

# Chemical Reactions of Omeprazole and Omeprazole Analogues. V. The Reaction of *N*-Alkylated Derivatives of Omeprazole Analogues with 2-Mercaptoethanol

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Omeprazole, 5-methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinylmethylsulfanyl)-1*H*-benzimidazole, analogues substituted on the benzimidazole nitrogen, but lacking the methoxy group in the benzimidazole part, have been studied. In dilute HCl (in the absence of 2-mercaptoethanol, Hβ) the *N*-methylated analogue is much more stable than omeprazole (INN name). The results obtained in the presence of Hβ have been used to show that an acid-catalyzed attack of Hβ on the sulfenamide (D<sup>+</sup>) from omeprazole is the major reaction in the formation of the disulfide HEβ<sup>+</sup> in 0.1 M HCl and that the direct reaction to HEβ<sup>+</sup> from the sulfenic acid HC<sup>+</sup> is of minor importance. The interconversion of two diastereomeric *N*-alkylated compounds has also been studied.

It has been shown<sup>1</sup> that a sulfenamide (D<sup>+</sup>) plays an important role as a reactive intermediate in the reaction of omeprazole (HA) in the presence of 2-mercaptoethanol (Hβ). The reaction between the sulfenamide and 2-mercaptoethanol, giving rise to the disulfide HEβ<sup>+</sup>, also serves as a model reaction for the enzyme-inhibition reaction taking place in the parietal cell.<sup>2</sup> The formation of the sulfenamide has been postulated to take place via the sulfenic acid HC<sup>+</sup>.<sup>2</sup>

In the present part of the series the results from compounds substituted on the benzimidazole nitrogen are presented. The replacement of the hydrogen atom on one of the two nitrogen atoms of the benzimidazole ring with an alkyl group would be expected to prevent the transformation of the sulfenic acid MeC<sup>+</sup> into the corresponding sulfenamide. Such compounds could thus be used to gain information about the importance of the intermediate HC<sup>+</sup>.

*Description of the compounds studied.* Methylation of omeprazole gives a mixture of two positional isomers (the OCH<sub>3</sub> group in the 5- or 6-position). This mixture is somewhat difficult to separate, and in order to avoid this difficulty compound MeA, in which the OCH<sub>3</sub> group is lacking, was prepared. MeA is a much more stable a compound than omeprazole, as seen (below) in the very slow reaction of MeA in the absence of Hβ in dilute HCl. However, this stability is not due to the slow conversion of MeA to MeC<sup>+</sup>, since, if β-mercaptoethanol is added to a solution of MeA

in dilute HCl, a rapid transformation into the corresponding MeEβ<sup>+</sup> occurs. The rate constant for this reaction increases with an increase in [Hβ] and [H<sub>3</sub>O<sup>+</sup>], but in a rather complicated way.

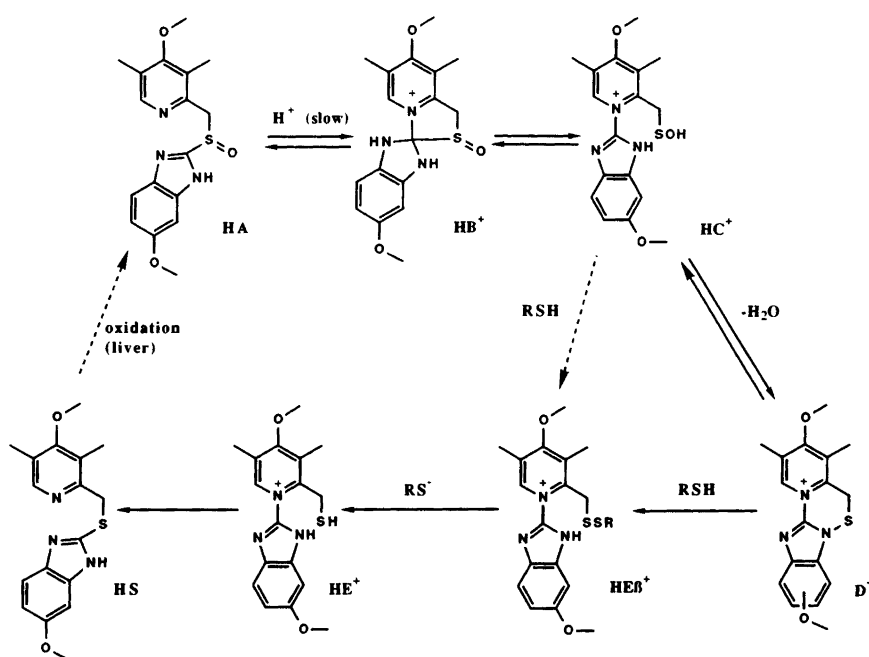
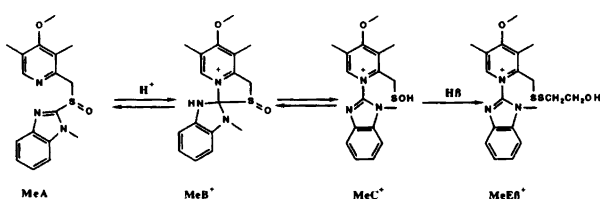
The reason for the relatively high stability of MeA is therefore that the reverse reaction MeC<sup>+</sup> → MeA is faster than the forward reaction MeA → MeC<sup>+</sup>. At equilibrium the compound is therefore present mainly as MeA. This is in contrast with the behaviour of omeprazole where the equilibrium in an acidic solution is shifted in the direction of D<sup>+</sup>, which is present to about 80 % at equilibrium.

*Description of the reaction system.* For MeA we have to consider the reactions in Scheme 2. By analogy with the reactions of omeprazole with Hβ in acidic solution,<sup>1</sup> this reaction can be treated as MeA + H<sup>+</sup>  $\xrightarrow{H\beta}$  MeEβ<sup>+</sup>. Using the steady-state approximation on MeC<sup>+</sup>, we obtain eqn. (1).

$$-\frac{d[\text{MeA}]_{\text{tot}}}{dt} = \frac{d[\text{MeE}\beta^+]}{dt} = \frac{k_{\text{MeAC}}[\text{H}\beta][\text{MeA}]_{\text{tot}}}{\frac{k_{\text{MeCA}}}{k_{\text{MeCE}\beta}} + [\text{H}\beta]} = k_{\text{obs}} [\text{MeA}]_{\text{tot}} \quad (1)$$

$$k_{\text{obs}} = \frac{k_{\text{MeAC}} [\text{H}\beta]}{\frac{k_{\text{MeCA}}}{k_{\text{MeCE}\beta}} + [\text{H}\beta]}$$

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Scheme 1. RSH = (a) HOCH<sub>2</sub>CH<sub>2</sub>SH (Hβ); (b) Enz<sub>3</sub>-SH.

Scheme 2.

If Hβ is used in excess over MeA and can be considered as constant during the experiment, the reaction can be treated as first-order in MeA, and  $k_{\text{obs}}$  is readily obtained as the pseudo first-order rate constant valid at this concentration of Hβ and HCl. If the reaction is run at a fixed concentration of HCl, but with different concentrations of Hβ, the constant  $k_{\text{MeAC}}$  and the ratio  $k_{\text{MeCA}}/k_{\text{MeCEβ}}$  can be obtained by non-linear regression of  $k_{\text{obs}}$  against [Hβ]. The constants thus obtained are valid at that particular HCl concentration. It should be noted that in the case of omeprazole,  $k_{\text{obs}}$  is independent of [Hβ].<sup>3</sup> In practice,  $k_{\text{MeAC}}$  and  $k_{\text{MeCA}}/k_{\text{MeCEβ}}$  are calculated directly from the total set of  $t$ , [Hβ], [MeA] and [MeEβ<sup>+</sup>] values using our general least-squares program (Appendix 1 in Ref. 1) with no intermediate calculation of  $k_{\text{obs}}$ .

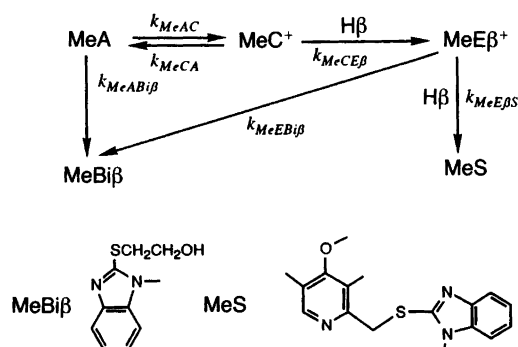
At pH values above 3 the reaction scheme above had to be revised according to Scheme 3. MeBiβ could be obtained either directly from MeA or from the disulfide MeEβ<sup>+</sup>. The compounds MeBiβ and MeS could not be detected in runs at pH 1 and 2.

MeBiβ is more readily observed in the presence of MeEβ<sup>+</sup> than HBiβ in the presence of HEβ<sup>+</sup>. The reason for this is that the formation of HEβ<sup>+</sup> (MeEβ<sup>+</sup>) always com-

petes with the formation of HBiβ (MeBiβ). It is reasonable to assume that the formation of both HBiβ and MeBiβ proceeds with about the same rate constant. From our experiments we have seen that the rate of formation of HEβ<sup>+</sup> at pH 3–4 is much higher than that of MeEβ<sup>+</sup>. This means that the ratio [HBiβ]/[HEβ<sup>+</sup>] will be much lower than that of [MeBiβ]/[MeEβ<sup>+</sup>] and HBiβ is much more difficult to detect than MeBiβ.

From the total set of  $t$ , [Hβ], [MeA], [MeEβ<sup>+</sup>], [MeBiβ] and [MeS] values, the rate constants given in Scheme 3 were determined using our general least-squares program (Appendix 1 in Ref. 1). The results obtained at a number of HCl concentrations are given in Table 1.

*pH dependence of the rate constants.* To understand the variations of  $k_{\text{MeAC}}$  and  $k_{\text{MeCA}}/k_{\text{MeCEβ}}$  with [HCl], we have to consider the protolytic behaviour of MeA and MeC<sup>+</sup> in acidic solutions. MeA is a weak base which in dilute acid



Scheme 3.

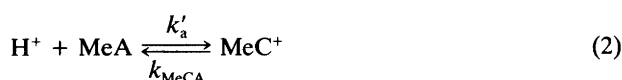
Table 1. Reaction of MeA with 2-mercaptoethanol.<sup>a</sup>  $T = 37^\circ\text{C}$ . Ionic strength 0.1 M.

[HCl]/M	$k_{\text{MeAC}}/10^{-2} \text{ s}^{-1}$	$(k_{\text{MeCA}}/k_{\text{MeCE}\beta})/10^{-4} \text{ M}$	$k_{\text{MeAB}\beta}/10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	$k_{\text{MeEB}\beta}/10^{-5} \text{ s}^{-1}$
0.1	9.80(13)	2.8(1)		
0.01	3.52(12)	8.2(4)		
0.001	1.12(2)	30.0(1)	9.3(7)	2.4(2)
0.0003	0.36(1)	47(2)	9.6(2)	1.6(1)
0.0012	2.74(18)	62(4)	6(2)	2.7(4)
0.00018	0.33(30)	68(8)	9.7(7)	2.0(8)

<sup>a</sup>At least 3 concentrations of H $\beta$  were used for each [HCl]. The highest and lowest [H $\beta$ ] usually differed by a factor of 100.

protonates to the acid HMeA<sup>+</sup> ( $pK_a = 3.65$ ). At very low pH values this species can in turn be protonated on the benzimidazole ring system to give the form H<sub>2</sub>MeA<sup>2+</sup>. Using the methods outlined in Ref. 4 we can estimate the  $pK_a$  value for this equilibrium to be about 0.2.

MeC<sup>+</sup> contains a sulfenic acid group with the estimated  $pK_a$  of ca. 9.<sup>4</sup> The rate constant  $k_{\text{MeAC}}$  is defined with the total concentration  $[\text{MeA}]_{\text{tot}}$  as the standard. If, by analogy with the reactions with HA, we assume that the reaction  $\text{MeA} \rightarrow \text{MeC}^+$  is an acid-catalyzed reaction, eqn. (2) is valid. We can write eqn. (3) where  $X_{\text{MeA}}$  is the fraction of  $[\text{MeA}]_{\text{tot}}$  present as the uncharged form MeA. With the aid



of eqn. (3),  $k'_a$  is readily calculated from  $k_{\text{MeAC}}$ , as long as we can neglect the uncatalyzed reaction  $\text{MeA} \rightarrow \text{MeC}^+$ . This has been done in Table 2.

$$k_{\text{MeAC}}[\text{MeA}]_{\text{tot}} = k'_a[\text{MeA}][\text{H}_3\text{O}^+] = k'_a[\text{MeA}]_{\text{tot}}[\text{H}_3\text{O}^+]X_{\text{MeA}} \quad (3)$$

From the first 4 entries, which contain no buffer compounds (except HCl), we can see a fifteen-fold increase in  $k'_a$  with a 333-fold increase in  $[\text{H}_3\text{O}^+]$ . By analogy with the case for the reaction  $\text{HA} \rightarrow \text{HC}^+$ ,<sup>1</sup> this is interpreted as the existence of an acid-catalyzed conversion  $\text{MeB}^+ \rightarrow \text{MeC}^+$  in addition to an uncatalyzed conversion. Using the procedure outlined in Ref. 1, we can calculate the mechanistic constants  $\log[k_{\text{MeAB}}(2)/\text{M}^{-1} \text{ s}^{-1}] = 2.85 \pm 0.03$ ,  $k_{\text{MeBC}}(2)/$

$k_{\text{MeBC}}(1) = 428 \pm 139$ ,  $k_{\text{MeBA}}/k_{\text{MeBC}}(1) = 18 \pm 4$ . The ratio  $k_{\text{MeBC}}(2)/k_{\text{MeBC}}(1)$  is substantially higher than that of  $k_{\text{BC}}(2)/k_{\text{BC}}(1) \approx 20$ . This means that the acid-catalyzed reaction for the conversion  $\text{MeB}^+ \rightarrow \text{MeC}^+$  is detectable at lower acid concentrations than is that for the conversion  $\text{HB}^+ \rightarrow \text{HC}^+$ . Consequently the last two entries in Table 2 have somewhat higher  $k'_a$  values than expected from their HCl concentrations. These solutions contain buffer acids in addition to HCl and we have interpreted the difference as a general acid catalysis by the buffer acids on the step  $\text{MeB}^+ \rightarrow \text{MeC}^+$ , but have not studied this in detail. The position of the equilibrium [eqn. (2)] should be independent of pH and buffer acids. We thus obtain eqn. (4). The subscript e in

$$k'_a/k_{\text{MeCA}} = [\text{MeC}^+]_e/([\text{H}_3\text{O}^+][\text{MeA}]_e) = [\text{MeC}^+]_e/([\text{HMeA}^+]_e K_{\text{HMeA}^+}) \quad (4)$$

eqn. (4) indicates equilibrium. Since we have both  $k'_a$  and  $k_{\text{MeCE}\beta}$  we can readily calculate the ratio  $R$ , which together with eqn. (4) gives eqn. (5). Since the fraction  $[\text{MeC}^+]_e/$

$$R = k_{\text{MeCE}\beta}(k'_a/k_{\text{MeCA}}) = k_{\text{MeCE}\beta}[\text{MeC}^+]_e/([\text{HMeA}^+]_e K_{\text{HMeA}^+}) \quad (5)$$

$([\text{HMeA}^+]_e K_{\text{HMeA}^+})$  is independent of pH and the presence of buffer acids, any variation in  $R$  is due to a variation in  $k_{\text{MeCE}\beta}$ . From Table 2 we can see that  $R$  decreases with decreasing  $[\text{H}_3\text{O}^+]$ , and that the ratio  $R/[\text{H}_3\text{O}^+]$  is constant for all solutions where exact measurements are possible. In 0.1 M HCl, the reaction is so rapid that accurate measure-

 Table 2. Acid-catalyzed reaction of MeA with H $\beta$ . The uncatalyzed reaction has been neglected.

$[\text{H}_3\text{O}^+]/\text{M}$	$X_{\text{MeA}}$	$k'_a/\text{s}^{-1}$	$R/(10^4 \text{ M}^{-2} \text{ s}^{-1})^a$	$(R/[\text{H}_3\text{O}^+])/(10^7 \text{ M}^{-1} \text{ s}^{-1})^a$
0.1	0.00193	508(7)	180(2)	1.80(2)
0.01	0.0216	163(6)	19.9(7)	1.99(7)
0.001	0.183	61(1)	2.0(1)	2.0(1)
0.0003	0.427	28(1)	0.6(2)	2.0(1)
0.0012	0.157	145(9)	2.4(2)	1.97(16)
0.00018	0.554	33(3)	0.49(5)	2.70(3)

<sup>a</sup> $R$  is the ratio defined by eqn. (5).

ments are very difficult, and in the last solution contributions from an uncatalyzed reaction  $\text{MeA} \rightarrow \text{MeB}^+$  cannot be excluded. Even if the two extremes are included, a more than 500-fold decrease in  $[\text{H}_3\text{O}^+]$  results in only a 1.5-fold increase in  $R/[\text{H}_3\text{O}^+]$ . The reaction  $\text{MeC}^+ \rightarrow \text{MeE}\beta^+$  is thus acid catalyzed in the pH region 1 to at least 4. This is in contrast with the reaction  $\text{D}^+ \rightarrow \text{HE}\beta^+$ , which is acid catalyzed at pH values less than 2 and base catalyzed at higher pH values.<sup>5</sup> This difference probably reflects the fact that the ylide form  $\text{E}\beta^\pm$  (with a  $\text{p}K_a \approx 6$ ) is a much better leaving group in the attack of  $\text{D}^+$  with  $\beta^-$  than is that of the  $\text{OH}^-$  group in the attack of  $\text{MeC}^+$  by  $\beta^-$ .

*Evidence against  $\text{HC}^+$  as an intermediate in the reaction  $\text{D}^+ + \text{H}\beta \rightarrow \text{HE}\beta^+$ .* Since the reaction  $\text{MeC}^+ + \text{H}\beta \rightarrow \text{MeE}\beta^+$  is acid catalyzed, the question arises as to whether the reaction  $\text{D}^+ + \text{H}\beta \rightarrow \text{HE}\beta^+$  goes via  $\text{HC}^+$  in the region where it is acid catalyzed. Indications against this can be found in the following way.

For the reaction  $\text{D}^+ \rightarrow \text{HA}$  we have found<sup>6</sup> the rate constant  $k_{\text{DA}} = 1.34 \times 10^{-3} \text{ s}^{-1}$  in 0.1 M HCl. The reactions  $\text{D}^+ \rightleftharpoons \text{HC}^+$  are very rapid compared with the reactions  $\text{HC}^+ \rightleftharpoons \text{HA}$ , and we thus have a fully developed equilibrium  $\text{D}^+ \rightleftharpoons \text{HC}^+$  throughout the reaction  $\text{D}^+ \rightarrow \text{HA}$ .

In Ref. 1 we defined  $k_{\text{DA}}$  according to eqn. (6). From the numerical value for  $k_{\text{DA}}$  we thus obtain eqn. (7). For the

$$k_{\text{DA}} = \frac{k_{\text{CA}} k_{\text{DC}}}{k_{\text{CA}} + k_{\text{CD}}} \approx \frac{k_{\text{CA}} k_{\text{DC}}}{k_{\text{CD}}}; k_{\text{CA}} \ll k_{\text{CD}} \quad (6)$$

$$k_{\text{CA}} = 1.34 \times 10^{-3} \text{ s}^{-1} k_{\text{CD}}/k_{\text{DC}} \quad (7)$$

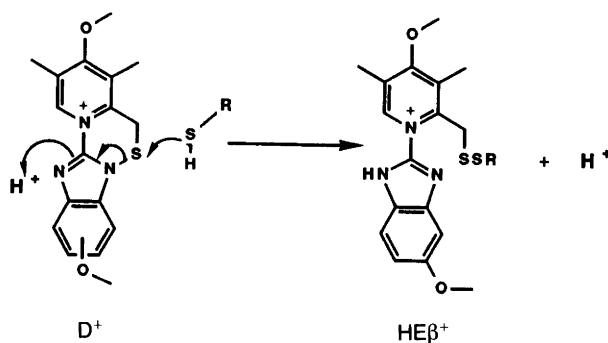
reaction  $\text{MeA} + \text{H}\beta \rightarrow \text{MeE}\beta^+$  we found  $k_{\text{MeCE}\beta}/k_{\text{MeCA}} = 3.5 \times 10^3 \text{ M}^{-1}$  in 0.1 M HCl (Table 1). We can expect that the removal of the  $\text{NCH}_3$  group does not change this ratio very much. We can thus expect that  $k_{\text{CE}\beta}/k_{\text{CA}} \approx 3.5 \times 10^3 \text{ M}^{-1}$ . If the value for  $k_{\text{CA}}$  in eqn. (7) is introduced, we obtain eqn. (8). The left-hand side equals  $k_{\text{DE}\beta}$  [compare

$$k_{\text{CE}\beta} k_{\text{DC}}/k_{\text{CD}} = 4.8 \text{ M}^{-1} \text{ s}^{-1} \quad (8)$$

with eqns. (6) and (7)] for a reaction of  $\text{D}^+ + \text{H}\beta$  proceeding via  $\text{HC}^+$  (where  $k_{\text{CD}} \gg k_{\text{DE}\beta}[\text{H}\beta]$ ). The rate constant measured in 0.1 M HCl ( $\mu = 0.1 \text{ M}$ ) is, however,  $360 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>7</sup> The reaction via  $\text{HC}^+$  can thus only explain about 1% of the total rate observed. This means that an acid-catalyzed attack of  $\text{H}\beta$  on  $\text{D}^+$  is the dominant mechanism for the formation of  $\text{HE}\beta^+$  in 0.1 M HCl. The mechanism for this reaction is given in Scheme 4.

The conclusion that  $\text{HE}\beta^+$  is formed mainly from  $\text{D}^+$  in 0.1 M HCl does not mean that  $\text{D}^+$  is much more reactive than  $\text{HC}^+$ . The most likely explanation is that both reactions have about the same rate constant, but that  $\text{D}^+$  is present in a much higher concentration than  $\text{HC}^+$ .

The deduction above is based on the assumption  $k_{\text{CE}\beta}/k_{\text{CA}} \approx k_{\text{MeCE}\beta}/k_{\text{MeCA}}$ . The conclusions drawn are correct even if  $k_{\text{CE}\beta}/k_{\text{CA}} < k_{\text{MeCE}\beta}/k_{\text{MeCA}}$ . The constants  $k_{\text{CE}\beta}$  and  $k_{\text{MeCE}\beta}$



Scheme 4.

should differ very little since the reaction occurs at atoms far away from the  $\text{NH}$  or  $\text{NCH}_3$  group. An indication in this direction is found in the reaction of  $\text{HE}\beta^+$  with  $\beta^-$  in which the effect of substituents in the benzimidazole ring on the rate constant is small, if any.<sup>5</sup> The assumption is thus correct if we can demonstrate that  $k_{\text{CA}} \geq k_{\text{MeCA}}$ . The rate-limiting step in the reaction  $\text{HC}^+ \rightarrow \text{A}$  is the step  $\text{HC}^+ \rightarrow \text{B}$ .<sup>1</sup> This is an attack on the electron-deficient 2-carbon atom of the benzimidazole ring by the electron pair of the  $\text{S}$  atom. The expected effect of a  $\text{CH}_3$  group on the  $\text{N}$  atom is to reduce the rate of this reaction both for steric reasons and for its slight electron-releasing effect. We thus have good reason to believe that  $k_{\text{CA}} \geq k_{\text{MeCA}}$ . This is in good agreement with the finding that  $\log k_{\text{AB}}(2) = 3.48$  is higher than  $\log k_{\text{MeAB}}(2) = 2.85$ . Both reactions are attacks on the 2-carbon atom of the benzimidazole ring.

*Reactions in the absence of  $\text{H}\beta$ .* In the absence of  $\text{H}\beta$  there is a slow degradation of  $\text{MeA}$  in dilute HCl. This is believed to be due to a conversion of  $\text{MeC}^+$  into the products  $\text{Me}_2\text{F}^+$  and  $\text{Me}_2\text{CE}^{2+}$  by analogy with the conversions of  $\text{D}^+$  into  $\text{H}_2\text{F}^+$  and  $\text{H}_2\text{CE}^{2+}$ .<sup>6</sup> Kinetically the reaction is of the type given in Scheme 5. The reaction  $\text{MeC}^+ \rightarrow \text{Me}_2\text{F}^+$  is a first-order reaction, whereas  $\text{MeC}^+ \rightarrow \text{Me}_2\text{CE}^{2+}$  is second order in  $\text{MeC}^+$ . Since  $[\text{H}_3\text{O}^+]$  is constant in a run, the reaction  $\text{MeA} \rightleftharpoons \text{MeC}^+$  can be treated as a pseudo-first-order reaction in  $\text{MeA}$  with the rate given in eqn. (9).

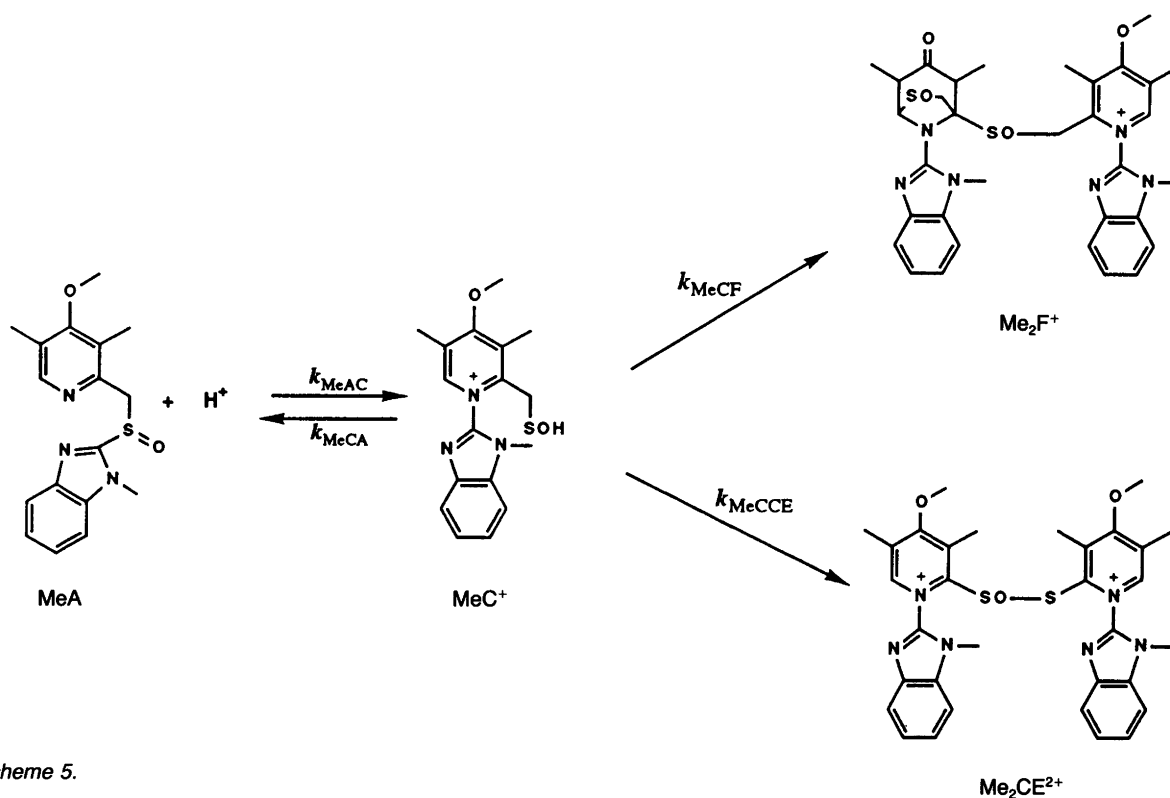
$$d[\text{MeA}]_{\text{tot}}/dt = -k_{\text{MeAC}}[\text{MeA}]_{\text{tot}} + k_{\text{MeCA}}[\text{MeC}^+] \quad (9)$$

The reaction  $\text{MeA} \rightleftharpoons \text{MeC}^+$ , is very fast compared with the reactions  $\text{MeC}^+ \rightarrow \text{Me}_2\text{F}^+$  and  $\text{MeC}^+ \rightarrow \text{Me}_2\text{CE}^{2+}$ , and has been followed in 0.1 and 0.01 M HCl where  $[\text{HMeA}^+] \approx [\text{MeA}]_{\text{tot}}$ .

From the equilibrium  $\text{MeA} \rightleftharpoons \text{MeC}^+$ , eqn. (10) can be

$$[\text{MeC}^+] = [\text{HMeA}^+] k_{\text{MeAC}}/k_{\text{MeCA}} \quad (10)$$

deduced. The steady-state approximation on  $\text{MeC}^+$  gives eqn. (11). If eqns. (10) and (11) are introduced into eqn. (9) we obtain eqn. (12).



Scheme 5.

$$d\text{MeC}^+/\text{dt} = k_{\text{MeAC}}[\text{MeA}]_{\text{tot}} - k_{\text{MeCA}}[\text{MeC}^+] - k_{\text{MeCF}}[\text{MeC}^+] - 2k_{\text{MeCCE}}[\text{MeC}^+]^2 = 0 \quad (11)$$

$$d[\text{MeA}]_{\text{tot}}/\text{dt} = -k_{\text{MeCF}}(k_{\text{MeAC}}/k_{\text{MeCA}})[\text{MeA}]_{\text{tot}} - 2k_{\text{MeCCE}}(k_{\text{MeAC}}/k_{\text{MeCA}})^2[\text{MeA}]_{\text{tot}}^2 \quad (12)$$

This is readily integrated to eqn. (13) in which  $k_{\text{MeAF}} = k_{\text{MeCF}}(k_{\text{MeAC}}/k_{\text{MeCA}})$  and  $k_{\text{MeACE}} = k_{\text{MeCCE}}(k_{\text{MeAC}}/k_{\text{MeCA}})^2$ .

$$[\text{MeA}]_{\text{tot}} = \frac{[\text{MeA}]_{\text{tot}}^0 k_{\text{MeAF}} e^{-k_{\text{MeAF}}t}}{k_{\text{MeAF}} + 2k_{\text{MeACE}} [\text{MeA}]_{\text{tot}}^0 (1 - e^{-k_{\text{MeAF}}t})} \quad (13)$$

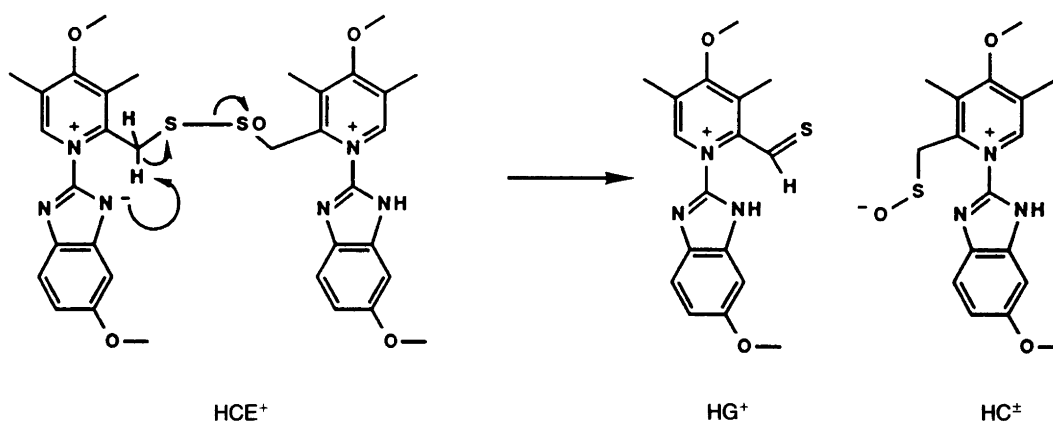
From a set of  $([\text{MeA}], t)$  values at two different starting concentrations ( $10^{-4}$  and  $10^{-5}$  M), the values  $k_{\text{MeAF}}$  and  $k_{\text{MeACE}}$  can be calculated by our least-squares method. The results are given in Table 3.

The first-order reaction should involve the 4-methoxy group in the pyridine ring giving a pyridone as the primary product, which can then undergo the same type of reactions as in the first-order reactions of  $\text{D}^+$  to  $\text{H}_2\text{F}^+$ .<sup>1,6</sup> The rate constant for the reaction  $\text{MeA} \rightarrow \text{Me}_2\text{F}^+$  is of the same magnitude as that of the reaction  $\text{D}^+ \rightarrow \text{H}_2\text{F}^+$  and that of  $\text{HE}\beta^+$  to the corresponding pyridone compound, which is some indication that all of the reactions are of a similar type. Since the structure-activity relationships of these re-

 Table 3. Reaction of MeA in 0.01 M HCl and 0.09 M NaCl in the absence of 2-mercaptoethanol.  $T = 37^\circ\text{C}$ .

$t/\text{min}$	$[\text{MeA}]/10^{-5}$ M		$t/\text{min}$	$[\text{MeA}]/10^{-5}$ M	
	Found	Calc. <sup>a</sup>		Found	Calc. <sup>a</sup>
0		9.89	0		0.989
10	7.50	7.47	31	0.893	0.886
20	6.00	6.00	60	0.793	0.807
30	5.06	5.01	120	0.680	0.678
45	3.98	4.01	210	0.543	0.543
75	2.87	2.85	300	0.448	0.449
113	2.00	2.08	392	0.383	0.379

<sup>a</sup>Calculated using the assumption  $-d[\text{A}]/\text{dt} = (k_{\text{MeAF}} + k_{\text{MeACE}}[\text{MeA}])[\text{MeA}]$ , where  $k_{\text{MeAF}} = 8.8(3) \times 10^{-6} \text{ s}^{-1}$  and  $k_{\text{MeACE}} = 5.34(4) \text{ M}^{-1} \text{ s}^{-1}$  were determined according to Appendix 1 in Ref. 1.



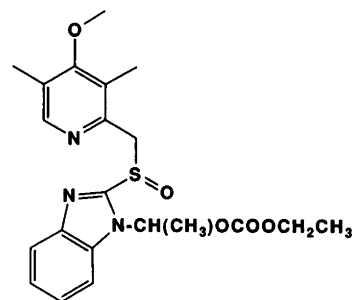
Scheme 6.

actions with a  $\text{OCH}_3$  group with sterically restricted resonance are difficult to predict, a more exact analysis along this line was not undertaken. The reaction  $\text{MeA} \rightarrow \text{Me}_2\text{CE}^{2+}$  has about the same rate constant as the reaction  $\text{D}^+ \rightarrow \text{HCE}^{2+}$ ,<sup>6</sup> indicating that they are of similar type. However, the  $\text{CH}_3$  group on the nitrogen atom in  $\text{Me}_2\text{CE}^{2+}$  prevents a rapid conversion analogous to that given for  $\text{H}_2\text{CE}^{2+} \rightarrow \text{HG}^+ + \text{HC}^+$  (Scheme 6).  $\text{Me}_2\text{CE}^{2+}$  can therefore be expected to be much more stable than  $\text{H}_2\text{CE}^{2+}$ .

The reaction  $\text{MeA} \rightarrow \text{Me}_2\text{CE}^{2+}$  was studied by both UV and NMR spectroscopy. When 10  $\mu\text{l}$  of concentrated aqueous  $\text{DCl}$  were added to 1.441 mg  $\text{MeA}$  in 0.500 ml  $\text{CD}_3\text{OD}$  and the reaction followed at 32 °C at 530 nm, the curve in Fig. 1 was obtained. This demonstrates that a fairly stable compound is obtained in about 15 min. An examination of the same solution by NMR spectroscopy at different times and temperatures indicates that at -40 °C the protonated form  $\text{HMeA}^+$  is obtained, which is stable for some hours at this temperature. When the temperature is increased to 0 °C the sulfenic acid methyl ester is rapidly formed. The reaction is essentially complete after 25 min at 0 °C. After about 1 h at room temperature the thiosulfinate  $\text{Me}_2\text{CE}^{2+}$  is the main product. The main side reactions are the methanolysis of the methoxy group to give methanol, and ex-

change of the acidic  $\text{CH}_2$  protons for deuterium. The NMR spectra obtained during the reactions are complicated by a number of dynamic processes that take place, due to restricted rotations of different groups. The NMR interpretations of the reaction  $\text{MeA} \rightarrow \text{Me}_2\text{CE}^{2+}$  will be published in a separate article.

*An N-alkylated analogue with a chiral alkyl group.* If the  $\text{NCH}_3$  group in  $\text{MeA}$  is replaced by an alkyl group containing an asymmetric carbon atom two diastereoisomeric compounds ( $\text{RA}$ ,  $\text{RA}'$ ) are obtained since the  $\text{SO}$  group is also a chiral center. With chromatography we were able to obtain two fractions: one contained 85 % of one isomer  $\text{RA}$  and 15 % of the other isomer  $\text{RA}'$ . The second fraction



contained 95 % of  $\text{RA}'$  and 5 % of  $\text{RA}$ . From measurements on these fractions,  $\text{p}K_a$  values could be calculated for the pure compounds (see the Experimental). In this way we obtained  $\text{p}K_a(\text{RA}) = 3.52$  and  $\text{p}K_a(\text{RA}') = 3.86$ .

In acidic solution these two forms are interconvertible. The stereochemical change must occur at the  $\text{SO}$  group, and, from what is known about sulfenic acids,<sup>8</sup> it probably occurs at the  $\text{SO}$  group of the sulfenic acid  $\text{RC}^+$ . We thus have the reaction  $\text{RA} \rightleftharpoons \text{RC}^+ \rightleftharpoons \text{RA}'$ . Since the concentrations of  $\text{RA}$  and  $\text{RA}'$  can be measured by HPLC, the kinetics of the reaction can be followed. If 2-mercaptoethanol is added to the solution, we can still see an interconversion but also a decrease in the total concentration  $[\text{RA}] + [\text{RA}']$ , probably due to the formation of the correspond-

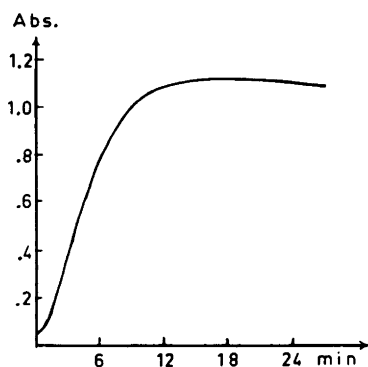
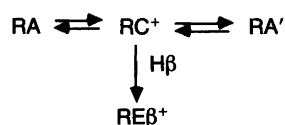


Fig. 1. Absorbance at 530 nm of a solution containing 1.441 mg of  $\text{MeA}$ , 0.5 ml  $\text{CD}_3\text{OD}$  and 10  $\mu\text{l}$  of concentrated aqueous  $\text{DCl}$  in a 1 cm cell as a function of time.  $T = 32^\circ\text{C}$ .

ing  $\text{RE}\beta^+$  compound. In this case we have the reactions shown in Scheme 7.



Scheme 7.

Using the steady-state approximation on  $\text{RC}^+$  we obtain the differential eqns. (14).

$$d[\text{RA}]/dt = - (a + b)[\text{RA}] + a'[\text{RA}'] \quad (14a)$$

$$d[\text{RA}']/dt = - (a' + b')[\text{RA}'] + a[\text{RA}] \quad (14b)$$

in which we have

$$a = k_{\text{RAC}}/N, \quad a' = k_{\text{RA}'\text{C}}(k_{\text{RCA}}/k_{\text{RCA}'})/N, \quad b = k_{\text{RAC}}(k_{\text{RCE}\beta}/[\text{H}\beta])/k_{\text{RCA}}/N$$

$$b' = k_{\text{RA}'\text{C}}(k_{\text{RCE}\beta}/[\text{H}\beta])/k_{\text{RCA}'}/N, \quad N = k_{\text{RCA}}/k_{\text{RCA}'} + 1 + k_{\text{RCE}\beta}/k_{\text{RCA}}$$

This set of differential equations can be integrated to eqns. (15) and (16) in which  $\lambda_1$  and  $\lambda_2$  are given by eqns. (17) and (18).

$$[\text{RA}] = \{[(a' + b' - \lambda_1)[\text{RA}]_0 + a'[\text{RA}']_0]e^{-\lambda_1 t} - [(a' + b' - \lambda_2)[\text{RA}]_0 + a'[\text{RA}']_0]e^{-\lambda_2 t}\}/(\lambda_2 - \lambda_1) \quad (15)$$

$$[\text{RA}'] = \{[(a + b - \lambda_1)[\text{RA}']_0 + a[\text{RA}]_0]e^{-\lambda_1 t} - [(a + b - \lambda_2)[\text{RA}']_0 + a[\text{RA}]_0]e^{-\lambda_2 t}\}/(\lambda_2 - \lambda_1) \quad (16)$$

$$\lambda_1 = \{(a+a'+b+b') + [(a+a'+b+b')^2 - 4(a+b)(a'+b') + 4aa']^{1/2}\}/2 \quad (17)$$

$$\lambda_2 = \{(a+a'+b+b') - [(a+a'+b+b')^2 - 4(a+b)(a'+b') + 4aa']^{1/2}\}/2 \quad (18)$$

From a set of ( $t$ ,  $[\text{RA}]$ ,  $[\text{RA}']$ ) values the constants  $k_{\text{RAC}}$ ,  $k_{\text{RA}'\text{C}}$ ,  $[\text{RA}]_0$  and  $[\text{RA}']_0$  can be calculated together with the ratios  $k_{\text{RCE}\beta}/k_{\text{RCA}'}$ , by the general method given in Appendix 1, Ref. 1. The experimental material did not allow the simultaneous calculation of both  $k_{\text{RAC}}$  and the ratio  $k_{\text{RCA}}/k_{\text{RCA}'}$ . A calculation based on the assumption that  $k_{\text{RAC}}/k_{\text{RCA}'}$  was the same with and without  $\text{H}\beta$  gave  $k_{\text{RCA}}/k_{\text{RCA}'}$  values close to 1. This value was therefore used in our final calculations which are given in Table 4. The following should be noted. All runs except two were performed starting with  $\text{RA}'$ . The agreement between the results from comparable runs starting from  $\text{RA}$  and  $\text{RA}'$  is gratifying.

In agreement with previous findings,  $k_{\text{RAC}}$  is independent of  $[\text{H}\beta]$  and depends only on  $[\text{H}^+]$ . The ratio  $k_{\text{RAC}}/k_{\text{RA}'\text{C}}$  should be equal to the ratio  $X_{\text{RA}}/X_{\text{RA}'}$ , i.e., the ratio between the concentration of the unprotonated form of  $\text{RA}$  and  $\text{RA}'$ , respectively, if the difference in  $\text{p}K_a$  values were the only factor of importance for the difference in rate constants. As seen in Table 4 the ratios ( $k_{\text{RAC}}/k_{\text{RA}'\text{C}}$ ) are only slightly higher than half the ratios  $X_{\text{RA}}/X_{\text{RA}'}$ , indicating that steric factors are also of some importance for the differences in rate. The rate constant  $k_{\text{RCA}'}$  should be independent of  $[\text{H}\beta]$ , which means that the ratio  $k_{\text{RCE}\beta}/k_{\text{RCA}'}$  should also be independent of  $[\text{H}\beta]$ .

Table 4. Epimerization of  $\text{RA}$ ,  $\text{RA}'$  in the absence and in the presence of 2-mercaptoethanol.  $T = 37^\circ\text{C}$ , ionic strength 0.05 M. The calculated values in the table are obtained using the assumption  $k_{\text{RCA}} = k_{\text{RCA}'}$ .

$[\text{H}^+]/\text{M}$	$[\text{H}\beta]/\text{M}$	$k_{\text{RAC}}/k_{\text{RA}'\text{C}}$	$k_{\text{RAC}}/10^{-3} \text{ s}^{-1}$	$(k_{\text{RCE}\beta}/k_{\text{RCA}'})/10^2 \text{ M}^{-1}$	$X_{\text{RA}}^a$	$X_{\text{RA}}/X_{\text{RA}'}$
0.01	0 <sup>b</sup>	1.28(2)	2.83(7)		0.03	2.13
	0	1.27(5)	2.8(2)			
	0.001 <sup>b</sup>	1.6(1)	4.5(8)	7(1)		
	0.001	1.7(1)	3.9(2)	10(1)		
	0.003	2.1(4)	7(2)	5(1)		
0.001	0	1.16(2)	1.9(1)		0.23	1.90
	0.001	1.24(4)	2.1(2)	1.2(1)		
$10^{-4.13}$	0	0.70(3)	0.68(5)		0.79	1.25
	0.001	0.76(1)	0.77(3)	0.42(2)		
	0.01	0.67(2)	0.67(2)	0.36(2)		
$10^{-5.14}$	0	0.55(4)	0.19(2)		0.98	1.03
	0.01	0.49(3)	0.23(2)	1.34(7)		
$10^{-7.14}$	0	0.55(7)	0.09(2)		1.00	1.00
	0.01	0.54(6)	0.12(2)	2.8(4)		

<sup>a</sup> $\text{p}K_a(\text{RA}) = 3.53$ ,  $\text{p}K_a(\text{RA}') = 3.86$ . For  $\text{RA}$  and  $\text{RA}'$  see the text. <sup>b</sup>Run started with  $\text{RA}$ .

Table 5. Determination of  $pK_a$  values for the two diastereomeric forms RA and RA'.  $T = 37^\circ\text{C}$ , ionic strength 0.1 M.

$A_0/M$	$A_0'/M$	$S_0/M$	$\text{pH}_0$	$\text{pH}_1$	$[\text{H}_3\text{O}^+]/M$	$([\text{HRA}^+] + [\text{HRA}'^+])/M$	$Y$
8.003	1.412	4.263	3.45	4.010	1.236	3.027	2.449
8.003	1.412	4.669	3.38	3.917	1.427	3.242	2.271
8.003	1.412	5.075	3.35	3.879	1.580	3.495	2.212
0.468	8.897	4.263	3.43	4.170	0.817	3.446	4.221
0.468	8.897	4.669	3.40	4.112	0.954	3.715	3.895
0.468	8.897	5.075	3.35	4.047	1.073	4.002	3.729

## Experimental

The HPLC method used was the same as that described in Ref. 1.

*Determination of  $pK_a$  values for RA and RA'.* The instruments and the procedures for the measurements were in all essential details identical with those described in Ref. 4.

*Theory.* For a mixture of two uncharged bases RA and RA' eqns. (19) and (20) are valid.

$$[\text{RA}][\text{H}_3\text{O}^+]/[\text{HRA}^+] = K_{\text{RA}} \quad (19)$$

$$[\text{RA}'][\text{H}_3\text{O}^+]/[\text{HRA}'^+] = K_{\text{RA}'} \quad (20)$$

These can be rearranged to eqns. (21) and (22) in which  $\text{RA}_0 = [\text{HRA}^+] + [\text{RA}]$   $\text{RA}'_0 = [\text{HRA}'^+] + [\text{RA}']$ . If eqn. (21) is added to eqn. (22) we obtain eqn. (23). This is the fundamental equation for the calculation of  $K_{\text{RA}}$  and  $K_{\text{RA}'}$ .

$$[\text{HRA}^+]/[\text{H}_3\text{O}^+] = \text{RA}_0/([\text{H}_3\text{O}^+] + K_{\text{RA}}) \quad (21)$$

$$[\text{HRA}'^+]/[\text{H}_3\text{O}^+] = \text{RA}'_0/([\text{H}_3\text{O}^+] + K_{\text{RA}'}) \quad (22)$$

$$([\text{HRA}^+] + [\text{HRA}'^+])/[\text{H}_3\text{O}^+] = \text{RA}_0/([\text{H}_3\text{O}^+] + K_{\text{RA}}) + \text{RA}'_0/([\text{H}_3\text{O}^+] + K_{\text{RA}'}) \quad (23)$$

We started with 9.5 ml of a solution of known concentration  $S_0/0.95$  of HCl in 0.1 M NaCl. The pH value of this solution is  $\text{pH}_0$ . We then added 0.5 ml of a solution of a mixture of RA and RA' which were in excess over the acid. The electroneutrality condition gives rise to eqn. (24).

$$[\text{HRA}^+] + [\text{HRA}'^+] = [S_0] + [\text{OH}^-] - [\text{H}_3\text{O}^+] \quad (24)$$

(Owing to the dilution from 9.5 ml to 10.0 ml the concentration of the acid added is not  $S_0/0.95$  but  $S_0$ ). Since the mean activity coefficient is not changed by the addition of

$$[\text{H}_3\text{O}^+] = (S_0/0.95)10^{(\text{pH}_0 - \text{pH})} \quad (25)$$

the base we obtain eqn. (25). The  $[\text{OH}^-]$  can also be calculated from the pH reading, but can be neglected in the present case since it is very small compared with  $S_0 - [\text{H}_3\text{O}^+]$ . We can now readily calculate the left hand side of eqn. (23) which we call  $Y$ . From a set of ( $[\text{H}_3\text{O}^+]$ ,  $\text{RA}_0$ ,  $\text{RA}'_0$ ,  $Y$ ) values we can calculate  $K_{\text{RA}}$  and  $K_{\text{RA}'}$  by non-linear regression using our general method.<sup>1</sup> The results of the calculation based on the values in Table 5 are  $pK_{\text{RA}} = 3.519(8)$  and  $pK_{\text{RA}'} = 3.861(4)$ .

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