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2-((2-PYRIDYLMETHYL)SULFINYL)BENZIMIDAZOLES: ACID SENSITIVE SUICIDE INHIBITORS OF THE PROTON TRANSPORT SYSTEM IN THE PARIETAL CELL

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Summary: 2-((2-Pyridylmethyl)sulfinyl)benzimidazoles, selective inhibitors of the H^+/K^+-ATPase in the parietal cells of the stomach, have been investigated concerning their chemical behaviour in acidic medium. Protonation of the sulfoxide and subsequent elimination of water forms a sulfenium ion or a chemical equivalent thereof. If no external nucleophiles are present, a rearrangement process takes place. In the presence of mercaptans, the sulfenium ion is trapped giving rise to a variety of products. On the basis of these results, a mechanistic scheme is proposed for the inactivation of the H^+/K^+-ATPase by these compounds.

The discovery and development of H_2-antagonists has been a major breakthrough in the treatment of peptic ulcers. More recently, another class of compounds with potent and selective gastric antisecretory activity has become the center of interest (1): the 2-((2-pyridylmethyl)sulfinyl)benzimidazoles. These compounds are being developed by Hässle AB, Sweden, and also, more recently, by Byk-Gulden in Germany. The prototypes are shown in Fig. 1.

These compounds have a unique pharmacological profile: they are potent antisecretory agents with a long duration of action (2) and with high organ selectivity (3). They block the H^+/K^+-ATPase of the parietal cell, the "proton pump" which is responsible for the transport of gastric acid into the lumen of the stomach (4). However, the instability of the compounds at the pH of their target organ together with the discrepancy between plasma half life and duration of action (2) suggests that degradation products rather than the sulfoxides themselves are the "active principle". This hypothesis

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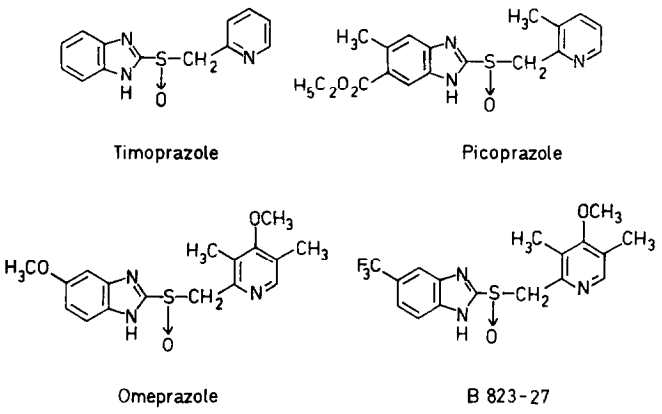


Figure 1

has recently been proposed independently from us by B. Wallmark et al. (5), and also by A. Brändström and B. Wallmark (6).

In order to elucidate the structure of this "active principle", we treated timoprazole under conditions which resemble the physiological condition in the stomach (0.1n HCl, room temperature). The solution turned yellow almost instantly, and after 30 min. a dark violet solid could be isolated as the main product of decomposition (Figure 2). The elemental analysis showed that the compound had lost one molecule

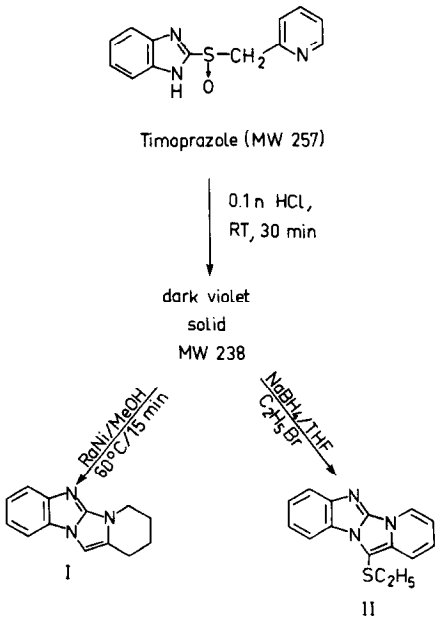
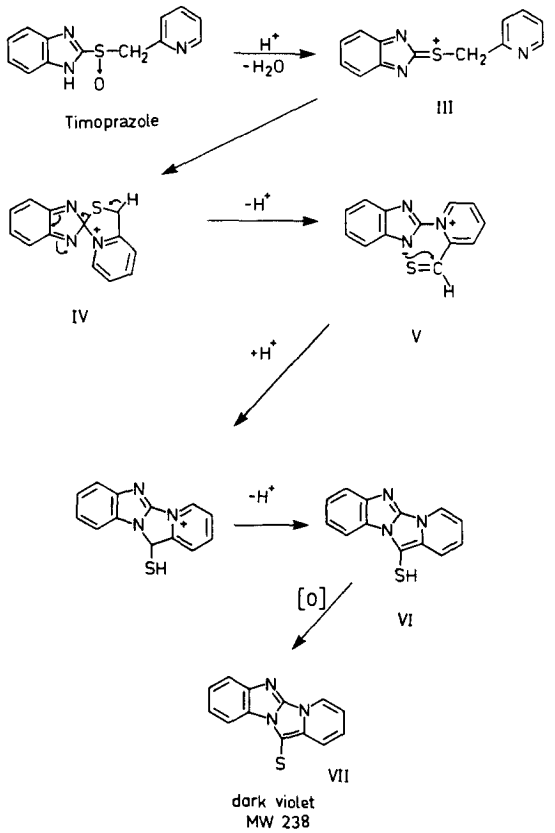


Figure 2

of water. No reasonable NMR could be obtained, and the molecular ion in the MS was always one mass unit too low. The first clues came from two derivatives of this decomposition product: treatment with Raney nickel in methanol rapidly decolorized the solution and yielded a compound with the so far unknown tetracyclic pyrido(1,2-c)imidazo-(1,2-a)-benzimidazole skeleton (I). On the other hand, reduction with $NaBH_4$ in methanol and subsequent alkylation with ethyl bromide gave a thioether derivative (II) which indicated that the sulfur was in position 12 of this tetracycle. Both structures were confirmed by X-ray analysis.

This information together with other chemical evidence, which will be published separately (7), enable us to propose a mechanism for the acid catalyzed rearrangement (scheme 1).



Scheme 1

After protonation at the sulfoxide oxygen and elimination of H_2O a sulfenium ion III is formed which is attacked intramolecularly by the pyridine nitrogen to yield the spiro compound IV. Fragmentation in the indicated manner liberates thioaldehyde V which rapidly reacts with the benzimidazole nitrogen to form a rather unstable thiole VI, which cannot be isolated under the usual conditions. The isolated compound VII is most probably a radical, which explains the dark color, the missing proton in the MS and the negative results of the NMR spectroscopy. ESR studies did not give unambiguous results, probably due to solubility problems.

The idea of a rather unstable thiol compound or a radical being the "active principle" of timoprazole was quite attractive, because it would explain the mentioned properties of this class of compounds: since the rearrangement is acid catalyzed, the active principle is liberated selectively in the parietal cells. Reaction of the instable thiol or the radical with protein structures may inactivate the enzyme by cleavage of -SS-bonds or by a radical mechanism. Timoprazole and related compounds can thus be considered as acid-sensitive 'suicide inhibitors'.

This hypothesis was supported by another observation (Figure 3). Reduction of the radical VII by $NaBH_4$ and subsequent reaction with acetic anhydride yielded the compound VIII, which as an active ester lost the acetyl group on the TLC-plate or on standing in methanol and

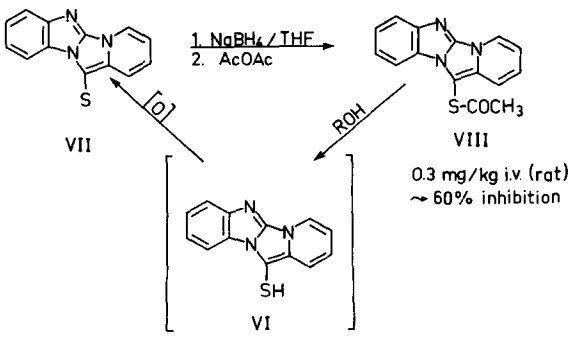


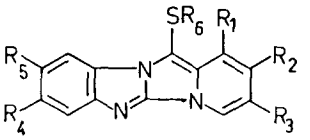
Figure 3

formed via the instable thiol VI the radical VII back. This process should also happen in the presence of acid in the parietal cell. If the thiol or the radical were the active principle, compound VIII should show some antisecretory activity. After i.v.-administration in the rat, VIII is indeed indistinguishable from timoprazole in potency and duration of action.

However, after i.d.- or i.p.-administration the antisecretory activity of compound VIII is much weaker than timoprazole. Probably the compound decomposes in the presence of body fluids or tissues, before it can reach its destination, the parietal cell. Our efforts to synthesize related compounds in order to find thiol protecting groups which are cleaved more selectively in acidic medium will also be described separately (7).

The antisecretory in-vivo- and in-vitro-activity of the radical corresponding to omeprazole as well as of some derivatives could surprisingly not be compared to omeprazole (Table 1).

TABLE 1



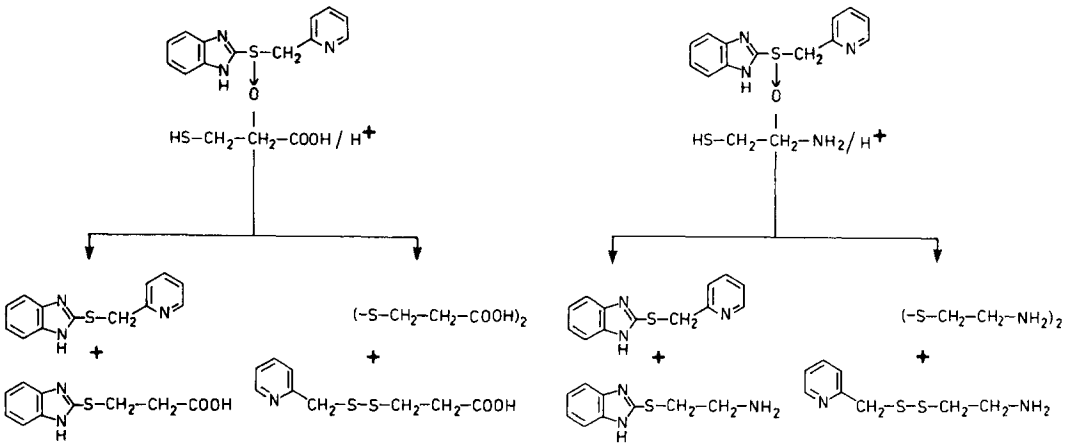
R_1	R_2	R_3	R_4	R_5	R_6	in-vivo-activity[8] % inhibition (5mg/kg i.p.) (rat)	in-vitro-activity[9] IC_{50} (µmol/l)
H	H	H	H	H	•	10	11.4
H	H	H	H	H	$COCH_3$	15	9.5
H	H	H	H	H	Boc	30	6.7
H	H	H	H	H	benzoyl	13	13.0
H	OCH_3	H	H	H	Boc	9	100.0
CH_3	OCH_3	CH_3	H	OCH_3	•	35	22.3
CH_3	OCH_3	CH_3	H	H	Boc	10	N.D.
	Timoprazole					70	6.0
	Omeprazole					94	0.5

N.D. = not determined

With these findings in mind we had to recheck our original hypothesis, since obviously the acid-catalyzed rearrangement products contribute only a minor part, if any, to the antisecretory profile of omeprazole. In the body, omeprazole is transformed into the active compound in the acidic compartments of the parietal cell (3), conditions which do not closely resemble the conditions in the test tube. In the test tube no peptide or protein structures are present which might provide potential nucleophiles in their side chains. The proposed rearrangement mechanism involves several unstable intermediates which should be highly reactive towards nucleophiles (i.e. sulfenium-ion, thioaldehyde). We therefore reexamined the acid catalyzed rearrangement process in acidic medium in the presence of nuleophiles such as -SH, $-NH_2$, $-COO^-$, which may also be part of peptide side chains. Cysteine is an amino acid which provides all three potential nucleophiles. If omeprazole is treated with 0.1 n HCl in the presence of an excess of cysteine, no radical is formed. On the other hand, if cystine is used instead of cysteine, the colored rearrangement products are formed in the same way as before. This observation clearly indicates that a free SH-group is necessary to prevent rearrangement.

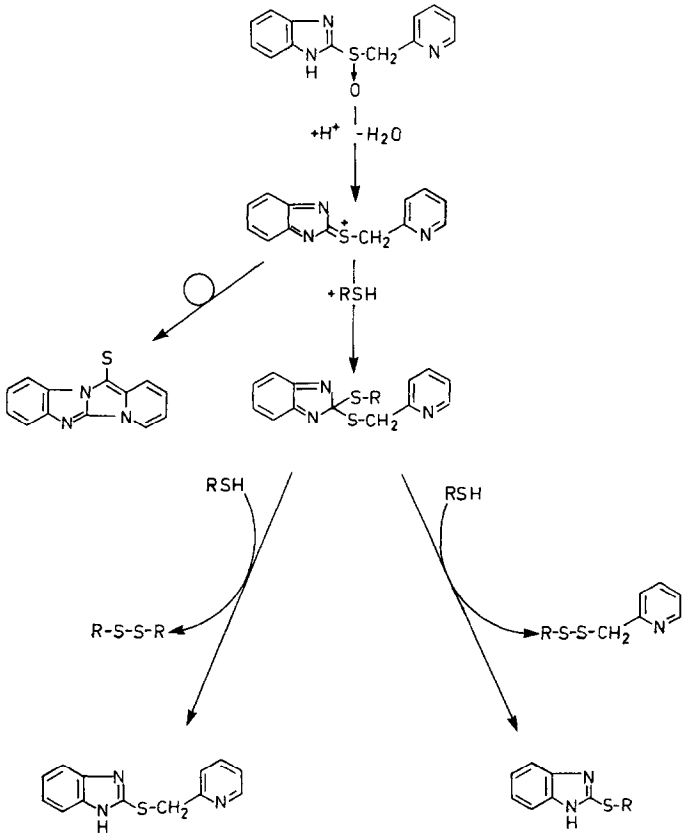
For reasons of handling of the reaction products we chose 3-mercaptopropionic acid resp. cysteamine as a nucleophile in the acid catalyzed activation reaction and identified the reaction products (see Scheme 2).

Based on these results, a reaction scheme (Scheme 3) can be proposed. The initial step in all cases is protonation of the sulfoxide (which explains the selectivity of the compounds for acidic compartments in the body and the inactivity of the corresponding thioethers and sulfones) and subsequent elimination of water to form a sulfenium ion or a chemical equivalent thereof. If no external nucleophiles are present, this ion is attacked intramolecularly by the pyridine



Scheme 2

nitrogen to form the above mentioned rearrangement products, which may inactivate the enzyme by a radical mechanism or by the cleavage of -SS-bonds. In the presence of mercaptans RSH, a dithioketal intermediate is formed. Either sulfur group can be attacked by a



Scheme 3

second molecule of RSH giving rise to the formation of the thioether corresponding to the sulfoxide and the symmetrical disulfide RSSR (see also similar proposal in (6)), or the thioether 2-benzimidazolyl-SR and the unsymmetrical disulfide 2-picolyl-SSR. Driving force for this process is obviously the rearomatization of the benzimidazole system.

In the case that RSH originates from the active site of the H^+/K^+-ATPase, the formation of the oxidized enzyme RSSR, of the dithioketal-derivative or of benzimidazolyl-SR resp. 2-picolyl-SSR might explain the longlasting inhibition of the proton transport system by timoprazole and related compounds.

A similar mechanistic rational has been proposed for the sulfoxide moiety in the inhibition of peptidyl transferase by sparsomycin (10).

Whether all of the mentioned pathways (to different extents) are responsible for the selective antisecretory activity or whether it is exclusively one, is subject of further investigations presently underway in our laboratories.

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