion.

The reaction $HB^+ \rightarrow HC^+$ involves the breakage of a C–S bond and an N–H bond with the simultaneous formation of an O–H bond and a C–N double bond. The transfer of the proton from the nitrogen atom to the oxygen atom can be mediated by the solvent water, but also by any acid present in the solution. In solutions containing no acids other than H_2O and H_3O^+ we thus have the two possibilities depicted in Scheme 4.



Scheme 4.

For k_{BC} we thus have eqn. (21). We now have the three constants necessary for the calculation of k_{AD} and obtain eqn. (22).

Table 1. Summary of rate data for the reaction of omeprazole at different acid concentrations. Temperature 37 °C.

$[H^+]$/M	Method	$k_{AD}/10^{-2}\,s^{-1}$	k_{AD} (calc/$10^{-2}\,s^{-1}$)[c]
0.5	[a]	2.26±0.06	2.26
0.2	[a]	1.50±0.14	1.54
0.1	[a]	1.13±0.04	1.13
0.03	[a]	0.78±0.02	0.75
0.01	[a]	0.63±0.06	0.63
0.003	[a]	0.54±0.04	0.57
0.001	[a]	0.50±0.04	0.52
0.0003	[a]	0.46±0.06	0.42
$7.11 \cdot 10^{-5}$	[b]	0.25	0.23
$7.27 \cdot 10^{-6}$	[b]	0.039	0.037
$7.11 \cdot 10^{-7}$	[b]	0.0055	0.0043
$7.27 \cdot 10^{-8}$	[b]	0.0012	0.00082
$7.11 \cdot 10^{-8}$	[b]	0.0011	0.00081
$1.3 \cdot 10^{-9}$	[b]	0.00014	0.00017

[a] Spectrophotometric method at μ = 0.5 M. k_{AD} is obtained according to Appendix 2. Means and standard deviations of at least 3 runs. [b] Obtained from HPLC runs at μ = 0.05 M in the presence of β-mercaptoethanol (10^{-3} M). [c] See text. Obtained according to eqn. (22) using

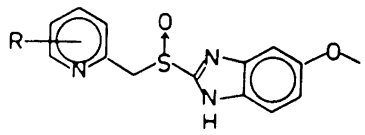
$pK^*_{H_3A^{2+}} = -0.21$ $pK_{H_2A^+} = 3.98$ $pK_{HA} = 8.7$

$a = 12.9 \pm 1.2$

$b = 13.7 \pm 2.1$, $\log [k_{AB}(2)/M^{-1}s^{-1}] = 2.90 \pm 0.06$

$\log [k_{AB}(1)/s^{-1}] = -4.18 \pm 0.08$.

Table 2. Mechanistic rate constants for compounds of the type:



No.	R	$pK^*_{H_3A^{2+}}$	$pK_{H_2A^+}$	pK_{HA}	a/M^{-1}	b	log [$k_{AB}(2)$/ M^{-1} s^{-1}]	log (k_a/ M^{-1} s^{-1})	log (k_n/ s^{-1})[a]
1	H	−0.38	2.84	8.55[b]	1.9 ±0.4	1.9 ± 0.1	0.43±0.03	−0.03±0.02	very low
2	5-CH_3	−0.32	3.26[b]	8.62[b]	2.1 ±0.4	2.1 ± 0.1	0.93±0.03	0.44±0.02	–
3	4-CH_3	−0.27	3.64	8.65	1.6 ±0.2	1.6 ± 0.1	1.45±0.02	1.03±0.01	−5.9
4	4,5-$(CH_3)_2$	−0.21	4.05	8.72[b]	1.9 ±0.2	1.9 ± 0.4	2.00±0.01	1.55±0.01	−5.6
5	3,5-$(CH_3)_2$	−0.25	3.77	8.70[b]	13 ±3	22 ±10	2.38±0.20	1.01±0.03	−5.2
6	3,4-$(CH_3)_2$	−0.19	4.21	8.74[b]	20 ±1	12 ± 1	2.83±0.02	1.71±0.01	−4.8
7	3,4,5-$(CH_3)_3$	−0.14	4.56	8.81	19 ±3	11 ± 1	3.21±0.04	2.14±0.02	−4.5
8	4.OCH_3	−0.19	4.27[b]	8.76[b]	0.9*±0.1	0.9 ± 0.1	2.57±0.03	2.29±0.01	−5.2
9	4-$OCH(CH_3)_2$	−0.16	4.42	8.78[b]	very low	very low	2.27±0.01	2.27±0.01	−5.2
10	4-OCH_3,5-C_2H_5	−0.15	4.47[b]	8.79[b]	0.4*±0.1	0.4*± 0.1	2.58±0.04	2.45±0.01	−4.5
11	3-CH_3,4-OCH_3	−0.10	4.83	8.83	9 ±1	3.8 ± 0.4	3.66±0.04	2.98±0.01	−4.4
12	3-$CH(CH_3)_2$,4-OCH_3	−0.10	4.83	8.83[b]	17 ±3	13 ± 3	4.04±0.09	2.89±0.03	−4.0
13	3,5-$(CH_3)_2$,4-OCH_3	−0.21	3.98	8.70	13 ±1	14 ± 2	2.91±0.06	1.74±0.01	−5.4

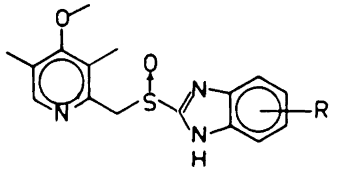
* *a* and *b* assumed to be equal. [a] Calculated from single determination at pH 7. [b] Calculated value.[3]

$$k_{BC} = k_{BC}(2)[H_3O^+] + k_{BC}(1) \quad (21)$$

$$k_{AD} = X_{HA}\{k_{AB}(2)[H_3O^+] + k_{AB}(1)\} = \frac{\frac{k_{BC}(2)}{k_{BC}(1)}[H_3O^+] + 1}{\frac{k_{BA}}{k_{BC}(1)} + \frac{k_{BC}(2)}{k_{BC}(1)}[H_3O^+] + 1} \quad (22)$$

Eqn. (22) should thus be able to account for the variation of k_{AD} with pH over a considerable pH range. This equation contains four unknown parameters $k_{AB}(2)$, $k_{AB}(1)$, $k_{BC}(2)/k_{BC}(1)$ and $k_{BA}/k_{BC}(1)$ all of which can be calculated from a set of experimental k_{AD}, and $[H_3O^+]$ values. This has been done for omeprazole and the results are given in Table 1. Eqn. (22) satisfactorily explains the variation of k_{AD} over the total pH scale up to at least pH 8. A summary

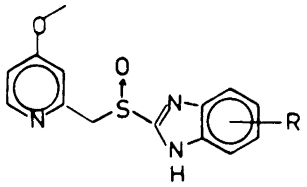
Table 3. Mechanistic rate constants for compounds of the type:



No.	R	$pK^*_{H_3A^{2+}}$	$pK_{H_2A^+}$	pK_{HA}	a/M^{-1}	b	log [$k_{AB}(2)$/ s^{-1} M^{-1}]	log (k_a/ s^{-1} M^{-1})	log (k_n/ s^{-1})[a]
1	5,6-$(OCH_3)_2$	+0.25	3.99	8.56	4.2± 0.3	4.2± 0.3	2.26±0.02	1.54±0.01	−6
2	5,6-$(CH_3)_2$	+0.47	4.01[a]	9.08	5 ± 2	15 ± 11	3.24±0.32	2.03±0.17	−4.86±0.07
3	4,7-$(CH_3)_2$	+0.11	4.00[a]	9.01[a]	12 ± 3	12 ± 4	3.06±0.14	1.96±0.03	−4.87±0.07
4	5-OCH_3	−0.21	3.98	8.70	13 ± 1	14 ± 2	2.91±0.06	1.74±0.01	−5.35±0.08
5	5-CH_3	+0.14	4.00	8.90[a]	13 ± 1	23 ± 5	3.34±0.11	1.96±0.02	−5.10±0.05
6	5-$C(CH_3)_3$	+0.06	3.99[a]	9.01	16 ± 1	9.4± 0.3	2.87±0.01	1.85±0.01	−5.2
7	H	−0.12	4.01	8.71	21 ± 5	40 ± 19	3.48±0.23	1.87±0.05	–
8	5-$CH_2CH_2OCH_3$	+0.05	4.00	8.70	22 ± 1	26 ± 1	3.28±0.02	1.84±0.01	–
9	6-CH_2OH	−0.24	3.92	8.50	29 ± 8	26 ± 6	3.17±0.11	1.74±0.07	−4.8
10	6-$NHCOCH_3$	−0.48	3.90[a]	8.35	31 ± 8	39 ± 11	3.18±0.13	1.58±0.07	–
11	5-CH_3,6-COOH	−1.83	3.82	8.73	42 ± 6	300 ±186	4.08±0.29	1.60±0.04	−4.7
12	6-CF_3	−1.80	3.78[a]	7.55	57 ±13	420 ±389	3.95±0.44	1.33±0.07	−3.80±0.04
13	5-CH_3,6-$COOCH_3$	−1.83	3.82	7.88[a]	57 ± 4	260 ± 45	3.86±0.08	1.44±0.02	−3.67±0.03
14	6-NO_2	−3.4	3.67[a]	6.78[a]	154 ±53	–	–	0.59±0.14	−2.09±0.01

[a] Calculated value.[3]

Table 4. Mechanistic rate constants for compounds of the type:



No.	R	$pK^*_{H_3A^{2+}}$	$pK_{H_2A^+}$	pK_{HA}	a/M^{-1}	b	log [$k_{AB}(2)$/ s^{-1} M^{-1}]	log (k_a/ s^{-1} M^{-1})	log (k_n/ s^{-1})[a]
1	5,6-$(CH_3)_2$	0.50	4.33[a]	9.13[a]	very low	very low	2.58±0.01	2.58±0.01	–
2	5-OCH_3	−0.17	4.27[a]	8.76[a]	0.9*±0.1	0.9*± 0.1	2.57±0.03	2.29±0.01	−5.16
3	5-$C(CH_3)_3$	0.11	4.31[a]	9.01[a]	1.2*±0.2	1.2*± 0.2	2.81±0.03	2.47±0.01	−4.98
4	H	−0.09	4.27	8.73[a]	2.6 ±0.2	2.6 ± 0.2	2.90±0.01	2.34±0.01	−5.09
5	5-OCH_3,6-F	−0.41	4.20	8.27	3.2 ±0.6	1.9 ± 0.4	2.27±0.05	1.80±0.004	–
6	5-F	−0.89	4.22	8.44[a]	6.2 ±0.5	5.3 ± 0.5	2.81±0.04	2.01±0.01	−5.12
7	5-Cl	−0.94	4.25	8.05[a]	8.2 ±0.4	14 ± 1	3.20±0.04	2.02±0.004	−4.90
8	5-Br	−0.97	4.23	7.99[a]	7.1 ±0.7	(44 ±34)	(3.65±0.34)	2.00±0.01	−4.82
9	5-OCF_2CF_2H	−1.24	4.15[a]	7.90[a]	8 ±1	11 ± 3	2.88±0.12	1.78±0.02	−4.93
10	5-CH_3,6-$COCH_3$	−2.08	4.16[a]	8.00[a]	11 ±1	13 ± 1	3.21±0.04	2.06±0.01	–

[a] Calculated value.[3]

of constants obtained with other analogues of omeprazole is given in Tables 2–4.

The calculation of all four parameters from a set of (k_{AD},[H_3O^+]) values is, however, not always possible. This is readily appreciated if eqn. (22) is rewritten as eqn. (23).

$$k_{AD} = X_{HA}\,([H_3O^+]k_a + k_n) \cdot F \qquad (23)$$

$$F = 1 + \frac{\underline{a} \cdot b\,[H_3O^+]}{\underline{a} \cdot [H_3O^+] + b + 1} \qquad \underline{a} = \frac{k_{BC}(2)}{k_{BC}(1)} \qquad b = \frac{k_{BA}}{k_{BC}(1)}$$

$$k_a = \frac{k_{AB}(2)}{b+1} \qquad k_n = \frac{k_{AB}(1)}{b+1}$$

Let us first assume that $F \simeq 1$, which is valid when the constants $\underline{a}$ and b are small. It will be demonstrated later that this occurs when the benzimidazole ring is substituted by electron-releasing groups and when the pyridine ring is alkoxy-substituted in the 4-position and unsubstituted in the 3-position. For these compounds we also have $[H_3O^+] \cdot k_a \gg k_n$ and $K_{HA} \ll H_3O^+$ if pH ≤ 3. Eqn. (23) now becomes eqn. (24).

$$k_{AD} = \frac{k_a\,[H_3O^+]}{1 + \dfrac{[H_3O^+]}{K_{H_2A^+}}\;1 + \left(\dfrac{[H_3O^+]}{K^*_{H_3A^{2+}}}\right)} \qquad (\underline{a} \text{ and } b \text{ small}) \quad (24)$$

For compounds of this type we will see a decrease in k_{AD} with an increase in [H_3O^+] and the equation can be used to calculate k_a and $K^*_{H_3A^{2+}}$ by non-linear regression. The values for $K^*_{H_3A^{2+}}$ thus obtained can then be used to estimate $K^*_{H_3A^{2+}}$ values for other compounds by the method outlined in part III. This has been done and the $pK^*_{H_3A^{2+}}$ values presented in Tables 1–4 were obtained in this way.

For compounds where $F > 1$ the variation of k_{AD} with [H_3O^+] is, to a large extent, dependent on the variation of F with [H_3O^+]. The constants $\underline{a}$ and b can often be calculated from a set of (k_{AD}, [H_3O^+]) values.

In Fig. 2 a plot of log F against pH is given for values of $\underline{a} > 1$ M^{-1} and $b \geq 1$. From the figure we see that a

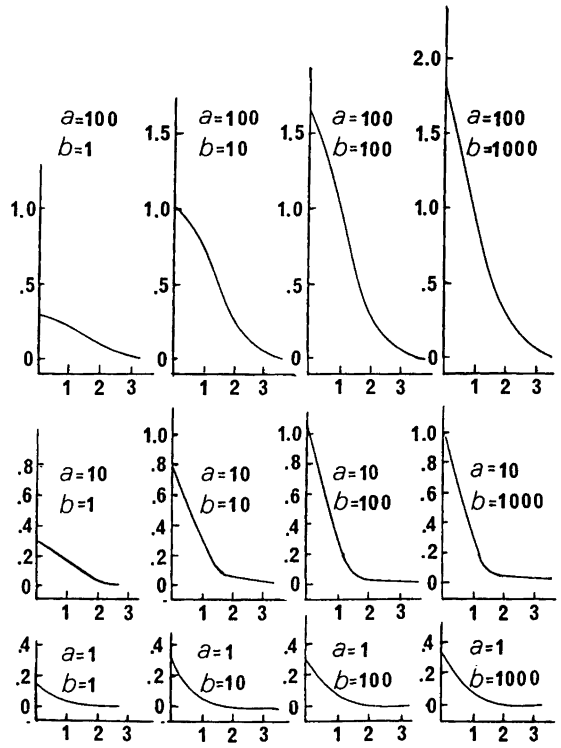


Fig. 2. Variation of log F (y axis) with pH (x axis) for some values of a/M^{-1} and b. The symbols, a and b are explained in the text.

significant variation in F with pH is observed only when $\underline{a} \cdot b \geq 1\ M^{-1}$.

It is, however, not always possible to calculate $\underline{a}$ and b even if there is a significant variation of F with $[H_3O^+]$. This can be seen from the two limiting values for F obtained from eqn. (23) and given in eqn. (25). The first equation

$$F \sim 1 + \underline{a}\,[H_3O^+] \quad b > \underline{a}/M^{-1} \gg 1$$
$$F \sim 1 + \underline{a}b\,[H_3O^+] \quad b \sim \underline{a}/M^{-1} < 1 \qquad (25)$$

indicates that high values of b obtained by the calculation will be very uncertain. This occurs when the benzimidazole ring contains a strong electron-withdrawing group (Table 3). In no case, however, is there any problem with the determination of k_a, and when the measurements extended to neutral solutions, even k_n can be calculated. One example with a very high b value is given in Table 5.

The second part of eqn. (25) means that when $\underline{a}$ and b are low only the product $\underline{a} \cdot b$ is available from a regression of k_{AD} against $[H_3O^+]$ and not the individual values. From Table 2 we can see that $\underline{a}$ and b are small when a 3-methyl group in the pyridine ring is lacking and particularly when there is an alkoxy group in the 4-position in the same ring. We can also see that for small yet measurable values of $\underline{a}/M^{-1}$ and b their numerical values are the same. This is therefore assumed to be true when the individual values are so small that they are no longer available, thus enabling a calculation of individual values from their product. The values thus obtained are marked with an asterisk in Tables 2 and 4.

The pH profile of each of the different compounds is thus determined by the rate constants involved and the pK_a values of the compound in question. This is illustrated in Fig. 3, which gives the concentrations of the different protonated forms of omeprazole as a function of pH. In the same figure the logarithm of the total observed rate constant is presented together with the contributions from the uncatalyzed reaction, the proton-catalyzed reaction and the particular reaction that occurs at very low pH values.

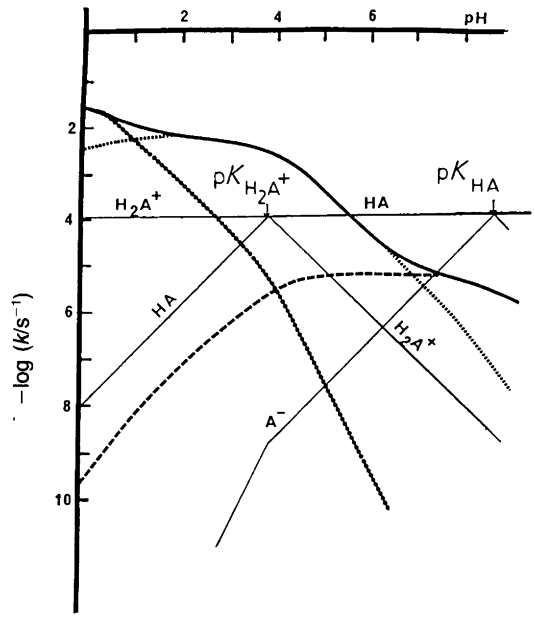


Fig. 3. Reaction of omeprazole in the presence of β-mercaptoethanol. $T = 37\,°C$. The contributions to the observed rate constant (———) from the uncatalyzed reaction (-----), the proton-catalyzed reaction (.....) and the reaction occurring at very low pH values (.....) are given.

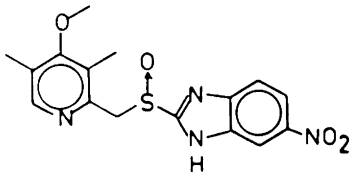
Substituent effects on the rate constants

Under neutral and moderately acidic conditions ($F = 1$), eqn. (23) is simplified to eqn. (26).

$$k_{AD} = X_{HA}([H_3O^+] \cdot k_a + k_n) \qquad (26)$$

Thus k_n and k_a are the rate constants for the forward conversions of omeprazole and analogues to the reactive species, the sulfenic acid, HC^+ and the sulfenamide, D^+, under neutral and moderately acidic conditions. k_a and k_n are very important constants, which together with $pK_{H_2A^+}$ and pK_{HA} determine the conversion rates of omeprazole and analogues over a wide pH range. Eqn. (26) has been used to calculate k_a and k_n for a variety of compounds with different substituents in the pyridine ring or in the benzimidazole ring. The results are presented in Tables 6–9. The small differences in the constants in these tables from those

Table 5. Found and calculated rate constants for the reaction of β-mercaptoethanol with

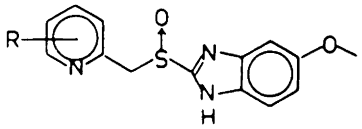


at different pH values. $T = 37\,°C$.

$[H^+]/M$	μ/M	k_{oba}/s^{-1} [a]	k_{calc}/s^{-1} [b]
0.1	0.1	0.01397	0.01383
0.03	0.03	0.00452	0.00468
0.00589	0.0084	0.00215	0.00219
0.00263	0.0043	0.00212	0.00205
0.000279	0.0010	0.00417	0.00420
0.0000820	0.00075	0.00643	0.00603
0.0000196	0.0021	0.00723	0.00717
0.00000611	0.0030	0.00760	0.00738
0.00000154	0.0033	0.00660	0.00700
0.00000105	0.0040	0.00653	0.00672

[a] Obtained from HPLC runs in the presence of β-mercaptoethanol (10^{-3} M). [b] Eqn. (23) is used with the approximation $F = 1 + a[H_3O^+]$, $pK^*_{H_2A^{2+}} = -3.4$, $pK_{H_2A^+} = 3.67$, $pK^*_{HA} = 6.54 \pm 0.09$, $\log k_a = 0.59 \pm 0.14$, $\log k_n = -2.09 \pm 0.01$, $a = 154 \pm 53$. From pK^*_{HA} we can calculate $pK_{HA} = 6.78$.

Table 6. The effect of substituents in the pyridine ring on the rate constants for compounds of the type:



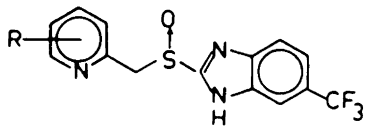
R	$pK_a(1)$	$pK_a(2)$	log (k_a/M^{-1} s^{-1})		log (k_n/s^{-1})
			Exp.	Calc.[a]	Exp.
H	2.84	8.55[b]	−0.26	−0.20	very small
4-CH_3	3.64	8.65	0.88	1.02	−5.9
5-CH_3	3.26[c]	8.62[b]	0.36	0.44	
5-C_2H_5	3.12[c]	8.60[b]	0.36	0.23	
4-CH_3S	3.78	8.69[b]	1.49	1.23	
4-$(CH_3)_2CHO$	4.42	8.78[b]	2.34	2.21	−5.1
4-C_6H_5O	3.56	8.66[b]	1.02	0.90	−5.7
3,5-$(CH_3)_2$	3.77	8.70[b]	0.97	1.22	−5.2
4,5-$(CH_3)_2$	4.05	8.72[b]	1.43	1.64	−5.6
3,4-$(CH_3)_2$	4.21	8.74[b]	1.60	1.89	−4.8
3-CH_3,4-OCH_3	4.83	8.83	2.87	2.83	−4.4
4-OCH_3,5-C_2H_5	4.47	8.79	2.56	2.28	−4.5
3,4,5-$(CH_3)_3$	4.56	8.81	2.19	2.42	−4.5
3,5-$(CH_3)_2$,4-CH_3O	3.98	8.70	1.74	1.54	−5.1
3,5-$(CH_3)_2$,4-C_2H_5O	3.98	8.70	1.60	1.54	
3,5-$(CH_3)_2$,4-CH_2=$CHCH_2O$	3.93	8.72[b]	1.42	1.46	
3,5-$(CH_3)_2$,4-CH≡CCH_2O	3.64	8.67[b]	1.09	1.02	
3,5-$(CH_3)_2$,4-$CH_3OCH_2CH_2O$	3.91	8.71[b]	1.45	1.43	
3,5-$(CH_3)_2$,4-$C_6H_5CH_2CH_2O$	3.91	8.71	1.37	1.43	
3,5-$(CH_3)_2$,4-$(CH_3)_2$,4-$(CH_3)_2CHCH_2O$	3.91	8.71[b]	1.45	1.43	

[a] log (k_a/M^{-1} s^{-1}) = −4.525 + 1.523 $pK_a(1)$. [b] Calculated from: pK_{HA} = 8.55 − 0.14 Δpy(R), see part III.[3] [c] Calculated from: $pK_{H_2A^+}$ = 2.79 − Δpy(R), see part III.[3]

in Tables 2–4 are due to measurements under different experimental conditions μ = 0.5 M and pH 0.3–3 in Tables 2–4 and μ ≤ 0.05 M and pH values >3 in Tables 5–9.

In the deduction of the rate equations for the conversion of $HA \rightarrow HC^+$ we have assumed that it proceeds via a short-lived intermediate HB^+, which can be regarded as a

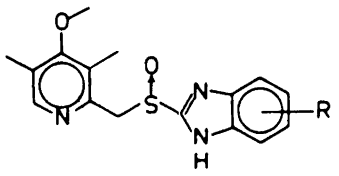
Table 7. The effect of substituents in the pyridine ring on the rate constants for compounds of the type:



R	$pK_a(1)$[a]	$pK_a(2)$[b]	log (k_a/M^{-1} s^{-1})		log (k_n/s^{-1})
			Exp.	Calc.[c]	Exp.
H	2.62	7.41	−0.68	−0.70	−4.96
3-CH_3	3.25	7.50	0.25	0.25	−4.07
4-CH_3O	3.97	7.61	1.66	1.34	−4.15
3,4-$(CH_3)_2$	4.01	7.62	1.11	1.40	−3.31
4,5-$(CH_3)_2$	3.85	7.59	0.98	1.16	−4.25
3-CH_3,4-CH_3O	4.60	7.71	2.34	2.30	−2.91
3,5-$(CH_3)_2$,4-CH_3O	3.78	7.55	1.33	1.10	−3.74

[a] Calculated according to: $pK_a(1)$ = 2.62 − Δpy(R), see part III.[3] [b] Calculated according to: $pK_a(2)$ = 7.41 − 0.14 Δpy(R), see part III.[3] [c] log (k_a/M^{-1} s^{-1}) = −4.62 + 1.51 $pK_a(1)$. [d] Experimental value.

Table 8. Found and calculated rate constants for the non-catalyzed reaction of β-mercaptoethanol with compounds of the type:



No.	R	pK_a		ΔP(R)		log (k_n/s^{-1}	
		1	2	R^1	R^2	Exp.[a]	Calc.[b]
1	5-H	4.01	8.71	0	0	−5.03	−4.93
2	5-CH_3	4.00	8.90[c]	−0.20	−0.10	−5.11	−5.09
3	5-C_2H_5	3.98[d]	8.89	−0.21	−0.07	−4.81	−5.05
4	5-$(CH_3)_2CH$	3.98[d]	8.91[c]	−0.24	−0.16	−4.95	−5.12
5	5-Cl	3.85	8.02[c]	0.59	0.88	−4.28	−4.16
6	5-CH_3O	3.98	8.70	−0.21	0.35	(−5.23)	−4.67
7	6-CF_3	3.78	7.55	1.05	1.32	−3.74	−3.74
8	6-NO_2	3.67[d]	6.78	1.64	2.84	−2.09	−2.22
9	6-CH_3CO	3.84	7.71[c]	0.75	1.95	−3.09	−3.08
10	6-CH_3SO	3.75[d]	7.34	1.22	1.71	−3.30	−3.36
11	5,6-$(CH_3)_2$	4.01[d]	9.08	−0.38	−0.38	−4.82	−5.30
12	5,7-$(CH_3)_2$	4.00[d]	9.03	−0.50	−0.20	(−4.76)	−5.20
13	4,7-$(CH_3)_2$	4.00[d]	9.02[c]	−0.32	−0.32	(−4.82)	−5.24
14	5,6,7-$(CH_3)_3$	4.03[d]	9.24[c]	−0.60	−0.48	(−4.93)	−5.44
15	4,5,7-$(CH_3)_3$	4.03[d]	9.21[c]	−0.60	−0.42	(−4.95)	−5.40
16	5-CH_3,6-$COOCH_3$	3.83	7.90[c]	0.55	1.64	−3.56	−3.39

[a] Values within parentheses excluded from the regression. [b] Calculated according to: log k_n = −4.93 + 0.96 $\Delta P(R^2)$ + log(1+10$^{a(0.97+0.96)}$) − log(1+10$^{a(0.97)}$); a = $\Delta P(R^1) - \Delta P(R^2)$. [c] $pK_a(2)$ = 8.41 − 0.97 $\Delta P(R^1)$ + log(SF). [d] $pK_a(1)$ = 3.95 − 0.15 ΔBi.

model for a transition state of the rate-limiting step in this reaction. This gives us a means of predicting the effect of substituents on the two constants k_a and k_n.

The effect of substituents in the pyridine ring on the rate constants. In the reaction HA → HB^+ a positive charge is formed on the pyridine nitrogen atom. We can thus expect a close relationship between this reaction and the protonation of the compound, in which reaction a positive charge is also created on the pyridine nitrogen atom. A plot of log k_a against $pK_{H_2A^+}$ should thus give a straight line. This is also roughly the case, which is seen from the results presented in Tables 6 and 7. A more careful examination of a plot of log(k_a/$M^{-1}s^{-1}$) against $pK_{H_2A^+}$ (Fig. 4) using the more accurate values in Table 2 reveals that we have two classes of compounds. One class with only alkyl groups in the pyridine ring fitting one line and another class of compounds also containing a 4-alkoxy group fitting another line parallel to the first line, but about 0.3 log units above it. This is interpreted as some type of resonance operating with the 4-alkoxy group. When the resonance of this group is hindered by 3- and 5-methyl groups the compounds approach the line of compounds without 4-alkoxy groups. (Several compounds of this type can be found in Table 6).

We can also see that all compounds containing only alkyl groups fall on the same line regardless of whether they have a 3-CH_3 group or not. This is in sharp contrast with the effect of a 3-CH_3 substituent on $k_{AB}(2)$ (see below).

Unfortunately, the measurements of k_n are subject to

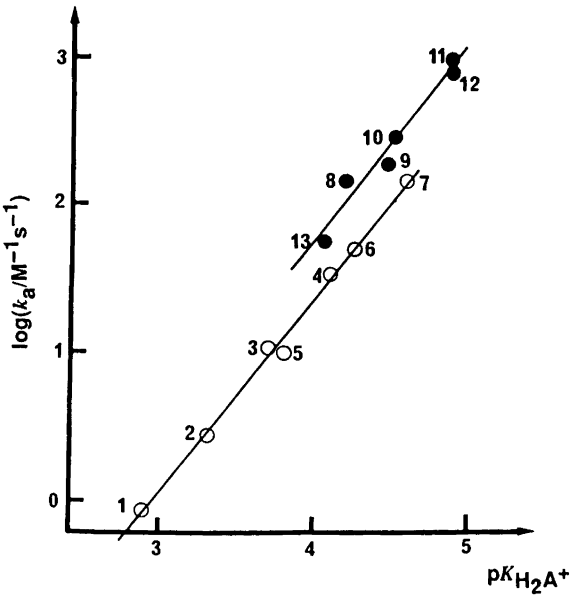
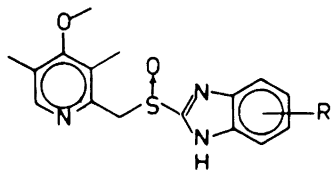


Fig. 4. The promoting effect of a 4-alkoxy substituent on the pyridine ring of omeprazole analogues. The substituent in the benzimidazole ring is 5-OCH_3.

Table 9. Found and calculated rate constants for the non-catalyzed reaction of β-mercaptoethanol with compounds of the type:



No.	R	pK_a		ΔP(R)		σ(R)		log (k_a/M^{-1} s^{-1})	
		1	2	R^1	R^2	R^1	R^2	Exp.[a]	Calc.[b]
1	H	4.01	8.71	0	0	0	0	1.86	1.79
2	5-CH_3	4.00	8.90[c]	−0.28	−0.10	−0.14	−0.06	1.97	1.91
3	5-C_2H_5	3.98[d]	8.89	−0.21	−0.07	−0.13	−0.18	1.93	1.92
4	5-$(CH_3)_2CH$	3.98[d]	8.91[c]	−0.24	−0.16	−0.13	−0.08	1.94	1.92
5	5-$(CH_3)_3C$	3.99[d]	9.01	−0.39	−0.12	−0.15	−0.09	1.87	1.93
6	5-Cl	3.85	8.02[c]	0.59	0.88	0.24	0.37	1.33	1.37
7	5-Br	3.84[d]	8.01	0.63	0.97	0.27	0.37	1.30	1.35
8	5-CH_3O	3.98[d]	8.70	−0.21	0.35	−0.12	0.10	1.74	1.74
9	5-C_2H_5O	3.94[d]	8.61	−0.13	0.34	−0.24	0.10	1.65	1.82
10	6-CH_3CONH	3.90[d]	8.35[c]	0.30	0.42	0	0.14	1.58	1.69
11	6-NO_2	3.67[d]	6.78[c]	1.64	2.84	0.71	0.81	0.59	0.74
12	6-CH_3CO	3.84	7.71[c]	0.75	1.95	0.36	0.47	1.33	1.19
13	6-CH_3SO	3.75[d]	7.34	1.22	1.71	0.49	0.52	1.08	1.12
14	6-CF_3	3.78[d]	7.55	1.05	1.32	0.46	0.54	1.33	1.12
15	5-CH_3,6-$COOCH_3$	3.83	7.88[c]	0.55	1.64	0.18	0.33	1.45	1.38
16	5,6-$(CH_3)_2$	4.01[d]	9.06	−0.38	−0.38	−0.19	−0.19	2.00	2.03
17	5,7-$(CH_3)_2$	4.00[d]	9.03	−0.50	−0.20	−0.20	−0.12	(1.92)	1.98[e]
18	4,7-$(CH_3)_2$	4.00[d]	9.02[c]	−0.32	−0.32	−0.12	−0.12	(1.97)	1.94[e]
19	5,6,7-$(CH_3)_3$	4.03[d]	9.24[c]	−0.60	−0.48	−0.26	−0.26	(2.08)	2.12[e]
20	4,5,7-$(CH_3)_3$	4.03[d]	9.21[c]	−0.60	−0.42	−0.26	−0.18	(2.03)	2.06[e]

[a] Values within parentheses excluded from the regression. [b] Calculated according to: $\log k_a = 1.79 - 1.3\,\sigma(R^2) - \log(1+10^{(0.97a-1.3b)}) - \log(1+10^{0.97a})$; $a = \Delta p(R^1) - \Delta p(R^2)$, $b = \sigma(R^1) - \sigma(R^2)$. [c] $pK_a(2) = 8.41 - 0.97\,\Delta P(R^1) + \log(SF)$, see part III.[3] [d] $pK_a(1) = 3.95 - 0.15\,\Delta Bi$, see part III.[3] [e] Assuming σ(*ortho*-CH_3) = −0.06.

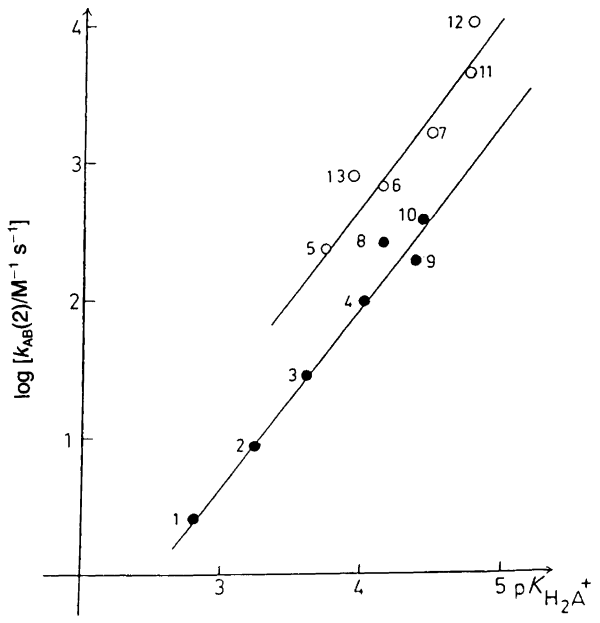
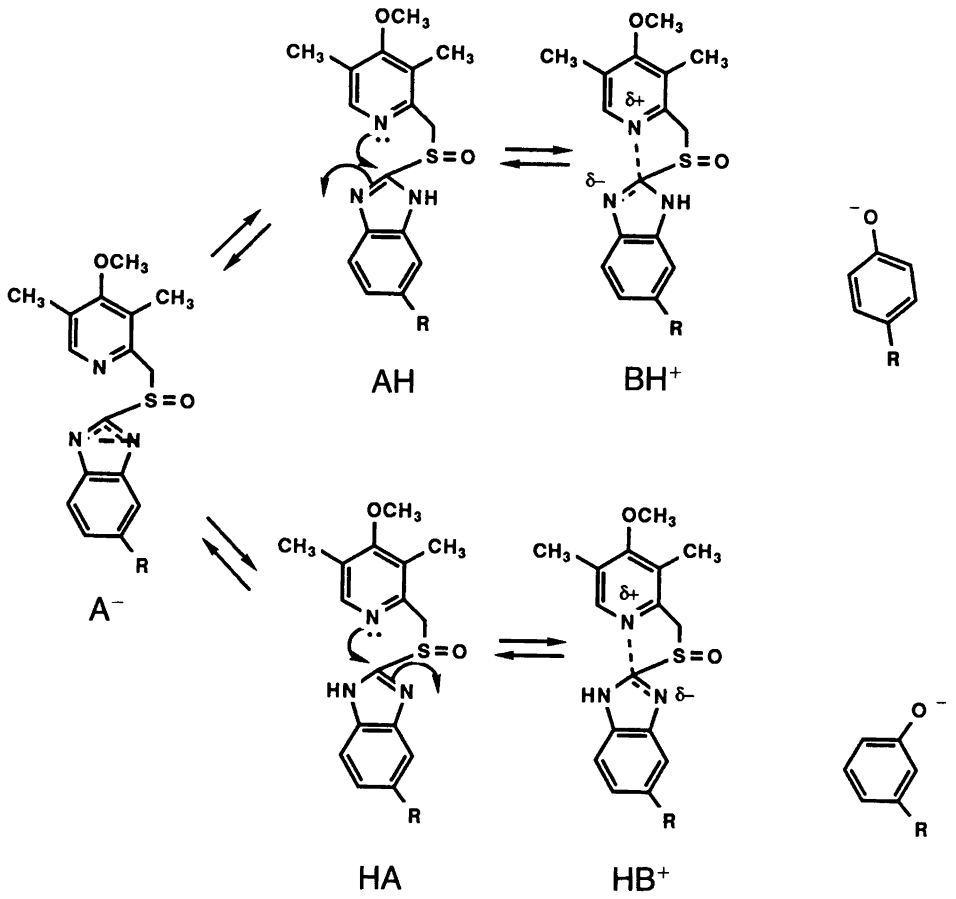


Fig. 5. The promoting effect of a 3-methyl substituent in the pyridine ring of omeprazole analogues. The substituent in the benzimidazole ring is 5-OCH_3.

many types of error due to side-reactions, and the results presented in Table 6 are too uncertain to be used in such a plot. The k_n values in Table 7 are probably more correct, but are too few to allow a definitive conclusion. However, a specific promoting effect of a 3-CH_3 group on this reaction was observed. This is probably steric in origin, and in agreement with the usual concept that CH_3 groups favor ring-closure reactions. In this case it probably promotes the formation of the conformer with the benzimidazole ring in a position suitable for attack of the pyridine nitrogen.

The peculiar effect of a 3-CH_3 substituent is also seen when $\log[k_{AB}(2)/M^{-1}s^{-1}]$ is plotted against $pK_{H_2A^+}$ (Fig. 5) using the values in Table 2. The compounds fall into two classes: one with and one without a 3-CH_3 substituent. A 3-CH_3 substituent increases $k_{AB}(2)$ by a factor of about 6 over that caused by the increase in $pK_{H_2A^+}$.

Another strong steric effect can be observed when a methyl group is introduced into the 6-position of the pyridine ring. When such a substituent is present, no reaction, or a very slow one, can be observed. An inspection of space filling molecular models shows that a 6-CH_3 substituent should experience strong steric interference with the imidazole ring in the formation of HB^+.



Scheme 5.

The ratio $k_{BC}(2)/k_{BC}(1)$ is increased by a factor of about 10 by the introduction of a 3-CH_3 group in the pyridine ring and slightly reduced by the introduction of a 4-alkoxy group (Table 2). The effect of the 3-CH_3 group is probably of steric origin. It is still higher if a 3-$CH(CH_3)_2$ group is introduced instead of the 3-CH_3 group.

The effect of substituents on the benzimidazole ring on the rate constants. As with the prediction of the pK_{HA} values (part III[3]) we have to consider the reaction of two tautomeric compounds HA and AH, which are in equilibrium with the common anion A^-. The total rate observed is equal to the sum of the rates of the two acids. For the non-catalyzed reaction we thus have eqn. (25). In part III[3] we deduce eqn. (26). Dividing eqn. (25) by [HA] and introducing eqn. (26) gives eqn. (27).

$$k_n(\text{obs})([\text{HA}]+[\text{AH}]) = k_n(\text{HA})[\text{HA}] + k_n(\text{AH})[\text{AH}] \quad (25)$$

$$K_{HA}[\text{HA}] = K_{AH}[\text{AH}] \quad (26)$$

$$k_n(\text{obs})\left(1+\frac{K_{HA}}{K_{AH}}\right) = k_n(\text{HA})\left(1+\frac{k_n(\text{AH})\,K_{HA}}{k_n(\text{HA})\,K_{AH}}\right) \quad (27)$$

We can calculate K_{HA} and K_{AH} from the structure, and if we also can predict $k_n(\text{HA})$ and $k_n(\text{AH})$, we can predict $k_n(\text{obs})$. In the reaction HA is converted into the zwitterion $B^{\pm}$ and AH to the isomeric zwitterion $B^{\mp}$ (Scheme 3). A substituent that will decrease the anionic charge on the N atom of $B^{\pm}$ can be expected to increase the rate of conversion of HA into $B^{\pm}$ in the same way that it will increase the strength of the acid AH with the substituent in the corresponding position.

If, as in part III,[3] we associate HA with the structurally similar phenol PhR^1 and AH with PhR^2 we obtain eqns. (28) and (29).

$$pK_{HA} = pK_0 - \Delta P(R^1)\varrho \quad pK_{AH} = pK_0 - \Delta P(R^2)\varrho \quad (28)$$

$$\log[k_n(\text{HA})/\text{s}^{-1}] = \log[k_0/\text{s}^{-1} + \Delta P(R^2)\varrho'] \quad (29a)$$

$$\log[k_n(\text{AH})/\text{s}^{-1}] = \log[k_0/\text{s}^{-1} + \Delta P(R^1)\varrho'] \quad (29b)$$

Note that pK_{HA} is a function of $\Delta P(R^1)$ and $\log[k_n(\text{HA})/\text{s}^{-1}]$ is a function of $\Delta P(R^2)$. If we now introduce eqns. (28) and (29) into eqn. (27) we obtain eqn. (30). Using this

$$k_n(\text{obs}) = k_0 10^{\varrho'\Delta P(R^2)}\,\frac{1+10^{(\varrho+\varrho')[\Delta P(R^1)-\Delta P(R^2)]}}{1+10^{\varrho[\Delta P(R^1)-\Delta P(R^2)]}} \quad (30)$$

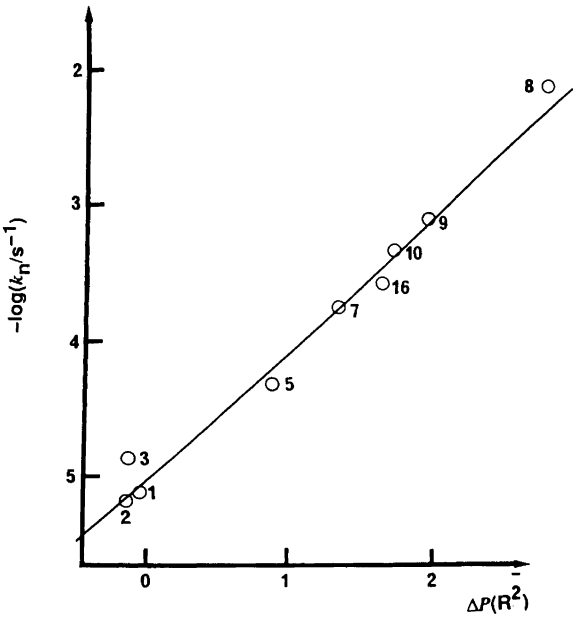


Fig. 6. The effect of benzimidazole substituents on the uncatalyzed reaction (k_n). For the meaning of $\Delta P(R^2)$ see the text.

equation, the two unknown parameters k_0 and ϱ' can be calculated by non-linear regression (Appendix 1) using observed k_n(obs) values and tabulated ΔP(R) values. This has been done, and the results are presented in Table 8.

It is difficult to obtain accurate measurements of low k_n values owing to the occurrence of side reactions and experimental difficulties in experiments in neutral solution with a duration of 8 h or more. This makes the k_n values for all alkyl-substituted compounds very uncertain. The compounds with a substituent in the 4 or 7 position should be excluded from a regression since there might be additional steric effects involved. The 5-CH_3O might be a real outlier in the regression. This group is a notorious troublemaker in regression analysis. All compounds for which reliable k_n values are obtained fit eqn. (30) very well.

When the indices R^1 and R^2 are selected such that $\Delta P(R^1) < \Delta P(R^2)$, eqn. (30) is very well approximated by eqn. (31).

$$\log[k_n(\text{obs})/\text{s}^{-1}] \sim \log(k_0/\text{s}^{-1}) + \Delta P(\text{R}^2)\varrho' \qquad (31)$$

A plot of $\log[k_n(\text{obs})/\text{s}^{-1}]$ against $\Delta P(R^2)$ (the higher of the two values) should thus give a straight line. The deviation caused by the approximation of eqn. (30) to eqn. (31) is ≤ 0.05 log units. A plot of this type is given in Fig. 6. This can be used for a rapid and good estimate of $\log(k_n/\text{s}^{-1})$ values for new analogues.

As for the non-catalyzed transformation, we must consider the reaction of the two isomers HA and AH in the acid-catalyzed reaction with the rate constant k_a(obs). In this case, a positive charge is generated in the transition state in the formation of HB^+ from HA (or AH) and H^+ (Scheme 4). If this is compared with the distribution of a negative charge in the benzoate anion the analogy is obvious.

Also as for the non-catalyzed reaction, we have eqns. (32)–(34) where σ is the Hammett's sigma value. Note

$$\text{p}K_{\text{HA}} = \text{p}K_0 - \Delta P(\text{R}^1)\varrho$$

$$\text{p}K_{\text{AH}} = \text{p}K_0 - \Delta P(\text{R}^2)\varrho \qquad (32)$$

$$\log[k_a(\text{HA})/\text{M}^{-1}\text{s}^{-1}] = \log(k_0/\text{M}^{-1}\text{s}^{-1}) + \sigma(\text{R}^2)\varrho' \qquad (33)$$

$$\log[k_a(\text{AH})/\text{M}^{-1}\text{s}^{-1}] = \log(k_0/\text{M}^{-1}\text{s}^{-1}) + \sigma(\text{R}^1)\varrho' \qquad (34)$$

that $\text{p}K_{\text{HA}}$ is connected to R^1, whereas $\log[k_a(\text{HA})/\text{M}^{-1}\text{s}^{-1}]$ is connected to R^2 and that ϱ' should be negative, since a positive charge is generated in the reaction but a negative one in the dissociation of the benzoic acid.

In the same way as for the non-catalyzed reaction we obtain eqn. (35) from which this the unknown parameters ϱ' and k_0 can be calculated by non-linear regression. The results are given in Table 9. The fit for the first 16 compounds is excellent. The bottom four compounds in the Table all contain '*ortho*' substituents and are therefore excluded from the regression calculation. Their rate constants are all in good agreement with the reasonable value $\sigma(\textit{ortho}\text{-}CH_3) = -0.06$.

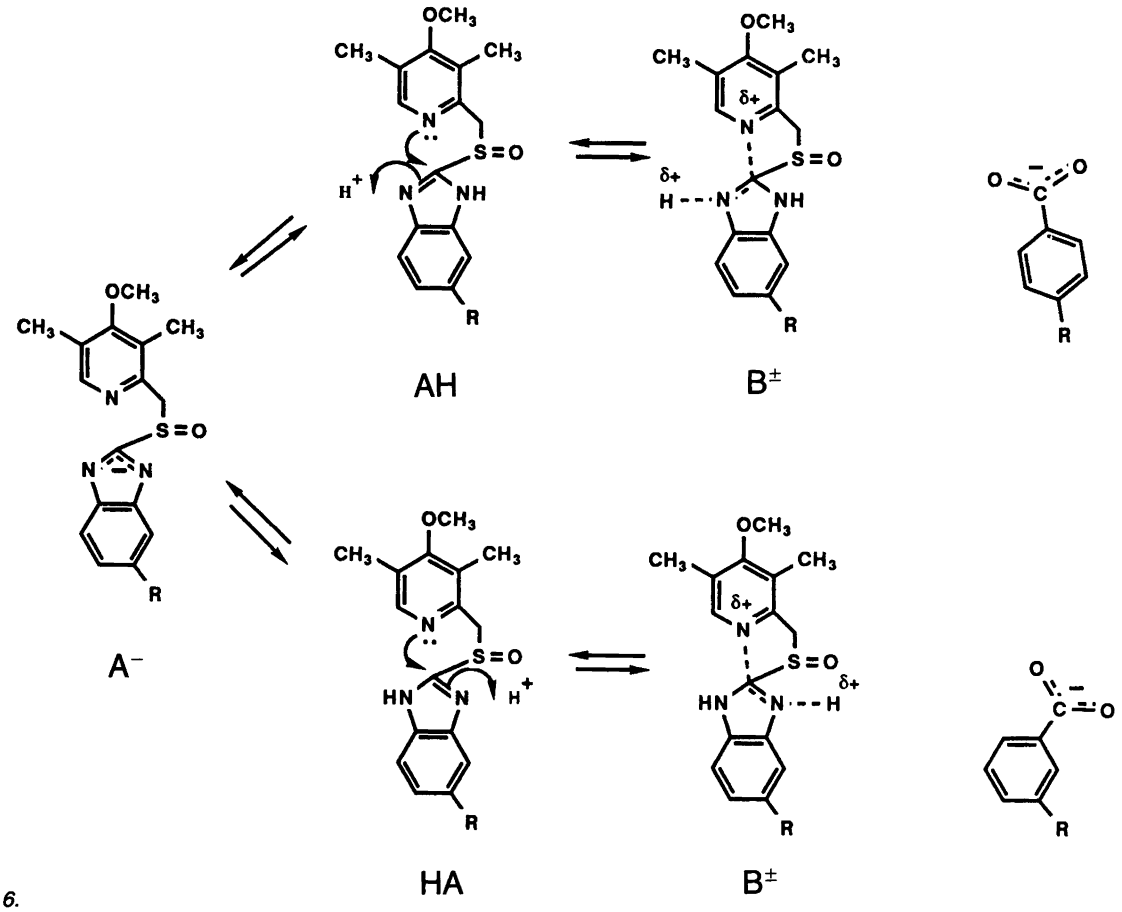
$$k_a(\text{obs}) = k_0 \cdot 10^{\varrho'\sigma(\text{R}^2)} \frac{1+10^{\{[\Delta P(\text{R}^1)-\Delta P(\text{R}^2)]+[\sigma(\text{R}^1)-\sigma(\text{R}^2)]\}\varrho'}}{1+10^{[\Delta P(\text{R}^1)-\Delta P(\text{R}^2)]}} \qquad (35)$$

Exactly as for k_n(obs), eqn. (35) can be approximated by eqn. (36), where (R^2) is the higher of the two σ values. This plot is seen in Fig. 7 and can be used as a rapid and good estimate of $\log(k_a/\text{M}^{-1}\text{s}^{-1})$ for new compounds.

$$\log[k_a(\text{obs})/\text{M}^{-1}\text{s}^{-1}] \sim \log(k_0/\text{M}^{-1}\text{s}^{-1}) + \sigma(\text{R}^2)\varrho' \qquad (36)$$

It is interesting to compare the effect of substituents on k_a and $k_{AB}(2)$ (Table 3). When an electron-withdrawing group is introduced into the benzimidazole ring, k_a is decreased whereas $k_{AB}(2)$ is increased. The explanation for the increase in $k_{AB}(2)$ is probably that in the transition state for the conversion $HA \rightarrow HB^+$ the attack of the electron pair is almost finished whereas that of the proton is just starting. This is in total disagreement with the tentative mechanism given by Senn-Bilfinger *et al.*[4]

The substituent effect on $k_{BC}(2)/k_{BC}(1)$ Tables 2 and 4 can be understood in the following way. The dihydrobenzimidazole ring system in HB^+ is a reduced aromatic-ring system and thus more electron-rich than is the benzimidazole ring system in HC^+. A conversion of HB^+ into HC^+ will thus be more difficult the stronger the electron-withdrawing effect of the substituent on the benzimidazole ring. The need for a catalyst for the conversion will be higher, and the ratio $k_{BC}(2)/k_{BC}(1)$ will increase with the electron-withdrawing power of the group. This is also found to be the case.



Scheme 6.

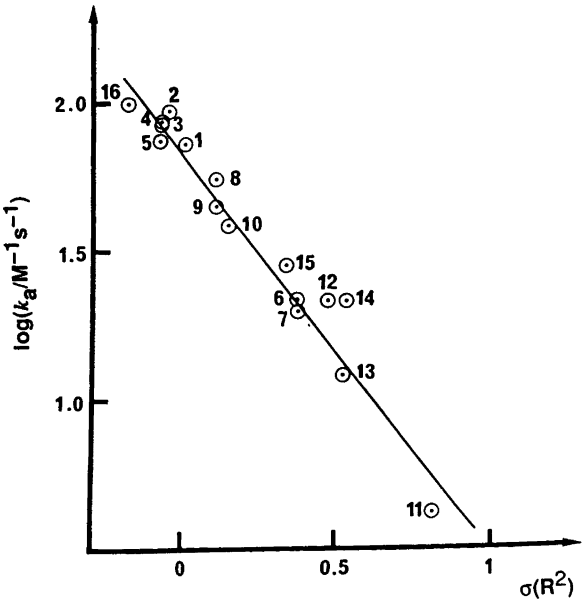


Fig. 7. The effect of benzimidazole substituents on the proton-catalyzed reaction (k_a). For the meaning of $\sigma(R^2)$ see the text.

The substituent effect on $k_{BA}/k_{BC}(1)$ can be accounted for in the following way. Mechanistically, the reactions associated with the constants k_{BA} and $k_{BC}(2)$ are very similar. In both we have an attack of H_2O on the NH group in the benzimidazole ring and (in both) the leaving-group formation is assisted by a proton. [The reverse reaction to the conversion $HB^+ \rightarrow HA$ has the rate constant $k_{AB}(2)$]. We can thus expect that k_{BA} and $k_{BC}(2)$ will have roughly the same substituent dependence and an electron-withdrawing group in the benzimidazole ring can thus be expected to increase $k_{BA}/k_{BC}(1)$. From Tables 3 and 4 we can see that $k_{BA}/k_{BC}(1)$ increases somewhat more rapidly than does $k_{BC}(2)/k_{BC}(1)$ when the electron-withdrawing effect of the benzimidazole substituent increases. This difference is small and our present understanding of the mechanism does not allow a meaningful explanation.

Conclusions

The measurements or predictions of pK_a values (see part III)[3] and rate constants (present work) enable us to calculate the rate by which any compound within this class at any pH value is converted into the corresponding sulfenamide. The only thing one has to do is to put the proper figures into the general eqn. (23) or, under neutral and moderately acidic conditions, into the simplified one [eqn. (24)].

Experimental

General. The reactions were followed using a liquid chromatographic system or a photometric method. Minor variations in the procedures were necessary depending upon the particular reaction studied. The specific procedures will be given in the corresponding part of the series.

Instruments and chemicals. The liquid chromatograph consisted of a Beckman 112 pump or a LKB 2152 pump, a Rheodyne 7010 injection valve with a 100 µl loop and an LDC spectromonitor III UV detector or a Waters 441 detector operating at 280 nm. The separation columns were of precision-bore stainless steel (150×4 or 125×4 mm), either home-packed or packed by the manufacturer with Lichrosorb RP8, 5 µm. A pre-column, Brownlee OS–GU, RP-8, 30×4.6 mm was also used. The mobile phase was 35–60 % acetonitrile in phosphate buffer pH 6.5–7.5 ($\mu = 0.025$ M), flow rate 1 ml min^{-1}. The photometer was a Beckman DU-7 UV–VIS spectrophotometer with a thermostatted cell holder.

2-Mercaptoethanol was from Serva or Roth, and acetonitrile (HPLC grade) was from Fisons. Buffer substances (analytical grade) were from Merck.

Procedures

HPLC method in the absence of 2-mercaptoethanol, pH = 1. HCl (0.1 M, 4.0 ml) was thermostatted at 37 °C. A solution of omeprazole in methanol (150 µl) was added. The resulting concentration of omeprazole was 1×10^{-5} M. The reaction was stopped by the addition of 100 µl of 2-mercaptoethanol, (1×10^{-3} M), and 1000 µl of phosphate buffer pH 6 ($I = 1.0$ M), to which 0.4 mmol of NaOH had been added. A 100 µl aliquot was injected onto the separation column. Runs at other pH values were performed under similar conditions. With a Brownlee OS–GU, RP-8, 5 µm (30×4.6 mm ID) pre-column, a LiChrosorb RP-8, 5 µm (125×4 mm ID) column and the mobile phase 35 % acetonitrile, 10 % buffer pH = 7.0, $\mu = 0.25$ M and 55 % water, the retention times were HA = 7.3 min, $HE\beta^+$ = 9.0 min, H_2F^+ = 11.4 min and HS = 17.1 min. Since $HE\beta^+$ has $pK_a \simeq 6$ its retention time is very sensitive toward changes in pH and ionic strength and small changes in these parameters can change its retention time by several minutes. The same is true for H_2F^+ (part VI[5]).

HPLC method in the presence of 2-mercaptoethanol. The reaction of omeprazole (10^{-5} M) was allowed to take place in a buffer solution of desired pH in the presence of 2-mercaptoethanol. At various time intervals, samples were withdrawn and analyzed by means of HPLC.

Spectrophotometric method. When omeprazole in a 10^{-5} M solution is treated with dilute HCl the reversible formation of D^+ occurs. The reaction can be followed by repetitive scanning of the UV spectrum since D^+ has a UV maximum at 355 nm, at which wavelength omeprazole is transparent.

The method consists of adding 50 µl of a solution of omeprazole in methanol to 3.0 ml of dilute HCl, thermostatted to 37 °C. The resulting concentration of omeprazole was 10^{-5} M. The increase of the absorbance at 355 nm was followed until equilibrium was reached.

Formation of HA from D^+ in 0.001 M HCl. In this experiment we used a freshly prepared PF_6^- salt of D^+ formed from the omeprazole analogue lacking the OCH_3 group in the benzimidazole ring in order to avoid problems due to the existence of two isomers as in the case of D^+ prepared from omeprazole. A stock solution of the salt was prepared from 1.4 mg of the pure salt in 5 ml of methanol. 50 µl of the stock solution were added to 5 ml of 0.001 M HCl at 37 °C. After 3 min, 80 µl of 0.1 M 2-mercaptoethanol were added and the mixture was injected as soon as possible onto an HPLC column and analyzed for HA and $HE\beta^+$, which were obtained in the ratio 9:73 with retention times of 3.7 and 6.4 min. In a corresponding experiment in 0.01 M HCl, the ratio was found to be 7:77 after 1.5 min. In order to prove that no HA was initially present, 50 µl of the stock solution were added to a mixture of 5 ml of 0.001 M HCl and 80 µl of 0.1 M 2-mercaptoethanol. The resulting solution was injected as rapidly as possible onto the HPLC column. No trace of HA was seen, but a large peak corresponding to $HE\beta^+$. By using HA instead of D^+ in the last experiment we demonstrated that less than 10 % of HA had reacted to give $HE\beta^+$ under the same conditions.

Appendix 1

The calculation of rate constants

General principles and application to first-order reactions. In the kinetic investigations needed for this project, we have, on several occasions, been involved with highly complex kinetic problems for which we had to develop our own solutions. Our general least-squares computer program* has been very helpful in this work owing to its simplicity and high flexibility. The possibility of tackling complex kinetic problems with simple methods is usually unknown to organic chemists, and we will thus take this opportunity to explain our method.

Rate constants are usually best calculated by multiple regression using the least-squares method. A good description of this method can be found in Ref. 6, and programs for calculations are available for most computers. They usually handle the problem of calculating the parameters $b_0, b_1, \ldots b_p$ when one variable Y is linearly dependent on several variables $x_1, x_2 \ldots x_p$, as in eqn. (A1:1). This is done by minimizing the sum of the squares of n observations of Y_k, eqn. (A1:2).

* The program was written in BASIC for an ABC 802 personal computer and may be obtained upon request.

$$Y_k - b_0 = b_1X_{k,1} + b_2X_{k,2} + \dots b_pX_{k,p} = \sum_{i=1}^{p} b_iX_{k,i} \quad \text{(A1:1)}$$

$$\sum_{k=1}^{n} \left(Y_k - b_0 - \sum_{i=1}^{p} b_iX_{k,i} \right)^2 \quad \text{(A1:2)}$$

All that is needed is a table of Y_k values measured at different values of $x_1, x_2, \dots x_p$.

In kinetic practice Y_k is seldom a linear function of time and other variables. However, non-linear functions can easily be converted into linear functions that can be solved by this method. This possibility has already been pointed out by Gauss,[7] and is used in almost every iterative least-squares program, including ours. The basic principle of our program is explained in the following using the very simple reaction $A \rightarrow B \rightarrow C$ as an example.

For a system $A \xrightarrow{a_1} B \xrightarrow{a_2} C$ where the reactions are all first order, we can immediately present the following differential eqn. (A1:3), where a_1 and a_2 are first-order rate

$$\frac{d[A]}{dt} = -a_1[A], \frac{d[B]}{dt} = a_1[A] - a_2[B], \frac{d[C]}{dt} = a_2[B] \quad \text{(A1:3)}$$

constants. The solutions to these differential equations in a general case will be of the type (A1:4), where $c_1, c_2, \dots c_l$ are known constants such as initial concentrations, pK_a values, pH values, and so on. $a_1, a_2 \dots a_m$ are rate constants or other constants to be determined, t is time and $Y_k(t)$ is the concentration of the species Y_k at the time t.

$$Y_k(t) = F_k(a_1, a_2 \dots a_m, c_1, c_2 \dots c_l, t) \quad \text{(A1:4)}$$

In the case of first-order reactions the conversion of eqns. of type 3 into those of type 4 is readily done by integration, preferably using the operator method (Laplace or Laplace–Carson). A short and easily understandable description of this method has been presented by Regårdh[8] (6 pages) and by Rodiguin and Rodiguina[9] (16 pages). (At first sight the solutions presented by Rodiguin and Rodiguina look discouraging, but even their most complex equations are readily handled by most personal computers). When second-order reactions are involved, a simple integration is usually not possible, but as described later $F_k(t)$ is also readily obtained in these cases.

In our example, eqn. (A1:3) is readily transformed into eqn. (A1:5) using the table of Laplace transforms in Ref. 7, assuming $[B]_0 = [C]_0 = 0$. Here $\overline{A}$ is the transformation of [A] and so on. Eqn. (A1:5) is algebraically solved for $\overline{A}$, $\overline{B}$ and $\overline{C}$ to give eqn. (A1:6). From the table with transforms (Ref. 7) we can see that the transformations in eqn. (A1:6) correspond to the equations in eqn. (A1:7).

$$s\overline{A} = [A]_0 - a_1\overline{A}, \; s\overline{B} = a_1\overline{A} - a_2\overline{B}, \; s\overline{C} = a_2\overline{B} \quad \text{(A1:5)}$$

$$\overline{A} = \frac{[A]_0}{s+a_1}, \; \overline{B} = \frac{a_1[A]_0}{(s+a_1)(s+a_2)}, \; \overline{C} = \frac{a_1a_2[A]_0}{s(s+a_1)(s+a_2)} \quad \text{(A1:6)}$$

$$F_1(t) = [A] = [A]_0 \cdot e^{-a_1t}$$

$$F_2(t) = [B] = [A]_0 \cdot \frac{a_1}{a_2 - a_1}(e^{-a_1t} - e^{-a_2t}) \quad \text{(A1:7)}$$

$$F_3(t) = [C] = [A]_0\left[1 + \frac{1}{a_2 - a_1}(a_2e^{-a_1t} - a_1e^{-a_2t})\right]$$

These and other equations of type (A1:4) are not linear functions of the parameters to be calculated and therefore have to be transformed before they are used in eqn. (A1:1). The next step in the calculation is therefore a linearization.

Given an initial estimate of the rate constants $a_i \sim a_i(0)$, we can develop $F_k(t)$ around the point $a_1(0), a_2(0), \dots a_m(0)$. If the guess is good, only terms of the first degree are needed to give a good approximation. The approximation is better the closer $a_i(0)$ is to a_i. We thus obtain eqn. (A1:8) where $F_k(0,t)$ is the value of $F_k(t)$ evaluated at $a_1(0), a_2(0), \dots a_m(0)$, $\Delta a_i = a_i - a_i(0)$ and $\delta F_k(0,t)/\delta a_i$ is the partial derivative with respect to a_i evaluated at the same point and readibly obtained using eqn. (A1:9). In this equation $F_{k,i}(0,t)$ is the value of $F_k(t)$ at $a_1(0), a_2(0) \dots a_i(0+\varepsilon_i), \dots a_m(0)$. ε_i is a small value [conveniently $\varepsilon_i = 10^{-6}\, a_i(0)$].

$$F_k(t) \simeq F_k(0,t) + \sum_{i=1}^{m} \left(\Delta a_i \frac{\delta F_k(0,t)}{\delta a_i} \right) \quad \text{(A1:8)}$$

$$\frac{\delta F_k(0,t)}{\delta a_i} = [F_{k,i}(0,t) - F_k(0,t)]/\varepsilon_i \quad \text{(A1:9)}$$

Eqn. (A1:8) is identical with eqn. (A1:1) if $Y_k - b_0$ is replaced by $Y_k(t) - F_k(0,t)$, b_i is replaced by Δa_i and $X_{k,i}$ is replaced by $[F_{k,i}(0,t) - F_k(0,t)]/\varepsilon_i$. Using the least-squares minimization procedure Δa_i can thus be obtained, and the new estimate $a_i(1) = \Delta a_i + a_i(0)$, which should be more correct, can be used in a new calculation to give $a_i(2)$, and so on until Δa_i values smaller than a specified magnitude are obtained. In most cases the convergence is very rapid provided that the kinetic model is correct and that the $a_i(0)$ values are resonably correct, which often means that they are of the correct power of 10.

Before we continue with the actual example $A \rightarrow B \rightarrow C$, a few words must be said about eqns. (A1:1) and (A1:2). In a correct statistical treatment the variables $X_{k,i}$ should be orthogonal (independent of each other), and the experimental errors of Y_k should have a normal distribution. In practice this is seldom fulfilled. A least-squares calculation using eqn. (A1:1) always gives b_i values as an answer, often combined with a variance–covariance matrix and standard deviations of the calculated parameters. The results are the more correct the more the theoretical requirements are fulfilled. To give useful results all experimental errors in Y_k should at least be of the same magnitude. In the present case, this means that the absolute value of $Y_k(t) - F_k(t)$ should be of the same magnitude for all values of t and k. If they are not, a transformation (normalization) should be

Table A1:1. The reaction of 10^{-5} M omeprazole with 0.0108 M β-mercaptoethanol at pH 4.26 and 37°C. $[A]_0 = 1.026\times10^{-5}$ M. Starting values of the parameters: a_1 = 1.000E−01; a_2 = 1.200E−02; B_m = 4.000E+03.

$\Delta a_1/a_1$	$\Delta a_2/a_2$	$\Delta B_m/B_m$	$\Delta C_m/C_m$
−3.94E−02	1.12E−01	5.60E−02	−5.31E−02
1.31E−03	6.19E−03	2.83E−03	3.52E−03
1.48E−05	4.97E−05	−2.26E−06	−5.96E−06
−9.14E−08	6.75E−07	9.40E−08	−1.54E−07
2.81E−09	1.08E−08	1.79E−09	−3.53E−09
−3.57E−12	2.16E−10	4.21E−11	−6.63E−11

X/min	[A]/10^{-5} M		B/integration units		[C]/10^{-5} M	
	Found	Calc.	Found	Calc.	Found	Calc.
0.000E+00	1.026E+00	1.026E+00	0.000E+00	0.000E+00	0.000E+00	0.000E+00
3.000E+00	7.570E−01	7.688E−01	9.920E+02	1.040E+03	6.000E−03	4.959E−03
9.250E+00	4.330E−01	4.215E−01	2.273E+03	2.326E+03	4.200E−02	3.815E−02
1.600E+01	2.090E−01	2.202E−01	2.953E+03	2.915E+03	1.000E−01	9.243E−02
3.000E+01	5.300E−02	5.728E−02	2.992E+03	3.016E+03	2.320E−01	2.206E−01
5.000E+01	6.000E−03	8.367E−03	2.502E+03	2.476E+03	3.970E−01	3.871E−01
7.050E+01	0.000E+00	1.165E−03	1.933E+03	1.905E+03	5.270E−01	5.219E−01
1.220E+02	0.000E+00	8.223E−06	9.720E+02	9.567E+02	7.290E−01	7.356E−01
1.750E+02	0.000E+00	5.025E−08	4.670E+02	4.696E+02	8.390E−01	8.449E−01

a_1 = 9.618E−02 a_2 = 1.343E−02 B_m = 4.236E+03 C_m = 9.502E−06.
SD(a_1) = 1.18E−03 SD(a_2) = 2.21E−04 SD(B_m) = 3.81E+01 SD(C_m) = 8.38E−08.

performed before applying the least-squares method. The meaning of this is well illustrated in our example.

For the reaction of omeprazole in the presence of 2-mercaptoethanol at 37 °C and pH 4.26 we have measured (in integration units) the HPLC peaks of three compounds corresponding to A, B and C. For A and C the integration units could be converted into concentrations by a comparison with reference peaks. This was not considered practical for B, but we assumed (on good grounds) that the concentration was directly proportional to the peak area in integration units. The results are presented in Table A1:1. This reaction is of the type A→B→C, and eqn. (A1:7) should in principle be valid. We could, however, not exclude the possibility of side reactions occurring involving A. If they do occur, the formulas are still correct if $[A]_0$ in $F_2(t)$ is replaced by B_m where B_m is the integration units of B that would have been obtained if $a_2 = 0$ and if $[A]_0$ in $F_3(t)$ is replaced by C_m, which is the maximum concentration of C obtainable. We thus obtain eqn. (A1:7a).

$$F_1(t) = [A] = [A]_0 \cdot e^{-a_1 t}$$

$$F_2(t) = [B] = B_m \cdot \frac{a_1}{a_2 - a_1}(e^{-a_1 t} - e^{-a_2 t}) \qquad \text{(A1:7a)}$$

$$F_3(t) = [C] = C_m\left[1 + \frac{1}{a_1 - a_2}(a_2 e^{-a_1 t} - a_1 e^{-a_2 t})\right]$$

Four parameters, a_1, a_2, $a_3 = B_m$ and $a_4 = C_m$ thus have to be calculated in the regression analysis. This should present no problem if we can use all the 3×9 measurements. For this purpose we have to use $Y_1(t) - F_1(t)$, $Y_2(t) - F_2(t)$ and $Y_3(t) - F_3(t)$ in the same least-squares equation. This is not practicable since they are of very different magnitudes. $Y_1(t) - F_1(t)$ and $Y_3(t) - F_3(t)$ can be expected to be of magnitude 10^{-7} and $Y_2(t) - F_2(t)$ of the magnitude 10. If used together in eqn. (A1:1) the values $Y_2(t) - F_2(t)$ will totally outweigh all other values. A normalization is therefore necessary before use in eqn. (A1:1). For this purpose $Y_1(t) - F_1(t)$ and $[F_{1,i}(0,t) - F_1(0,t)]/\varepsilon_i$ are divided by $[A]_0$, whereas $Y_2(t) - F_2(0,t)$ and $[F_{2,i}(0,t) - F_2(0,t)]/\varepsilon_i$ are divided by B_m and $Y_3(t) - F_3(0,t)$ and $[F_{3,i}(0,t) - F_{3,i}(0,t)]/\varepsilon_i$ are divided by C_m. All are now of the same magnitude and can be used in eqn. (A1:1).

To start the calculation we need $a_i(0)$ values. We readily obtain $a_i(0) = 0.1$ from the slope of a plot of ln[A] against t. The value $a_2(0) = 0.012$ can be obtained by the numerical integration method described by Brändström and Strandlund[10] from the slope of a plot of $[C]/C_m$ against $\int_0^t [B]/B_m dt$. With no calculation we can also guess that $a_3(0) = B_m = 4000$ and $a_4(0) = C_m = 10^{-5}$. These starting values and the t and $Y_k(t)$ values were fed into a computer and $a_1 - a_4$ were determined according to the principles given above.

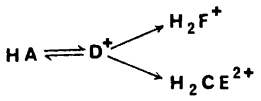
From the calculated standard deviations in a_i an indication of the correctness of the kinetic model and of the a_i values could be obtained but a good deal of caution is recommended. It should be remembered that, mathematically, there is an unlimited number of functions that fit the points.

In Table A1:1 the $\Delta a_i/a_i$ values obtained after each calcu-

lation are presented, together with the final a_i values with their standard deviations, as well as calculated values of [A], [B] and [C].

We can see that the fit of the points to the functions is very good, and that all four parameters are obtained with a standard deviation of about 1 %.

Application to second-order reactions. When second-order reactions are involved, the resulting set of differential equations is only in rare cases resolvable into elementary functions, but nonetheless the principle described above can be used. The crucial point in the calculation is to obtain $F_k(0,t)$ and $F_{k,i}(0,t)$ values, which may readily be obtained directly from the differential equations by the 'small increment' method. This is best illustrated by an example. In the reaction of omeprazole, HA, we observed the following reactions in Scheme 7. All of these reactions are first-order with the exception of $D^+ \rightarrow H_2CE^{2+}$ which appears to be second order in D^+.



Scheme 7.

We have the differential eqns. (A1:10) where [A] is the total concentration of HA in all its protolytic forms and [D] the concentration of D^+. If we start with pure HA and measure [A] and [D] as functions of time, we have initially $[A] = [A]_0$ and $[D]_0 = 0$. Eqn. (A1:10) is also valid in the form of eqn. (A1:11)

$$\frac{d[A]}{dt} = -a_1[A] + a_2[D] \qquad \text{(A1:10)}$$

$$\frac{d[D]}{dt} = a_1[A] - a_2[D] - (a_3[D] + a_4[D]^2)$$

$$\begin{aligned} \Delta[A] &= (-a_1[A] + a_2[D])Dt \\ \Delta[D] &= -\Delta[A] - (a_3[D] + a_4[D]^2)Dt \end{aligned} \qquad \text{(A1:11)}$$

where Dt is a time interval short enough to give correct results. Usually $Dt = [t(N) - t(N-1)]/100$, where $t(N)$ and $t(N-1)$ are two consecutive time values in the measurements. $F_1(0,t) = [A]$ and $F_2(0,t) = [D]$ are readily obtained by iteration using eqn. (A1:12). In the same way, $F_{1,i}(0,t)$ and $F_{2,i}(0,t)$ can be computed and we thus have everything needed for a calculation.

$$\begin{aligned} &\text{Start } [A] = [A]_0,\ [D] = 0,\ t = 0 \\ &\Delta[A]_i = (-a_1[A]_i + a_2[D]_i)Dt \\ &\Delta[D]_i = -\Delta[A]_i - (a_3[D]_i + a_4[D]_i^2)Dt \\ &t_{i+1} = t_i + Dt \\ &[A]_{i+1} = [A]_i + \Delta[A]_i \\ &[D]_{i+1} = [D]_i + \Delta[D]_i \end{aligned} \qquad \text{(A1:12)}$$

Finally a few words should be said about the use of measurements on several compounds with different starting conditions in the same calculation. In every case tested where this has been done, a considerable stabilization in the computation of the constants has been obtained, provided that a proper normalization of the values has been undertaken. In many cases the problem cannot be solved in other ways. To take one example from the reaction $A \rightarrow B \rightarrow C$, it is obvious that the measurements on A alone give no information about a_2. The measurement on B alone gives two constants but which of them is a_1 cannot be derived from this experiment alone. A measurement on A and B does not reveal whether anything is happening to C, and so on.

In the reaction $D^+ \rightarrow H_2CE^{2+}$ mentioned above, the second-order nature of the reaction is not revealed unless the measurements are performed using at least two different starting concentrations.

Appendix 2

The calculation of rate constants by the photometric method

The photometric method described in the Experimental section has been used to complement the HPLC method in the study of rapid reactions at low pH values. In this method omeprazole or an analogue is added to an excess of dilute HCl and the UV absorption spectrum recorded at different times. Spectral changes due to the reaction $HA \rightarrow D^+$ occur, as is demonstrated in Fig. A2:1. If the absorbance at about 355 nm is followed as a function of time, curves of the types given in Fig. A2:2 are obtained. At about 355 nm D^+ has a flat absorption peak, whereas HA has very little or no absorption.

This method has sometimes been used to measure k_{AD} values in dilute HCl solutions. It is a very rapid method and several compounds and concentrations can be measured in one day. With this method, k_{AD} values can be calculated from a set of absorbance versus t values without, or with only a few, additional measurements.

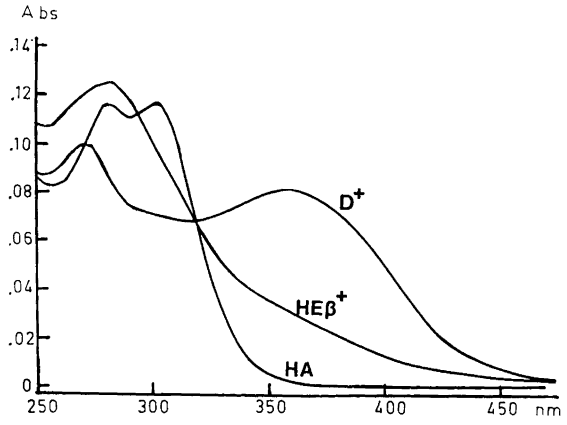


Fig. A2:1. Spectral changes due to the reaction $HA \rightarrow D^+$.

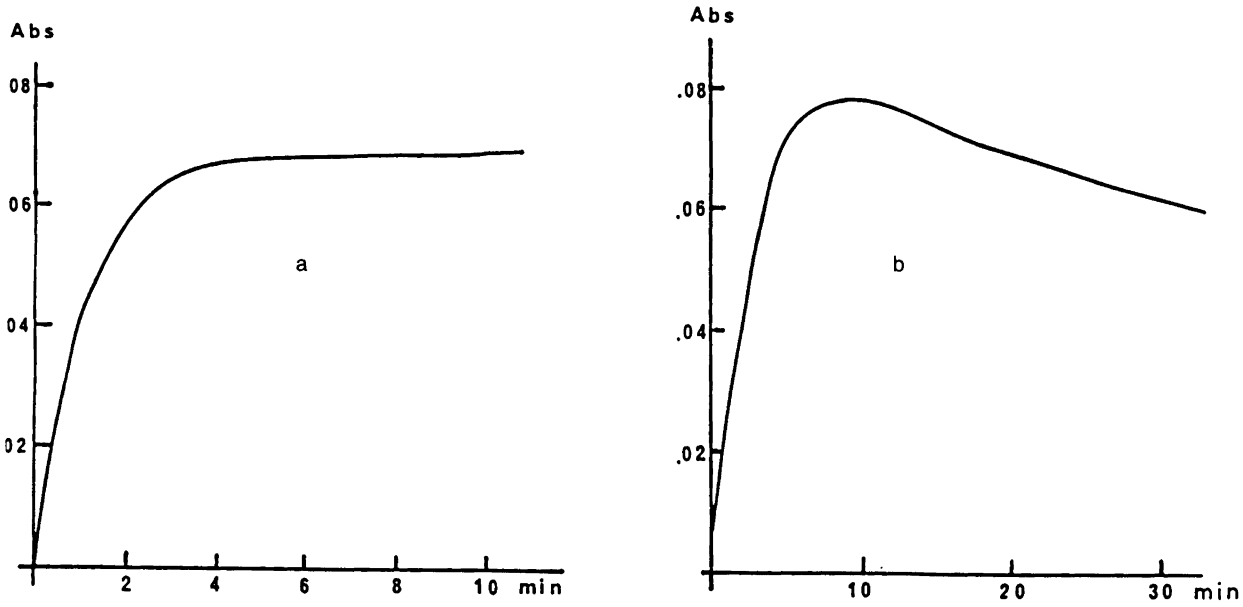


Fig. A2:2. Time dependence of the absorbance at 355 nm for compounds of the type: a, R = 3,4-$(CH_3)_2$, 10^{-5} M in 0.5 M HCl; b, R = 3,5-$(CH_3)_2$, 4-OCH_3, 10^{-5} M in 0.001 M HCl, μ = 0.05 M.

Depending on the substituents and the pH value, two types of curves are observed. In the first type [(a) seen in Fig. A2:2], absorbance tends asymptotically toward an equilibrium value Abs_e. In the second type (b), the absorbance passes through a maximum value and then slowly decreases, indicating that secondary reactions of D^+ (or HA) occur.

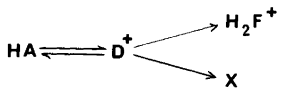
A curve of the first type indicates a reversible first-order reaction without disturbing side reactions. For such a reaction eqn. (A2:1) is valid.

$$Abs = Abs_e - (Abs_e - Abs_0)e^{-(k_{AD}+k_{DA})t} \qquad \text{(A2:1)}$$

From a set of (Abs, t) values the three parameters Abs_0, Abs_e and $(k_{AD} + k_{DA})$ are readily calculated by non-linear regression. For omeprazole and a few analogues we found by HPLC measurements that k_{DA}/k_{AD} was independent of the pH (in the pH range 1 to 3) and fairly low (0.12–0.40). The same was assumed to be true for all other analogues studied in the present investigation and is in agreement with the fact that a catalyst does not change the position of an equilibrium. The determination by HPLC of k_{DA}/k_{AD} at one pH value was thus assumed to be sufficient. Using this ratio the value k_{AD} was readily calculated from $(k_{AD} + k_{DA})$. As long as the ratio k_{DA}/k_{AD} is low, even a moderate error in k_{DA}/k_{AD} will not seriously affect the k_{AD} value thus obtained.

When the absorbance versus time curves are of type (b) illustrated in Fig. A2:2, some secondary reactions of D^+ have occurred and the method given above cannot be used.

Instead of the simple reaction $HA \rightarrow D^+$ we now have to consider the much more complicated situation in Scheme 8, where the absorbance may be due to absorptions from D^+, H_2F^+ and various compounds X. In principle it should still be possible to express the absorbance as a function of starting concentrations, rate constants, time and ε values for all compounds involved and calculate all these parameters from a set of absorbance versus t values by non-linear regression by a method similar to that described in part VI.[5] However, the use of one single dependent variable Abs will make many of the parameters strongly interdependent, and thus the parameters obtained will be very uncertain. Another method must therefore be used.



Scheme 8.

A solution of omeprazole (0.994×10^{-5} M) in 0.01 M HCl has been carefully studied by both HPLC and spectrophotometric methods (part VI[5]). A plot of [HA], $[D^+]$ (measured as $[HE\beta^+]$) and Abs/ε_D against t, together with the calculated values of [HA], and $[X] = [HA]_0 - [HA] - [D^+]$ is given in Fig. A2:3.

The curves for $[D^+]$ and Abs/ε_D agree exactly from the start up to about 4 min. After this the difference between Abs/ε_D and $[D^+]$ increases with time. This clearly indicates that besides D^+ there are also other compounds that absorb at 355 nm. We now make the *mathematical approximation* that there is only one additional compound X with the molecular absorptivity ε_X. In the figure we have plotted [X]. Since $Abs_t = [D^+]_t \varepsilon_{D^+} + [X]_t \varepsilon_X$ we can readily calculate ε_X to be 5550 which explains exactly all Abs_t values up to ca. 30 min. In this case the mathematical approximation that there is only one compound X formed is very good al-

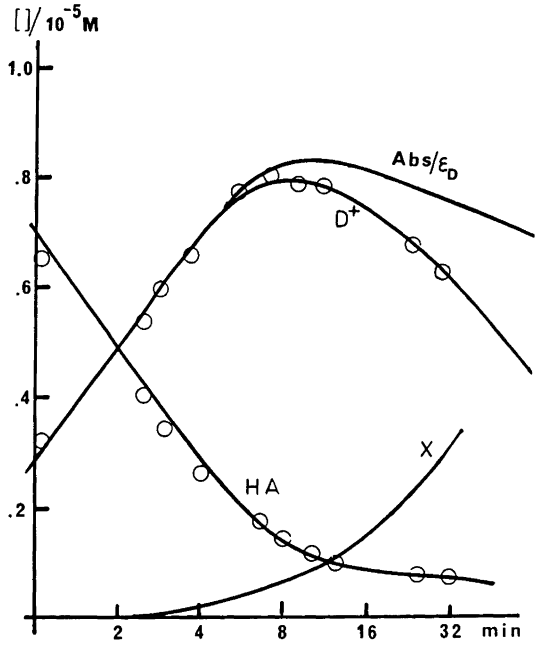


Fig. A2:3. Plot of [HA], $[D^+]$, Abs/ε_D and [X] versus t. The curves correspond to calculated values according to the text.

though we know that several absorbing compounds are formed in the reaction. The approximation will be the better the lower the contribution of X to the total absorption.

If we therefore limit the useful observations for the calculations to those from the start up to the maximum absorbance value, we can expect the approximation to be correct in the majority of cases.

The mathematical approximation introduced means that we have simplified the problem to a reaction of the type in eqn. (A2:2), for which we can measure *Abs* and derive eqn. (A2:3).

$$A \underset{k_{DA}}{\overset{k_{AD}}{\rightleftharpoons}} D \xrightarrow{k} X \qquad k_{DA} = R \cdot k_{AD} \qquad \text{(A2:2)}$$

$$Abs = [D]\varepsilon_D + [X]\varepsilon_X \qquad \text{(A2:3)}$$

The main reactions of D^+ are a second-order reaction to a thiosulfinate and, if a 4-OCH_3 group is present in the pyridine ring, also a first-order hydrolysis of this group. This first-order reaction is rather slow and is accompanied by only a slight change in absorbance. As a practical approximation we can therefore assume that X is formed by a second-order reaction from D^+. The constants k_{AD}, ε_D are obtained by iterations using the eqn. (A2:4) in our general program (Appendix 1). In these calculations it is necessary to introduce a measured or estimated value for R, and, in addition to k_{AD} and ε_D, we obtained the auxiliary constants k and ε_X which are of very limited interest.

$$A_0 = [HA]_0,\ D_0 = 0,\ Dt = [t(N) - t(N-1)]/100 \qquad \text{(A2:4)}$$

$$a_i = k_{AD}[HA]_i Dt,\ b_i = R \cdot k_{AD}[D^+]_i Dt,\ c_i = k[D^+]_i^2 \cdot Dt$$

$$[HA]_{i+1} = [HA]_i - a_i + b_i,\ [D^+]_{i+1} = [D^+]_i + a_i - b_i - c_i$$

$$[X]_{i+1} = [X]_i + c_i\ Abs_t = [D^+]_t\varepsilon_D + [X]_t\varepsilon_X$$

When k is small this method gives k_{AD} values which are identical with those obtained from a curve of type (a) in Fig. A2:2. In all cases where the spectrophotometric method was tested against the HPLC method, the resulting values for k_{AD} were the same.

The ε_D values obtained are remarkably constant for all compounds and can be used as an indication that the calculation is correct. The spectrophotometric method failed in only two cases, both for reactions in 0.001 M HCl and with pyridines of low pK_a. In all other cases, the fit of the experimental points to the theoretical curves was excellent, with a standard deviation of the same magnitude as the spectrophotometric reading error.

The rate constant k_{AD} can also be obtained by measuring the change in absorbance with time when omeprazole or an analogous compound is treated with Hβ in a dilute HCl solution. We thus have the reactions $HA \rightarrow D^+ \rightarrow HE\beta^+$ in which the first step is rate determining and the last step is rapid.

The absorbance changes from that of HA to that of $HE\beta^+$ and is usually a simple first-order reaction. The rate constant k_{AD}, is obtained from a set of (Abs, t) values using eqn. (A2:5) and our general method for calculation. In this

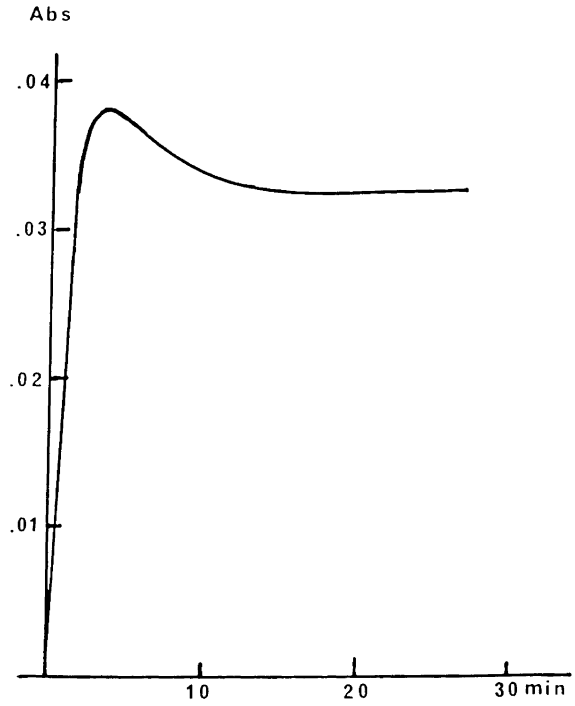


Fig. A2:4. Absorbance at 355 nm versus time for a solution of omeprazole (10^{-5} M) and 2-mercaptoethanol (10^{-4} M) in 0.01 M HCl.

way, we obtain k_{AD} as well as Abs_0 and Abs_∞. This method is free from the assumptions used in the previous method, but is hampered by the low ε value of HEβ$^+$. The method is usually free from complications as long as it is used for acidic solutions, since the complicating reactions, consisting of the formation of S and the pyridine analogue

$$Abs_t = Abs_0 + (Abs_\infty - Abs_0)\, e^{-k_{AD}t} \qquad \text{(A2:5)}$$

of HEβ$^+$, are very slow. However, complications occur if the speed of the reaction D$^+ \rightarrow$ HEβ$^+$ is strongly reduced by conducting the reaction at a pH value that gives a small rate constant for this reaction in combination with a low concentration of Hβ. If omeprazole (10^{-5} M) is treated with Hβ (10^{-4} M) in 0.01 M HCl, a maximum on the absorbance versus time curve (Fig. A2:4) is obtained before it reaches its asymptotic value for the absorbance of HEβ$^+$. In this case, the two steps in the reaction occur with comparable rates and some accumulation of D$^+$ with an ε value about four times that of HEβ$^+$ occurs. The reaction can be treated as a two-step reaction with the rate constants k_{AD} and ($k_{DE\beta} \cdot [H\beta]$) and both constants can in principle be calculated from a set of (Abs, t) values. Although the fit of the experimental points to the theoretical curve is excellent, this is not a good method for the measurements of these constants, since most of the kinetic information is present in the not very pronounced maximum.

A spectrometric method was also used for the measurement of the very rapid reaction D$^+$ + Hβ$\rightarrow$ HEβ$^+$. Even in this case the absorption at about 355 nm was followed, since HEβ$^+$ has a much lower absorptivity than D$^+$. This reaction is discussed in part IV.[1]

References

1. Brändström, A., Lindberg, P., Bergman, N.-Å., Tekenbergs-Hjelte, L. and Ohlson, K. *Acta Chem. Scand. 43* (1989) 577.
2. Brändström, A., Lindberg, P., Bergman, N.-Å., Alminger, T., Ankner, K., Junggren, U., Lamm, B., Nordberg, P., Erickson, M., Grundevik, I., Hagin, I., Hoffmann, K.-J., Johansson, S., Larsson, S., Löfberg, I., Ohlson, K., Persson, B., Skånberg, I. and Tekenbergs-Hjelte, L. *Acta Chem. Scand. 43* (1989) 536.
3. Senn-Bilfinger, J., Krüger, U., Sturm, E., Klemm, K., Kohl, B., Rainer, G., Blake, T. J., Darkin, D. W., Ife, R. J., Leack, C. A., Mitchell, R. C., Pepper, E. S., Salter, C. J., Viney, N. J., Huttner, G. and Zsolnai, L. *J. Org. Chem. 52* (1987) 4582.
4. Brändström, A., Bergman, N.-Å., Grundevik, I., Johansson, S., Tekenbergs-Hjelte, L. and Ohlson, K. *Acta Chem. Scand. 43* (1989) 569.
5. Brändström, A., Lindberg, P., Bergman, N.-Å., Grundevik, I., Tekenbergs-Hjelte, L. and Ohlson, K. *Acta Chem. Scand. 43* (1989) 595.
6. Davies, O. L. Ed., *Statistical Methods in Research and Production,* Oliver and Boyd, London and New York 1958, Chap. 8.
7. Gauss, C. F. *Theoria Motus Corporum Caelestium,* Hamburg 1809.
8. Regårdh, C.-G. *Farmakokinetik,* Apotekarsocieteten, S-111 81 Stockholm 1973.
9. Rodiguin, N. M. and Rodiguina, E. N. *Consecutive Chemical Reactions. Mathematical Analysis and Development,* van Nostrand, New Jersey 1964, Appendix 1.
10. Brändström, A., Strandlund, G. and Lagerström, P.-O. *Acta Chem. Scand., Ser. B 34* (1979) 467.

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