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Communications to the Editor

The Mechanism of Action of the Gastric Acid Secretion Inhibitor Omeprazole

Sir:

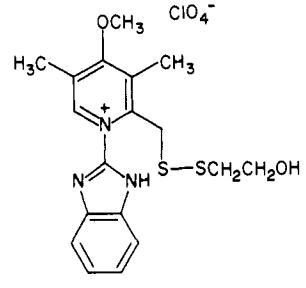
Omeprazole (1), a potent antiulcer agent,[1,2] is presently under extensive clinical evaluation. This compound and several close analogues are effective inhibitors of gastric acid secretion in the rat, dog, and man,[3-5] by being inhibitors of the gastric H^+,K^+-ATPase[6] (unlike acid secretion inhibitors such as cimetidine). The enzyme H^+,K^+-ATPase is responsible for gastric acid production and is located in the secretory membranes of the parietal cell.[7,8] Omeprazole itself is not an active inhibitor of this enzyme, but it is transformed within the acid compartments of the parietal cell into the active inhibitor, close to the enzyme.[9]

We now report the isolation and structure of a new sulfenamide (4) from acid decomposition of omeprazole (1) and propose, in contradiction to two recent investigations,[10,11] that sulfenamide 4, or the corresponding unstable sulfenic acid 3, is the active inhibitor formed in vivo from omeprazole (1).[12]

Chemistry. To obtain information about the mechanism of action at a molecular level we undertook an extensive study of the acid degradation of omeprazole (1). We also studied some structural analogues to avoid unnecessary isomer problems.

Of crucial importance to this work was the great simplification of decomposition by adding β-mercaptoethanol to the acid medium before addition of omeprazole (1). This caused only two compounds to form: the sulfide 8 and, according to ^1H NMR [PF_6^- salt: δ (500 MHz, $CDCl_3$) 2.5 (s, 6 H), 2.65 (t, 2 H), 3.6 (t, 2 H), 3.85 (s, 3 H), 4.2 (s, 2 H), 4.3 (s, 3 H), 7.05 (dd, 1 H), 7.1 (d, 1 H), 7.6 (d, 1 H), 8.35 (s, 1 H)], an adduct with β-mercaptoethanol (5).[13]

At high dilution (10^{-5} M) in dilute hydrochloric acid (0.001–0.5 M) the conversion of omeprazole (1) into an intermediate 4 (taking 5–15 min to completion at 37 °C) could be followed kinetically by UV. When a slight excess of β-mercaptoethanol was added to the resulting solution, a very rapid conversion of 4 into the above-mentioned adduct 5 could be followed kinetically by UV. The three UV spectra recorded before the addition of acid, 15 min after the addition of acid, and after the addition of β-mercaptoethanol are identical with those presented in ref 10. We were also able to obtain crystals for X-ray analysis of the β-mercaptoethanol adduct (ClO_4^- salt from methanol) generated from the omeprazole analogue 2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, resulting in the disulfide structure 9,[14] containing a pyridinium cation.



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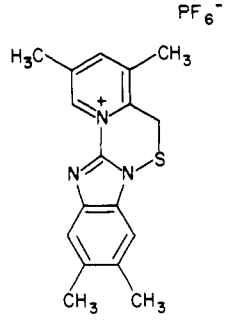

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On decomposition of omeprazole (1) in methanol instead of water, the intermediate 4 was significantly more stable, even at moderate concentrations. When a 0.1 M solution of 1 in 0.2 M methanolic HCl was allowed to stand at room temperature for 7 min and then a 50% excess of 50% HBF_4 or 70% HPF_4 was added, crystals of the corresponding salts of 4 were obtained in a yield of about 45%. ^1H NMR spectra (in acetonitrile) of these salts were characterized by a very low shift (δ 9.3) for the proton in

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(3) Larsson, H.; et al. *Gastroenterology* **1983**, *85*, 900.
(4) Brändström, A.; Lindberg, P.; Junggren, U. *Scand. J. Gastroenterol.* **1985**, *108* (Suppl.), 15.
(5) Lind, T.; Cederberg, C.; Ekenved, G.; Haglund, U.; Olbe, L. *Gut* **1983**, *24*, 270.
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(7) Smolka, A.; Helander, H. F.; Sachs, G. *Am. J. Physiol.* **1983**, *245*, G589.
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(9) Wallmark, B.; Brändström, A.; Larsson, H. *Biochim. Biophys. Acta* **1984**, *778*, 549.
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(12) Reported for the first time at the ASPET–ACS meeting in Boston, MA, August 18–22, 1985. See: Brändström, A. *Pharmacologist* **1985**, *27*, 104. Also reported (during the preparation of this manuscript) at the 3rd SCI–RSC Medicinal Chemistry Symposium in Cambridge, UK, September 15–18, 1985: Wallmark, B.; Brändström, A.; Lindberg, P. Abstract S 16. At the same symposium the sulfenamide and the β-mercaptoethanol adduct structures were presented, independently of us, also by another group: Figala, V.; et al. Abstract P 20.
(13) Anal. ($C_{19}H_{24}N_3O_3S_2PF_6$) H, N; C: calcd, 46, 26; found, 44.88.
(14) Anal. ($C_{18}H_{22}N_3O_6S_2Cl$) C, H, N, S. The X-ray investigation was performed by Anders Svensson, Leif Andersson, and Lennart Sjölin (unpublished results).

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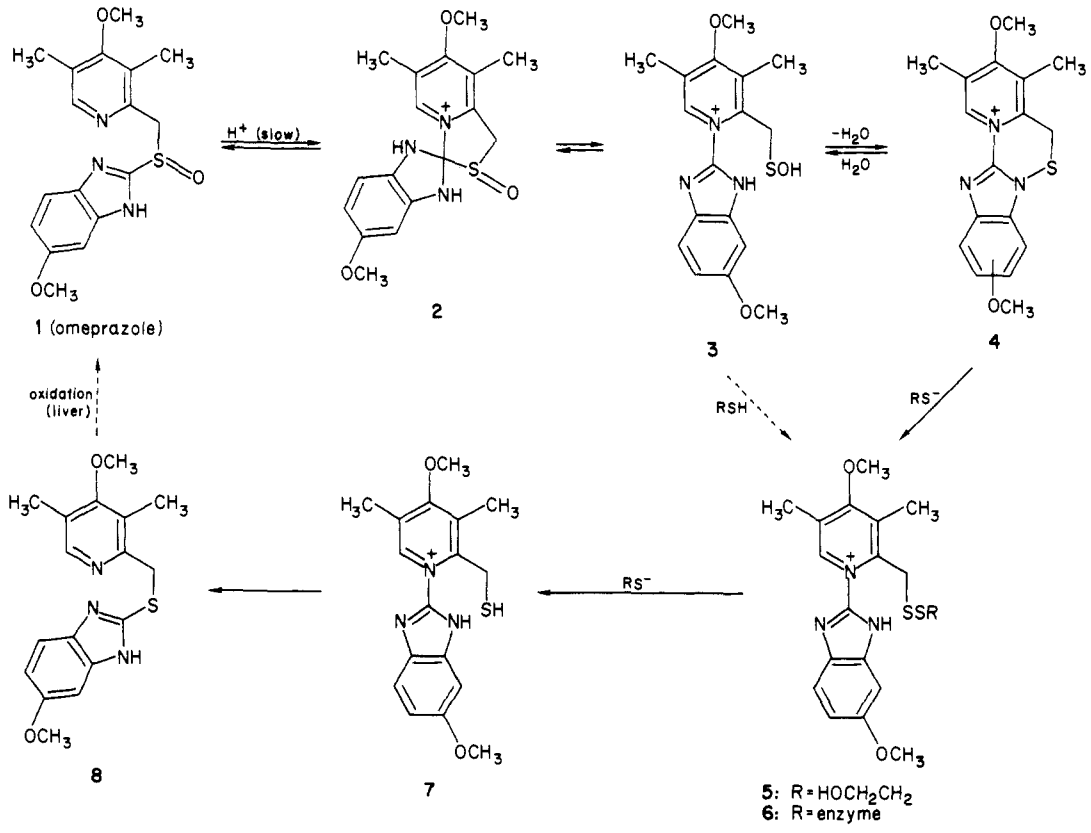
Scheme I



the 6-position of the pyridine ring. An X-ray investigation of a PF_6^- salt of an analogue to compound **4** revealed the cyclic sulfenamide structure **10**.[15] The sulfenamide intermediate **4**[16] from omeprazole (**1**) is a mixture of two isomers, which can easily be seen from the ^1H NMR spectrum [$AuCl_4^-$ salt: δ (500 MHz, CD_3CN) 2.5 (s, 3 H), 2.6 (s, 3 H), 3.9 (s, 3 H), 4.3 (s, 3 H), 4.85 (s, 2 H), 7.05 and 7.15 (2 dd, total 2 H), 7.1 and 7.3 (2 d, total 1 H), 7.7 and 7.5 (2 d, total 1 H), 9.25 and 9.3 (2 s, total 1 H)].

The reaction mechanism we propose for the acid transformation of omeprazole (**1**) to the sulfenamide isomers **4** is outlined in Scheme I. The reaction is reversible and goes via the spiro intermediate **2** and the sulfenic acid **3**. The reversibility was firmly proved by kinetic measurements in both directions, i.e., starting from **1** or **4**. An equilibrium solution contains **1** and **4** in the ratio 1:10 (HPLC measurements; unpublished results). The formation of the spiro intermediate **2** in the rate-limiting step is supported by kinetic measurements. The rate constants obtained for omeprazole analogues are strongly dependent on substituents in the pyridine ring, indicating that a positive charge is created on the pyridine nitrogen atom in the rate-limiting step. In the decomposition of a 1 mM solution of **1** in 0.5 mM HCl, it was observed that the pH increased at a rate corresponding to the consumption of one proton per molecule of **1** in the rate-limiting step, in good agreement with the mechanism proposed. The spiro intermediate **2** is a dihydrobenzimidazole, with a pronounced tendency to undergo aromatization, thus forming the sulfenic acid **3** (which we were unable to isolate) by a C–S bond cleavage. The subsequent formation of the sulfenamide **4** is in accordance with known reactions between sulfenic acids and amines.[17] Likewise, the reaction of **4** with β-mercaptoethanol to form adduct **5** is now easily understood, since sulfenamides or sulfenic acid derivatives in general are known to react with mercaptans to form disulfides.[17] The adduct **5** may then react with a second molecule of β-mercaptoethanol in a base-catalyzed (unpublished results) reaction to form the sulfide **8**, probably via the unstable mercaptan **7**, resulting from an S–S bond cleavage during simultaneous formation of the disulfide of β-mercaptoethanol.

The introduction of a methyl group in the 6-position of the pyridine ring of omeprazole analogues results in compounds stable in acid solution. This supports the suggested mechanism, since space-filling models show that the 6-methyl group will experience a strong steric interference with the imidazole ring, which prevents the formation of the spiro intermediate **2**.

Biochemistry and Pharmacology. When the isomeric sulfenamide mixture **4** was added to an isolated H^+,K^+-ATPase preparation,[18] we observed an instantaneous block of the enzyme with an IC_{50} value of 0.6×10^{-6} M. With the concentrations of omeprazole and enzyme used above, the extent of block is increased with increasing time of contact with the enzyme and with decreasing pH and will under no conditions exceed 50%.

A mercapto group is known to be involved in the inhibiting reaction,[19] and therefore the transformations described in Scheme I can be considered as a model of the reactions in the acid compartments of the parietal cell

(15) Anal. ($C_{17}H_{18}N_3SPF_6$) C, H, N. The X-ray investigation was performed by Staffan Sundell and Max Lundmark (unpublished results).

(16) The IUPAC names of the two isomers are 2,4-dimethyl-3,9- and 2,4-dimethyl-3,10-dimethoxy-5*H*-pyrido[1′,2′:4,5][1,2,3]-thiadiazino[2,3-*a*]benzimidazol-13-ium tetrachloroaurate. Anal. ($C_{17}H_{18}N_3O_2S{\cdot}AuCl_4$) C, H, N, O, S.

(17) Hogg, D. R. In *Comprehensive Organic Chemistry*; Barton, D., Ullis, W. D., Eds.; Pergamon: London, 1979; Vol. 3, p 261.

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when omeprazole inhibits the H^+,K^+-ATPase enzyme. This formation of a disulfide is a fundamentally different mechanism of action of omeprazole than those previously proposed by Im et al.[10] and by Rackur et al.[11]

In vitro the enzyme–adduct complex **6** reacts with β-mercaptoethanol to form the sulfide **8** and the enzyme–β-mercaptoethanol complex enzyme–$SSCH_2CH_2OH$, in which form the enzyme is still blocked. This complex then slowly reacts with β-mercaptoethanol to the free enzyme enzyme–SH.[19] It is not clear whether, under in vivo conditions, glutathione or any other endogenous mercaptan can form a corresponding enzyme–mercaptan complex and furthermore react in a second step to generate the free enzyme and the disulfide of glutathione. Recovery of the enzyme activity might require synthesis of the enzyme de novo.

Since the sulfenamide **4** and the sulfenic acid **3** are permanent cations, neither possible active inhibitors can probably penetrate the secretory membrane of the parietal cell. This fact and maybe also the permanent-cation character of the enzyme–inhibitor complex **6** might be important factors for the in vivo inhibiting effect of omeprazole (**1**).

The sulfide **8** is formed from omeprazole in isolated gastric glands.[9] This is probably also the case in the gastric mucosa in vivo. We have also demonstrated that the sulfide **8** in vivo is oxidized to the sulfoxide omeprazole (**1**), thus closing the cyclic reaction process. However, in the in vivo situation, due to simultaneous metabolic processes, probably only a fraction of the total omeprazole dose may complete this cyclic process.

Factors contributing to the specificity of the inhibition of gastric acid secretion by omeprazole are as follows: (1) It is a well-known fact that weak bases concentrate in acidic compartments. Omeprazole is a weak base and therefore concentrates in acidic compartments.[9] (2) The parietal cell containing the enzyme H^+,K^+-ATPase is the only cell in the body with a low pH value. The low pH value causes the conversion of omeprazole into the active inhibitor close to the enzyme that produces the acid. (3) The active inhibitors, the sulfenamide **4**, and the sulfenic acid **3** are permanent cations with limited possibilities to penetrate membranes.

Registry No. **1**, 73590-58-6; **2**, 102283-09-0; **4**·$AuCl_4^-$ (3,9-$(OMe)_2$), 102283-06-7; **4**·$AuCl_4^-$ (3,10-$(OMe)_2$), 102283-08-9; **5**, 102283-11-4; **8**, 73590-85-9; **9**, 102260-42-4; **10**, 102260-44-6; HO-$(CH_2)_2$SH, 60-24-2.

Supplementary Material Available: X-ray data on compounds **9** and **10** (7 pages). Ordering information is given on any current masthead page.

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Articles

Streptonigrin. 1. Structure–Activity Relationships among Simple Bicyclic Analogues. Rate Dependence of DNA Degradation on Quinone Reduction Potential

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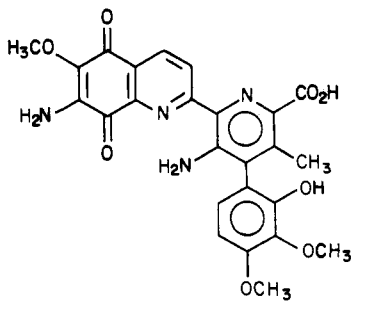
A series of simple aza and diaza bicyclic quinones related to the AB ring system of streptonigrin (**1**) have been synthesized and tested in vitro for their ability to degrade DNA under conditions similar to those used with the parent drug. The results obtained from a study of 22 quinones indicate that there is a quantitative linear relationship between their reduction potentials and the rate at which they degrade DNA under identical conditions in vitro. Almost all of the synthetic substances were superior to **1** in their DNA-degrading ability.

I. Introduction

Streptonigrin (**1**) is an antitumor agent that has seen only limited use as an anticancer agent because of its toxicity[1] but continues to receive attention because of interest in its ability, common to a number of quinone antibiotics, to degrade[2] DNA. This ability has been demonstrated both in vivo and in vitro. The drug is lethal to *Escherichia coli* and to human leucocytes and in both cases DNA degradation[3,4] is observed. These effects seem to be



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(2) For a recent review, see: Lown, J. W. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; Chapter 9, pp 283–314.

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