Chemical Reactions of Omeprazole and Omeprazole Analogues. I. A Survey of the Chemical Transformations of Omeprazole and its Analogues#

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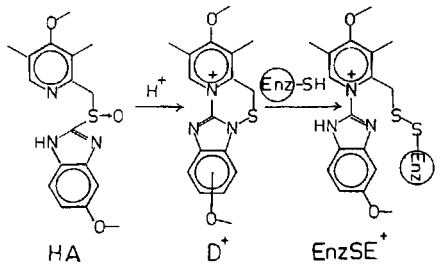
The chemical reactions of the H^+,K^+-ATPase inhibitor omeprazole, 5-methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinylmethylsulfinyl)-1*H*-benzimidazole, and analogues of omeprazole have been studied. An overview of the reactions both in the presence and absence of 2-mercaptoethanol is given.

5-Methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinylmethylsulfinyl)-1*H*-benzimidazole known under the INN name omeprazole (HA), a potent antiulcer agent,[6,7] is at present undergoing extensive clinical evaluation. This compound and several close analogues are effective inhibitors of gastric acid secretion in the rat, dog and man.[8–10] Unlike currently used acid-secretion inhibitors such as cimetidine they act by being inhibitors of gastric H^+, K^+ ATPase.[11] This enzyme is responsible for gastric acid production, and is located in the secretory membranes of the parietal cells.[12,13] Omeprazole itself is not an active inhibitor of this enzyme, but is transformed within the acid compartments of the parietal cells into the active inhibitor, close to the enzyme.[14]

The active inhibitor has been shown to be a cyclic sulfenamide D^+ (two isomers), which reacts with mercapto-groups in the enzyme with the formation of a disulfide complex ($EnzSE^+$), thus inactivating the H^+, K^+-ATPase (Scheme 1). These reactions leading to the blockade have been studied both *in vivo* and *in vitro*.[15]

Omeprazole has a very high specificity in its action, due to the following factors:

1. Omeprazole is a *weak base* that accumulates in the cells of the body with the lowest pH, the parietal cells.
2. It is *converted into the active blocker* in the acidic region close to the target enzyme.
3. In the neutral part of the body omeprazole has *good stability* with very slow conversion into the active blocker. The minute quantities that might be produced there are immediately rendered harmless by reaction with the endogenous thiol glutathione.
4. The active blocker is a *permanent cation* which cannot easily penetrate the cell membranes of the parietal cells and other cells.



Scheme 1.

For a correct understanding of the underlying chemical conversions an extensive investigation of the reactions of omeprazole has been performed. This includes isolation, structure elucidations and physico-chemical characterization of both intermediates and products, as well as a thorough kinetic investigation of omeprazole and some of its close analogues. References to accompanying papers that include thorough treatments of specific problems are given in parentheses. Since many of the results have been obtained from highly complex kinetic investigations, a special appendix (part II[1]) is devoted to the problem of calculating rate constants.

The reactions were carried out both in the presence of

* With reference to the following articles.[1–5]

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thiols, where the conversions are fairly simple, and without added thiols where the reactions are much more complex.

Symbols. In this and the following papers we have used symbols HA, HB^+, HC^+ etc. for specific compounds. The same symbols are also used for classes of compounds differing only in the substitutes of the pyridine ring and the benzimidazole ring. Symbols containing two or more letters are also used in the same way. Here each letter refers to a residue with that general structure. A compound H_2CE^{2+} thus means a molecule in which an HC^+ residue is combined with an HE^+ residue (usually by a S–S link). The Greek letter β in a formula indicates a $HOCH_2CH_2S$ residue. In the text, Hβ means the molecule $HOCH_2CH_2SH$. In the Schemes both the symbols and the structures will be given whenever possible.

When a specific form of a compound is considered this is indicated by the addition of protons and charges. In the reaction of omeprazole as an acid able to release a proton from the benzimidazole NH group, the reaction is symbolized by the reaction $HA \rightleftharpoons A^- + H^+$. Omeprazole can also accept a proton on either the pyridine ring or the benzimidazole ring. This is symbolized by the reactions

$$HAH^+ \rightleftharpoons HA + H^+ \rightleftharpoons H_2A^+$$

Acid decomposition of omeprazole in the presence of an added thiol

Isolation and identification of the disulfide $HE\beta^+$. The reaction of omeprazole in an acidic aqueous solution in the presence of Hβ proceeds according to Scheme 2, i.e. the main products formed are the disulfide $HE\beta^+$, the sulfide HS and the disulfide ββ.

The reaction HA → HS via the disulfide $HE\beta^+$ is formally a reduction of the sulfoxide HA to the sulfide HS by Hβ, with the simultaneous formation of the disulfide ββ. The structure of the intermediate $HE\beta^+$ has been determined by X-ray crystallography using an analogue of omeprazole (part II[1]).

Kinetically, the transformation HA → $HE\beta^+$ appears to be a first-order reaction in which the rate is proportional to the concentration of HA, but independent of the concentration of Hβ up to the millimolar range. This indicates that the reaction proceeds through an intermediate that is very reactive towards SH groups. This intermediate, D^+, will be discussed below.

At higher concentrations of Hβ, a side reaction,

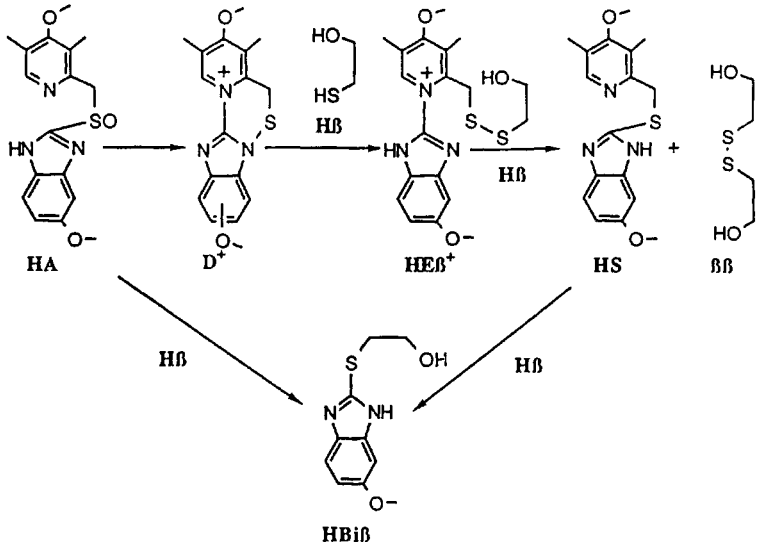
$$HA \xrightarrow{H\beta} HBi\beta,$$

first order in Hβ and HA, is observed (Scheme 2). The structure of HBiβ has been confirmed by direct synthesis. HBiβ can also be formed from the sulfide HS by the reaction

$$HS \xrightarrow{H\beta} HBi\beta \text{ (part IV[3])}$$

Under the conditions used in the ordinary kinetic procedures in this paper ([Hβ] = 10^{-4} to 10^{-3} M), the formation of HBiβ is an unimportant side reaction that can usually be neglected.

If *threo*-1,4-dimercapto-2,3-butanediol (DTT) is used instead of Hβ the only compounds detected (by HPLC) are the starting material HA and the sulfide HS. A compound corresponding to $HE\beta^+$ is thus missing and the rate of formation of HS is exactly equal to the rate of the disappearance of HA. A reasonable explanation for this is that the compound, of type $HE\beta^+$, formed has a SH group in a position perfect for a very rapid intramolecular reaction to give HS and the internal disulfide of DTT, which is also formed. (We have occasionally made use of this reaction to prepare HS for calibration purposes, and for the identification of the peaks corresponding to HS and $HE\beta^+$ in the HPLC chromatograms).

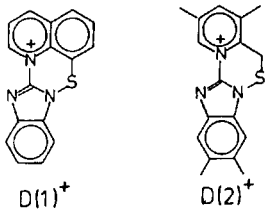


Scheme 2.

Isolation and identification of the key intermediate D^+. The kinetic finding that the formation of $HE\beta^+$ from HA in the presence of $H\beta$ is a first-order reaction with a rate indpendent of the concentration of $H\beta$, indicates that there has to be at least one intermediate, which very rapidly reacts with $H\beta$. Thus, at high dilution (10^{-5} M) in hydrochloric acid, the conversion of HA into an intermediate D^+ could be followed kinetically, both directly by UV spectroscopy and indirectly by HPLC, after having trapped the intermediate as $HE\beta^+$, by the addition of buffer and a slight excess of $H\beta$.

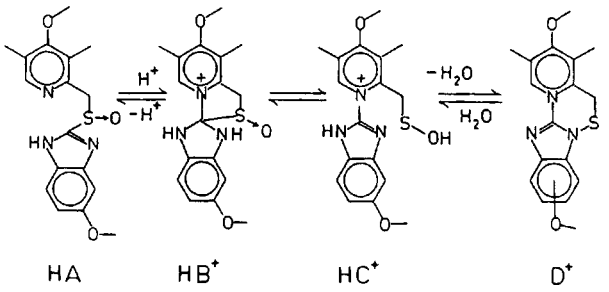
Although the concentration of $HE\beta^+$ is not exactly equal to the concentration of D^+ (part VI[5]), this method enabled us to find conditions for an efficient conversion of HA into D^+.

The intermediate D^+ was significantly more stable if omeprazole was dissolved in methanolic, instead of aqueous HCl. On addition of an acid with a lipophilic anion such as $HClO_4$, HBF_4, HPF_6, or $HAuCl_4$, the corresponding salt of D^+ precipitated from the solution and was obtained in excellent yield. However, the salt of D^+ from omeprazole was found to be a mixture of two isomers owing to the presence of the CH_3O group in the benzimidazole ring. In order to obtain a single compound, symmetrically substituted analogues of omeprazole were used. Thus, we prepared PF_6^- salt of $D(1)^+$ and $D(2)^+$ (Scheme 3) from which we obtained crystals good enough for X-ray crystallography. The X-ray analyses[16] confirmed the permanent cation-containing cyclic sulfenamide structure for D^+.



Scheme 3.

Mechanism for the formation of the sulfenamide D^+. The reaction mechanism we propose for the acid transformation of HA → D^+ is a nucleophilic attack of the pyridine nitrogen on the electron deficient 2-carbon atom of the benzimidazole ring with the assistance of an attack of a proton on the doubly bonded nitrogen atom of the benzimidazole ring. The dihydrobenzimidazole HB^+ thus formed has a high tendency for aromatization. It will react by S–C bond fission to form the sulfenic acid HC^+, or alternatively (see below), by breaking the N^+–C bond to return back to the sulfoxide HA. The reaction $HB^+ \rightarrow HC^+$ can occur with or without the assistance of a proton. The sulfenic acid HC^+, like other sulfenic acids,[17] is a very reactive compound towards nucleophiles and is converted into D^+ in a rapid reversible reaction via an intramolecular attack by the NH group of the benzimidazole. This equilibrium is displaced far to the side of D^+. According to the kinetic data, the reverse reaction of HC^+, i.e., a nucleophilic attack of the sulfenic acid group on the highly electron deficient 2-carbon of the benzimidazole ring, returning to HB^+, also occurs. Thus, the total reaction HA ⇌ HB^+ ⇌ HC^+ ⇌ D^+ (Scheme 4) is fully reversible, and the equilibrium is displaced towards the sulfenamide D^+.



Scheme 4.

A rigorous proof of the reversibility of HA ⇌ D^+ was obtained by dissolving a pure salt of D^+ in dilute HCl. By means of HPLC we demonstrated that this solution contained no HA at the beginning, but after 3 min about 10 % HA was obtained. The composition of the solution is thus almost the same as if we had started form pure HA (part II[2]).

Evidence for the spiro compound HB^+ and the sulfenic acid HC^+ as intermediates. Since none of the intermediates HB^+ or HC^+ has so far been isolated, their existence and structures are totally based on kinetic and mechanistic considerations. Apart from all the kinetic data, providing evidence for the existence of two intermediates, other mechanism-based evidence is available to support the two proposed structures HB^+ and HC^+.

When during the measurement of the pK_a of omeprazole, (part III[2]) two moles of omeprazole were added to one mole of HCl, to give a solution about 10^{-4} M in omeprazole, the rapid increase in pH, due to the neutralization, was followed by a slower increase in pH. The second slow rise in pH indicates that protons are consumed in the initial step of the degradation of omeprazole. A careful analysis showed that one proton is consumed per molecule of omeprazole degraded. This observation clearly indicates the formation of a quaternary pyridinium compound in the first, rate-determining step, thus providing support for the suggested structure of the intermediate HB^+.

Another observation supporting the formation of a pyridinium compound in the rate-determining step is that the logarithm of the second-order rate constant, first order in HA and first order in H_3O^+, for the degradation of omeprazole analogues with different substituents in the 3, 4 and /or 5 positions in the pyridine ring increases linearly with the pK_a of the compound.

A nucleophilic attack of the pyridine nitrogen on the electron-deficient 2-carbon atom in the benzimidazole ring

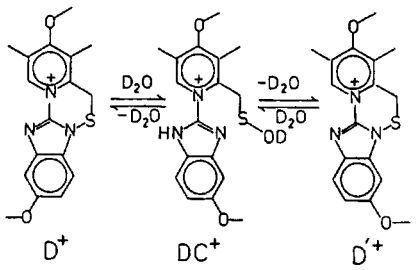
system to form this pyridinium-containing spirocyclic Meisenheimer complex would clearly be facilitated by simultaneous protonation on the doubly bonded benzimidazole nitrogen atom. The ordinary, relatively low nucleophilicity of a pyridine nitrogen atom is here compensated for by the favored steric position for an intramolcular reaction. When the nucleophilic character of the pyridine nitrogen or the favorable steric situation is absent, no reaction occurs. Thus, on elimination of the nucleophilic character by substitution of the pyridine nitrogen atom by making the *N*-oxide or by *N*-methylation, the degradation is inhibited. The same is true for the unfavorable steric situation when the CH_2SO group is moved from the 2- to the 3- or 4-position in the pyridine ring.

The introduction of a methyl group into the 6-position of the pyridine ring blocks the degradation. This also supports the suggested mechanism, since molecular models reveal that the 6-CH_3 group will experience a very strong steric interaction with the imidazole ring. This prevents the formation of the spiro intermediate HB^+. All these observations are in agreement with the assumption that HB^+ is formed in the first step of the degradation of HA.

The formation of the sulfenic acid HC^+ from the spiro intermediate HB^+ in the transformation HA → D^+ is quite logical. The elimination of sulfenic acids from certain sulfoxides is a well-known reaction,[17] and should be particularly favorable in this case due to the aromatization of the benzimidazole. Moreover, sulfenic acids are notoriously unstable compounds and are known to react very rapidly with nucleophiles, e.g. with nitrogen nucleophiles to form sulfenamides.[17] Hence, an intramolecular reaction between the sulfenic acid group in HC^+ with a benzimidazole nitrogen to form the sulfenamide D^+ should be very favorable.

However, sulfenamides also react with different nucleophiles (cf. the reaction with Hβ to $HE\beta^+$ above). Thus, reaction with water gives the reverse reaction $D^+ \rightarrow HC^+$. The reaction $HC^+ \rightarrow D^+$ is also an expected reaction since sulfenic acids are good sulfur nucleophiles and are also known to add reversibly to double bonds to form sulfoxides, a reaction which has been used for trapping sulfenic acids[17] (cf. also the reversible intramolecular addition–elimination reaction of the sulfenic acid moiety in the sulfoxides of penicillins[18]).

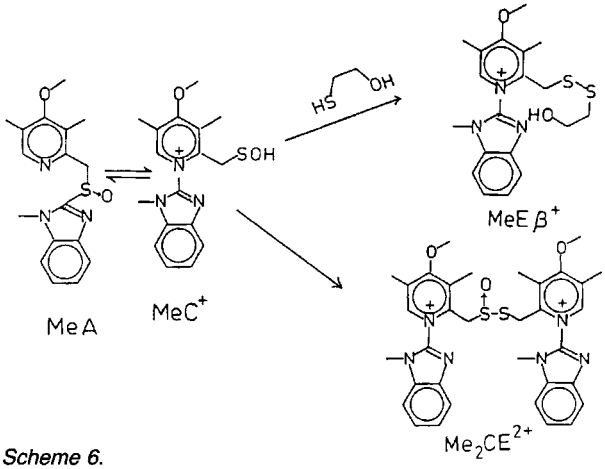
The reversible reaction $HC^+ \rightleftharpoons D^+$ has been studied by ^1H NMR spectroscopy. A crystalline perchlorate of D^+, obtained from a methanolic solution of omeprazole on treatment with $HClO_4$, is a mixture of two geometrical isomers depending on the position of the OCH_3 group in the benzimidazole ring. In CD_3CN no interconversion of these isomers occurs and the ^1H NMR spectrum is in agreement with a 2:3 mixture of isomers, for which an assignment of all protons can be made. In D_2O no sharp peaks are obtained for the benzimidazole protons at room temperature. At −25 °C in CD_3OD the ^1H NMR signals for these protons are as sharp as in CD_3CN. This indicates that there is a rapid interconversion of the two isomers in D_2O and CD_3OD at room temperature but not at −25 °C. From the CD_3OD experiment a pseudo first-order rate constant for the interconversion of the two isomers can be estimated to be about $10^2\ s^{-1}$. The interconversion of the two isomers must involve the breakage of the N–S bond and the formation of a new N–S bond. The obvious intermediate in such a process is the sulfenic acid DC^+ in D_2O and the corresponding sulfenic methyl ester in CD_3OD (Scheme 5).



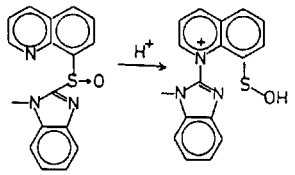
Scheme 5.

The NMR experiment also demonstrates that the equilibrium $HC^+ \rightleftharpoons D^+$ is displaced far to the side of D^+ in methanol and probably also in water. The same type of experiment has also been reported by Senn-Bilfinger *et al.*[19]

A methyl group on the benzimidazole nitrogen efficiently blocks the formation of the sulfenamide D^+ from HA by blocking the step $HC^+ \rightarrow D^+$. It also dramatically changes the rate of decomposition of HA (part V[4]). Thus, *N*-methylated omeprazole MeA is stable in acidic solutions at low concentrations. However, in the presence of Hβ, the disulfide $MeE\beta^+$ is formed, even more rapidly than $HE\beta^+$ is formed from omeprazole under the same conditions. Moreover, at higher concentrations in the absence of Hβ there is a significant rate of decomposition of MeA. The only reasonable explanation is that the sulfenic acid MeC^+ is formed in a fast equilibrium with the sulfoxide MeA, but the equilibrium is displaced far to the side of MeA. In the presence of Hβ, however, the sulfenic acid reacts fast enough with Hβ to compete with the reverse reaction, $MeC^+ \rightarrow$ MeA, and MeC^+ is trapped as the disulfide



Scheme 6.



Scheme 7.

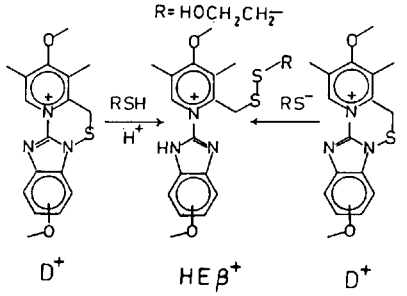
$MeE\beta^+$. The dimerization of MeC^+ to a thiosulfinate Me_2CE^{2+} is second order in MeC^+, and at low concentrations of MeC^+ it is thus very slow and cannot be seen. The compound MeA, therefore appears to be stable if low starting concentrations of MeA are used. At higher concentrations in the absence of Hβ, the concentration of the sulfenic acid is higher and the rate of the second-order reaction increases so that this reaction can compete with the first-order reaction back to MeA, MeC^+ will thus undergo dimerization to the thiosulfinate Me_2CE^{2+} (Scheme 6; for further details of the NCH_3 analogues see part V[4]).

It may be concluded that the decomposition chemistry of *N*-methylated sulfoxides described above provides firm support for the existence of a sulfenic acid intermediate.

If the carbon atom in the CH_2SO chain of the *N*-methylated sulfoxide is included in an aromatic ring, as in the compound in Scheme 7, the corresponding sulfenic acid will be considerably stabilized.[17] In this case, an $AuCl_4^-$ salt could be isolated from the decomposition. The ^1H NMR spectrum was in agreement with that expected from the sulfenic acid structure, but contained insufficient information to be conclusive. Unfortunately we have not been able to obtain crystals good enough for an X-ray crystallographic structure confirmation.

Reaction of the sulfenamide D^+ with Hβ: a model reaction for the enzyme-inhibition reaction

Base- and acid-catalyzed reactions. The UV spectrum of D^+ is significantly different from HA and $HE\beta^+$ and can therefore be used to follow the reaction $D^+ + H\beta \rightarrow HE\beta^+$. In this way we have demonstrated that D^+ is extremely reactive towards thiolates, RS^-, i.e. in a *base-catalyzed* reaction with RSH. Interestingly, D^+ also reacts very rapidly with RSH in an *acid-catalyzed* reaction (Scheme 8) (part IV[3]).



Scheme 8.

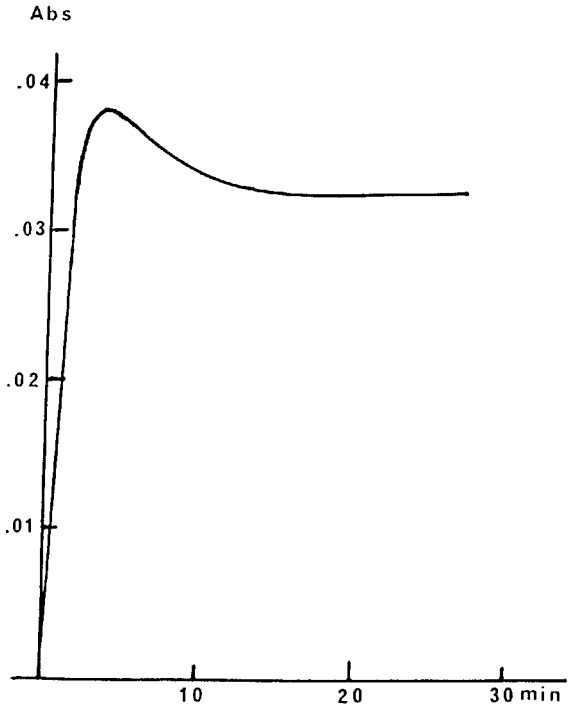


Fig. 1. Absorbance at 355 nm versus time for a solution of omeprazole (10^{-5} M) and 2-mercaptoethanol (10^{-4} M) in 0.01 M HCl.

Thus the rate of the second-order reaction of D^+ with Hβ has a minimum at pH 2. At this pH, in the reaction of HA to D^+ in the presence of a low concentration of Hβ (10^{-4} M), the rate of formation of D^+ from HA will be of the same magnitude as the rate of the further reaction of D^+ with Hβ. This can be seen in Fig. 1 where the absorbance of the solution at 355 nm is followed as a function of time. The spectra of HA, βE and D^+ are given in Appendix 2 in Part II.[1]

At the start of the reaction we have HA with zero absorbance and at the end, $HE\beta^+$ with absorbance = 0.033. The curve has a broad maximum at about 3 min since the molar absorptivity of D^+ is higher than that of $HE\beta^+$. This maximum disappears if the concentration of Hβ is increased or if the pH is increased or decreased. At the normal concentrations of Hβ (10^{-3} M) in the studies of the reaction of HA → D^+, the rate of the reaction of D^+ with Hβ can be regarded as very high compared with that of the reaction HA → D^+.

The enzyme reaction. The fact that the reaction between D^+ and Hβ is acid-catalyzed at pH < 2 means that D^+ can react with the SH groups of H^+, K^+–ATPase in a rapid reaction even at the low pH of the acidic compartment of the parietal cell. This might be of outstanding importance to the biological effects. Since there is rapid transport of the acid secretion from the parietal cell, the molecules of D^+ formed have a very short time to react with the SH groups of the enzyme before they are transported away (and eventually converted into the sulfide HS by the reac-

tion with SH groups in the acidic stomach contents, and then with more SH groups in the neutral contents of the intestine).

The rate of the transformation of omeprazole and its analogues into the corresponding sulfenamides D^+

The rate of formation of D^+ from HA and its variation with substituents in the pyridine and benzimidazole ring is of fundamental importance for the biological effects of omeprazole and its analogues, and has therefore been extensively studied (part II[1]).

The kinetics observed in the presence of Hβ using HPLC or a photometric method. We preferred to study the rate of the reaction HA → D^+ in the presence of Hβ for two reasons. Firstly, the addition of Hβ to the reaction medium resulted in considerable simplification of the HPLC analyses. The only peaks observed under these conditions were those corresponding to HA, $HE\beta^+$ and HS. In rare cases, a small peak corresponding to HBiβ was also observed. If Hβ is absent the HPLC traces are very complicated. Secondly, the addition of Hβ converts the reversible reaction HA ⇄ D^+ into an irreversible reaction HA → $HE\beta^+$ with the same rate as that of the forward reaction HA → D^+. Kinetically, an irreversible reaction is much simpler to study than a reversible one.

The reaction HA → $HE\beta^+$ → HS was usually followed by HPLC, which enabled measurements of all the three components as a function of time. This had a stabilizing effect in the calculation of the rate constants and therefore resulted in rate constants of good reproducibility. (Appendix 1 in part II[1]). In a few cases, a photometric method with Hβ was used. (Appendix 2 in part II[1]). This method enables measurements of rate constants for rapid reactions.

The study, using the considerably simplifying addition of Hβ, enabled us to investigae the kinetics of the reaction HA → D^+ as well as of the steps HA ⇄ HB^+ and HB^+ ⇄ HC^+, but resulted in loss of information about the reactions of HC^+ and D^+. For the necessary study of the reactions of these compounds in the absence of Hβ, methods for measuring the concentrations of D^+ (or, more exactly, the equilibrium mixture D^+ ⇄ HC^+) have been developed. Extensive discussions of the reactions involved in the transformations HA ⇄ HB^+ ⇄ HC^+ ⇄ D^+ and of the reaction $HE\beta^+$ → HS can be found separately (parts II[1] and part IV[3]).

It has been demonstrated (part II[1]) that the rate-limiting step in the conversion of HA into D^+ is the step HA → HB^+. The rate of the reaction HA → D^+ is, however, also dependent on the fraction of HB^+ formed from HA that is converted into HC^+ (the rest goes back to HA). This fraction is pH dependent since the reaction HB^+ → HC^+ can be both uncatalyzed and acid-catalyzed, whereas the reaction HB^+ → HA is uncatalyzed.

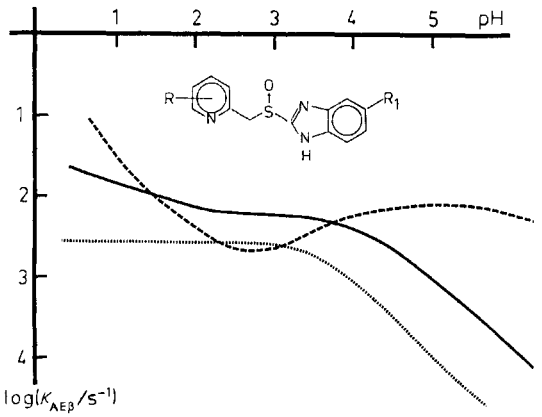


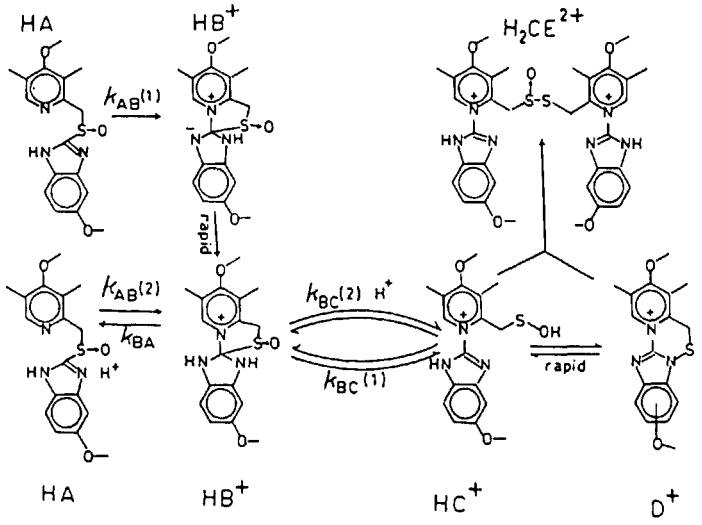
Fig. 2. Rate constants versus pH for omeprazole (——) and two analogues: R = 3,5-$(CH_3)_2$, 4-OCH_3, R_1 = NO_2 (-----); R = 4,5-$(CH_3)_2$, R_1 = OCH_3 (·····).

pH *Dependence of the reaction.* The kinetic data show that the rate constant for the reaction HA → $HE\beta^+$ (also representing the rate constant for the reaction HA → D^+) is strongly dependent on the pH of the solution, which can be seen from a plot of the logarithm of the first-order rate constant, $\log(k_{AE\beta}/s^{-1})$ versus pH of omeprazole and two analogues (Fig. 2).

It can also be seen from Fig. 2 that the rate constants for the different omeprazole analogues HA are quite dependent on the substitution pattern in the two aromatic rings. The substituents in the pyridine have a large influence on the *position* of the curve in the diagram, while the substituents in the benzimidazole ring have a large influence on the *shape* of the curve. Our proposed reaction mechanism, based on careful kinetic analyses, is shown in Scheme 9.

These analyses revealed three regions in the pH dependence of the reaction. At very low pH (<2) the reaction is acid catalyzed because both HA → HB^+ and HB^+ → HC^+ are acid catalyzed. In the pH region 2 < pH < pK_a (1) the reaction HA → HB^+ is acid catalyzed and the reaction HB^+ → HC^+ uncatalyzed. At high pH both the reaction HA → HB^+ and HB^+ → HC^+ are uncatalyzed. Depending upon the relative magnitudes of the rate constants involved, different compounds show different pH profiles.

Variation of the substituents in the pyridine ring. From the mechanistic investigation of the conversions HA ⇄ HB^+ ⇄ HC^+ ⇄ D^+, we have seen that the reaction HA → D^+ at pH values above 2 is well approximated by a reaction A ⇄ D that passes through a transition state similar to HB^+. The reaction can be both uncatalyzed (rate constant k_n) and acid catalyzed (rate constant k_a). A positive charge is formed on the pyridine nitrogen atom in both the acid-catalyzed reactions and in the uncatalyzed reaction. The same occurs on protonation. Substituents in the pyridine



Scheme 9.

ring should therefore have the same influence on both reactions, and the reaction rates should correlate well with the $pK_{H_2A^+}$ values of the pyridine ring. This is also the case, which has been demonstrated by high regression coefficients (part II[1]). The pK_{H_2A} values have been measured or calculated (part III[2]).

Variation of the substituents in the benzimidazole ring. A partial positive charge is formed on the benzimidazole N-3 nitrogen atom in the acid-catalyzed reaction and a partial negative charge is formed in the uncatalyzed reaction. Substituents in the benzimidazole should therefore be expected to have the opposite influence on the two reactions. This is also the case. However, the influence is much more pronounced in the uncatalyzed reaction. Thus, strongly electron-withdrawing groups such as NO_2, $SOCH_3$ and CF_3 in the 5-position of the benzimidazole ring have a strong influence on rate constants of the non-catalyzed reaction, which, in turn, gives very low neutral stabilities to these compounds, whereas the opposite is true for electron-donating groups.

For medical use as gastric-acid inhibitors the low neutral stability caused by, for instance, NO_2, $SOCH_3$ and CF_3 is undesirable, since it means that, apart from the pharmaceutical stability problems, the transformation into the sulfenamide D^+, a reactive sulfhydryl reagent, will take place outside the parietal cell *in vivo*. In contrast, the 5-OCH_3 group in omeprazole gives rise to a high reaction rate of the desired acid-catalyzed reaction and a very low rate of the undesired uncatalyzed reaction.

Correlation studies of the effect of different substituents in the benzimidazole ring on the two reaction pathways are described in part II.[1]

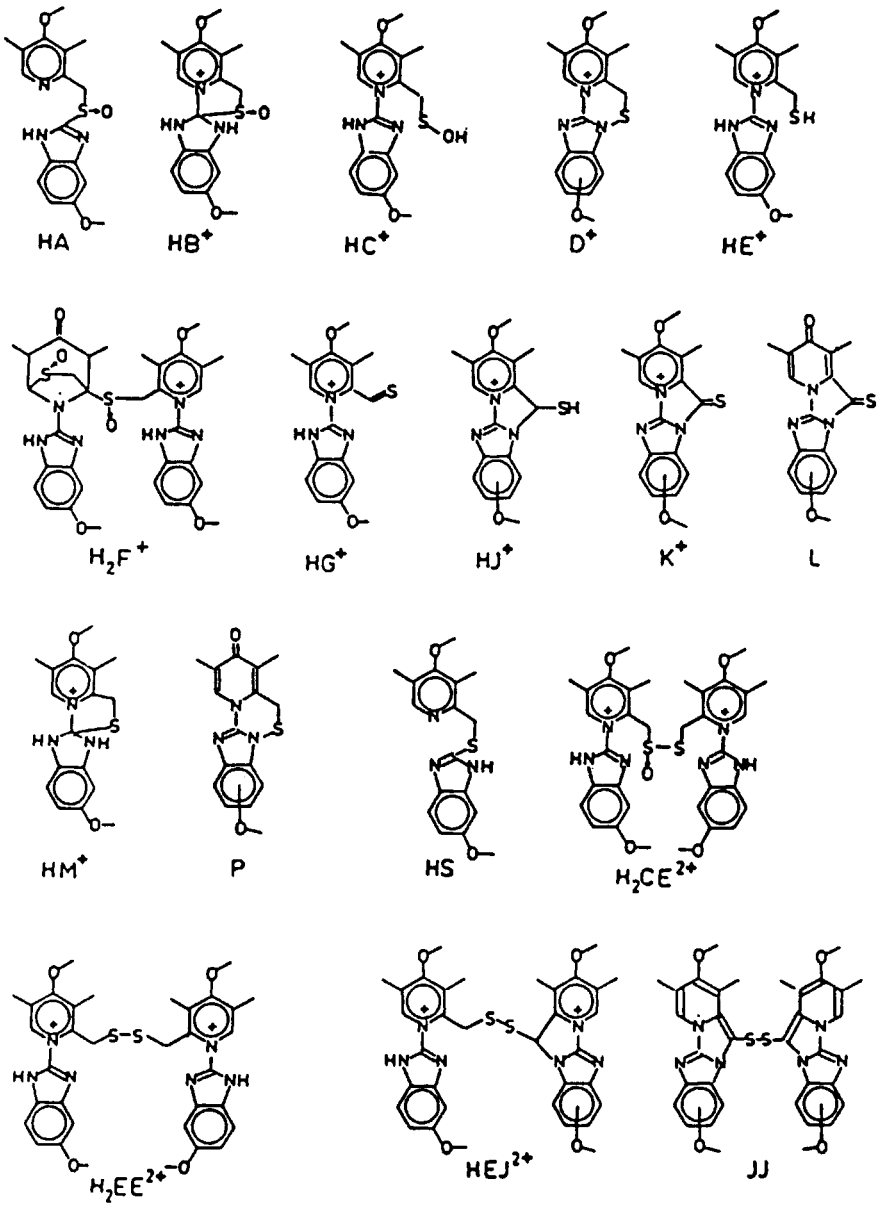
Calculation of the observable rate constant. By using the correlation methods described in part II[1] we can, directly from a given structure, calculate $pK_{H_2A^+}$, pK_{HA} and the rate constants k_a and k_n for the reactions responsible for the transformation of the compound HA to the reactive sulfenamide D^+. This means that, for each analogue, we can calculate the observed rate constant k_{obs} (part II[1]) and thus the quantity of active inhibitor produced for each combination of time and pH.

Correlation between reaction rates and effects in vitro *on the enzyme.* By testing a large number of omeprazole analogues HA on an isolated H^+, K^+–ATPase preparation and comparing the inhibiting effects, we have shown that the only important factor affecting inhibiting capacity is the *amount* of sulfenamide D^+ formed from the sulfoxide HA during the experiment, and this is independent of the structure of D^+ (within the limits tested). Apparently, this is due to the very high reactivity of the sulfenamide D^+ toward the SH groups of the enzyme, which means that to block a given quantity of enzyme, the equivalent quantity of drug must be converted into the active sulfenamide D^+.

Transformation of omeprazole and its analogues in aqueous solution in the absence of added nucleophiles

Main reaction pathways. In the absence of Hβ, the acid-catalyzed degradation of omeprazole in aqueous solution is quite complicated. By using preparative degradations combined with extensive kinetic investigations under different conditions we have established the occurrence of structure-types D^+, JJ, L, H_2EE^{2+} and HS. The structures of these and some other intermediates are given in Scheme 10.

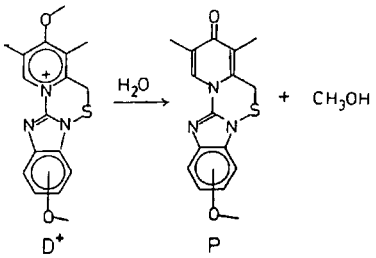
Stability of the sulfenamide D^+ at different pH values and concentrations. From an examination of the concentration dependence of the stability of D^+ at different pH values, we have found that D^+ decomposes at pH > 4 at rates that rapidly increase with increased pH. At pH = 2 the rate of decomposition of D^+ is about the same in 10^{-5} M and 10^{-6}



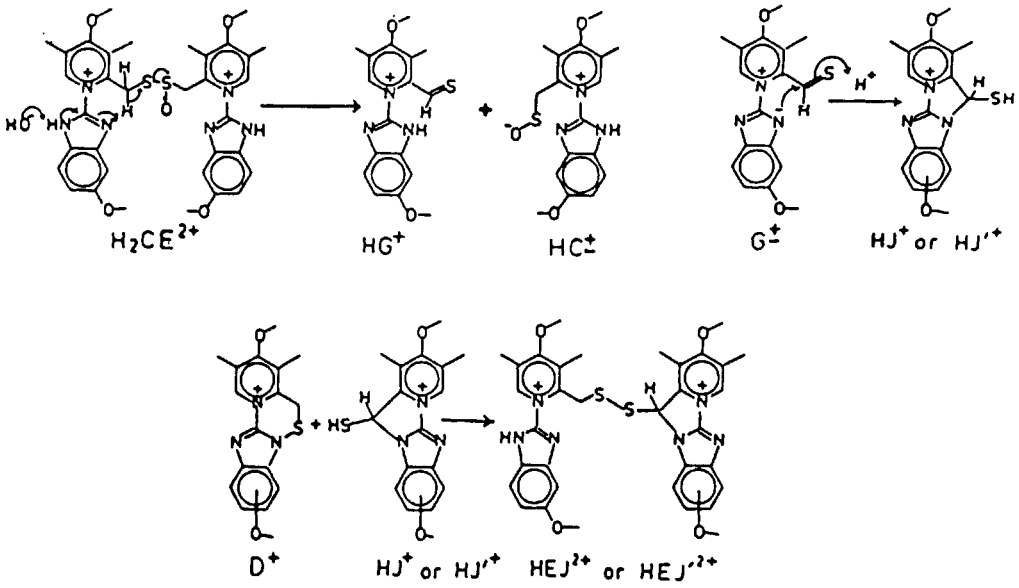
Scheme 10.

M solutions, and somewhat higher in a 10^{-4} M solution. At pH = 1 the rate of decomposition of D^+ is higher than at pH 2 and also more concentration dependent. This indicates that also in this reaction of D^+ there is a rate minimum at about pH 2, similar to that found for the reaction of D^+ with Hβ. This can be explained by the fact that, in this conversion, the attacking species are also sulfur-containing nucleophiles formed in the subsequent reactions.

A first-order reaction to compound H_2F^+. For solutions of the sulfenamide D^+ in the presence of [HCl] ≥ 0.01 mol l^{-1}, a first-order decomposition predominates. The first rate-determining step in this reaction is the cleavage of the 4-methoxy group. This occurs by an aryl–oxygen cleavage and not a methyl–oxygen cleavage, since methanol is formed and not methyl chloride. The same type of cleavage was observed for HEβ+ and HS, and by analogy with these reactions we can state that the first reaction from D^+ is the formation of the pyridone analogue P (Scheme 11), which cannot be stable in the reaction mixture. The final product



Scheme 11.



Scheme 12.

formed by this first-order degradation of D^+ is H_2F^+. The formation of H_2F^+ was followed by HPLC. Speculations about the structure of H_2F^+ and its formation are given in part VI.[5]

A second-order reaction to give the thiosulfinate H_2CE^{2+}. One of the most characteristic reactions of sulfenic acids is their ready dimerization to thiosulfinates.[20] This is a consequence of the high reactivity of sulfenic acids towards sulfur-containing nucleophiles and the fact that the anion of a sulfenic acid is a strong nucleophile. In this case, we can expect the anion of HC^+ to attack D^+ in a second-order reaction with the formation of the thiosulfinate H_2CE^{2+}. This is in good accordance with our kinetic data for the disapperance of D^+ from aqueous solutions. An acid-catalyzed reaction between HC^+ and D^+ to H_2CE^{2+} is also very probable, since it is a direct analogue of the acid-catalyzed formation of $HE\beta^+$ from D^+ and $H\beta$. The thiolsulfinate H_2CE^{2+} is, however, also a very reactive compound, and we have not been able to isolate any compound of this structure. The closest we have come to that is an NMR spectrum of a dilute solution of an *N*-methylated derivative of H_2CE^{2+} [cf. below and in the *N*-alkyl section, part V[4]]. The existence of the thiosulfinate H_2CE^{2+} has also been proposed by Figala *et al.*[15]

Further reactions from the thiosulfinate H_2CE^{2+}, via the thioaldehyde HG^+ to the tetracyclic thiol HJ^+ and the disulfide HEJ^{2+}. Thiosulfinates containing the group $-CH_2-S-SO-$ are known to undergo elimination very readily.[20] For CE this reaction leads to the thioaldehyde HG^+ and the sulfenate anion of $HC^{\pm}$, shown in Scheme 12.

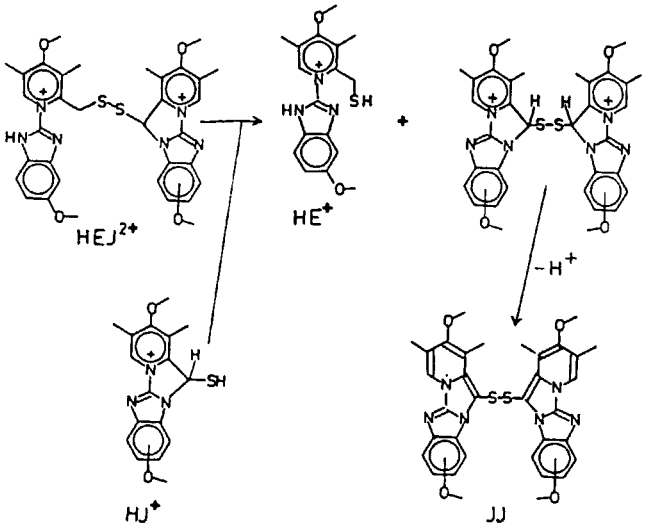
The thioaldehyde HG^+, probably after intial loss of the proton on the nitrogen atom, rapidly undergoes an intramolecular addition to form the thiol HJ^+. This will then, of course, react rapidly with HC^+ or D^+ to form the asymetric disulfide HEJ^{2+}. Although the existence of this intermediate is strongly supported by the kinetic data, we have been unable to isolate either HG^+ or HEJ^{2+}, or to detect them by HPLC. HEJ^{2+} can, however, be trapped by $H\beta$ and analyzed as βJ^+, see below.

If a CH_3 group is present on one of the benzimidazole nitrogen atoms, the conversion of HG^+ into HJ^+ is prevented, and the interconversion $H_2CE^{2+} \rightleftharpoons HG^+ + HC^+$ might well be displaced towards H_2CE^{2+}. This might be the explanation for the observation that a ^1H NMR spectrum of the NCH_3 analogue of H_2CE^{2+} from omeprazole can be obtained. The NCH_3 derivative of H_2CE^{2+} is, however, not very stable. This is expected since there is a multitude of reactions by which thiosulfinates can be converted into other compounds.[21]

From the kinetics we have found that the disulfide HEJ^{2+} is very reactive towards SH-containing compounds. It thus reacts with $H\beta$ at about the same rate as does D^+. This means that if HJ^+ is formed in solutions containing comparable quantities of D^+ and HEJ^{2+}, HJ^+ might react as easily with HEJ^{2+} to form JJ + HE^+ as with D^+ to form HEJ^{2+} (Scheme 13).

Isolation and identification of a disulfide JJ. When a high concentration of timoprazole, the unsubstituted analogue of omeprazole, is treated with dilute HCl, a deeply red crystalline compound precipitates. Its structure has been determined by X-ray crystallography,[16] which revealed the symmetrical disulfide structure JJ(1).

In independent work by Rackur *et al.*, this compound has

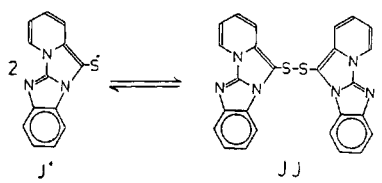


Scheme 13.

been described as the stable free radical J·.[15n] This indicates an equilibrium between the two compounds.

This equilibrium seems to explain the lack of sharp signals in the ^{1}H NMR spectrum of JJ(1), as well as the strong MS peak at *m/e* 238, the molecular ion of the radical.[19]

The radical J· has an 18-electron ring system which is probably stable ($n = 4$ in Hückel's $4n + 2$ rule). This might be one explanation of why the MS peak corresponding to J· appears in the mass spectrum of omeprazole and a variety of its reaction products. It is, e.g., the base peak in HEβ$^+$. This appearance of J· in the mass spectra of various compounds reduces the possibility of using the otherwise powerful LCMS method of obtaining important structural information. In this connection it should also be pointed out that in the ^{1}H NMR spectra of the intermediates obtained in the degradation of omeprazole, there are only a few proton signals which contain important structural information. [The signals from the proton(s) on the carbon atom adjacent to the S atom, the proton in the 6 position of the pyridine ring, and, to some extent, the 3 aromatic protons in the benzimidazole ring]. This reduces the possibility of using the otherwise powerful ^{1}H NMR technique for obtaining conclusive structural information. These two facts, together with the high reactivity of the intermediates and the presence of positive charge(s) and acidic proton(s) with a pK_a value around 6, which complicates the HPLC separations, explain why the structure elucidution of the intermediates was an unusually difficult task. We were therefore forced to use kinetic and mechanistic considerations to obtain information about the structure of several intermediates.



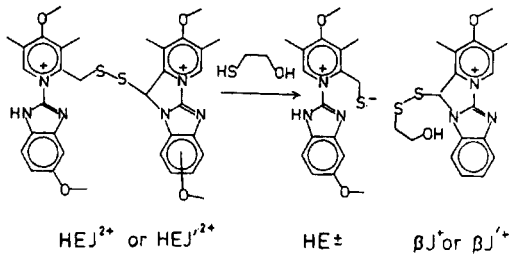
Scheme 14.

On treatment of omeprazole with HCl as described above for timoprazole, no disulfide JJ could be isolated. According to a TLC screening of the acid (0.11 M HCl) decomposition of a large number of omeprazole analogues with many different substitution patterns in both the benzimidazole and pyridine rings, it was apparent that all the compounds formed significant quantities of disulfides of type JJ, except for those with a 3-CH_3 group in the pyridine ring, such as omeprazole. However, the same study performed at pH 4 clearly indicated the formation of JJ even for the 3-CH_3 substituted pyridyl analogues. The existence of the radical J· corresponding to omeprazole has been described by Rackur *et al.*,[15n] but not the exact conditions used for its formation.

A dark blue compound or mixture of compounds is formed and precipitates at pH ~ 5 in solutions of omeprazole more concentrated than 10^{-4} M. This blue precipitate was not well characterized, in spite of several attempts; it is not stable in solution. The ^{1}H NMR spectrum of this material shows very broad signals, from which the signals due to CH_3 and OCH_3 groups appear as sharp peaks.

This indicates that the solution probably includes the disulfide JJ, which should contain three different isomers. We have some NMR indications that the blue precipitate is partly converted back into omeprazole when allowed to stand. A possible explanation for this may be that it contains a polymer, an oligomer or perhaps a trimer, of HG$^+$ since it is well known that thioaldehydes readily undergo polymerization.[22] This would indicate that all reactions from HA to HG$^+$ are reversible, which seems to be quite possible.

Cleavage of the disulfide HEJ^{2+} with formation of the thioamide compound K$^+$ and HC$^+$. If a buffer at pH ~ 6.5

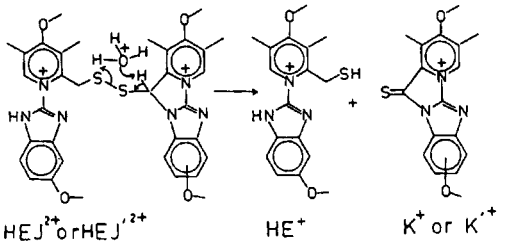


Scheme 15.

containing a slight excess of Hβ is added to a solution containing HEJ^{2+}, a rapid reaction occurs leading to the formation of βJ^+ and $HE^{\pm}$ (Scheme 15).

The formation of βJ^+ can be followed by HPLC. Owing to the presence of the CH_3O group in the benzimidazole ring, βJ^+ exists as two isomers, which are formed in about the same quantity. The transformation of the disulfide HE^{2+} into βJ^+ and $HE^{\pm}$ by Hβ has been used to trap HEJ^{2+} in the kinetic studies. We have, however, not been able to isolate βJ^+ or $\beta J'^+$.

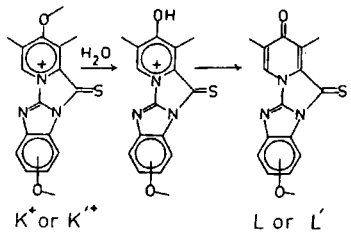
In solution, before being trapped with Hβ, HEJ^{2+} slowly undergoes an elimination reaction to give the thioamide K^+ (Scheme 16). The intermediate K^+ is strongly activated



Scheme 16.

towards nucleophilic attack on the pyridine ring, and by analogy with compound D^+, it may undergo an attack by water in the 4-position, followed by the elimination of methanol and a proton, to form the uncharged pyridonethioamide L (Scheme 17).

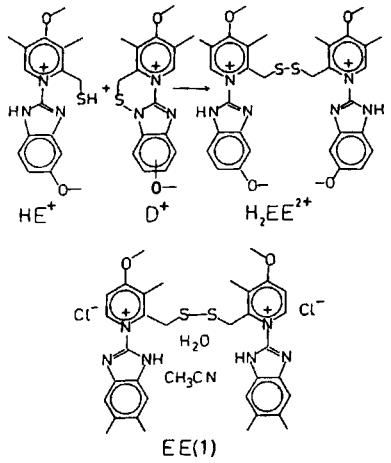
Owing to the presence of the OCH_3 group in the benzimidazole ring, L exists in two isomeric forms. It is not a protolyte under normal conditions and it is only sparingly soluble in water. It therefore precipitates as a deeply orange amorphous soild from solutions of omeprazole in dilute acids. It can thus be readily obtained, albeit in low yield. It is slowly decomposed in solution. It probably undergoes hydrolysis of the thioamide linkage (and it also reacts with Hβ). The hydrolysis reaction is the probable reason for the unexpectedly low yield (< 5 %) of precipitated L on acid treatment of omeprazole. The kinetic study, of the formation of L from HA via D^+ and HEJ^{2+}, involving the trapping of HEJ^{2+} with Hβ (see above) and determination of the amounts of HS (formed from HE^+; see below), $HE\beta^+$ and βJ^+, indicate that a much higher yield of L might be obtained at pH 4 (part VI[5]).



Scheme 17.

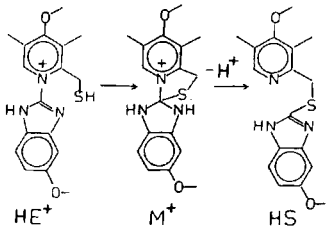
We have at present been unable to obtain crystals of L or L' from omeprazole or any analogue suitable for X-ray structure determination. Their NMR spectra are, however, in agreement with the proposed structure L and L' (part VI[5]).

Formation of the disulfide H_2EE^{2+}. The decomposition of HEJ^+ gives HE^+ and K^+. The zwitterion $HE^{\pm}$ can undergo three very fast reactions. It can react with a proton to form HE^+, it can undergo an intramolecular nucleophilic attack on the 2-benzimidazole carbon atom to give the sulfide HS, and it can undergo a reaction with D^+ to give the symmetrical disulfide H_2EE^{2+} (Scheme 18). The same product can also be formed from HE^+ and D^+ in an acid-catalyzed reaction and in fact a salt of H_2EE^{2+} is the main product formed when omeprazole is dissolved in dilute HCl and the reaction mixture treated with HPF_6. The structure of another salt EE(I) has been confirmed by X-ray crystallography.[16]



Scheme 18.

The reaction of the thiolate anion of $HE^{\pm}$ with the 2-benzimidazole carbon atom to give the sulfide HS is analogous to the reaction of the sulfenic anion $HC^{\pm}$ to form HA, or in the case of the *N*-methylated analogue of omeprazole MeC^+ to MeA (Scheme 19). By analogy with the reaction $MeC^+ \rightarrow$ MeA the equilibrium $HE^+ \rightleftharpoons$ HS is shifted far to the right. In the equilibrium MeA $\rightleftharpoons$ MeC^+ the product



Scheme 19.

MeC^+ can undergo a dimerization reaction to the thiosulfinate Me_2CE^{2+}. This product may then undergo a variety of reactions, and the result is that MeA can be transformed in a second-order reaction into a variety of products. No corresponding reaction is possible with HE^+, and HS is therefore a stable compound. It is formed in the transformation of HA under a variety of conditions.

The formation of H_2EE^{2+} should be a second-order reaction, first order in HE^+ and first order in D^+, whereas the formation of HS should be a first-order reaction in HE^+, independent of the concentration of D^+. This means that the formation of H_2EE^{2+} is favored at the expense of the formation of HS when the concentration of D^+ is increased. This is in agreement with the observation that H_2EE^{2+} is the main product of HA formed in a 0.1 M solution of HA in HCl, whereas very little H_2EE^{2+} is formed in a 10^{-5} M solution of HA in the same solvent.

Reactions of H_2EE^{2+}. The difference in structure between H_2EE^{2+} and H_2CE^{2+} is the oxygen atom on one of the sulfur atoms. We can thus expect a similarity in the reactions of these two compounds, especially in reactions where this oxygen is not involved or is only marginally involved.

In dilute solutions ($c = 10^{-5}$ M), very little H_2EE^{2+} is formed and we can therefore neglect its reactions in these solutions. In concentrated solutions where H_2EE^{2+} is the main product, we must consider the reactions of H_2EE^{2+}. In these solutions we can expect that an elimination is the dominant reaction. This should proceed with the formation of HG^+ and HE^+. These compounds are not stable under the reaction conditions, as mentioned above, but react further. The compound HE^+ thus reacts with D^+ to give a new molecule of H_2EE^{2+} and HG^+ reacts further via HJ^+, HEJ^{2+}, and K^+ to L, which is the product isolated in addition to H_2EE^{2+}, in the treatment of a 0.1 M solution of HA with HCl. Since our interest is in the reactions of HA under physiological concentrations, we did not study the reactions of H_2EE^{2+} in any detail, except for its reactions with Hβ which are described in part IV.[3]

Methods for the preparation of starting marerials and intermediates

A very large number of compounds have been prepared in this project, starting with the preparation of the parent compound timoprazol in 1974. The syntheses of a vast number of sulfides and sulfoxides are described in our patents and patent applications, especially the comprehensive GB Pat 2134523.

After our discovery of the very strong and selective antisecretory effect of omeparazole in 1980 about one hundred patent applications on analogues have appeared from our competitors. The following might be of interest for the preparative methods contained therein: Eur. Pat. Appl. 85108034.1, Eur. Pat. Appl. 85306600.9, Eur. Pat. Appl. 85307928.3, Eur. Pat. Appl. 87104619.9.

The synthesis of sulfenamides of type D^+ is described in our US Pat. 4,636,499.

The synthesis of various disulfides are described in our US Pat. Appl. 788,768.

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