Chemical Reactions of Omeprazole and Omeprazole Analogues. VI. The Reactions of Omeprazole in the Absence of 2-Mercaptoethanol

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The reactions of omeprazole, 5-methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinylmethylsulfinyl)-1*H*-benzimidazole, and some omeprazole analogues in acidic solution in the absence of 2-mercaptoethanol have been studied. The very complex spectrum of reactions also include the reactions of various degradation products of omeprazole.

The addition of 2-mercaptoethanol (Hβ) has been shown greatly to simplify the reactions of omeprazole (HA) in acidic solutions.[1] At the same time, much of the information regarding the behaviour of the reactive intermediates was lost. The results obtained in the presence of thiols have, however, been invaluable in our attempts to tackle the more complex reactions that occur in their absence.

After an extensive investigation we have arrived at a reaction sequence which describes the products formed in a quantitative or at least semi-quantitative manner. The complexity of these reactions is due to the occurrence of sulfenic acids and their derivatives as intermediates. These compounds are notorious in organic chemistry for the complexity of their reactions.[2,3] We will therefore start with a discussion of the reactions that can be predicted for HC^+ and D^+, and a short description of the experimental observations made in relation to these reactions.

Expected reactions of compound D^+ in acidic solutions

Reactions of the OCH_3 group in the pyridine ring. The OCH_3 group in the 4-position of the pyridinium cation in D^+ can be expected to be hydrolytically labile. To demonstrate this, we have studied the hydrolytic behaviour of some compounds related to D^+.

The first compound tested was the sulfide HS. In 0.1 M HCl HS is fully protonated and stable at 37 °C for 100 h. If HS is heated in 2 M HCl it is, however, slowly demethylated to the pyridone analogue (U), which has been isolated in good yield.

Another analogue tested was $HE\beta^+$, which is slowly demethylated in dilute HCl to the pyridone analogue (V). This reaction is very clean and no foreign peaks could be detected in the HPLC traces.

A kinetic investigation of the reaction was performed in 0.1, 0.01 and 0.001 M HCl. The results obtained in the 0.001 M solution are given in Table 1, and a summary of

Table 1. Reaction of the disulfide $HE\beta^+$ in 0.001 M HCl at 37 °C. Ionic strength 0.05 M.

	$[HE\beta^+]$[a]		[V][a]	
t/h	Obs.	Calc.[b]	Obs.	Calc.[c]
0.03	452.2	447.7	6.600	6.215
0.38	443.9	445.9	9.2	8.718
0.72	446.9	444.1	11.00	11.14
1.07	434.4	442.2	14.10	13.62
1.31	437.7	441.0	15.60	15.32
2.33	439.9	435.7	23.60	22.48
3.81	426.3	428.2	32.80	32.71
5.33	429.8	420.6	43.20	43.04
6.19	408.3	416.3	48.2	48.80
7.70	415.4	409.0	57.81	58.77
9.22	398.3	401.7	69.1	68.63
10.74	390.9	394.6	78.4	78.32
12.25	390.3	387.6	87.8	87.77
13.77	384.1	380.7	96.9	97.12
15.29	370.9	374.0	107.4	106.3
16.80	366.8	367.3	114.4	115.3
18.32	361.9	360.8	123.0	124.1
19.84	356.8	354.4	131.5	132.8
22.25	338.3	344.5	146.5	146.3
23.27	343.5	340.4	153.8	151.9

[a] Values given are in integration units. [b] Calculated according to: $[HE\beta^+] = [HE\beta^+]_0 e^{-k_{obs}t}$, $[HE\beta^+]_0 = 448(1)$ integration units, $k_{obs} = 3.28(8)\times10^{-6}$ s^{-1}. [c] Calculated according to: $[V] = A(1)(1 - e^{-k_{obs}t}) + A(2)$, where $A(1) = [V]_\infty - [V]_0 = 607(16)$ integration units. $A(2) = 6(1)$ integration units, where $A(2)$ is due to the initial presence of some V.

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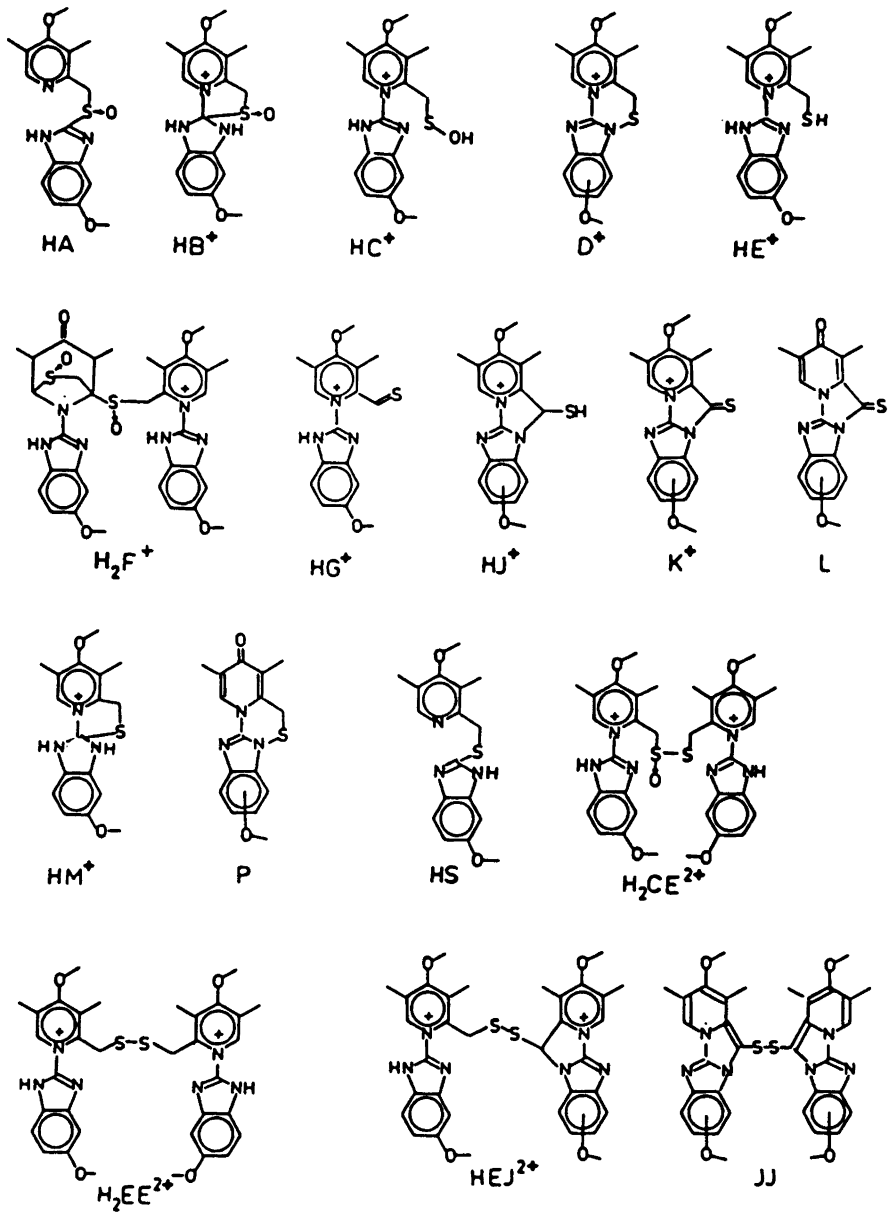


Scheme 1.

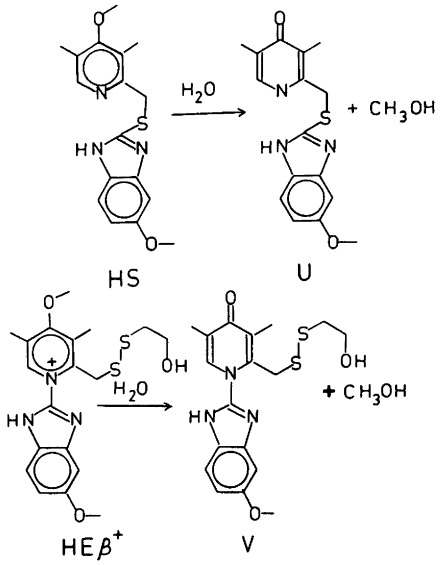
the rate constants obtained at three different concentrations of HCl is given in Table 2.

The formation of methanol when omeprazole is dissolved in 0.1 M HCl can be detected by head-space GC. Demethylation of protonated omeprazole probably does not occur, since the omeprazole analogue with a methyl group in the 6-position of the pyridine ring has been found to be stable under these conditions. This compound does not react to give the pyridinium compounds HB^+, HC^+ or D^+. The formation of methanol is thus with all probability the result of a reaction of D^+. In kinetic experiments with 10^{-5} M D^+ in 0.1 M HCl, we have found that a good portion of D^+ is decomposed by a first-order reaction with the formation of a compound, here called H_2F^+, which is readily detected by HPLC. The first-order nature with respect to D^+ is seen from the kinetics which also demonstrate that we have a competing reaction, second-order in D^+, which gives other products. In accordance with this finding, the fraction of H_2F^+ formed from a 10^{-4} M solution of omeprazole in 0.1 M HCl is much less than that from a 10^{-5} M solution of omeprazole in 0.1 M HCl. This makes it very difficult to isolate H_2F^+, and its structure is therefore based on the following speculations.

Table 2. Summary of rate constants for the reaction of $HE\beta^+$ in dilute HCl at 37 °C.

[HCl]/M	0.1[a]	0.01[b]	0.001[b]
$k_{obs}/10^{-6}\ s^{-1}$	5.2(2)	3.5(2)	3.28(8)

[a] μ = 0.1 M. [b] μ = 0.05 M.



Scheme 2.

H_2F^+ cannot be the pyridone analogue of D^+, since only one isomer could be detected in the HPLC trace. Moreover, the pyridone structure contains an α,β-unsaturated carbonyl group. Compounds containing such a group are used as trapping reagents for sulfenic acids,[2,3] and we can expect that HC^+ (or D^+) will react rapidly with an initially formed pyridone.

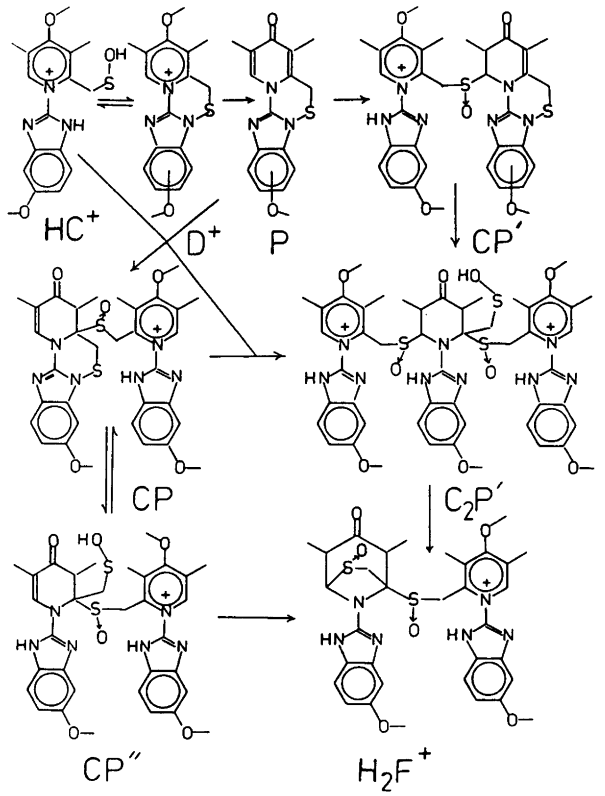
We now consider what can be expected to occur when the pyridone sulfenamide is first generated from D^+. Although the pyridone sulfenamide contains two reactive groups (an α,β-unsaturated group and a masked sulfenic acid), an intramolecular reaction is not possible since the two reactive groups are well separated in the planar or almost planar pyridone ring. In an intermolecular reaction the pyridone sulfenamide P in equilibrium with the corresponding sulfenic acid cannot compete with the sulfenic acid HC^+ in equilibrium with the sulfenamide D^+, which is in great excess. One molecule of HC^+ thus reacts with one molecule of the pyridone sulfenamide (Scheme 3). The point of attack on the pyridone sulfenamide is however an open question. It may occur at the 2- or at the 6-position. When the attack has occurred, the former pyridone ring is no longer planar. If we assume that the attack occurs in the 2-position, the sulfenic acid group of the compound just formed is now in an excellent position for an intramolecular attack at the 6-position and compound H_2F^+ may be obtained.

The same compound, or more correctly one of its stereoisomers, may also be obtained if HC^+ attacks the pyridone sulfenamide in both the 2- and the 6-position and the sulfoxide group in the 6-position is then released by an attack of the sulfenic acid group in the former pyridone sulfenamide.

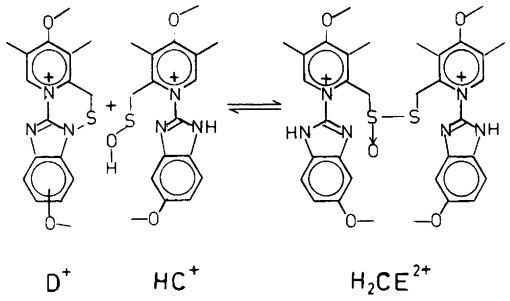
Second-order reactions of D^+. The sulfenamide D^+ is in equilibrium with the sulfenic acid HC^+. Sulfenic acids are known readily to undergo dimerization to give thiosulfinates.[2,3] We have demonstrated that a thiosulfinate is a probable reaction product when the *N*-methylated derivative of omeprazole is dissolved in 0.1 or 0.01 M HCl.[4] From what is known about the reactions of D^+ with thiols we can also make a semi-quantitative prediction of the rate of reaction of D^+ to give a thiosulfinate H_2CE^{2+}. The background of this is the great similarity we can expect between the reaction of HC^+ with D^+ and that of Hβ with D^+.

The pK_a of Hβ (9.27) is almost the same as that predicted for the sulfenic acid proton of HC^+ (pK_a = 8.9).[5] The resulting anions both contain a strongly nucleophilic sulfur atom that can be expected to react with D^+ in reactions with almost the same very high rate. At pH 3 we have found a rate constant of Hβ with D^+ which is 1.7×10^3 M^{-1} s^{-1}.[6] At pH 4.3 the reaction should be 20 times faster, and the rate constant will thus be 3.4×10^4 M^{-1} s^{-1}. We can expect the same rate constant for HC^+, but if D^+ contains only about 3 % of HC^+ (lower limit 1 %[4] and upper limit ca. 10 % as HC^+ could not be detected in an NMR spectrum of D^+), the rate constant for the reaction of D^+ to H_2CE^{2+} is expected to be about $0.03\times3.4\times10^4$ M^{-1} s^{-1} = 10^3 M^{-1} s^{-1}. The rate constant observed is 1.5×10^3 M^{-1} s^{-1}, in good agreement with the predicted one.

D^+ can also be expected to react with HC^+ in an acid-catalyzed reaction exactly analogous to that with Hβ. For the reaction of D^+ with Hβ in 0.1 M HCl (μ = 0.1 M) we



Scheme 3.



Scheme 4.

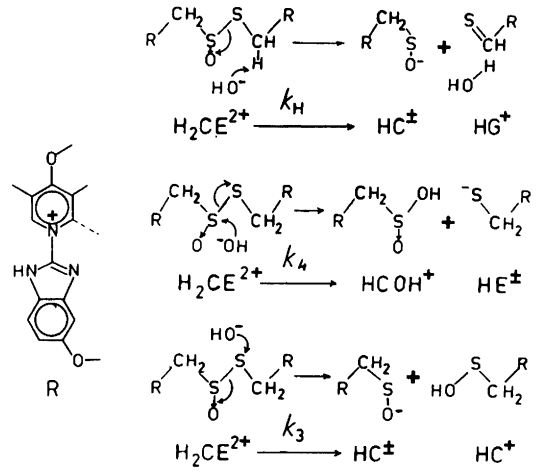
have measured a rate constant of 360 M^{-1} s^{-1}.[7] If the reactivity of HC^+ is the same as that of Hβ even in this reaction, and D^+ contains 3% of HC^+, we can predict a rate constant for the reaction $D^+ \rightarrow H_2CE^{2+}$ equal to 10. The rate constant found is 2.5 M^{-1} s^{-1} and thus almost of the magnitude predicted.

With this background we can confidently accept the proposal that H_2CE^{2+} is formed in a second-order reaction of D^+ (Scheme 4).

Expected reactions of H_2CE^{2+}

The thiosulfinate H_2CE^{2+} could be expected to undergo three types of primary reaction with water or OH^- (Scheme 5).[2,3] The resulting compounds, denoted $HC^{\pm}$, $HE^{\pm}$, $HCOH^+$ and HG^+, may undergo subsequent reactions. These reactions will be discussed in more detail below in connection with the isotope-effect studies. The elimination may also proceed by an acid-catalyzed concerted mechanism (Scheme 6). For more details of the reaction $H_2CE^{2+} \rightarrow HC^+ + HG^+$, see below.

Another characteristic reaction of thiosulfinates is their reaction with thiols such as Hβ. Under most conditions, the concentration of H_2CE^{2+} is too low to enable a study of its reactions with Hβ. In the study of *N*-methylated derivatives MeA of HA,[4] however, we observed by means of NMR spectroscopy that thiosulfinates were formed from a

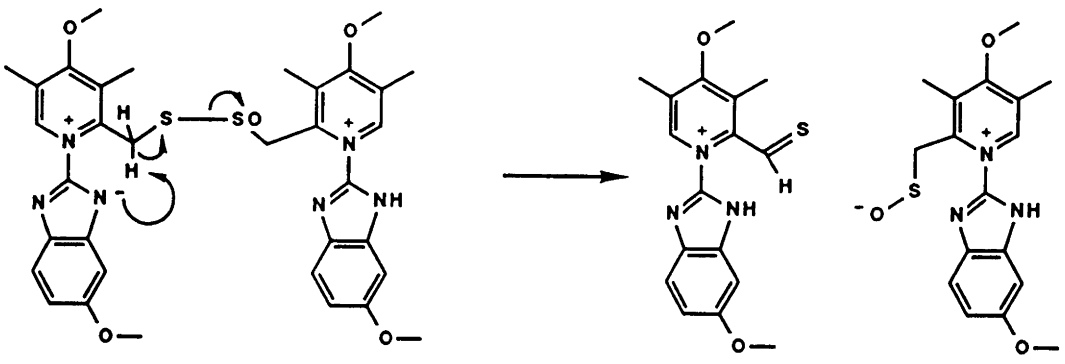


Scheme 5.

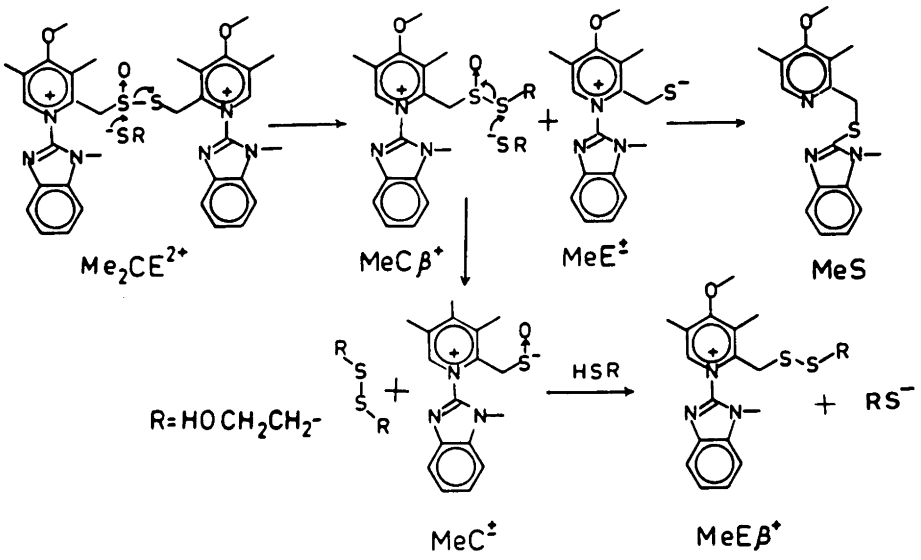
10^{-3} M solution of MeA in 0.1 M DCl. When such a solution was added to a buffer containing a slight excess of Hβ, equivalent amounts of MeS and probably $MeC\beta^+$ were formed. The thiosulfinate $MeC\beta^+$ does not contain the very good leaving group MeE^+ and thus reacts much more slowly with Hβ than Me_2CE^{2+} does. In this reaction $MeE\beta^+$ is formed to some extent. We thus have the reactions in Scheme 7.

The elimination of H_2CE^{2+} gives HC^+ and the thioaldehyde HG^+. Thioaldehydes usually exist as polymers. A polymerization can be expected to be a second- or higher-order reaction. Such a reaction will thus be very concentration dependent. We have found that at a starting concentration of omeprazole $> 10^{-4}$ M a dark blue precipitate is formed that probably contains such a polymer of HG^+. At a pH value of about 5 with an initial concentration of omeprazole $> 10^{-3}$ M, a very high percentage of omeprazole is converted into this precipitate. In solutions with a concentration $<10^{-4}$ M, very little of this compound is formed and we have to consider other reactions.

The pK_a of the NH group in HG^+ can be estimated to be about 6.5. The corresponding anion is thus very readily formed. Its nitrogen atoms are situated in an excellent



Scheme 6.

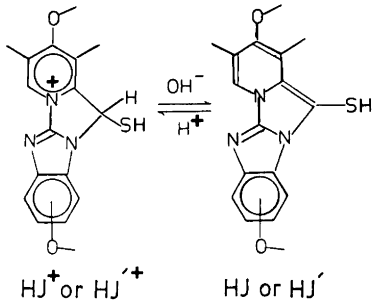


Scheme 7.

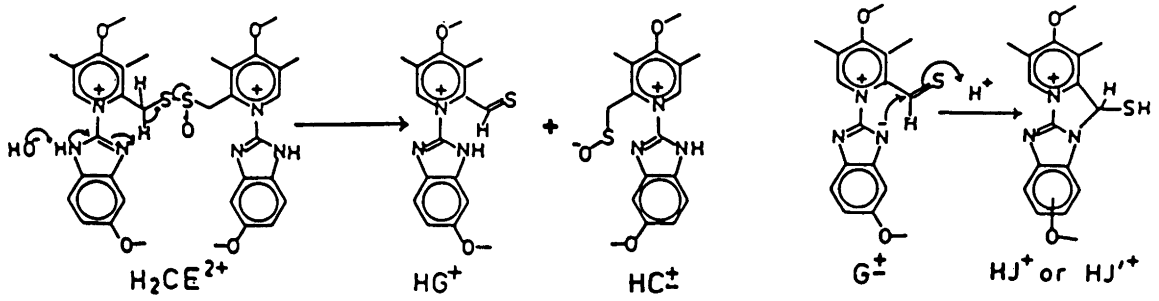
position for a reaction with the C=S group and two thiols, HJ^+ and HJ'^+, depending on which nitrogen atom reacts, can be expected to be formed (Scheme 8). A substituent in a position that makes NH a stronger acid will increase the concentration of the anion. However, it also makes it less reactive in the reaction with the C=S group. These two effects can be expected to cancel and HJ^+ and HJ'^+ will be formed in the same quantities. This is in agreement with the findings for omeprazole at pH 4.26.

Expected reactions of HJ^+ and HJ'^+

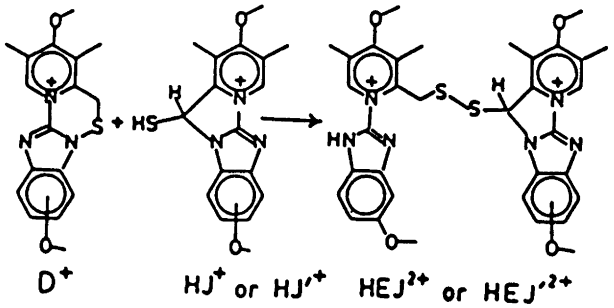
HJ^+ and HJ'^+ contain an acidic CH group in the ring just formed. The pK_a value of this CH group is difficult to predict with any certainty. The presence of the two strongly electron-withdrawing groups, the pyridinium ring and the benzimidazole ring, however, seems to enable the release of the CH proton at a reasonably low pH value. We can thus expect that HJ^+, HJ'^+ and their derivatives are involved in equilibria of the type given in Scheme 9. HJ^+ and HJ'^+ are thiols and as such very reactive toward D^+ and HC^+. We can thus expect the reaction in Scheme 10 to occur, especially as HC^+ is formed in the same reaction as HG^+.



Scheme 9.



Scheme 8.



Scheme 10.

Expected reactions of HEJ^{2+} and HEJ'^{2+}

HEJ^{2+} and HEJ'^{2+} are disulfides, and as such they can undergo elimination.[2] Even in this case a concerted acid-catalyzed mechanism is possible (Scheme 11). In fact, this seems to be the dominant mechanism for the elimination in this case, since the rate of conversion of HEJ^{2+} was found to be higher the lower the pH of the solution.

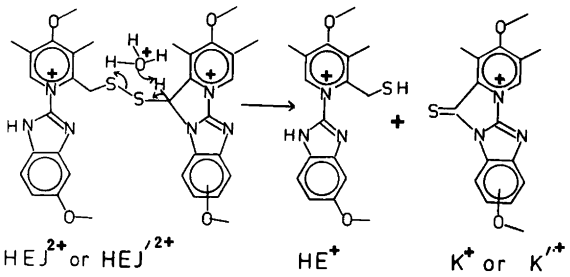
It has not been possible to identify peaks corresponding to HEJ^{2+} and HEJ'^{2+} in the HPLC chromatogram, although HEJ^{2+} and HEJ'^{2+} seem to be rather stable on the basis of structural considerations. The possible reason for this is that the charges on HEJ^{2+} (and HEJ'^{2+}) and the two protons available for protolytic reactions, one on the nitrogen of the benzimidazole ring of the HE^+ part of the molecule and the other on the acidic CH group of HJ^+, give rise to complications in the chromatographic properties of the compounds. The presence of HEJ^{2+} and HEJ'^{2+} can, however, be detected by the addition of Hβ, which rapidly converts HEJ^{2+} and HEJ'^{2+} into βJ^+, $\beta J'^+$ and HE^+. The compounds βJ^+ and $\beta J'^+$ are readily detected with HPLC chromatography. The high reactivity of HEJ^{2+} and HEJ'^{2+} with thiols is discussed in part IV of this series.[7]

Since this reactivity is of the same magnitude as that of D^+ with Hβ we can expect that the thiols HJ^+ and HJ'^+ also react with HEJ^{2+} and HEJ'^{2+} with the formation of JJ, JJ',J'J' and HE^+. A compound of this structure [JJ(1)] is obtained on treatment of timoprazole, the unsubstituted analogue of omeprazole, with 0.1 M HCl (Scheme 12). The structure was confirmed by X-ray analysis.[8] The same compound has been described as a free radical J'.[9]

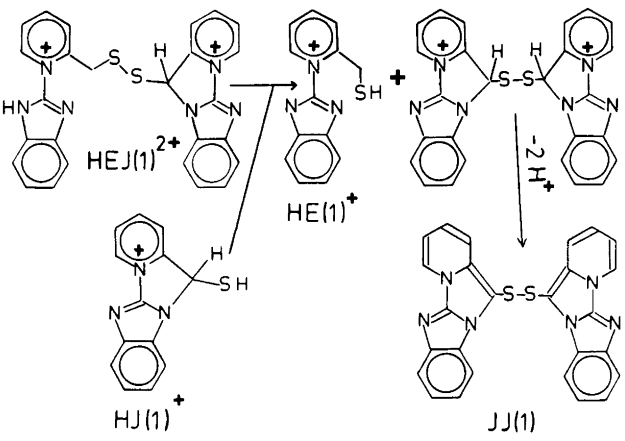
Expected reactions of the thioamides K^+ and K'^+

The CH_3O group in the pyridine ring of K^+ (or K'^+) will readily undergo hydrolysis to give an OH group due to the activation from the positive charge and the thioamide group. The resulting compound is very rapidly transformed into the pyridone L (or L') (Scheme 13). This reaction might also be described as an S_N2 attack of H_2O on the carbon of the methoxy group with the formation of the excellent leaving group L (or L').

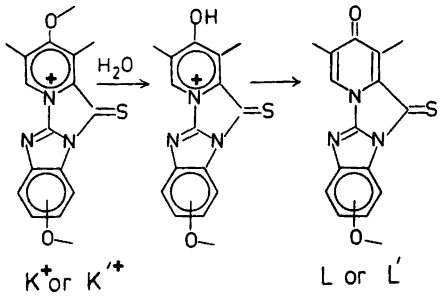
These compounds are the first uncharged compounds to be expected in the second-order degradation of D^+. The loss of the charge decreases the solubility in water to such an extent that L and L' precipitate when acidic solutions of omeprazole are allowed to stand and are thus readily ob-



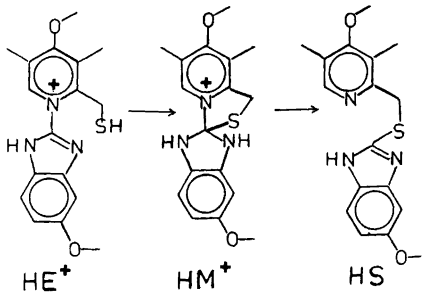
Scheme 11.



Scheme 12.



Scheme 13.



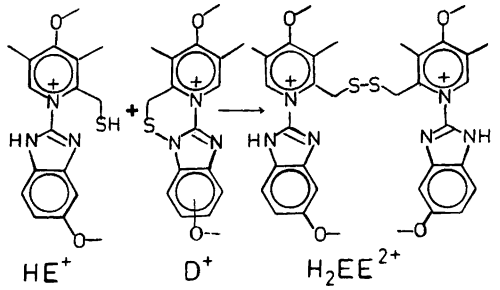
Scheme 15.

tained. L and L′ from omeprazole have been obtained in pure forms by LC, and could thus be used as references in HPLC for quantification of the responses of the corresponding peaks in the chromatogram. However, we have not been able to obtain crystals good enough for an X-ray structure determination of L, L′ or any of their analogues. We must therefore rely on NMR spectroscopy in the structure determination, see below.

L and L′ are not the final products in the degradation of omeprazole since they still contain reactive groups, such as the thioamide function that may be hydrolyzed, and the pyridone structure that may add HC^+ or D^+. However, these further reactions have not been studied in detail.

Expected reactions of HE^+

The thiol HE^+ can be formed in the eliminations of H_2CE^{2+}, HEJ^{2+} and HEJ'^{2+}. Since a thiol reacts very rapidly with HC^+ or D^+, we can expect that HE^+ will undergo the reaction $D^+ + HE^+ \rightarrow H_2EE^{2+}$. A salt of H_2EE^{2+} is, in fact, obtained in good yield by stirring a 0.04 M solution of omeprazole in 0.1 M HCl containing 40 % methanol for 75 min. A mixture of L and L′ was filtered off and the PF_6^- salt of H_2EE^{2+} was then precipitated by the addition of HPF_6 (Scheme 14).



Scheme 14.

The SH group in HE^+ is in an excellent position for a reaction with the 2-C atom in the benzimidazole ring, giving HS according to the reactions in Scheme 15. This explains the occurrence of HS in the solutions.

Two competing reactions can thus be expected, the rates of which can be expressed as

$$dH_2EE^{2+}/dt = k_{EEE}[D^+][HE^+] \quad dHS/dt = k_{ES}[HE^+]$$

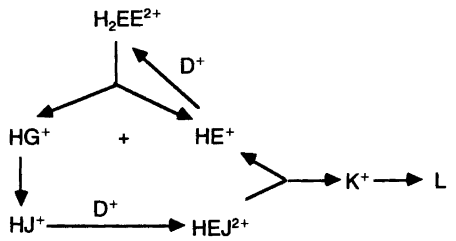
The fraction of HE^+ going to HS, Fr(HS), is thus at any moment

$$Fr(HS) = k_{ES}/(k_{EEE}[D^+] + k_{ES}) = 1/(1 + [D^+]k_{EEE}/k_{ES})$$

Fr(HS) thus decreases with an increase in $[D^+]$. This is in agreement with the observation that H_2EE^{2+} is obtained in good yield when omeprazole is decomposed in acidic concentrated solutions, whereas HS is the *main product* from dilute solutions.

Expected reactions of H_2EE^{2+}

The disulfide H_2EE^{2+} may as such undergo an elimination to give $HE^+ + HG^+$, a reaction which probably is acid catalyzed. Our kinetic measurements are performed in dilute solutions where the rate of formation of H_2EE^{2+} is low. We therefore have no clear indications for the occurrence of this reaction. However, it might be an important source of L and L′ formed in concentrated solutions of omeprazole in 0.1 M HCl (Scheme 16).



Scheme 16.

The two separated positive charges and two acidic protons on the nitrogen atoms of the benzimidazole ring in H_2EE^{2+} will make an HPLC determination difficult and the peak corresponding to H_2EE^{2+} is very broad. However, H_2EE^{2+} will react with Hβ in the reaction $H_2EE^{2+} + H\beta \rightarrow HE\beta^+ + HS$. This reaction is found to be much slower than the reaction of D^+ with Hβ. It should thus be possible to differentiate between D^+ and HC^+ on the one hand and H_2EE^{2+} on the other.[7]

Methods used for the quantitative determination of the concentration of D^+

Since D^+, or more exactly the equilibrium mixture HC^+/D^+, is of central importance in the degradation of omeprazole, several attempts have been devoted to find a quantitative method for the determination of D^+.

Addition of excess of Hβ and a buffer (Method A). The first method attempted was to add a slight excess of Hβ and a buffer to a solution containing D^+. The resulting solution was then injected, as rapidly as possible, onto an HPLC reversed-phase column and the concentration of $HE\beta^+$ was measured using a standard curve. There are, however, several problems with this method.

Firstly, there is a time lag from the moment when the reaction is stopped to that at which the solution is injected onto the HPLC column. Even by proper handling it is difficult to decrease this to much less than 30 s. During this time the quantity of HA converted into D^+ and the quantity of $HE\beta^+$ converted into HS must be negligible. If the acceptable limit is 1 % conversion of HA to D^+, the pH of the solution must be >5. The conversion of $HE\beta^+$ to HS, on the other hand, is so slow at all reasonable pH values and Hβ concentrations that it presents no problem. The requirements are thus fulfilled as soon as the pH > 5 after the addition of the buffer. The conversion of D^+ into $HE\beta^+$ at that pH is very complete, provided that Hβ is in excess over D^+.

Secondly, $HE\beta^+$ must not, or at least only to a negligible extent, be formed from compounds other than D^+ or HC^+. The critical compounds are H_2EE^{2+} and to some extent H_2CE^{2+}, which both give $HE\beta^+$ on the addition of Hβ. The method thus gives the sum $D^+ + HC^+ +$ some $H_2CE^{2+} + H_2EE^{2+}$. Correct values for $D^+ + HC^+$ are thus obtained only when the concentrations of H_2EE^{2+} (and H_2CE^{2+}) are negligible.

Spectrophotometric method (Method B). The fact that D^+ has a strong absorption at 355 nm, whereas HA and HS are transparent, can be used in another method to measure D^+. The selectivity is however very low, since H_2CE^{2+}, HEJ^{2+}, H_2F^+, L and L′ all absorb at 355 nm. The method has been used for kinetic measurements, and is further discussed, Appendix 2 in Ref. 10.

Modified spectrophotometric method (Method C). The selectivity of Method B can be considerably increased by measuring the absorbance at 355 nm before and immediately after the addition of a slight excess of Hβ. The difference in absorbance at 355 nm, after correction for volume changes, is due to the very rapid conversion (within a few seconds) of D^+ ($\varepsilon = 12\,700\ M^{-1}\ cm^{-1}$) into $HE\beta^+$ ($\varepsilon = 3\,000\ M^{-1}\ cm^{-1}$). However, three other reactions which are fast enough to be taken into consideration can also be expected.

Firstly, a change in pH could cause a change in absorbance. Such a change is probably small for most of the compounds present in the solution and can be completely eliminated by maintaining the same pH before and after the addition of Hβ.

Secondly, the reaction of H_2CE^{2+} with Hβ is probably fast enough to be taken into consideration. However, it may be expected that it is accompanied by very little change in the absorbance, and it has therefore been neglected.

Thirdly, the reaction $HEJ^{2+} + H\beta \rightarrow HE^+ + \beta J^+$ is also a very fast reaction,[7] but the change in the absorbance is probably very small and has therefore been neglected.

The reaction $H_2EE^{2+} + H\beta \rightarrow HE^+ + HE\beta^+$ is not fast enough to disturb the measurements. This method is thus selective for D^+ as long as the pH is the same before and after the addition of Hβ.

Addition of Hβ and a buffer followed by acetylcysteine (Method D). In the final method, we added Hβ and a buffer to give a pH of about 4.3 and a concentration of Hβ of 10^{-4} M. After 2 s acetylcysteine (HAccys) was added to give a concentration of 10^{-2} M. The solution was then injected as soon as possible onto a reversed-phase HPLC column and analyzed for $HE\beta^+$.

This method converts D^+ into $HE\beta^+$ and the presence of H_2CE^{2+} and H_2EE^{2+} are not disturbing. HEJ^{2+} and HEJ'^{2+} react fast enough, but do not give $HE\beta^+$ and therefore do not interfere. The method thus gives the $[D^+]$ but is somewhat too cumbersome to be used as a standard method and was only rarely used. However, a variation of this method has been used to study the rate of the reactions $HEJ^{2+} + H\beta \rightarrow HS + \beta J^+$ and $\beta J^+ + HAccys \rightarrow AccysJ^+ + H\beta$. The results obtained with the three methods A–C on a 10^{-5} M solution of omeprazole in 0.01, 0.001 M HCl and at pH 4.26 are given in Figs 1(a)–(c).

One can see that all methods give almost the same results in the HCl solutions for the first 10 min. After this time they begin to deviate. Since all errors involved tend to give values of $[D^+]$ that are too high, we can immediately conclude that the method giving the lowest value is the most correct.

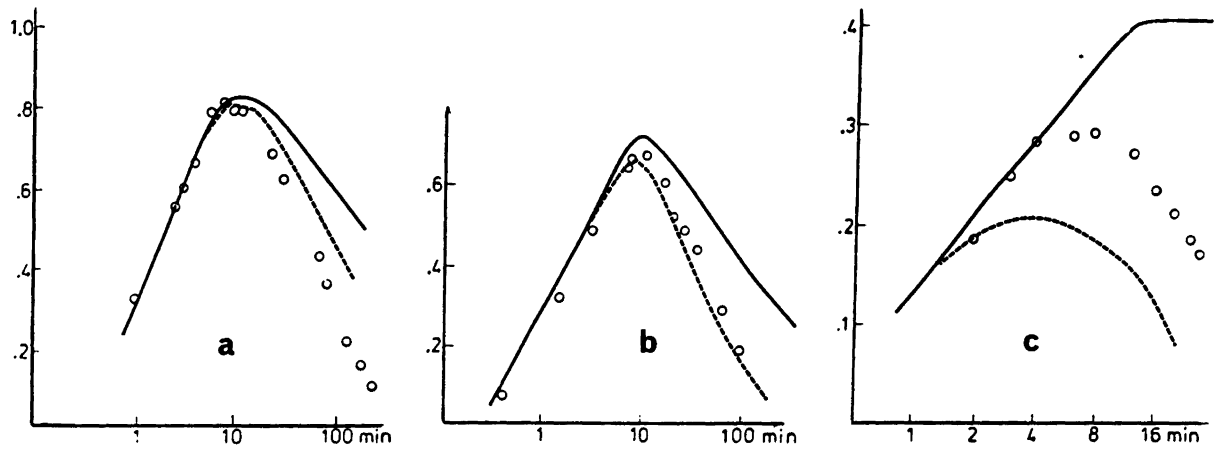


Fig. 1. Comparison between Method A (○), Method B (———) and Method C (- - - -) in the determination of D^+ formed from omeprazole after different times at (a) $[H^+]$ 0.01 M, (b) $[H^+]$ = 0.001 M and (c) pH = 4.26.

Analysis of kinetic behavior in solutions of different pH

Reactions in 0.01 M HCl. In 0.01 M HCl the measurements of $HE\beta^+$ give the most correct values of $[D^+]$. The somewhat higher values obtained by Method C are interpreted as being the result of a small decrease in absorbance caused by the presence of H_2F^+ when the buffer is added to increase the pH value to about 4. The much higher values obtained by Method B are interpreted as a result of the presence of H_2F^+.

A quantitative determination of the rate constants for the reactions in Scheme 17 is based on the following stoichiometric considerations.

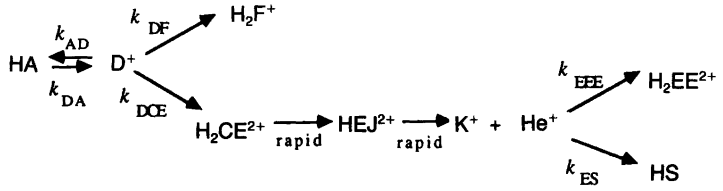
For each equivalent of H_2F^+ formed two moles of D^+ are consumed. The conversion H_2CE^{2+} into HEJ^{2+} is, stoichiometrically, a rearrangement since one mole of HC^+ is first produced and then consumed in the next step (see above, Expected Reactions of H_2CE^{2+}). One mole of D^+ is also consumed for each mole of H_2EE^{2+} formed from HE^+. In the reaction of H_2EE^{2+} with Hβ one mole of $HE\beta^+$ and one mole of HS are formed. This means that for each mole of H_2CE^{2+} formed and converted, one mole of HS is formed. (In part directly from HE^+ and in part via H_2EE^{2+}).

The concentration of $HE\beta^+$ found is the concentration of D^+ + that of H_2EE^{2+}. (H_2CE^{2+} is slowly formed and very rapidly decomposed, and is thus assumed to be present in only negligible concentrations).

The absorbance at 355 nm (*Abs*) is assumed to be the sum of the absorbance of D^+ with $\varepsilon_D = 1.27\times10^4\ M^{-1}\ cm^{-1}$ and that of H_2F^+ with ε_F. The rapid change in absorbance (ΔAbs) is assumed to be the sum of that caused by D^+ with $\Delta\varepsilon_D = 0.97\times10^4\ M^{-1}\ cm^{-1}$ and that caused by H_2F^+ with $\Delta\varepsilon_F$.

In the experiments we followed the concentrations of HA, $HE\beta^+$, H_2F^+ and HS, as well as *Abs* and ΔAbs as functions of time. The 'calculated' values of these quantities for use in the least-squares calculations are obtained by iterations from $t = 0$ to t in eqn. (1) and calculations from eqn. (2).

$$da_i = k_{AD}[HA]_i\,\Delta t \quad db_i = k_{DA}[D^+]_i\,\Delta t$$
$$dc_i = k_{DF}[D^+]_i\,\Delta t \quad dd_i = k_{DCE}([D^+]_i)^2\,\Delta t$$
$$de_i = dd_i/(1 + [D^+]_i\,k_{EEE}/k_{ES}) \text{ (see above, Expected Reactions of } HE^+) \qquad (1)$$



Scheme 17.

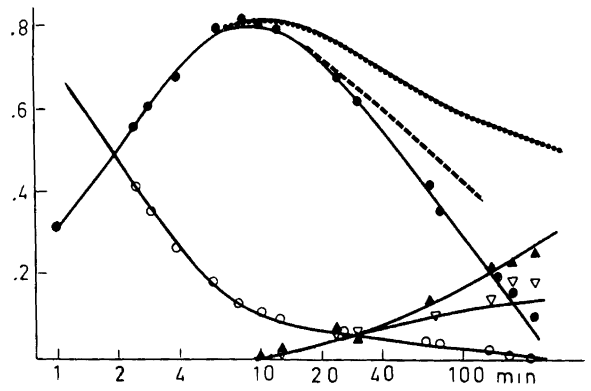


Fig. 2. Reaction of omeprazole in 0.01 M HCl. The relative concentrations of HA (○), HEβ$^+$ (●), HS (▽) and H_2F^+ (▲) are plotted vs. time. Curves correspond to calculated concentrations. For the *Abs* (.....) and Δ*Abs* (-----) curves experimental points are very close together and very close to the curves and are therefore not given.

$$[HA]_{i+1} = [HA]_i - da_i + db_i$$
$$[D^+]_{i+1} = [D^+]_i + da_i - db_i - 2dd_i - (dd_i - de_i) - 2dc_i$$
$$[H_2F^+]_{i+1} = [H_2F^+]_i + dc_i$$
$$[HS]_{i+1} = [HS]_i + dd_i$$
$$[HE\beta^+]_{i+1} = [D^+]_{i+1} + dd_i - de_i$$
$$Abs_t = 1.27\times10^4[D^+]_t + [H_2F^+]_t\varepsilon_F \quad (2)$$
$$\Delta Abs_t = 0.97\times10^4[D^+]_t + [H_2F^+]_t\Delta\varepsilon_F$$

In order to normalize the experimental values they are divided by the corresponding A_0 value, which is the starting concentration of omeprazole in each run. The values for *Abs* and Δ*Abs* are then divided by 10^4.

By the methods outlined in Appendix 1 in Ref. 10 we obtained the constants $k_{AD} = 5.86(9)\times10^{-3}$ s^{-1}, $k_{DA} = 7.0(5)\times10^{-4}$ s^{-1}, $k_{DCE} = 7.4(3)$ M^{-1} s^{-1}, $k_{DF} = 0.62(3)\times10^{-4}$ s^{-1}, $k_{EEE}/k_{ES} = 1.2(6)\times10^4$ M^{-1}, $\varepsilon_F = 1.9(1)\times10^4$ M^{-1} cm^{-1} and $\Delta\varepsilon_F = 0.68(8)\times10^4$ M^{-1} cm^{-1}. This is based on measurements performed on solutions with $A_0 \cong 10^{-5}$ M and $A_0 \cong 10^{-4}$ M.

The experimental values of different species in a 10^{-5} M solution of HA are given in Fig. 2. (The fit for $A_0 = 10^{-4}$ M is as good as that for $A_0 = 10^{-5}$ M). From the value of k_{EEE}/k_{ES} we can see that HE$^+$ is mainly converted into HS.

Reactions in 0.1 M HCl. The kinetic measurements on omeprazole in 0.1 M HCl can be treated in the same way as in 0.01 M HCl. Due to experimental difficulties the HPLC analyses were performed only in 10^{-5} M solutions, and no Δ*Abs* values were measured. In this way we obtained

$k_{AD} = 9.8(2)\times10^{-3}$ s^{-1}
$k_{DA} = 1.34(8)\times10^{-3}$ s^{-1}
$k_{DF} = 1.54(3)\times10^{-4}$ s^{-1}
$k_{DCE} = 2.45(8)$ M^{-1} s^{-1}
k_{EEE}/k_{ES} could not be determined $0.7(6)\times10^4$
$\varepsilon_F = 1.67(4)\times10^4$ M^{-1} cm^{-1}

We can see that ε_F is almost the same as that in 0.01 M HCl. The rate constants k_{AD}, k_{DA} and k_{DF} are about twice as high in 0.1 M HCl as in 0.01 M HCl, whereas k_{DCE} is about half that in 0.01 M HCl. An increase in pH above 2 can thus be expected to increase strongly the relative importance of the second-order reactions of D$^+$ over the first-order reactions. At pH 4.26 we could no longer detect the first-order reaction.

In 0.001 M HCl, H_2F^+, H_2CE^{2+} and H_2EE^{2+} might be expected to interfere with the determination of [D$^+$], and the system might be quite complicated. The detailed discussion will therefore be postponed until we have described the reactions taking place at pH ≅ 4.2.

Reactions at pH 4.2. When solutions of D$^+$ were prepared by dissolving omeprazole in 0.01 M HCl and a buffer was then added, we soon observed that the stability of D$^+$ in the solution rapidly decreased with increased concentration. This indicated that second-order reactions were involved.

In order to discover which reaction was second order, we added Hβ at different times to the solutions and measured the concentrations of HEβ$^+$, βJ$^+$ and βJ'$^+$, as integral *I*, for solutions with an initial concentration of omeprazole equal to 10^{-6}, 10^{-5} and 10^{-4} M. This is demonstrated in Fig. 3, which gives a plot of $A_0/I_{HE\beta^+}$ vs. time for three different starting concentrations A_0. If $I_{HE\beta^+}$ decreases according to a second-order reaction, three straight lines should be obtained with slopes that are proportional to A_0. This means that their proportions should be around 100:10:1, with the highest slope for the most concentrated solution. In the figure lines with slopes in these proportions have been drawn to fit the points. The figure demonstrates that the second-order concept is correct. However, complications occur since the reaction is coupled with other reactions.

The effect of the second-order conversion of D$^+$ into H_2CE^{2+} on the formation of HEJ^{2+} and HEJ'$^{2+}$ (measured as βJ$^+$ and βJ'$^+$), is demonstrated by the following. Assume the reactions can be described as in Scheme 18.

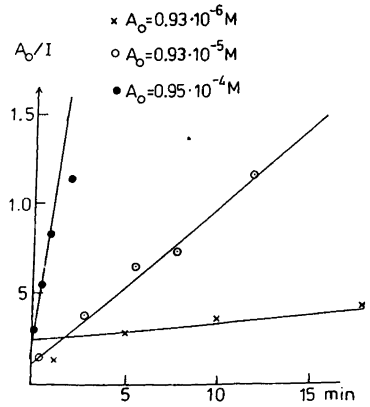
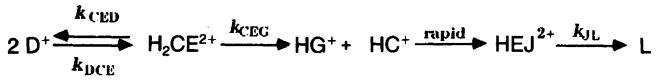


Fig. 3. Plot of $A_0/I_{HE\beta}$ for different A_0 values. $I_{HE\beta}$ is the integrated peak area for HEβ$^+$ obtained from HPLC measurements. The lines drawn have relative slopes 100:10:1.



Scheme 18.

If we apply the steady-state approximation on H_2CE^{2+} we obtain eqns. (3) and (4). We define k^* according to eqn. (5).

$$d[H_2CE^{2+}]/dt = k_{DCE}[D^+]^2 - k_{CEG}[H_2CE^{2+}] - k_{CED}[H_2CE^{2+}] = 0 \quad (3)$$

$$d[HEJ^{2+}]/dt = k_{CEG}[H_2CE^{2+}] - k_{JL}[HEJ^{2+}] = k_{DCE}k_{CEG}[D^+]^2/(k_{CEG} + k_{CED}) - k_{JL}[HEJ^{2+}] \quad (4)$$

$$k^*_{DCE} = k_{DCE}k_{CEG}/(k_{CEG} + k_{CED}) \quad (5)$$

Since we have $[HEJ^{2+}] = [\beta J^+]$, and make the assumption $[D^+] \cong [HE\beta^+]$ (inherent in the steady-state approximation), we obtain eqn. (6). If we measure in integration units and have $I_{\beta J} = R_{\beta J}[\beta J^+]$ and $I_{HE\beta} = R_{HE\beta}[HE\beta^+]$ we obtain eqn. (7), which is integrated to eqn. (8). This equation is of the type given in eqn. (9), where we can make the identifications according to eqn. (10).

$$d[\beta J^+]/dt = k^*_{DCE}[HE\beta^+]^2 - k_{JL}[\beta J^+] \quad (6)$$

$$dI_{\beta J}/dt = k^*_{DCE}R_{\beta J}(I_{HE\beta}/R_{HE\beta})^2 - k_{JL}I_{\beta J} \quad (7)$$

$$I_{\beta J} - I^0_{\beta J} = k^*_{DCE}R_{\beta J}/(R_{HE\beta})^2 \, {}_0\int^t (I_{HE\beta})^2 dt - k_{JL} \, {}_0\int^t I_{\beta J} dt \quad (8)$$

$$y = ax_1 + bx_2 + c \quad (9)$$

$$y = I_{\beta J}, x_1 = {}_0\int^t (I_{HE\beta})^2 dt, x_2 = {}_0\int^t I_{\beta J} dt, \quad a = k^*_{DCE}R_{\beta J}/(R_{HE\beta})^2, \; b = -k_{JL}, \; c = I^0_{\beta J} \quad (10)$$

Since the integrals are readily obtainable by numerical integration,[11] a regression of $I_{\beta J}$ against ${}_0\int^t (I_{HE\beta})^2 dt$ and ${}_0\int^t I_{\beta J} dt$ gives the constants a and b. We can see that b is independent of the scale used for $I_{HE\beta}$ and $I_{\beta J}$, and that a should be constant if the same scale is used for all measurements. The results of such a calculation are presented in Table 3.

Table 3. Rate constants for the formation and degradation of HEJ^{2+} and HEJ'^{2+} at three different starting concentrations of omeprazole. Rate constants were determined according to the text using a solution of D^+ prepared in a reaction of omeprazole in 0.01 M HCl (10 min) followed by the addition of a citrate buffer to give pH = 4.38. T = 37 °C.

Initial concentration of HA/M	HEJ^{2+}		HEJ'^{2+}	
	a	$k_{JL}/10^{-3}s^{-1}$	a	$k_{J'L'}/10^{-3}s^{-1}$
10^{-4}	68(30)	3.2(7)	54(22)	2.0(3)
10^{-5}	106(18)	2.2(2)	110(17)	1.5(2)
10^{-6}	287(44)	1.8(3)	254(39)	1.2(2)

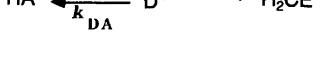
There is a slight but significant variation in the constants obtained, with all probability due to errors caused by the steady-state assumption, which means that the conversion $2D^+ \rightarrow H_2CE^{2+}$ is the rate-determining step in the conversion of D^+ into HEJ^{2+}. Since the rate of the reaction $2D^+ \rightarrow H_2CE^{2+}$ increases more rapidly with increasing $[D^+]$ than does that of the reaction $H_2CE^{2+} \rightarrow HEJ^{2+}$, the most correct values should be obtained in the most dilute solution. However, there is no doubt that the second-order step is found in the conversion of D^+ into H_2CE^{2+}, since no other assumption gives useful results. The constants k_{JL} and $k_{JL'}$ are also in good agreement with constants obtained by more correct methods.

The constant a contains arbitrarily chosen constants and its magnitude is thus uninteresting. It is, however, proportional to

$$k^*_{DCE} = k_{DCE}k_{CEG}/(k_{CEG} + k_{CED}).$$

The pH dependence of k_{DCE} and k_{CEG} can be predicted with some confidence, but not that of k_{CED} which is the rate constant for hydrolysis of the thiosulfinate H_2CE^{2+}. This reaction type is known to be very complicated,[2,3] which makes a prediction of the variation of k^*_{DCE} with pH very difficult. In the region where the reaction H_2CE^{2+} to D^+ is acid catalyzed and $k_{CED} > k_{CEG}$, at the same time as the reactions D^+ to H_2CE^{2+} and H_2CE^{2+} to HG^+ and HC^+ are base catalyzed, we can expect a very rapid increase in k^*_{DCE} with an increase in pH, as seen from the values obtained at pH 2 and pH 3 (see below Table 5).

The reaction $2D^+ \rightarrow H_2CE^{2+}$ can also be followed directly by a study of the system given in Scheme 19. Starting



Scheme 19.

from pure HA, or a mixture of HA and D^+, the concentration of HA is measured by HPLC and the concentration of D^+ by the Method C described above. A mixture of HA and D^+ at pH 4.15 was obtained by dissolving omeprazole in 0.01 M HCl. After 10 min at 37 °C a buffer was added to give pH 4.15. The starting value for $t = 0$ was obtained by adding a slight excess of Hβ and analyzing by HPLC. In this case $[D^+]_0 = [HE\beta^+]_0$, since all methods to measure $[D^+]_0$ give the same value for this solution. k_{AD} and k_{DCE} were calculated from the values of [HA] and $[D^+]$ at different times t using our general method.[10] The 'calculated' values of [HA] and $[D^+]$ for use in the least-squares calculations were obtained by iterations from $t = 0$ to t starting from $[HA]_0 = 0.133\times10^{-5}$ and $[D^+]_0 = 0.871\times10^{-5}$ using the formul

$$da_i = k_{AD}[HA]_i\Delta t, \; db_i = k_{DA}[D^+]_i\Delta t, \; dc_i = k^*_{DCE}([D^+]_i)^2\Delta t$$
$$[HA]_{i+1} = [HA]_i - da_i + db_i$$
$$[D^+]_{i+1} = [D^+]_i + da_i - db_i - 2dc_i$$

Table 4. Reactions of the sulfenamide D^+ at pH 4.15 and $T = 37\,°C$. $\varepsilon_D = 0.97\times10^4\ M^{-1}\ cm^{-1}$. Starting concentration of omeprazole 1.105×10^{-5} M.

t/min	*Abs* before addition of Hβ	*Abs* after addition of Hβ		Δ*Abs*	$[D^+]/10^{-5}$ M		$[HA]/10^{-5}$ M	
		Obs.	Corr.		Found	Calc.	Found	Calc.
0					0.871		0.133	
0.67	0.0849	0.0310	0.0313	0.0536	0.553	0.546	0.137	0.138
2.78	0.0630	0.0354	0.0368	0.0262	0.270	0.260	0.130	0.130
5.20	0.0557	0.0384	0.0388	0.0169	0.174	0.173	0.116	0.110
7.27	0.529	0.0400	0.0404	0.0125	0.129	0.140	0.091	0.094
9.53	0.510	0.0400	0.0404	0.0106	0.109	0.118	0.077	0.079
22.0	0.492	0.0410	0.0414	0.0078	0.080	0.101	0.064	0.064

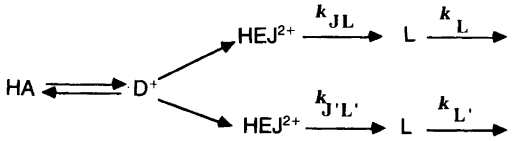
$k_{AD} = 2.3(4)\times10^{-3}\ s^{-1}$, $k_{DCE} = 9(2)\times10^2\ M^{-1}\ s^{-1}$.

The value $k_{DA} = 7.0\times10^{-4}\ s^{-1}$ was not calculated but taken from other measurements. The results of the measurements and calculations are presented in Table 4.

The reaction was also followed starting from pure HA by dissolving omeprazole in a buffer at pH 4.26, stopping the reaction by the addition of a buffer and Hβ, and injecting a sample onto a HPLC column. In another series, the reaction in the buffer solution containing omeprazole was stopped at different times by injecting samples onto the HPLC column and in a third type of experiment the concentration of D^+ was followed using Method C.

From the concentrations of HA and D^+ at different times we were able to calculate k_{AD}, k_{DA} and k_{DCE} by the method outlined above. In this way we obtained $k_{AD} = 2.24(6)\times10^{-3}\ s^{-1}$, $k_{DA} = 7(2)\times10^{-4}\ s^{-1}$, $k^*_{DCE} = 1.45(6)\times10^3\ M^{-1}\ s^{-1}$. These values are in good agreement with the results obtained starting from the mixture of HA and D^+ at pH 4.15. The value $k^*_{DCE} = 0.92\times10^3\ M^{-1}\ s^{-1}$ at pH 4.15 corresponds to a value $k^*_{DCE} = 1.18\times10^3\ M^{-1}\ s^{-1}$ at pH 4.26 if the reaction is base catalyzed.

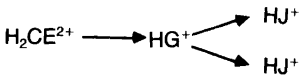
Since there are good reasons to believe that the reaction $D^+ \rightarrow H_2CE^{2+}$ is reversible, the k^*_{DCE} value should be interpreted as a rate constant for the net transformation of D^+ into H_2CE^{2+} and its reaction products. The total transformation is thus approximated by the reactions in Scheme 20 and the rate constants $k^*_{DCE} = k_{DEJ} + k_{DEJ'}$.



Scheme 20.

In the conversion of H_2CE^{2+} given in Scheme 21 the reaction $HG^+ \rightarrow HJ^+$ can be expected to occur with the same rate at pH 4.26 as the reaction $HG^+ \rightarrow HJ'$, since the same factor that increases the concentration of the anion of HG^+ also decreases its reactivity.

In the HPLC chromatograms obtained without Hβ, L and L′ are readily measured, but βJ^+ and $\beta J'^+$ are not seen.



Scheme 21.

By a combination of the two sets we can follow four components as functions of time.

Since HEJ^{2+} is decomposed about 33 % faster than HEJ'^{2+} (see below) we can readily detect which of the L peaks corresponds to which βJ^+ peak. From a set of βJ^+, $\beta J'^+$, L and L′ values at different times we can calculate the four rate constants as well as the response values for the four components by our general method. The 'calculated' values were obtained by iterations from $t = 0$ to t in the formulae in eqn. (11) starting from $[HA]_0 = 10^{-5}$ M and by using the formulae given in eqns. (12a)–(12d) we obtained

$$da_i = 2.24\times10^{-3}[HA]_i\,\Delta t \quad (11a)$$
$$db_i = 7.0\times10^{-4}[D^+]_i\,\Delta t \quad (11b)$$
$$dc_i = 1445\,([D^+]_i)^2\,\Delta t \quad (11c)$$
$$dd_i = k_{JL}[HEJ^{2+}]_i\,\Delta t \quad (11d)$$
$$de_i = k_{J'L'}[HEJ'^{2+}]_i\,\Delta t \quad (11e)$$
$$df_i = k_L[L]_i\,\Delta t \quad (11f)$$
$$dg_i = k_{L'}[L']_i\,\Delta t \quad (11g)$$
$$[HA]_{i+1} = [HA]_i - da_i + db_i \quad (11h)$$
$$[D^+]_{i+1} = [D^+]_i + da_i - db_i - 2dc_i \quad (11i)$$
$$[HEJ^{2+}]_{i+1} = [HEJ^{2+}]_i + 0.5dc_i - dd_i \quad (11j)$$
$$[HEJ'^{2+}]_{i+1} = [HEJ'^{2+}] + 0.5dc_i - de_i \quad (11k)$$
$$[L]_{i+1} = [L]_i + dd_i - df_i \quad (11l)$$
$$[L']_{i+1} = [L']_i + de_i - dg_i \quad (11m)$$

$$I_{\beta J} = R_{\beta J}[HEJ^{2+}] \quad (12a)$$
$$I_{\beta J'} = R_{\beta J'}[HEJ'^{2+}] \quad (12b)$$
$$I_L = R_L[L] \quad (12c)$$
$$I_{L'} = R_{L'}[L'] \quad (12d)$$

the constants given in eqns. (13a)–(13h).

$$k_{JL} = 1.28(7)\times10^{-3}\ s^{-1} \quad (13a)$$
$$k_{J'L'} = 0.96(5)\times10^{-3}\ s^{-1} \quad (13b)$$
$$k_L = 2.4(2)\times10^{-5}\ s^{-1} \quad (13c)$$
$$k_{L'} = 2.4(2)\times10^{-5}\ s^{-1} \quad (13d)$$

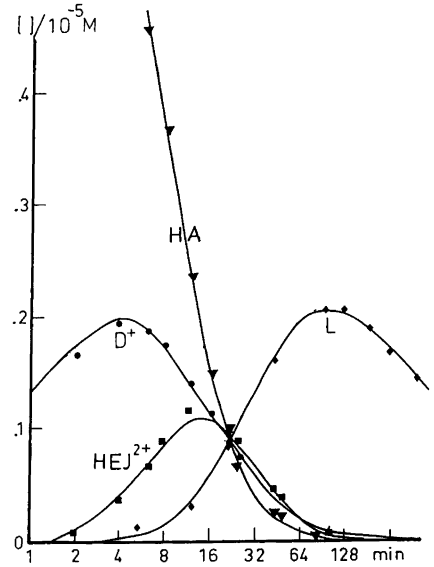


Fig. 4. Concentrations of HA, D^+, HEJ^{2+} and L vs. time for a reaction of omeprazole at pH = 4.26. T = 37 °C.

$$R_{\beta J} = 1.97(9)\times10^5 \quad (13e)$$
$$R_{\beta J'} = 2.04(8)\times10^5 \quad (13f)$$
$$R_L = 3.49(6)\times10^5 \quad (13g)$$
$$R_{L'} = 2.79(6)\times10^5 \quad (13h)$$

These values for R_L and $R_{L'}$ are comparable to those obtained by separate experiments on preparations of L and L' obtained from the mixture by preparative HPLC. These were $R_L = 3.70(12)\times10^5$, $R_{L'} = 2.99(6)\times10^5$, and thus close to those obtained by the regression. This demonstrates that the reactions studied are the main reactions and that side reactions only occur to the extent of about 6–7 %.

Fig. 4 gives the concentrations of HA, D^+, HEJ^{2+} and L from a solution of omeprazole in a buffer as functions of time. The concentrations of L' are almost identical with those of L.

Reactions in 0.001 M HCl. Using the observations at pH 4.26 as a basis, it is possible to tackle the reaction in 0.001 M HCl.

In the chromatograms we do not detect H_2F^+, and the interference from H_2F^+ in the determination of D^+ will thus be unimportant. We have also seen that the interference of H_2CE^{2+} with the determination of D^+ is moderate at pH 4.26 and almost non-existent at pH 2. We can thus assume that the interference at pH 3 will not be significant and that we can use the approximation $[D^+] = [HE\beta^+]$, i.e., the steady-state assumption used on H_2CE^{2+}. This is in agreement with the small differences between the 'concentrations of D^+' measured by Methods A and C in Fig. 1(b).

The reaction was followed starting with a 10^{-4} M solution of omeprazole in 0.001 M HCl (μ = 0.05 M). HA, $HE\beta^+$, βJ^+, $\beta J'^+$ were determined by HPLC. The solution also contained L and L', but it was not possible to determine them in the same run as βJ^+ and $\beta J'^+$. We used eqns. (14a)–(14m) in order to obtain 'calculated values' for use in our standard method of calculation.[10]

$$da_i = k_{AD}[HA]_i\,\Delta t \quad (14a)$$
$$db_i = k_{DA}[HE\beta^+]_i\,\Delta t \quad (14b)$$
$$dc_i = k^*_{DCE}([HE\beta^+]_i)^2\,\Delta t \quad (14c)$$
$$dd_i = k_{JL}[\beta J^+]_i\,\Delta t \quad (14d)$$
$$de_i = k_{J'L'}[\beta J^+]_i\,\Delta t \quad (14e)$$
$$[HA]_{i+1} = [HA]_i - da_i + db_i \quad (14f)$$
$$[HE\beta^+]_{i+1} = [HE\beta^+]_i + da_i - db_i - 2dc_i \quad (14g)$$
$$[\beta J^+]_{i+1} = [\beta J^+]_i + 0.5dc_i - dd_i \quad (14h)$$
$$[\beta J'^+]_{i+1} = [\beta J'^+]_i + 0.5dc_i - de_i \quad (14i)$$
$$I_{\beta J} = R_{\beta J}[\beta J^+] \quad (14j)$$
$$I_{\beta J'} = R_{\beta J'}[\beta J'^+] \quad (14k)$$
$$I_A = R_A[HA] \quad (14l)$$
$$I_{HE\beta} = R_{HE\beta}[HE\beta^+] \quad (14m)$$

In this way we obtained for deuteriated omeprazole the results $k_{AD} = 4.1(4)\times10^{-3}\ s^{-1}$, $k_{DA} = 8(6)\times10^{-4}\ s^{-1}$, $k^*_{DCE} = 128(25)\ M^{-1}\ s^{-1}$, $k_{JL} = 1.9(2)\times10^{-3}\ s^{-1}$, and $k_{J'L'} = 1.2(1)\times10^{-3}\ s^{-1}$.

For omeprazole we had fewer observations, but from a plot of the concentrations versus time we could see that the values for HA are identical with those for the deuterium compound. The same is valid for $HE\beta^+$, but the values obtained for βJ^+ ($\beta J'^+$) differed, in agreement with the isotope effect mentioned below. In the equations above we therefore introduced the values obtained for k_{AD} and k_{DA} together with all response values. The three remaining constants k^*_{DCE}, k_{JL} and $k_{JL'}$ were then calculated for the protium compound from the limited amount of data available. For the protium compound we thus obtained $k^*_{DCE} = 145(13)\ M^{-1}\ s^{-1}$, $k_{JL} = 1.48(9)\times10^{-3}\ s^{-1}$, $k_{J'L'} = 0.97(6)\times10^{-3}\ s^{-1}$. The apparent difference between the rate constants for the protium and deuterium compounds is hardly significant due to the limited amount of data.

Since a proton is probably involved in the rate-limiting step of the conversion of HEJ^{2+} into K^+, we might expect a large isotope effect for k_{JL} and $k_{J'L'}$, but we find no significant effect. This could be explained by a readily occurring proton exchange in HEJ^{2+} as explained above (under the heading: Expected Reactions of HEJ^{2+} and HEJ'^{2+}).

Reactions at pH 6.26. The reactions of D^+ were also followed at pH 6.26 by preparing D^+ from omeprazole and 0.01 M HCl for 10 min. A buffer was then added to bring the pH to 6.26. Aliquots were then withdrawn at intervals. The amount of remaining D^+ was determined by converting it into $HE\beta^+$ by the addition of a slight excess of Hβ. The mixture was then analyzed by HPLC. We observed that the reaction rate was strongly concentration dependent. The concentration of $HE\beta^+$ was thus reduced to half its original value in a few seconds when a 10^{-5} M solution of omeprazole was used. If a 10^{-6} M solution was used, this time was increased to about 1 min. This means that the rate-limiting step is a second-order reaction, and thus is probably the step $D^+ \rightarrow H_2CE^{2+}$. A calculation based on

Table 5. Summary of rate constants calculated for the decomposition of omeprazole at different pH values. Rate constants defined in the reaction scheme in the text.

pH	k_{AD}	k_{DA}	k_{DF}	k^*_{DCE}	k_{JL}	$k_{J'L'}$	k_L	$k_{L'}$	k_{EEE}/k_{ES}
	10^{-3} s^{-1}	10^{-4} s^{-1}	10^{-4} s^{-1}	M^{-1} s^{-1}	10^{-3} s^{-1}	10^{-3} s^{-1}	10^{-5} s^{-1}	10^{-5} s^{-1}	10^4 M^{-1}
1.06	9.8	13.4	1.54	2.5					
2.06	5.86	7.0	0.62	7.4					1.2
3.06	4.03	7		145	1.48	0.97			
4.26	2.24	7		1450	1.28	0.96	2.39	2.40	
6.26				1.5×10^4					

this assumption gave the constant $k^*_{DCE} = 1.5(2)\times10^4$ M^{-1} s^{-1} for the disappearance of HEβ$^+$. This is about ten times higher than the value for k^*_{DCE} obtained at pH 4.26, and is in agreement with a base-catalyzed mechanism for the conversion $D^+ \rightarrow H_2CE^{2+}$. The subsequent reactions of H_2CE^{2+} must be very fast, since otherwise H_2CE^{2+} would accumulate, and on the addition of Hβ it would give some HEβ$^+$. The disappearance of HEβ$^+$ would in that case not follow second-order kinetics.

Reactions at pH 5.25. The degradation of omeprazole has also been followed at pH 5.25. In this case L,L′ and HS are the dominant reaction products. On the addition of Hβ we observed that HEβ$^+$ and βJ$^+$ were present in low concentrations only and that L and L′ reacted with Hβ, to give a compound with a peak in the chromatogram close to the front. The area under this peak correlated well with the area under the L+L′ peaks. The concentration of HS was found to correlate well with the amount of L and L′ formed from HEJ^{2+} and HEJ'^{2+}. (The total amount of L and L′ plus the amounts of L and L′ decomposed in a slow first-order reaction). Due to the low concentrations of HEβ$^+$, βJ$^+$ and βJ′$^+$ observed (near the limits of detection), no other rate constants except k_{AD} could be calculated.

The rate constants obtained at different pH values are summarized in Table 5.

Isotope effect studies

The reaction $H_2CE^{2+} \rightarrow HG^+ + HC^+$ is the only reaction starting from H_2CE^{2+} that involves breaking of a C–H bond. If hydrogen in this bond is replaced by deuterium a difference in the rate of this step is expected, but not in the other steps. H_2CE^{2+} may be involved in the reactions in Scheme 22.

The differential equation according to eqn. (15) should be valid for the concentration of H_2CE^{2+}

$$d[H_2CE^{2+}]/dt = k_2[D^+]^2 + k_5[HG^+][HC^+] - (k_3 + k_4 + k_H)[H_2CE^{2+}] \quad (15)$$

If H_2CE^{2+} is present in low concentration and the reaction $HG^+ + HC^+ \rightarrow H_2CE^{2+}$ is negligible compared with the reaction $D^+ \rightarrow H_2CE^{2+}$ the steady-state approximation gives eqn. (16).

$$[H_2CE^{2+}] = k_2[D^+]^2/(k_3 + k_4 + k_H) \quad (16)$$

HEJ^{2+} and HEJ'^{2+} are the first observable compounds (in the form βJ$^+$ and βJ′$^+$) formed from H_2CE^{2+} and an isotope effect can be expected on their rates of formation. We denote the results obtained with the H and D compounds by the subscripts H and D, and limit our observations to the early stages of the reaction. Now we can neglect the rate of degradation of HEJ^{2+} and HEJ'^{2+} and we obtain eqns. (17) and (18). In the experiments we have observed that for

$$d[HEJ^{2+}]_H/dt = k_H[H_2CE^{2+}]_H = k_2k_H[D^+]^2_H/(k_3 + k_4 + k_H) \quad (17)$$

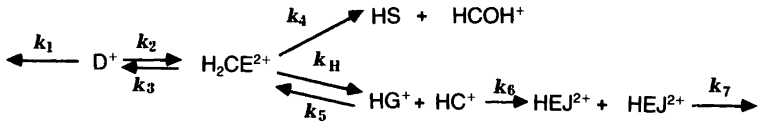
$$d[HEJ^{2+}]_D/dt = k_D[H_2CE^{2+}]_D = k_2k_D[D^+]^2_D/(k_3 + k_4 + k_D) \quad (18)$$

each reaction time t, the concentrations $[HE\beta^+]_H$ and $[HE\beta^+]_D$ are identical during the part of the reaction that is experimentally accessible. The same should thus be the case for $[D^+]_H$ and $[D^+]_D$, and we obtain eqn. (20). Depending on the relative size of $k_3 + k_4$ and k_H or k_D we can have the two extreme cases shown in eqns. (21) and (22).

$$(d[HEJ^{2+}]_D/dt)/(d[HEJ^{2+}]_H/dt) = k_D(k_3 + k_4 + k_H)/\{k_H(k_3 + k_4 + k_D)\} \approx [\beta J^+]_D/[\beta J^+]_H \quad (20)$$

$$k_3 + k_4 \gg k_H > k_D \quad (21)$$

$$k_3 + k_4 \ll k_D > k_H \quad (22)$$



Scheme 22.

Table 6. Ratio of concentrations D/H of βJ^+, $\beta J'^+$ and L, L' obtained under different experimental conditions.

pH	Exp. conditions[a]	*t*/s	Ratio for			
			βJ^+	$\beta J'^+$	L	L'
2.12	A, 10^{-5} M	1800	0.73	0.67	0.64	0.67
		3600	0.67	0.69	0.67	0.67
		5400	0.50	0.63	0.64	0.65
		7200	0.67	0.60	0.64	0.66
4.2	B, 10^{-5} M	30	0.84	0.88		
		60	0.87	0.90		
4.4	B, 10^{-4} M	2	0.73	0.68		
		5	0.57	0.57		
		10	0.81	0.67		

[a] A: Omeprazole or deuteriated compound dissolved in HCl or a buffer. After *t* s at 37 °C a ten-fold excess of Hβ and a buffer (pH 6.5) were added. The solution was then analyzed with reversed phase HPLC. B: Omeprazole or deuteriated compound dissolved in 0.01 M HCl and stored for 10 min at 37 °C. A buffer giving the pH value indicated was then added and after *t* s Hβ and a buffer were added and the solution analyzed as above.

In the first case, eqn. (21), we obtain $[\beta J^+]_D/[\beta J^+]_H = k_D/k_H$ and can expect an isotope effect with a minimum value of $k_D/k_H \approx 1/7$. In the second case, eqn. (22), we obtain $[\beta J^+]_D/[\beta J^+]_H = 1$ and cannot expect any isotope effect at all.

The results obtained under different conditions are given in Table 6, and we can see a significant isotope effect on the formation of HEJ^{2+} and HEJ'^{2+}. The $[\beta J^+]_D/[\beta J^+]_H$ ratio of about 0.7, introduced together with $k_H = 7k_D$, gives a value of $(k_3 + k_4) = 0.08\ k_H$. From measurements[2] on the hydrolysis of phenyl benzenethiosulfinate we know that k_3 and k_4, in the reaction with OH^-, have the same magnitude (140 M^{-1} s^{-1} and 170 M^{-1} s^{-1}) and the same is probably true for H_2CE^{2+}. This means that for the CH_2 compound, at most 4 % H_2CE^{2+} is converted into the sulfinic acid COH and HE^+, which in turn is converted into HS or H_2EE^{2+}. Since the reaction $HG^+ + HC^+ \rightarrow H_2CE^{2+}$ is neglected in the deduction above, this percentage may even be less. This means that the reaction $H_2CE^{2+} \rightarrow HEJ^{2+}$ can be expected to proceed with a high yield, which is in agreement with the experimental findings.

Since the rates of formation of L and L' are proportional to the concentrations of HEJ^{2+} and HEJ'^{2+}, respectively, the isotope effect on the relative concentrations of L and L' will be similar to that for HEJ^{2+} and HEJ'^{2+}, which is also observed at pH 2 (Table 6).

At pH 5.26 we can see a peak in the HPLC chromatogram corresponding to a compound much more hydrophilic than omeprazole showing D/H ≈ 1.7, a value which means that the corresponding compound is formed in a reaction that strongly competes with the reaction $H_2CE^{2+} \rightarrow HG^+$. The compound is thus probably the sulfinic acid $HCOH^+$ or some of its reaction products. The compound corresponding to the peak has not been identified.

Reaction of H_2CE^{2+} to HG^+

The isotope experiments showed a significant isotope effect in the formation of HEJ^{2+} and HEJ'^{2+} from D^+. The reason for this is that a C–H bond is broken in the step $H_2CE^{2+} \rightarrow HG^+$. A full isotope effect is, however, not obtained and the kinetically most important step in the conversion $D^+ \rightarrow HEJ^{2+} + HEJ'^{2+}$ is the step $D^+ \rightarrow H_2CE^{2+}$. This step is a second-order reaction in D^+ with a rate constant k_{DCE} = 1450 M^{-1} s^{-1} at pH 4.3 which means that the concentration of D^+ is reduced from 10^{-5} to one half this value in about 30 s. Since the step $H_2CE^{2+} \rightarrow HG^+$ must be faster than this it will have a half life of a few seconds. At pH 4.3 $[OH^-]$ is about 10^{-10} M. A reaction of H_2CE^{2+} with OH^- of this concentration must have a rate constant of at least 10^9 to proceed with that rate, which seems to be unrealistically high.

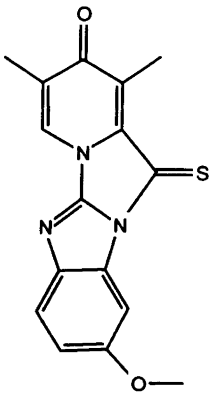
A weaker base may be present in a higher concentration, but should also react more slowly unless it is sterically very favoured. A sterically favoured base can, however, be found. From measurements on $HE\beta^+$ we know that the proton on the benzimidazole nitrogen atom can be released with a pK_a of 6.[5] A similar proton is present on the E part of H_2CE^{2+}. At pH 4.2 H_2CE^{2+} is thus present to more than 1 % as the anion in a rapid equilibrium with the main form. This anion is in perfect position for the abstraction of a proton in a reaction giving $HG^+ + HC^+$, as already demonstrated in Scheme 7. This explains the high rate of the reaction $H_2CE^{2+} \rightarrow HG^+$. It is also in agreement with the observation that the thiosulfinate from the *N*-methylated compound, which cannot form the corresponding anion, reacts much less readily to the corresponding thioaldehyde. In fact, the *N*-methylated thiosulfinate can be observed by NMR spectroscopy.[4]

Structural proofs for L and L'

The structural formulae of L and L' are given in Scheme 23. The structural assignments were based on the following observations. The two isomers were obtained from a reversed phase HPLC separation as one yellow compound and one pink compound. NMR data for the two compounds are given in Table 7.

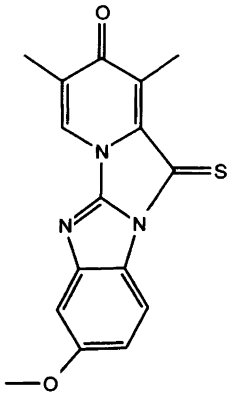
According to the structure given for L' the proton in the 10-position should be shifted strongly downfield due to the anisotropy of the C=S group. It should also appear as a doublet due to coupling with the proton in the 8-position (J_{obs} = 2.5 Hz). The proton in the 7-position should be found at a higher field than the proton in the 10-position and should appear as a doublet due to the coupling with the proton in the 8-position (J_{obs} = 8.7 Hz).

The proton in the 10-position of structure L should also appear at lower field than the proton in the 7-position in L.



L'
Pink

8-Methoxy-2,4-dimethyl-5-thioxo-5-hydro-pyrido [1',2':3,4]imidazo[1,2-*a*] benzimidazol-3-one



L
Yellow

9-Methoxy-2,4-dimethyl-5-thioxo-5-hydro-pyrido [1',2':3,4]imidazo[1,2-*a*] benzimidazol-3-one

Scheme 23.

However, in this case the coupling constants are J_{obs} = 8.9 Hz and J_{obs} = 2.4 Hz, respectively. The upfield shifting effect of the OCH_3 group on the signal from the proton in position 10 is greater when the OCH_3 group is in position 9. In agreement with this, the 10-proton in L' will appear at a higher field than that of compound L, and the proton in position 7 will appear at a higher field in L.

A further indication that the pink compound L' has the given structure comes from its colour. The electron pair of the OCH_3 group in L' is in conjugation with the double bonded nitrogen. This is not the case in the structure given for L. In accordance with this structure L' should absorb at higher wavelength than structure L, which is also the case.

Experimental

Preparation of the blue compound. Omeprazole (60 mg) was dissolved in a small volume of methanol and added to 200 ml of a buffer, pH 5.3. The resulting suspension was stirred for 20 h. The dark blue compound was then collected by filtration and dried at reduced pressure. The yield was 40 mg. This product is obviously not pure, but all attempts to purify it failed.

Preparation of $HE\beta^+$. Omeprazole (5 mmol) was dissolved in 50 ml of 0.2 M methanolic HCl at room temperature. After 10 min, 2-mercaptoethanol (5.1 mmol) was added, and after 5 min 6.3 mmol of 75 % $HClO_4$. The resulting mixture was carefully evaporated behind a safety shield. The residue was dissolved in CH_2Cl_2 and washed with three portions of water. On evaporation, an amorphous mass was obtained. This product was reasonably pure (> 95 %), as demonstrated by HPLC.

Preparation of H_2EE^{2+}. Omeprazole (1 g) was dissolved in 70 ml of 0.1 M HCl. After 75 min, a few milliliters of 70 % HPF_6 was added. The product was collected by filtration, washed with water and dried under reduced pressure. The yield was about 1 g. This product contained mainly H_2EE^{2+}, but also small amounts of other compounds. It slowly decomposed when allowed to stand. It is soluble in acetonitrile, chloroform and acetone and the solutions are stable for many hours. All attempts to obtain a pure product by recrystallization failed.

Preparation of JJ from timoprazole. Timoprazole (10 g, 0.039 mol) was stirred for 45 min with 500 ml of 0.1 M H_2SO_4. 3,5-Dimethylcyclohexyl hydrogen sulfate (10 g), dissolved in 500 ml of CH_2Cl_2, was added and the mixture was stirred for 30 min. The layers were separated and the CH_2Cl_2 layer was dried with Na_2SO_4 and added to a column containing 500 g of silica gel. The red compound was collected on the top and then eluated with CH_2Cl_2 containing 4 % CH_3OH by volume. The solvents were evaporated, to yield 4.2 g of the crude disulfide JJ. The product can be recrystallized from 2.5 l of methanol.

Preparation of deuteriated omeprazole. Omeprazole (1 g) was added to 5 ml of D_2O and 300 mg 40 % NaOD in D_2O were added. The solution was allowed to stand for 4 days (probably an unnecessarily long period). The solution was washed with an equal volume of $CHCl_3$, which was discarded. Another equal volume of $CHCl_3$ was added then the mixture was neutralized to pH = 9 with, vigorous

Table 7. NMR data for compounds L and L'. Chemical shifts are given in ppm downfield from Me_4Si. Solvent $CDCl_3$.

Pink compound (L')				Yellow compound (L)			
Group	δ / ppm	Type	J_{obs} / Hz	Group	δ / ppm	Type	J_{obs} / Hz
1-CH_3	2.62	s		1-CH_3	2.61	s	
3-CH_3	2.13	s		3-CH_3	2.12	s	
4-H	7.91	s		4-H	7.89	s	
7-H	7.47	d	8.7	7-H	7.12	d	2.4
8-H	6.93	q	2.5, 8.7	8-OCH_3	3.87	s	
9-OCH_3	3.88	s		9-H	6.86	q	2.4, 8.9
10-H	7.73	d	2.5	10-H	8.0	d	8.9

stirring, by the addition of about 2.9 ml of 1 M DCl. The layers were separated. The chloroform layer was dried and evaporated. About 10 ml of CH_3CN were added to the residue, which dissolved the oil, and white crystals separated when the solution was cooled in ice. The yield was 0.8 g and an analysis by NMR spectroscopy indicated 92.5 % isotopic purity in the CD_2SO group. Repetition of this procedure gave a product of 97 % isotopic purity in the CD_2SO group.

Preparation of L and L'. Omeprazole (1 g) was added to 20 ml of methanol. HCl (0.1 M, 50 ml) was then added with stirring and the mixture was stirred for 75 min. The orange product, consisting of L and L', was collected by filtration, washed with water and dried in the air. The yield was about 30 mg.

Separation of L and L'. The mixture of L and L' was dissolved in methylene chloride (5 mg ml^{-1}) and injected in 1 ml portions onto a preparative HPLC column (LiChrosorb SI 60, 7 μm, 250×10 mm). Mobile phase: CH_2Cl_2 with 0.3 % CH_3OH; flow rate 5 ml min^{-1}. The yellow compound (t_R 20 min) eluted first, followed by the pink compound (t_R 23 min). Base-line separation was achieved. The pure compounds were obtained after evaporation of the solvents at reduced pressure.

Reactions in dilute HCl. The procedure described in Ref. 10 was followed. The peak at t_R = 11.4 in the HPLC chromatogram was obtained with the same area even if Hβ was not added before the analysis.

Reactions at pH ca. 4.2. Degradation of the sulfenamide D^+. A solution of D^+ was prepared at 37 °C by adding 300 μl of a 3.3×10^{-4} M solution of omeprazole in methanol to 10 ml of 0.01 M HCl. After 10 min was added 1 ml of a solution of 0.1 mmol NaOH in a Tris-acetic acid buffer pH 4.3, μ = 1.7 M. The resulting solution was analyzed at different times *t* by Method A, B, C or D.

Methods for analysis of D^+. Methods A and B are described in Ref. 10. In Method C the absorbance at 355 nm of one portion of a solution was continuously followed as in Method B. At intervals, 3 ml portions of the solution were withdrawn and 30 μl of a 10^{-2} M solution of Hβ were added. The solutions were rapidly mixed and the absorbance at 355 nm followed for 1 min. The absorbances thus obtained were plotted against time after the addition of Hβ and the straight line part of the plot was extrapolated back to the time of addition of Hβ. This absorbance was corrected for the volume change and subtracted from the value for the absorbance at the same time without the addition of Hβ. If the pH of the original solution was different from 4.2, Hβ was dissolved in a strong buffer to give that pH on addition. In Method D 30 μl of a 10^{-2} M solution of Hβ were added to 3 ml of a solution of D^+ at pH ca. 4.2. After 2 s 30 μl of a 1 M solution of acetylcysteine were added. A portion of the resulting solution was injected as rapidly as possible onto a HPLC column and analyzed for its $HE\beta^+$ content.

Analysis for βJ^+, $\beta J'^+$, L, and L'. With a Brownlee OS-GU, RP-8, 5 μm (30×4.6 mm ID) precolumn and a Lichrosorb RP-8, 5 μm (125×4 mm ID) column and the mobile phase 45 % acetonitrile, 10 % of a phosphate buffer pH 7.0, μ = 0.25 M and 45 % water the retention times were L' 12.0 min, L 12.8 min, βJ^+ 13.8 min, and $\beta J'^+$ 14.4 min. When Hβ was added before the analysis, the areas for L and L' were considerably smaller than those obtained without Hβ, in agreement with the reactivity of L and L' towards Hβ. The peaks corresponding to βJ^+ and $\beta J'^+$ could, of course, not be observed without the addition of Hβ.

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