Chemical Reactions of Omeprazole and Omeprazole Analogues. IV. Reactions of Compounds of the Omeprazole System with 2-Mercaptoethanol

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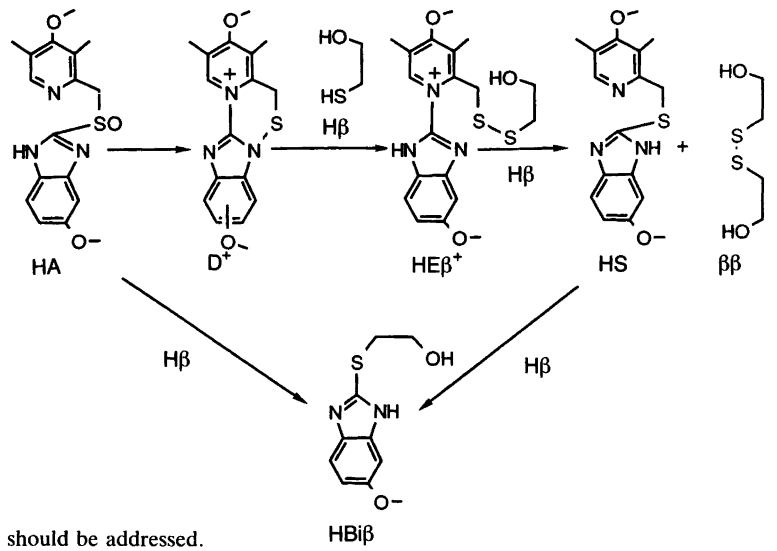
When omeprazole, 5-methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridinylmethylsulfinyl)-1*H*-benzimidazole, is dissolved in dilute HCl various reaction products are obtained. The addition of 2-mercaptoethanol greatly simplifies the complexity of these reactions. The reactions between 2-mercaptoethanol and some of the degradation products have been studied in detail.

The reaction of omeprazole in acidic solution in the presence of 2-mercaptoethanol (Hβ) proceeds according to Scheme 1.[1] The scheme is valid for omeprazole and for analogues of omeprazole. The symbols HA,D^+ etc. are therefore used for classes of compounds differing only in substituents. In this paper the reactions of D^+, and some disulfides with Hβ will be treated, as well as the reactions of HA and HS with Hβ at higher concentrations to form the compound HBiβ.

The reaction of the cyclic sulfenamide D^+ with 2-mercaptoethanol

Kinetic procedures. When omeprazole HA is dissolved in dilute HCl it is possible to follow its conversion into the cyclic sulfenamide D^+ by monitoring the UV absorption of the new ring. If Hβ is then added, a rapid conversion into $HE\beta^+$ occurs with the cleavage of this ring and a change in the UV spectrum back to one similar to that of HA. This reaction can thus be followed kinetically by UV. This has been done for omeprazole and a number of analogues at different concentrations of HCl.

In this type of experiment we have two difficulties. The first is that the reaction is very rapid. Since it is a second-order reaction between D^+ and Hβ, the rate of conversion of D^+ into $HE\beta^+$ is decreased by a decrease in the concentration of Hβ. At [Hβ] = 10^{-4} M the half-life of D^+ is a few seconds, and it is just possible to follow even the most rapid reaction by acting very rapidly in the mixing of reactants and insertion of the sample into the spectrophotometer. The second complication is that, for some compounds, the degradation of D^+ is rather fast. In this degradation reaction compounds with interfering UV spectra are formed. Since the reaction of D^+ with Hβ is usually about two



Scheme 1.

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orders of magnitude more rapid than any other of the reactions occurring, this problem can be resolved by adding Hβ at an appropriate time after the addition of HA to the acid, thus making the decrease in the UV absorbance optimal for the reaction to be studied.

Determination of k_{obs}. Since a tenfold excess of Hβ over D^+ was used, the reaction can be treated kinetically as a pseudo-first-order reaction in D^+ with the rate constant $k_{obs} = k[H\beta]$, where k is the second-order rate constant. The constant $k_{obs}$is obtained by non-linear regression of the UV absorbance, Abs, against t using eqn. (1). In the same re-

$$Abs = Abs_\infty + (Abs_0 - Abs_\infty)e^{-k_{obs}t} \quad (1)$$

gression Abs_0 and Abs_∞ are also obtained. These two parameters were used to detect serious side reactions. If such reactions do not occur, Abs_0 should be the absorbance at the start and Abs_∞ that at the end of the experiment.

For each compound tested, usually four HCl concentrations were used. At least three runs were performed at each HCl concentration.

pH dependence of k. A plot of log $(k/M^{-1}s^{-1})$ vs. log ([HCl]/M) for the reaction between Hβ and the sulfenamide from omeprazole is given in Fig. 1. We can see that the experimental points asymptotically approach two lines, one with slope −1 and one with slope +1. They thus fit

$$k = (k_a[H_3O^+] + k_b/[H_3O^+]) \quad (2)$$

eqn. (2) which is given as a solid curve in Fig. 1. This equation can thus be used to evaluate k_a and k_b from measurements of k_{obs} at different concentrations of HCl.

The dominant reaction at low [HCl] is recognized as a reaction of D^+ with β^-. Since Hβ has a concentration acidity constant of $K^* = 10^{-8.98}$ M (μ = 0.5 M, T = 37 °C),

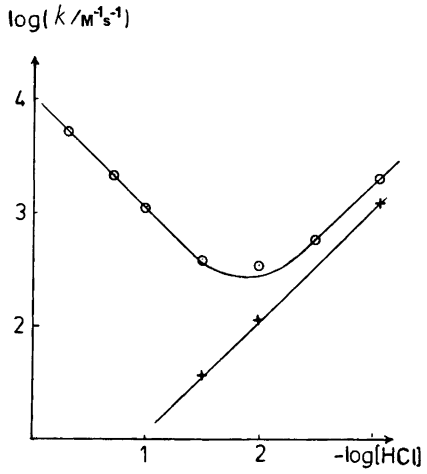


Fig. 1. pH dependence of the reaction between 2-mercaptoethanol and the sulfenamides D^+ (○) and $D(1)^+$ (+), respectively. For the structure of $D(1)^+$, see below.

Table 1. Rate data for reactions between some cyclic sulfenamides and 2-mercaptoethanol. T = 37 °C, μ = 0.5 M.

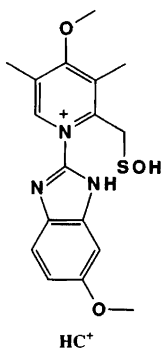
Substituents			
In pyridine	In benzimidazole	$k_{DE\beta}$/ $(10^9 M^{-1}s^{-1})^a$	k_a/ $(10^4 M^{-2}s^{-1})^a$
3,5-$(CH_3)_2$, 4-OCH_3	5-OCH_3	1.60(5)[b]	1.06(3)[b]
3,5-$(CH_3)_2$, 4-OCH_3	5-CH_3	1.5(1)	0.995(4)
3,5-$(CH_3)_2$, 4-OCH_3	5-H	1.78(7)	0.91(2)
3,5-$(CH_3)_2$, 4-OCH_3	5-CH_3, 6-$COOCH_3$	2.7(2)	0.50(2)
3,5-$(CH_3)_2$, 4-OCH_3	5-CF_3	3.2(1)	0.42(2)
H	5-OCH_3	3.3(2)	0.64(3)
5-CH_3	5-OCH_3	2.2(2)	1.10(5)
4-CH_3	5-OCH_3	2.4(2)	0.75(4)
4,5-(CH_3)	5-OCH_3	2.49(3)	1.08(1)
3,4,5-(CH_3)	5-OCH_3	1.18(7)	0.41(1)
3-CH_3, 4-OCH_3	5-OCH_3	0.99(9)	0.57(1)

[a] $k = \{k_{DE\beta}(K^*/[H_3O^+]) + k_a[H_3O^+]\}$, where $K^* = K_{RSH}/f^{\pm 2}$, $K_{RSH} = 10^{-9.22}$ (Ref. 2) and $f^{\pm} = 0.758$ (Ref. 3). [b] Standard deviations given are obtained in the non-linear least-squares regression and demonstrate how well the experimental values fit the mathematical model. The actual 'errors' are larger mainly due to spurious traces of disulfide in the 2-mercaptoethanol.

β^- is present as the fraction $10^{-8.98}/[H_3O^+]$ under the prevailing conditions and the mechanistic rate constant $k_{DE\beta}=k_b10^{8.98}$ is obtained, provided that the formation of β is not rate limiting. The values of $k_{DE\beta}$ and k_a for a few compounds are given in Table 1.

The magnitude of $k_{DE\beta}$ indicates that the rate of the reaction is diffusion controlled or nearly so. This means that it is difficult to tell whether the rate-determining step is the reaction between β^- and D^+ or the formation of β^- from Hβ in the acidic solution. If the formation of β^- is rate limiting, $k_{DE\beta}$ is still higher than that calculated above. In either case, the rate constant $k_{DE\beta}$ should be rather insensitive to variations of substituent in the two rings. From Table 1 it is seen that this is also the case.

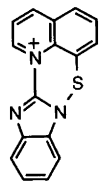
The dominant reaction at high [HCl] is an acid-catalyzed reaction of D^+ or the corresponding sulfenic acid HC^+ with Hβ. Since HC^+ and D^+ are very rapidly interconverted (this rate is at least 100 times faster than the reaction with Hβ),[4] it is difficult to tell which one of them is the reactive species. An answer to that question can, however, be ob-



HC^+

tained from other types of measurement. A comparison of the reaction between HA and Hβ with the corresponding reaction between the *N*-methylated derivative MeA and Hβ is illuminating, and indicates that the acid-catalyzed reaction is, mainly, a reaction between D^+, Hβ and a proton. A discussion of this point is given in connection with the kinetics of the *N*-alkylated compounds.[5] The acid-catalyzed reaction thus involves an attack on D^+ of both a proton and the electron pair of the sulfur atom. Since these two attacks have opposite electronic demands, the net effects of ring substituents of the two rings on the rate constant, k_a, are expected to be small. This is also the case, as shown in Table 1.

However, if we make a more drastic change in the structure of HA by including the carbon atom in the CH_2SO chain in an aromatic ring the sulfenamide $D(1)^+$ is obtained. We can expect that both $D(1)^+$ and the corresponding sulfenic acid will be strongly stabilized by resonance.



$D(1)^+$

(We made use of this in our first isolation of a crystalline salt of type $D(1)^+ PF_6^-$). Owing to the diffusion-controlled nature of the reaction of D^+ with β^- this stabilization does not show up in the rate constant $k_{DE\beta}$ for the base-catalyzed reaction of D^+. For the slower acid-catalyzed reaction, the resonance stabilization of $D(1)^+$ results in a strong decrease of the rate constant k_a. In fact no acid-catalyzed reaction was observed for the compound $D(1)^+$ (Fig. 1).

The reaction of D^+ with Hβ. A model reaction for the inhibition of the enzyme. The reaction of D^+ with Hβ is a model for the reaction of H^+,K^+-ATPase with D^+ that results in the inhibition of the enzyme.[6] Since D^+ is formed in the acidic compartment of the parietal cell, the contents of which are rapidly and continuously transported out from the cell as the acidic secretion, it is of fundamental importance for the *in vivo* effect that the reaction of D^+ with H^+,K^+-ATPase is very rapid. If the reaction is not rapid enough, a sizeable proportion of D^+ is transported away from the cell before it has time to react and is thus lost, probably in reactions with thiols, to give the sulfide as the final product.

The reaction of the disulfide $HE\beta^+$ with Hβ

The compound $HE\beta^+$ reacts with 2-mercaptoethanol with the formation of the disulfide $HOCH_2CH_2SSCH_2CH_2OH$ and the sulfide HS. In the kinetic investigations of the reaction of omeprazole HA and analogous compounds with Hβ the concentrations of HA, $HE\beta^+$ and sometimes even HS were followed as functions of time and pH.[1] These experiments were designed to give accurate values of the rate constant for the reaction $HA+H\beta \rightarrow HE\beta^+$, and the reaction conditions were often not suitable for an exact calculation of the rate constant for the reaction $HE\beta^+ + H\beta \rightarrow HS + \beta\beta$. The information obtained, however, gave a good picture even of this reaction, and sometimes very accurate measurements of the rate constants for the reaction $HE\beta^+ + H\beta \rightarrow HS + \beta\beta$ were obtained. Fig. 2 gives a good indication of the variations of the concentrations of the three components HA, $HE\beta^+$ and HS with time, obtained in a solution 10^{-5} M in HA and 10^{-3} M in 2 mercaptoethanol at four pH values.

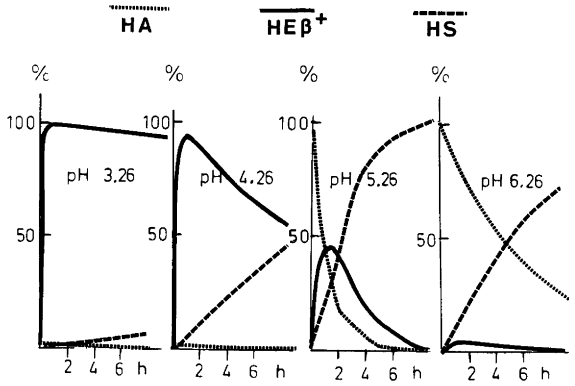


Fig. 2. Reaction between omeprazole (10^{-5} M) and 2-mercaptoethanol (10^{-3} M) at different pH values. $T = 37$ °C.

Comparison with the reaction HA + Hβ → HEβ⁺. Early investigations revealed two important differences between the two reactions $HA + H\beta \rightarrow HE\beta^+$ and $HE\beta^+ + H\beta \rightarrow HS + \beta\beta$:

1. The reaction $HA + H\beta \rightarrow HE\beta^+$ is a firstorder reaction with a rate independent of the concentration of Hβ, whereas the reaction $HE\beta^+ + H\beta \rightarrow HS + \beta\beta$ is a second-order reaction, first-order in Hβ and first-order in $HE\beta^+$.
2. The reaction $HA + H\beta \rightarrow HE\beta^+$ is mainly an acid-catalyzed reaction, whereas the reaction $HE\beta^+ + H\beta \rightarrow HS + \beta\beta$ is a base-catalyzed reaction.

Two mechanistic possibilities. For the base-catalyzed reaction $HE\beta^+ + H\beta \rightarrow HS + \beta\beta$ we have two possibilities:

1. A reaction between the acid form of $HE\beta^+$ and the base form of Hβ.
2. A reaction between the acid form of Hβ and the base form of $HE\beta^+$.

It is usually difficult to distinguish between these two possibilities by kinetic methods. In the present case, this can be done as follows. Consider a reaction of the first type. At a given pH value the observed pseudo-first-order rate constant, k_{obs}, in the presence of an excess of Hβ is given by eqn. (3).

$$k_{obs} = k[H\beta]_{tot} \frac{a_{H_3O^+}}{a_{H_3O^+} + K'_{HE\beta}} \frac{K'_{H\beta}}{a_{H_3O^+} + K'_{H\beta}} \quad (3)$$

In this equation, k is the true second-order rate constant and $K'_{HE\beta}$ and $K'_{H\beta}$ are the mixed protolytic constants of the two acids $HE\beta^+$ and $H\beta$. Since the reaction occurs by an attack at a sulfur atom, separated from the ring system by an $-S-CH_2-$ group, the influence of ring substituents on the rate constant is expected to be very small.

At a pH value of about 4.2, both $K'_{HE\beta}$ and $K'_{H\beta}$ are much smaller than $a_{H_3O^+}$, and eqn. (3) can be simplified to eqn. (4).

$$k_{obs} = k[H\beta]K'_{H\beta}/a_{H_3O^+} \quad (4)$$

Since $pK'_{H\beta}$ is constant, and even k is roughly constant, k_{obs} will also be constant at a given pH value, independent of the substituents in the ring. That this is the case is seen from Table 2, where k_{obs} is indeed remarkably constant. The variations in k_{obs} are, in fact, not significantly higher than those caused by the variations in the pH value of the solutions.

If the reaction is of the second type, a reaction of the acid form of $H\beta$ with the base form of $HE\beta^+$, the corresponding rate equation at low pH values is given by eqn. (5).

$$k_{obs} = k[H\beta]K'_{HE\beta}\, a_{H_3O^+} \quad (5)$$

Since $K'_{HE\beta}$ is not expected to be constant for the compounds in Table 2,[7] k_{obs} cannot be expected to be constant, which is at variance with the experimental facts. We thus have a good indication that the reaction is of the first type, involving the anion of $H\beta$ and the acid form of $HE\beta^+$.

Table 2. Pseudo first-order rate constants for the reaction $HE\beta^+ + H\beta \rightarrow$ HS of different compounds $HE\beta^+$ with 10^{-3} M 2-mercaptoethanol at 37 °C and pH values of ca. 4.2.

	Substituents		
pH	In pyridine	In benzimidazole	$k_{obs}/10^{-5}\,s^{-1}$
4.22	5-C_2H_5	5-OCH_3	1.83(8)
4.22	4,5-$(CH_3)_2$	5-OCH_3	1.55(7)
4.29	3,4,5-$(CH_3)_3$	5-OCH_3	1.83(7)
4.20	3,5-$(CH_3)_2$,4-OC_2H_5	5-OCH_3	1.862(7)
4.26	3,5-$(CH_3)_2$,4-OCH_3	5-OCH_3	2.040(7)
4.25	3,5-$(CH_3)_2$,4-OCH_3	–	2.38(5)
4.20	3,5-$(CH_3)_2$,4-OCH_3	5-Cl	2.30(1)
4.22	3,5-$(CH_3)_2$,4-OCH_3	5-$COCH_3$	2.50(3)
4.22	3,5-$(CH_3)_2$,4-OCH_3	5-$NHCOCH_3$	2.00(3)

[a]The standard deviations given are obtained from the non-linear least-squares regression and demonstrate how well the experimental values fit the mathematical model. The actual 'errors' in k_{obs} are larger and depend mainly on the error in the pH readings. These errors are of the order of 0.05 pH units, and correspond to a standard error in k_{obs} of about 12 %, which is of the same magnitude as the standard deviation (19 %) of k_{obs} in the table.

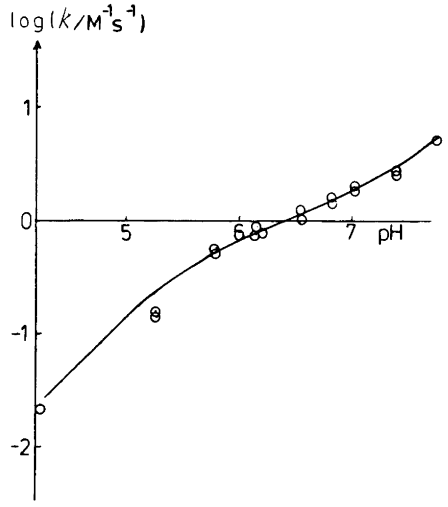


Fig. 3. pH dependence of the reaction $HE\beta^+ + H\beta$. The solid line corresponds to log k_{calc} where

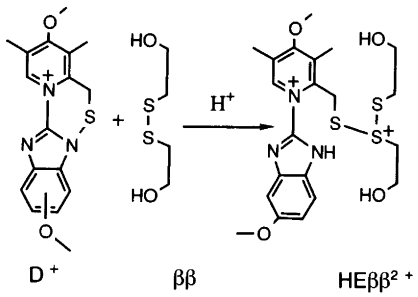
$$k_{calc} = \frac{1}{1 + [H_3O^+]/K'_{RSH}} \cdot \frac{k_{H\beta}[H_3O^+] + k_{\beta^-}K'_{HE\beta}}{K'_{HE\beta} + [H_3O^+]}$$

$K'_{RSH} = K_{RSH}/(f_\pm)^2 = 10^{-9.22}/0.793^2$, $[H_3O^+] = 10^{-pH}/f_\pm$

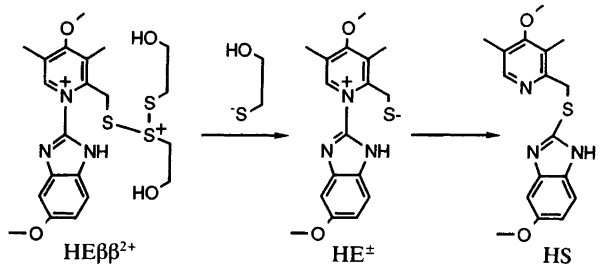
$k_{H\beta} = 1.9(5) \times 10^3\ M^{-1}\ s^{-1}$, $k_\beta = 0.98(4) \times 10^2\ M^{-1}\ s^{-1}$ and $K'_{HE\beta} = 10^{-5.82}$ were determined by non-linear least-squares regression according to Ref. 1.

pH dependence of k_{obs}. If the pH value of the solution increases, k_{obs} increases. At the same time, the concentration of $HE\beta^+$ in the reaction mixture starting from HA and $H\beta$ is strongly decreased (Fig. 2). Accurate values of the rate constants for the reaction $HE\beta^+ + H\beta \rightarrow$ HS are thus difficult to obtain in this way. The preferred way of obtaining these constants is to allow the reaction HA + $H\beta \rightarrow$ $HE\beta^+$ to proceed to about 99 % completion in 0.001 M HCl, which produces almost pure $HE\beta^+$. A buffer is then added and the reaction $HE\beta^+ + H\beta \rightarrow$ HS is then followed by HPLC. The results obtained with omeprazole are given in Fig. 3.

In the investigation of the reaction $HE\beta^+ + H\beta \rightarrow$ HS +

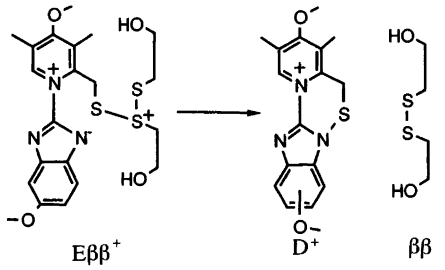


Scheme 2.



Scheme 3.

ββ by HPLC, we observed a small peak near to that corresponding to HS. The corresponding compound is probably formed from the disulfide ββ, which in this investigation was present in up to 4 mol% as an impurity of Hβ. In an acidic solution, we can expect the reaction in Scheme 2 to occur. This is in agreement with the high reactivity of sulfenic acid derivatives towards sulfur nucleophiles in acidic solution.[8] When the pH is increased, we can expect two pH-dependent reactions to occur. The first is an attack by the sulfide anion RS^- on the positively charged sulfur with the release of the excellent leaving group $HE^{\pm}$ which rapidly rearranges to HS according to Scheme 3.

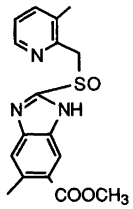


Scheme 4.

When the pH is increased to slightly above 6, the NH group of the benzimidazole ring releases its proton. The increased negative charge on the E residue strongly decreases its tendency to function as a leaving group, and facilitates the splitting off of ββ according to Scheme 4. The sulfenamide formed in this way then reacts rapidly with Hβ to give $HE\beta^+$. The reactions suggested above were confirmed by experiments in which pure ββ was used instead of Hβ containing some ββ.

The attack of 2-mercaptoethanol at the 2-position of the benzimidazole ring

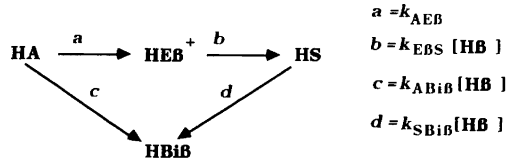
In the discussion of the reaction of omeprazole and analogous compounds in the presence of 2-mercaptoethanol it was mentioned that sometimes a few minor peaks were observed in the HPLC traces, indicating some side-reactions.[1] One such side reaction is the direct attack by 2-mercaptoethanol at the 2-position of the benzimidazole ring. This reaction could be neglected under most condi-



HA (2)

tions. However, during the investigation of picoprazole [HA(2)], our predecessor of omeprazole, we made some observations on its reaction with 2-mercaptoethanol which puzzled us for a long time. The reaction was studied by HPLC, and it was not until we discovered that the peak we had assigned to $HE\beta^+$ was composed of two peaks, corresponding to $HE\beta^+$ and HBiβ (Scheme 1), that we arrived at the solution of the problem.

Rate equations and determination of rate constants. In this experiment we have reactions according to Scheme 5, with the rate constants *a*, *b*, *c* and *d*. The solutions to the differential equations for this system of reactions are given in eqns. (6)–(9), where (*A*) is the area (proportional to [HA]) of the peak corresponding to HA and so on.



Scheme 5.

$$(A) = (A)_0 e^{-(a+c)t} \tag{6}$$

$$(E\beta) = (E\beta)_0 \frac{a}{b-a-c}\left(e^{-(a+c)t} - e^{-bt}\right) \tag{7}$$

$$(S) = -(S)_0 \frac{ab}{(a-b+c)(b-d)(d-a-c)}\left[(b-d)e^{-(a+c)t} + (d-a-c)e^{-bt} + (a-b+c)e^{-dt}\right] \tag{8}$$

$$(Bi\beta) = -(Bi\beta)_0 \frac{abd}{(a-b+c)(b-d)(d-a-c)}\left[\frac{b-d}{a+c}(1-e^{-(a+c)t}) + \frac{d-a-c}{b}(1-e^{-bt}) + \frac{a-b+c}{d}e^{-dt}\right] + (Bi\beta)_0 \frac{c}{a+c}e^{-(a+c)t} \tag{9}$$

At pH 4.26, this reaction was studied using three concentrations of Hβ: 1.02×10^{-4}, 1.039×10^{-3} and 9.82×10^{-3} M. From the HPLC chromatograms recorded at different times, the peak areas (A), (S) and ($E\beta$) + (Biβ) were determined and used in a non-linear least-squares analysis using two independent variables ($x_1=t$ and $x_2=$[Hβ], three dependent variables [$y_1=(A)$, $y_2=(E\beta)+(Bi\beta)$ and $y_3=(S)$], and 8 parameters [$(A)_0$, $(E\beta)_0$, $(S)_0$, $(Bi\beta)_0$, $k_{AE\beta}$, $k_{E\beta s}$, $k_{SBi\beta}$ and $k_{ABi\beta}$]. In this way we obtained

$k_{AE\beta} = 1.63(2)\ s^{-1}$
$k_{ABi\beta} = 0.75(3)\ M^{-1}\ s^{-1}$
$k_{SBi\beta} = 0.0030(2)\ M^{-1}\ s^{-1}$
$k_{E\beta S} = 0.046(2)\ M^{-1}\ s^{-1}$

This result was confirmed in a separate study of the reaction of the sulfide HS, corresponding to picoprazole [HA (2)], with 2-mercaptoethanol. We thus obtained $k_{SBi\beta} = 3.83(6) \times 10^{-3}\ M^{-1}\ s^{-1}$ at pH 4.26 and $1.30(3) \times 10^{-3}\ M^{-1}\ s^{-1}$ at pH 5.26. The value obtained at pH 4.26 agrees fairly well with that obtained on starting with picoprazole. The small difference might be due to an additional route for the formation of HBiβ, viz. by the rearrangement of HEβ$^+$ as described for the *N*-methylated compound.[5] However, the experimental material does not allow the calculation of an additional rate constant.

Mechanistic implications of the results obtained. The results are summarized in Fig. 4, where the curves for the four species HA, HEβ$^+$, HS and HBiβ (in this case for picoprazole), are given as functions of time at three concentrations of Hβ. We can see that at [Hβ] = 0.01 M the formation of HBiβ is the dominant reaction of picoprazole, at [Hβ] = 0.001 M it is a readily detectable side reaction, but at [Hβ] = 0.0001 M the formation of HBiβ is difficult to detect.

The formation of HBiβ either from HA or HS is the result of a nucleophilic reaction on the benzimidazole ring system (Scheme 1). From our knowledge of such reactions we can predict two possibilities. The first is an attack of Hβ on the protonated benzimidazole ring H_2A^+, while the second is an attack of β$^-$ on HA. From the pH dependence of the reaction of the sulfide (see above), we can see that the first mechanism is strongly predominant at these pH values. HA can be protonated at two different positions: on the pyridine ring to give the HAH$^+$ acid and on the benzimidazole ring to give the acid H_2A^+. Of these, HAH$^+$ is by far the major species present at equilibrium, and from the protonation equilibrium we have

$$[H_2A^+] = [HAH^+]\ K_{HAH}/K_{H_2A}.$$

When the pyridine ring is substituted by alkyl or alkoxy groups, K_{HAH} is greatly decreased and, since K_{H_2A} is changed very little, the ratio K_{HAH}/K_{H_2A} is greatly decreased. [HAH$^+$] is almost equal to the total concentration of HA at pH values near or below pK_{HAH} and is thus not

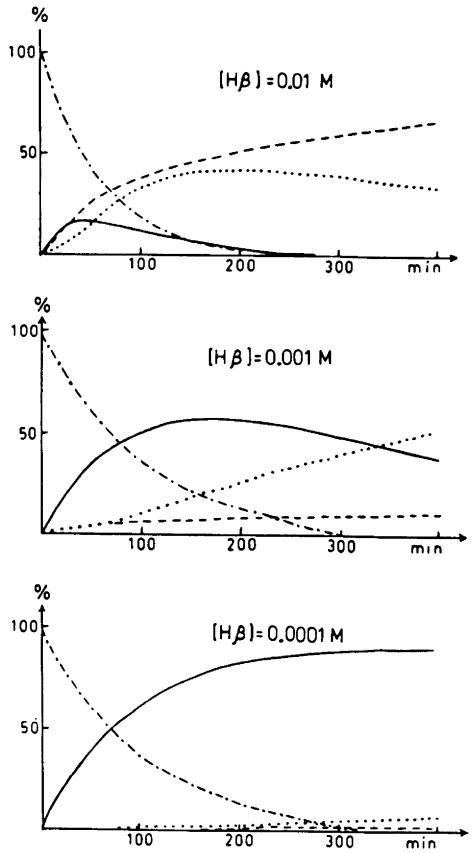
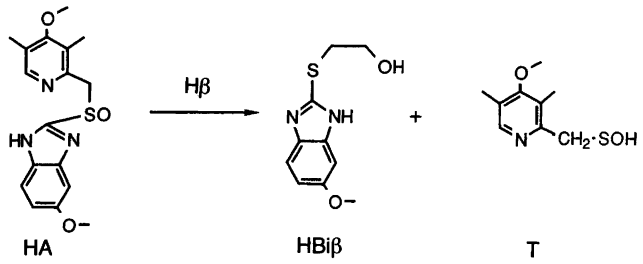


Fig. 4. Reaction of picoprazole [HA(2)] (——) at pH = 4.26 at three concentrations of Hβ. T = 37 °C. HEβ$^+$ (——), HBiβ (----) and HS (····) stand for the compounds formed from picoprazole.

influenced very much. The result is that alkyl and alkoxy substitution in the pyridine ring decreases the concentration of H_2A^+ in the acidic region, and thus the formation of HBiβ. This, and the fact that we are using [Hβ] = 10^{-3} M or [Hβ] = 10^{-4} M in our standard experiments, explains why the formation of HBiβ is usually not observed. A contributing factor is also that HA is converted into D$^+$ more rapidly the higher the pK_a of HAH$^+$. This shortens the reaction time for the reactions with alkyl and alkoxy substituted pyridines, and there is less time left for the formation of HBiβ. As soon as the conversion HA → D$^+$ is prevented, HA can be in contact with Hβ for a long time and the formation of HBiβ is readily observed. This is, e.g., observed when omeprazole is *N*-methylated.[5]

In addition to HBiβ, a substituted pyridinomethanesulfenic acid (T) should be formed in the reaction. As discussed in Ref. 8, sulfenic acids of this type undergo a variety of reactions. One of these reactions leads to the formation of a substituted pyridinomethyl disulfide. A product of this type has also been isolated from a reaction mixture in which we used a high concentration of Hβ in our attempts to prepare HEβ$^+$ from HA and Hβ by the addition of an acid to the mixture.

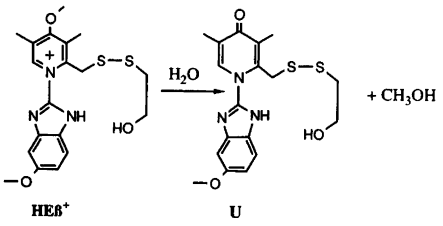


Scheme 6.

The hydrolytic reaction of the disulfide HEβ$^+$

In the study of the reaction HA → HEβ$^+$ → HS, we have assumed that HEβ$^+$ is a stable compound under the reaction conditions and does not undergo reactions other than those indicated. This has been found to be valid in all cases with the exception of the *N*-methylated compound at certain pH values.[5] In order to investigate whether side reactions occur with HEβ$^+$ from omeprazole during extended reaction times, we have studied, by means of HPLC, the stability of pure HEβ$^+$ in aqueous solutions at different pH values.

In 0.001 M HCl, HEβ$^+$ slowly undergoes a first-order reaction at 37 °C. (Scheme 7) with the rate constant 3.3 (1) × 10^{-6} s^{-1}, corresponding to a half-life of about 60 h. The reaction is very clean and no peaks other than that for the starting material and product (U) could be observed. This reaction is more fully described in Ref. 9. The reaction is slightly acid catalyzed and is thus somewhat faster at still lower pH values. In no case was it fast enough to disturb the measurements of the rate constant k_{AD}.



Scheme 7.

At pH 7.2 another type of decomposition occurs to give at least four different products which were not characterized. The first-order rate constant for the degradation of HEβ$^+$ is now 2.6(1) × 10^{-5} s^{-1}, corresponding to a half-life of about 7.5 h. It is thus so slow that it did not disturb any of the other measurements reported in this paper.

The reactions of some other disulfides with Hβ

Disulfides react with thiols with the formation of new disulfides and thiols.[10] Since some disulfides occur in the degradation of omeprazole, and others are generated by the addition of Hβ to the solutions, it was necessary to know certain facts about the rate of the disulfide–thiol interchange, especially as it was found that the rate constants differed by up to six powers of ten for different combinations of disulfide and thiol.

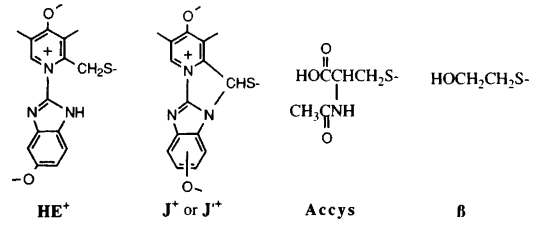
Owing to the huge differences in rate, different techniques had to be used for different reactions. These are described in the Experimental section. The results of a number of reactions are given in Table 3. From the table it is obvious that the rate constant depends on both the ease with which the sulfur atom is attacked and the ease with which the thiol is released. These observations and their implications will be demonstrated by a few comparisons.

In entries 1, and 2 and 6, we have the same leaving group, acetylcysteine (HAccys) (Scheme 8). The three disulfides are all attacked by Hβ, and the difference in rate must therefore be due to the ease of attack on the J$^+$ and J'$^+$ sulfur atoms on the one hand, and on the HE$^+$ sulfur atom on the other. A comparison between 6 and 8, both of

Table 3. Reactions of disulfides with sulfide at pH 4.26. *T* = 37 °C.

No.	Reaction[a]	*k*/M^{-1} s^{-1}	Method[b]
1	AccysJ$^+$ + Hβ → βJ$^+$ + HAccys	1.06(6)	C
2	AccysJ'$^+$ + Hβ → βJ'$^+$ + HAccys	0.70(5)	C
3	βJ$^+$ + HAccys → AccysJ$^+$ + Hβ	2.95(13)	C
4	βJ'$^+$ + HAccys → AccysJ'$^+$ + Hβ	2.36(14)	C
5	HEAccys$^+$ + Hβ → Accysβ + HS	4.8(1) × 10^{-3}	D
6	HEAccys$^+$ + Hβ → HEβ$^+$ + HAccys	0.5(1) × 10^{-3}	D
7	HEβ$^+$ + Hβ → ββ + HS	2.07(2) × 10^{-2}	E
8	H_2EE^{2+} + Hβ → HEβ$^+$ + HS	47(6)	F
9	HEJ^{2+} + Hβ → βJ$^+$ + HS	4.7(4) × 10^3 [c]	B
10	HEJ'$^{2+}$ + Hβ → βJ'$^+$ + HS	(3.4±0.5) × 10^3 [c]	B
11	D$^+$ + Hβ → HEβ$^+$	1.8 × 10^4	A

[a]For the meaning of the symbols see Scheme 8. [b]See the Experimental. [c]*T* = 20 °C.



Scheme 8.

which involve an attack on the HE^+ sulfur by Hβ, reveals that HE^+ is a very much better leaving group (giving HS in a rapid reaction) than is acetylcysteine. A combination of these two facts indicates that an attack of HEJ^{2+} by Hβ should occur on the readily attacked J^+ sulfur atom with release of the very good leaving group HE^+. The rate of this reaction should thus be very high which is indeed the case, as seen in reactions 9 and 10. This rate is of the same magnitude as the reaction of D^+ with Hβ (reaction 11). From a comparison between 1 and 2 or 3 and 4 we can see that the sulfur atom of J^+ is attacted somewhat more readily than is the sulfur atom of J'^+. A comparison between 5 and 7, both of which have HE^+ as the leaving group, indicates that the sulfur atom in Hβ is attacked about four times as readily as that of HAccys, and a comparison between 7, 8 and 9 reveals that the sulfur atoms of Hβ, HE^+ and J^+ are attacked in the proportions $1 : 10^3 : 6 \times 10^5$ (corrected for the difference in temperature), respectively. (A statistical factor of two is used for the attack of H_2EE^{2+}).

Experimental

The general LC system is described in Ref. 1.

Method A. This method was used in the study of the reactions of D^+ with Hβ to give $HE\beta^+$. A solution of D^+ was prepared at 37 °C by adding 50 µl of a 6×10^{-4} M solution of a compound of type HA in methanol to 3 ml of dilute HCl. The formation of D^+ was followed spectrophotometrically at 355 nm, as described in Ref. 1. Just before the maximum absorbance value was obtained, 30 µl of a 10^{-2} M aqueous solution of β-mercaptoethanol were added, and the readings at 355 nm were started as soon as possible (the half life of the reaction is only a few seconds). The rate constant was calculated by non-linear regression from a set of (*Abs, t*) values using the equation $Abs = Abs_\infty + (Abs_0 - Abs_\infty)e^{-k_{obs}t}$ and our standard method.[1] In this way, k_{obs}, Abs_0 and Abs_∞ are obtained, HCl concentrations ranging from 10^{-3} to 0.5 M with µ = 0.5 M. With concentrations of HCl outside these limits the reaction is too rapid to follow by this method.

Method B. This method was used in the study of the reactions $HEJ^{2+} + H\beta \rightarrow \beta J^+$ and $HEJ'^{2+} + H\beta \rightarrow \beta J'^+$.

The PF_6^- salt of the sulfenamide D^+ (2.5 mg) was dissolved in 10 ml of acetonitrile and the solution was stored at 0 °C. A 50 µl portion of this solution was added to 2.6 ml of a buffer, to give pH = 4.26, µ=0.05 M, T = 20 °C and $[D^+] = 10^{-5}$ M. This solution was stored at 20 °C for 3 minutes to give a maximal concentration of HEJ^{2+} and HEJ'^{2+}. A 25 µl portion of ca. 0.01 M Hβ was injected to give $[H\beta] = 10^{-4}$ M. After t seconds the reaction was stopped by the addition of 250 µl of 0.1 M acetylcysteine (AccysH), and the mixture was injected as rapidly as possible onto a reversed-phase HPLC column and analyzed for βJ^+ and $\beta J'^+$. With a LiChrosorb RP select B, 5µm (125 × 4 mm ID) (Merck) column and a mobile phase consisting of 45 % acetonitrile and 55 % water to which 20 ml of a buffer pH 5.9 µ = 0.05 M had been added to each 500 ml, the retention times were: HS 6.7 min, $HE\beta^+$ 8.5 min βJ^+ 12.7 min and $\beta J'^+$ 13.3 min. This system is very sensitive toward small changes and all measurements were performed on the same day using the same batch of mobile phase and column.

Both the areas A and the heights H were measured and converted into suitable units in order to obtain numbers of the same magnitude from both sets of measurements. The rate constant was calculated using our general method and the following equations, where $Y(1)$ and $Y(2)$ correspond to the observed peak height and peak area, respectively, and H_{resp} and A_{resp} are response factors. The results are given in Table 3 (reactions 9 and 10).

$$Y(1) = H_{resp}(1 - e^{-k_{obs}t})$$
$$Y(2) = A_{resp}(1 - e^{-k_{obs}t})$$

Method C. This method was used in the study of the conversions of $AccysJ^+$ and $AccysJ'^+$ into βJ^+ and $\beta J'^+$ and the reverse reactions. A 10^{-5} M solution of omeprazole in 0.01 M HCl was stored for 10 min at 37 °C to prepare a solution of D^+. To this solution was added strong buffer to a pH value of 4.26. This solution was stored for 2 min at 37 °C to obtain as much HEJ^{2+} and HEJ'^{2+} as possible. Acetylcysteine was added to give a 10^{-4} M solution, and after 2 s Hβ was added to give a 0.01 M solution. At t seconds after the addition of the Hβ-solution, the mixture was injected onto a reversed-phase HPLC column and analyzed for βJ^+ and $\beta J'^+$. Measurements of both heights (H) and areas (A) were made and the units were selected to give numbers of the same magnitude in both types of measurements. With a LiChrosorb 100 RP-8,5 µm (4 × 4 mm ID) (Merck) precolumn, a LiChrosorb RP-8 5 µm (125 × 4 mm ID) (Merck) column, and a mobile phase consisting of 45 % acetonitrile, 45 % water and 10 % buffer, pH 6.9, µ=0.25 M, the retention times were: HS 5.0 min, βJ^+ 9.7 min and $\beta J'^+$ 10.2 min.

The calculation of the rate constants were performed using our general method, using the formulas, as above, to give the value of k_{obs} and the responses H_{resp} and A_{resp}. In this way the results in Table 3 (reactions 1 and 2) were obtained for the formation of βJ^+ and $\beta J'^+$. The same method was also used for the reaction of $\beta J^+ \rightarrow AccysJ^+$,

Table 4. The reaction of 10^{-5} M H_2EE^{2+} with 10^{-4} M Hβ at pH 4.26 and 37 °C.

	I_{EE}		TH_S		$TH_{\beta E}$	
t/s	Found	Calc.	Found	Calc.	Found	Calc.
44	70.2	67.8	0.89	0.70	0.45	0.75
174	30.7	36.8	2.24	2.03	2.28	3.24
408	14.3	12.2	3.14	3.10	3.51	3.42
620	12.2	4.5	3.24	3.44	3.74	3.79

k_{obs} = 4.7(6) × 10^{-3} s^{-1} or k = 47(6) M^{-1} s^{-1} for the second-order reaction of Hβ and H_2EE^{2+} at pH 4.26.

and $\beta J'^{+} \rightarrow AccysJ'^{+}$, with the difference that, in this case, Hβ was added before acetylcysteine. The equations used for the calculation were:

$$Y(1) = H_{resp}\, e^{-k_{obs}t}$$
$$Y(2) = A_{resp}\, e^{-k_{obs}t}$$

and the results are given in Table 3 (reactions 3 and 4) for the reaction of βJ^{+} and $\beta J'^{+}$.

Method D. This method was used to study the conversion of $HEAccys^{+}$ into HS and $HE\beta^{+}$. A 10^{-5} M solution of $HEAccys^{+}$ was prepared by adding a concentrated solution of omeprazole in methanol to an aqueous solution containing, 10^{-3} M HCl and 10^{-4} M acetylcysteine. After 30 min at 37 °C, when the formation of $HEAccys^{+}$ was complete, a small volume of a strong buffer containing Hβ was added to give pH = 4.27 and [Hβ] = 10^{-2} M. The resulting solution was kept at 37 °C. At suitable intervals aliquots were withdrawn and analyzed for $HE\beta^{+}$ and HS by HPLC. The ratio $[HS]/[HE\beta^{+}]$ was always >10. Since we know that k_{obs} = 0.0124 s^{-1} for the reaction $HE\beta^{+} + H\beta \rightarrow HS$ under the same conditions, we can easily conclude that only a very small quantity of all HS formed is formed from $HE\beta^{+}$. The main quantity of HS is thus formed by the reaction $HEAccys^{+} + H\beta \rightarrow Accys\beta + HS$, and all $HE\beta^{+}$ is formed by the reaction $HEAccys^{+} + H\beta \rightarrow HE\beta^{+} + AccysH$. This enables a calculation of both constants by standard methods, and we obtain k = 4.8(1) × 10^{-3} M^{-1} s^{-1} for reaction (5) $HEAccys^{+} + H\beta \rightarrow Accys\beta + HS$, and k = 0.51(1) × 10^{-3} M^{-1} s^{-1} for the reaction (6) $HEAccys^{+} + H\beta \rightarrow HE\beta^{+} + AccysH$.

Method E. This method was used in the investigation of the reaction $HE\beta^{+} + H\beta \rightarrow HS + \beta\beta$. A solution of $HE\beta^{+}$ in 0.001 M HCl was prepared by adding 50 µl of a 6 × 10^{-4} M methanolic solution of HA and 30 µl of a 0.1 M aqueous solution of β-mercaptoethanol to 3 ml of 0.001 M HCl and storing the solution at 37 °C for 30 min. A concentrated buffer solution, containing a buffer of a suitable pH value and 3 × 10^{-3} mmol of NaOH, was then added. The pH value of the solution was measured and the reaction was followed by HPLC to measure the peaks corresponding to $HE\beta^{+}$ and HS. Besides these and a few very small peaks corresponding to trace impurities, one additional peak was observed near the sulfide peak, but only in the first sample taken after less than a minute. (See the text for an explanation). The rate constant was calculated from the areas $A_{E\beta}$ and A_S of the peaks of $HE\beta^{+}$ and HS at different times t using the equations

$$A_{E\beta} = A^{\circ}_{\beta E}\, e^{-k_{obs}(t-t_0)}$$
$$A_S = A^{\circ}_S + A^{\infty}_S\,(1 - e^{-k_{obs}(t-t_0)})$$

and our general method for calculation where A°_S, $A^{\circ}_{E\beta}$, A^{∞}_S and k_{obs} are obtained from the regression. The values $A^{\circ}_{E\beta}$ and A°_S corresponded well to the areas at the time t_0.

Method F. This method was used in our study of the reaction of the disulfide H_2EE^{2+} with Hβ. A 10^{-5} M solution of H_2EE^{2+} was prepared by dissolving the crude PF_6^{-} salt of H_2EE^{2+} in a small volume of acetonitrile and adding this solution to a citrate buffer (µ = 0.5 M) containing Hβ (10^{-4} M) to give a pH of 4.26. The solution was kept at 37 °C. Aliquots were taken at different times and injected onto a reversed-phase HPLC column. H_2EE^{2+} appeared as a very broad peak, retention time (t_R) 4–9 min. A small peak corresponding to omeprazole appeared at t_R 3.15 min, and peaks corresponding to $HE\beta^{+}$ and HS at t_R 4.4 and 5.8 min, respectively. Owing to the large overlap between the H_2EE^{2+} and HS peaks only approximate values were obtained for the corresponding integrals or peak heights. The best values were obtained for the integral (I_{EE}) of H_2EE^{2+} and the peak height (TH) of HS and HE^{+}. In spite of this difficulty rather good values for the rate constants could be calculated using our general method in Ref. 1. The 'calculated' values were obtained from:

$$I_{EE} = R_{EE}\, e^{-k_{obs}t}$$
$$TH_{E\beta} = R_{E\beta}(1 - e^{-k_{obs}t})$$
$$TH_S = R_S(1 - e^{-k_{obs}t})$$

The results are given in Table 4.

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