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IDENTIFICATION OF TWO MAIN URINARY METABOLITES OF [^{14}C]OMEPRAZOLE IN HUMANS

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ABSTRACT:

The excretion and metabolism of [^{14}C]omeprazole given orally as a suspension was studied in 10 healthy male subjects. An average of 79% of the dose was recovered in the urine in 96 hr, with most of the radioactivity (76% of dose) being eliminated in the first 24 hr. Pooled urine (0–2 hr) from five subjects, containing about 47% of the dose, was analyzed by reverse phase gradient elution LC with radioisotope detection. Omeprazole was completely metabolized to at least six metabolites. The two major metabolites were extensively purified by LC and their structures were determined by MS with derivatization and use of stable isotopes, ^{1}H NMR, and comparison with synthetic references. They were formed by hydroxylation of a methyl group in the pyridine ring, followed by further oxidation of the alcohol to the corresponding carboxylic acid. Both metabolites retained the sulfoxide group of omeprazole, rendering them as unstable as the parent compound at pH < 7. They accounted for approximately 28% (hydroxyomeprazole) and 23% (omeprazole acid) of the amount excreted in the 0–2-hr collection interval. Based on *in vitro* studies with the synthetic metabolites in isolated gastric glands, it is unlikely that M1 and M2 will contribute to the pharmacological effect of omeprazole in humans.

Omeprazole, a substituted 2-pyridinyl-2-sulfinyl benzimidazole, represents a new class of antisecretory agents. The compound effectively suppresses gastric acid secretion in rats, dogs, and humans (1, 2). An enteric coated formulation of omeprazole is presently under extensive clinical evaluation and has been shown to be superior to cimetidine (3) and ranitidine (4, 5) in the healing of duodenal ulcers. Promising results have been achieved with omeprazole in the management of Zollinger-Ellison patients (6, 7). Mechanistically, omeprazole differs from, for example, H_2 receptor blockers, by its ability to selectively inhibit gastric H^+,K^+-ATPase (8), the gastric proton pump of the gastric mucosa (9). The selectivity of omeprazole for inhibition of this enzyme is due to the fact that the protonated species of the lipid-permeable weak base (pK_a = 4) accumulates in the acidic secretory canaliculus of the parietal cell. Then, acidic conditions transform omeprazole into an inhibitor of the H^+,K^+-ATPase (10). By this mechanism, the active inhibitor is generated very close to its target enzyme (11, 12). Because the H^+,K^+-ATPase is uniquely located in the membrane that separates the acidic canaliculus of the parietal cell from the neutral compartments, activation of omeprazole will predominantly occur in the parietal cell (13).

A review of the pharmacodynamic and pharmacokinetic properties and therapeutic potential of omeprazole has been published (14). Omeprazole is a high clearance drug, with about 54% of an oral dose being available to the systemic circulation in humans. Previous studies in dogs, rats, and mice have demonstrated that omeprazole is extensively metabolized, with rapid elimination of metabolites by renal and biliary mechanisms (15). The structures of seven urinary metabolites observed after high oral doses of omeprazole to rats and dogs have been described (16). We now report on the renal elimination of metabolites after oral administration of [^{14}C]omeprazole to healthy subjects and the identification of the two major urinary metabolites. Steps were taken to prevent decomposition of the acid-sensitive parent compound and metabolites during drug administration, sample collection, and analysis.

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Materials and Methods

Chemicals. [^{14}C]Omeprazole was synthesized by reacting potassium [^{14}C]diethylxantogenate (from [^{14}C]carbon disulfide and KOH in ethanol) with 1,2-diamino-4-methoxybenzene. The product, [2-^{14}C]2-mercapto-5-methoxy-1*H*-benzimidazole, was treated with 2-chloromethyl-4-methoxy-3,5-dimethyl-pyridine. The resulting omeprazole-sulfide was oxidized with *m*-chloroperbenzoic acid to yield [^{14}C]omeprazole with the position of label as denoted in fig. 1. [2-^{13}C]2-Mercapto-5-methoxy-1*H*-benzimidazole was made by condensing [^{13}C]thiourea with 1,2-diamino-4-methoxybenzene. The rest of the ^{13}C synthesis was analogous to the ^{14}C synthesis.

M1, 5-methoxy-2-[[(4-methoxy-3-methyl-5-hydroxymethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (hydroxyomeprazole), was synthesized in 21 steps by an independent route, starting with 3-cyano-5,6-dimethylpyrid-2-one (17). The latter compound was formed as a mixture with 3-cyano-5-ethylpyrid-2-one, as known from published data (18). The isomers were later separated to yield pure 5-acetoxymethyl-2,3-dimethyl-4-nitropyridine-1-oxide. The above-mentioned mixture was treated with PCl_5 and oxidized with HNO_3/H_2SO_4 to give 2-chloro-5,6-dimethyl-nicotinic acid. Catalytic dehalogenation, acetylation, oxidation with H_2O_2, and nitration with NO_2BF_4 yielded, after chromatographic separation, the above-mentioned 5-acetoxymethyl-2,3-dimethyl-4-nitropyridine-1-oxide. ^{1}H NMR ($CDCl_3$): 2.13 ppm (s, 3H, $-COCH_3$), 2.36 ppm (s, 3H, $ArCH_3$), 2.56 ppm (s, 3H, $ArCH_3$), 5.14 ppm (s, 2H, $-CH_2-$), 8.38 ppm (s, 1H, ArH).

Successive treatment with sodium methoxide, *p*-nitrophenylacetate, acetic anhydride, and sodium hydroxide gave 2,5-bis-(hydroxymethyl)-4-methoxy-3-methylpyridine. Careful acetylation and chromatographic purification gave the key intermediate 2-acetoxymethyl-5-hydroxymethyl-4-methoxy-3-methylpyridine. ^{1}H NMR ($CDCl_3$): 2.13 ppm (s, 3H, $-COCH_3$), 2.28 ppm (s, $ArCH_3$), 3.88 ppm (s, 3H, $-OCH_3$), 4.75 ppm (s, 2H, $-CH_2OH$), 5.23 ppm (s, 2H, $—CH_2OOC—$, 8.48 ppm (s, 1H, ArH); ^{13}C NMR ($CDCl_3$): 10.5 ppm ($ArCH_3$), 20.6 ppm ($COCH_3$),

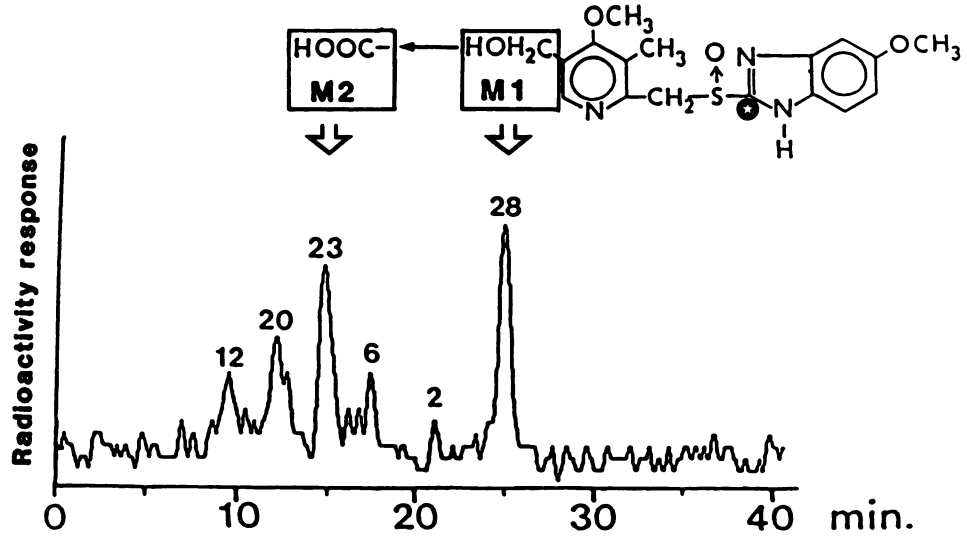


FIG. 1. *Metabolic pattern and structures of the two identified metabolites in pooled urine (0–2 hr) from five healthy subjects after an oral dose of 60 mg (25 μCi) of [^{14}C]omeprazole.*

The structure of parent drug is given in the *upper panel* of fig. 4. Metabolites were separated by reverse phase LC with gradient elution, followed by radioisotope detection. The *figures* above the peaks indicate the percentage of radioactivity present. The position of the ^{14}C label is denoted.

58.1 ppm (−O*C*H$_3$), 61.0 and 65.3 ppm (−*C*H$_2$−), 125.7, 129.3, 147.7, 154.7, and 163.9 ppm (Ar*C*), 170.6 ppm (−O*C*O−). This intermediate yields, if chlorinated and catalytically hydrogenated, 2-acetoxymethyl-4-methoxy-3,5-dimethylpyridine, which is identical with authentic material used in the synthesis of omeprazole.

Protection of the 5-hydroxymethyl group as the *o*-toluylester allowed for a selective hydrolysis of the 2-acetoxymethyl group. Chlorination and subsequent reaction with 2-mercapto-5-methoxy-1*H*-benzimidazole gave the sulfide, which was oxidized to sulfoxide. Hydrolytic removal of the *o*-toluylgroup completed the synthesis of M1.

M2, 5-methoxy-2-[[(5-carboxylate-4-methoxy-3-methyl-2-pyridinyl)-methyl]sulfinyl]-1*H*-benzimidazole, disodium salt (omeprazole acid, Na salt), was synthesized in 11 steps starting with 2,4-dihydroxy-5,6-dimethylnicotinic acid ethyl ester (19), which, after chlorination and selective dechlorination by catalytic hydrogenation, gave 4-chloro-5,6-dimethylnicotinic acid ethyl ester. Successive treatment with peracid, sodium methoxide, acetic anhydride, sodium methoxide, and $SOCl_2$ led to 6-chloromethyl-4-methoxy-5-methylnicotinic acid methylester. Treatment with 2-mercapto-5-methoxy-1*H*-benzimidazole gave a sulfide, which was oxidized to sulfoxide. Hydrolytic removal of the methyl ester group by base yielded the desired disodium salt of M2.

All solvents were of reagent grade and were used without further purification. Diazomethane was freshly prepared from Diazald (Aldrich Chemical Co., Millwaukee, WI) by distillation. Deuterated diazomethane (CD_2N_2) was produced according to the same method by slowly adding Diazald, dissolved in diethyl ester, to equal volumes of NaOD (2 mol/liter) and C_2H_5OD, followed by distillation. *N,O*-bis-(trimethylsilyl)-acetamide was purchased from Macherey-Nagel (Düren, Germany) and d_{18}-*N,O*,-bis-(trimethylsilyl)-trifluoroacetamide from Regis (Morton Grove, Il). Deuterated methanol (CD_3OD, 99.95%) for ^{1}H NMR measurements was obtained from Ciba-Geigy (Basel, Switzerland) and Insta-Gel from Packard (Zürich, Switzerland).

Drug Administration. The study was conducted in accordance with the "Declaration of Helsinki" as revised in Tokyo, Japan, 1975. After a full clinical examination, 10 healthy male subjects of median age 34 years (range, 27–39 years and median weight 74 kg (range, 65–84 kg) were included in the study. They were informed, both verbally and in writing, about the purpose and risks of the study and they gave their written consent to participate. The study was approved by the Ethics and Isotope Committees of the Medical Faculty of the University of Göteborg and was reviewed by the Swedish Board of Health and Welfare.

Alcohol and all medication, including over-the-counter drugs, were not permitted for 2 days before and during the experiment. The subjects arrived at the laboratory at about 8 a.m., having fasted since 10 p.m. the previous day. Control samples were collected before the administration of omeprazole.

A dose of 60 mg of omeprazole suspended in 50 ml of aqueous sodium bicarbonate solution (8 mmol/50 ml) was given orally. The bottle was then rinsed with 50 ml of the sodium bicarbonate solution and the liquid was ingested, to assure a complete intake of the dose. The suspension contained 25 μCi (925 KBq) of ^{14}C-labeled omeprazole [0.5 mg; radiochemical purity by LC (20) of 95%]. To protect the drug from exposure to the gastric acidic environment, 50 ml of the sodium bicarbonate solution was given orally 5 min before and at 10, 20, and 30 min after drug administration.

Five subjects collected all urine over 24-hr intervals into bottles containing 60 ml of 1 M Na_2CO_3. The urine volumes were recorded and 50 ml of each sample were stored frozen until determination of radioactivity.

The other five subjects collected all urine at intervals of 0–1, 1–2, 2–4, 4–6, 6–9, 9–12, 12–24, 24–48, 48–72, and 72–96 hr after omeprazole administration. Urine was collected in bottles containing 2.5 ml of 1 M Na_2CO_3 per hr of collection and the urine volumes were recorded. The entire volume collected during the first 12 hr and 50-ml aliquots during the rest of the experiment were stored frozen. The urine from these five subjects was used for determination of excreted radioactivity and for identification of metabolites.

Analytical Methods. For radioactivity measurements, the urine samples (0.5–3 ml) were mixed with 8 ml of Insta-Gel followed by liquid scintillation counting in a Mark III, model 6880 (Searle Analytic, Inc.) instrument, with external standard quench correction.

The liquid chromatographic system consisted of two pumps (Altex 110A; Berkeley, CA), which were controlled by a microprocessor (Altex 421). Samples were injected *via* a Rheodyne valve (Cotati, CA) fitted with a 250-μl loop.

For semipreparative gradient analysis of the urine, an 18-ml loop was connected to the injector. The columns used were LiChrosorb RP-8 (7 μm, 15 cm × 4.5 mm i.d.), Polygosil C_{18} (5 μm, 15 cm × 4.5 mm i.d.), Hibar SI-60 (7 μm, 25 cm × 10 mm i.d.), Hibar RP-8 (7 μm, 25 cm × 10 mm i.d.), and LichroPrep RP-8 (5–20 μm, 25 cm × 7.8 mm i.d.).

The absorbance of the eluate was measured at 302 nm using a Waters Lambda-Max 480 UV detector (Milford, MA) and recorded using a

Metrawatt Servogor 120 dual channel recorder (Nürnberg, Germany). The UV spectra of peaks eluting from the column were obtained with a Hewlett Packard 1040 diode array detector connected to a HP 85 microcomputer (Waldbronn, Germany). A Berthold LB 503 HPLC monitor (Wildbad, Germany) with a 200-μl glass scintillator cell was used to measure the eluting radioactivity from the column on-line. The data were stored on discs for further evaluation by a Berthold chromatography data system with an Apple computer. The eluting LC fractions were collected by a LKB 2211 Superrac (Bromma, Sweden) and a Kontron Sampler MSI 660 (Zürich, Switzerland) was used for repeated injections during purification of M2.

The ^{1}H NMR spectra were recorded at 500 MHz in a Bruker AM 500 instrument (Karlsruhe, Germany). All shifts were given in ppm by reference to d_4-methanol at 3.40 ppm. Spectra were recorded at both 24°C and 33°C to shift the broad H_2O signal, which interfered in the measurement of the metabolites.

Electron impact mass spectra of purified metabolites, reference substances, and their derivatives were recorded in a Finnigan MAT 44S quadrupole instrument (Bremen, Germany) operated at 70 eV. The samples, dissolved in methanol, were introduced into the ion source using a direct chemical ionization sample rod. They were evaporated according to the following temperature program: initial temperature, 20°C (for 30 sec); temperature gradient, 75°C/min; final temperature, 300°C.

The mass spectra were stored and normalized after background subtraction with a Finnigan MAT Spectro System model SS 300. Omeprazole and [^{13}C]omeprazole were used as references, to study their fragmentation pattern after electron impact.

Isolation of Metabolites. Pooled 0–2-hr urine was used in the work-up procedure shown schematically in fig. 2. This isolation procedure takes the acid sensitivity and the two dissociation constants of omeprazole (4.0 for the protonated pyridine and 8.7 for the benzimidazole NH group acting as an acid, respectively) into account.

The metabolic pattern was determined by injection of untreated urine onto a LiChrosorb RP-8 column. The metabolites were separated by gradient elution LC with mobile phases consisting of 10% methanol in phosphate buffer, pH 7.4 (ionic strength 0.05) (phase A), and 80% methanol in water (phase B). The gradient was 0–30% of phase B over 0–5 min, 30–60% B over 5–35 min, 60–100% B over 35–40 min, and 100–0% B over 40–50 min, at a flow rate of 0.8 ml/min.

Metabolite 1 (M1). Urine was extracted at pH 11 with 5 volumes of CH_2Cl_2 and the organic layer was discarded after phase separation. The remaining aqueous phase was extracted twice with 3 volumes of CH_2Cl_2 at pH 7. Both phases were analyzed by the gradient LC system used for metabolic profiling. M1 in the organic phase was purified further by semipreparative normal phase LC (Hibar SI-60 column) under isocratic conditions. The mobile phase (10% methanol in CH_2Cl_2) was used at a flow rate of 4.0 ml/min. The fraction (R_T~8.7 min) containing the metabolite was collected in 50 ml of methanol saturated with NH_3 gas. The solvents were evaporated in a Rotavapor at room temperature. The residue was dissolved in 45% methanol in water, which was also the mobile phase used in the second LC system. The sample was injected onto a Hibar RP-8 column operated at a flow rate of 4.0 ml/min. The radioactive peak (R_T~11.2 min) was collected and the solvent was evaporated to dryness with a Speed Vac concentrator. This sample was used to record the ^{1}H NMR, UV, and mass spectra. The reference compound had to be submitted to the same work-up procedure to give ^{1}H NMR shifts for the benzimidazole protons identical to those of the metabolite.

Metabolite 2 (M2). M2 was enriched from the aqueous phase remaining after extraction at pH 7. Samples (3 × 18 ml) were injected onto a LichroPrep RP-8 column and eluted by the gradient described above, at a flow rate of 2.8 ml/min. The fraction collector was controlled by the radioactivity detection system. The major fraction containing M2 (R_T~13.9 min) was purified further on a LiChrosorb RP-8 column with a mobile phase of 24% methanol in phosphate buffer, pH 7.4 (μ = 0.1), at a flow rate of 1.0 ml/min. The sample was repeatedly injected by the autosampler, which restarted the fraction collector at each injection. The

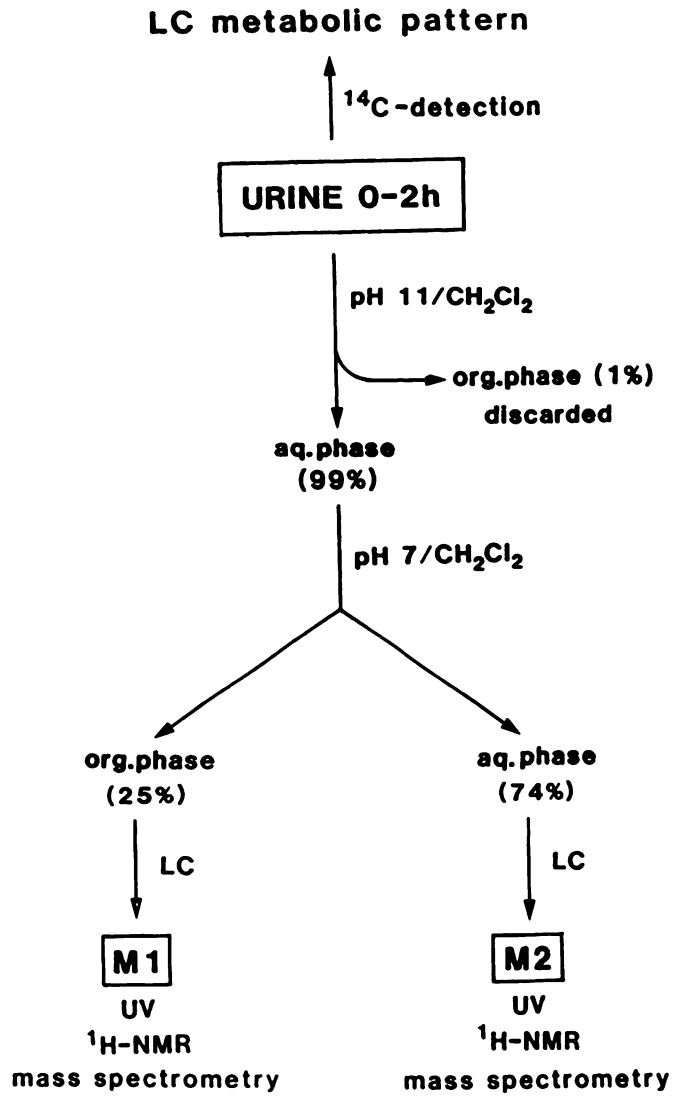


FIG. 2. *Metabolic profiling and isolation procedure for the two main metabolites of omeprazole in pooled human urine from five healthy subjects.*

M1 (hydroxyomeprazole) was purified by both normal phase and reverse phase LC, whereas M2 (omeprazole acid) was isolated in three different reverse phase systems. The distribution of radioactivity between the organic and aqueous phase is given as per cent of radioactivity in the urine sample.

fraction containing the purified metabolite (R_T~11.7 min) was concentrated in a Speed Vac concentrator but not to complete dryness. The sample was diluted with water and injected onto a Polygosil C_{18} column (R_T~8.8 min) to remove inorganic salts. Acetonitrile (4.5%) in water was used as eluting solvent, at a flow rate of 1.0 ml/min. After evaporation of the mobile phase, the metabolite was analyzed by ^{1}H NMR, UV, and mass spectrometry.

Derivatization of M1 and M2. Before mass spectrometric analysis, metabolite M1, or the synthetic reference compound, was dissolved in methanol, followed by evaporation of the solvent. Diazomethane in diethyl ether was added and the mixture was kept for 1 hr at room temperature. The solvent was evaporated, and the product was reacted with 30 μl of *N,O*-bis-(trimethylsilyl)-acetamide for 1 hr at ambient temperature. Before preparation of deuterated derivatives with CH_3OD, CD_2N_2, and d_{18}-*N,O,*-bis-(trimethylsilyl)trifluoroacetamide as reagents, the acidic proton in the metabolite was exchanged for deuterium by repeated evaporation of the sample dissolved in CH_3OD.

Metabolite M2, or the reference compound, was dissolved in methanol. After evaporation, diazomethane in diethyl ether was added. After 1 hr at room temperature, the solvent was evaporated and the metabolite was dissolved in methanol. The deuterated derivative was prepared accordingly.

Identification of the two metabolites was based on mass spectra (including derivatives), ^{1}H NMR, and UV spectra (UV spectra recorded on-line at an identical LC retention time) of the metabolites and their synthetic references.

Results

Urinary Excretion. The accumulated urinary excretion of total radioactivity is given in table 1. An average of 47% of the dose was excreted in the first 2 hr and 75% within 24 hr. About 80% of the dose was accounted for in urine in 96 hr.

Metabolic Pattern. The metabolic pattern of 0–2-hr pooled urine and the structures of the two identified metabolites are shown in fig. 1. Radioactivity measurements of the total effluent from the column demonstrated that no significant loss of radioactivity occurred during chromatography. No unchanged drug was detected (R_T=36 min), indicating that omeprazole was completely metabolized before renal excretion. At least six metabolites were separated. The most lipophilic metabolite, M1 (R_T~25 min), was the most abundant and was the only significant radioactive material extractable at pH 7. A different metabolic pattern might be obtained for urine collected over later intervals, because of quantitative differences in the rate of formation or elimination of metabolites. However, these samples were too dilute to be analyzed. The two main metabolites identified in this study corresponded to 28% (R_T~25 min) and 23% (R_T~15 min) of the radioactivity excreted in urine over 0–2 hr, equivalent to 13% and 11% of the given dose, respectively.

Metabolite M1. As shown in fig. 2, 25% of the radioactivity was extractable by CH_2Cl_2. Analysis of the extract by gradient LC demonstrated that the only significant radioactive fraction was M1 (fig. 1; R_T~25 min) and that no degradation had occurred during the extraction procedure. The extract contained about 1 mg of M1, which was purified by normal phase LC.

The ^{1}H NMR spectrum of M1 is shown in fig. 3, *upper panel.* Compared with the ^{1}H NMR spectrum of omeprazole, one of the two singlets (at 2.1 ppm) from the methyl groups in the spectrum of the parent drug was missing, indicating a metabolic reaction at this group. A new singlet, representing two protons, was shifted downfield to 4.74 ppm. These data were consistent

TABLE 1

Cumulative urinary excretion of radioactivity, as per cent of given dose, after oral administration of 60 mg (25 μCi) of [^{14}C]omeprazole to 10 healthy male subjects

Values represent observations for 5 (0–12 hr) or 10 (0–96 hr) subjects.

Time	Amount Excreted	
	Mean	SD
hr	*% of dose*	
0–1	18.3	12.0
0–2	46.5	6.2
0–4	61.7	6.2
0–6	68.1	6.2
0–9	72.5	5.9
0–12	74.8	5.9
0–24	75.3	6.6
0–48	78.0	6.7
0–72	78.9	6.7
0–96	79.2	6.6

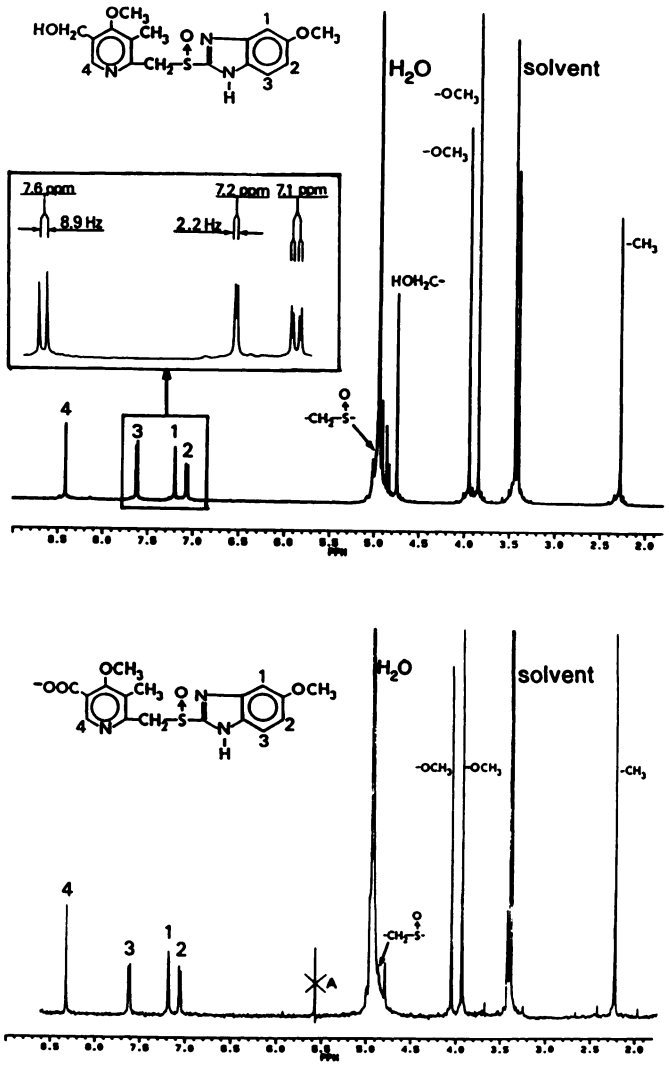


FIG. 3. *^{1}H NMR spectra and the structures of M1 (hydroxyomeprazole; upper panel) and M2 (omeprazole acid; lower panel) isolated from human urine.*

Deuterated methanol was used as solvent (probe temperature, 24°C).

with a hydroxylation reaction at one of the methyl groups. The $-CH_2SO-$ protons were detected as a quartet separated at 33°C from the broad H_2O signal originating from the residual water in the sample.

The electron impact mass spectrum of ^{13}C-labeled omeprazole was recorded, to elucidate the fragmentation pathways of the parent compound (fig. 4). The molecular weight of [^{13}C]omeprazole was increased to 346. Due to the position of the label in the benzimidazole, all ions still containing this ring system were shifted by 1 amu, compared with unlabeled omeprazole, *i.e.* all ions greater than *m/z* 165. The prominent fragments at *m/z* 297 (Fig. 4, *upper panel*) and 298 (*lower panel*) originated from the loss of the sulfoxide group from the M^+ ion with charge retention on the two aromatic rings after skeletal rearrangement (16). This unusual fragmentation pathway was highly indicative of the presence of the $-SO-$ group. Ions related to the substituted pyridine ring of omeprazole at *m/z* 150, 136, and 120 remained unchanged in the mass spectrum of [^{13}C]omeprazole and these fragments therefore represented a valuable tool in detecting structural changes at this site of the molecule.

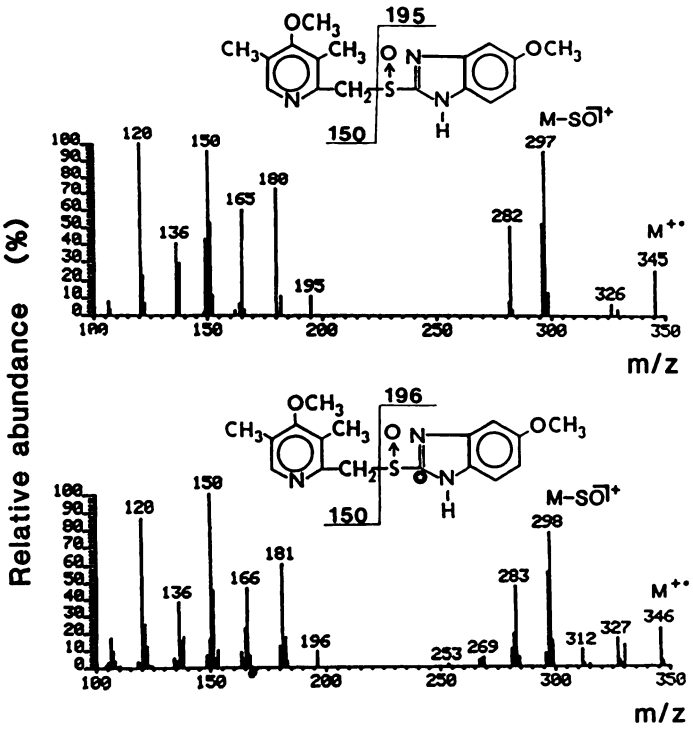


FIG. 4. *Electron impact mass spectra of omeprazole (upper panel)* and [^{13}C]omeprazole (*lower panel*).

The position of ^{13}C label is denoted.

The mass spectrum of underivatized M1 is shown in fig. 5 and was virtually identical to that of synthetic hydroxyomeprazole. The molecular ion at *m/z* 361 was 16 mass units higher than that of the parent drug, which indicated the incorporation of an oxygen into the molecule. The M − 48 fragment at *m/z* 313 confirmed that the sulfoxide group in the molecule was unchanged. The significant ions at *m/z* 120, 136, and 150 in the spectrum of omeprazole were shifted by 16 amu to 136, 152, and 166, which was consistent with hydroxylation of the pyridine moiety.

The mass spectra of derivatized M1 (fig. 6) showed a molecular ion at *m/z* 447 and at *m/z* 459 with deuterated reagents. The increase of the M^+ ion by 12 mass units in the spectrum of the deuterated derivative indicated that one methyl group and one trimethylsilyl group were added to the metabolite by derivatization. The ion at *m/z* 209 originated from the *N*-methylated benzimidazole part of the molecule and was increased to *m/z* 212 after treatment with deuterated reagents. Apparently, the methyl group introduced by derivatization was attached to one of the two nitrogen atoms in the benzimidazole moiety. The diagnostic ion at *m/z* 238 was derived from the modified pyridine part of the molecule, assuming that the pyridine fragment at *m/z* 150 (fig. 4) was increased by 16 amu by the metabolic hydroxylation and by 72 amu through silylation. Furthermore, this fragment was increased by 9 units to *m/z* 247 using deuterated reagents, indicating that the trimethylsilyl group was bonded to the hydroxymethyl group in the pyridine ring.

The UV spectra of the metabolite and the synthetic reference were identical to that of omeprazole. All compounds exhibited an absorption maximum at 302 nm, indicating that hydroxylation of the pyridine methyl group had a negligible influence on the absorbance properties.

Metabolite M2. Metabolite M2 (R_T~15 min; fig. 1) was not extracted into methylene chloride at pH 7 to 11, due to its acidic group, and degraded rapidly at pH below 7. Consequently, enrichment of M2 was based on reverse phase LC at neutral pH, permitting direct injection of aqueous samples. Semipreparative gradient LC enabled injection of large volumes of the urine. After complete extraction of hydroxyomeprazole, M2 represented the major radioactive peak in urine (see fig. 1 for comparison). Two additional reverse phase LC purification steps were required to isolate M2 of sufficient purity and to remove buffer constituents.

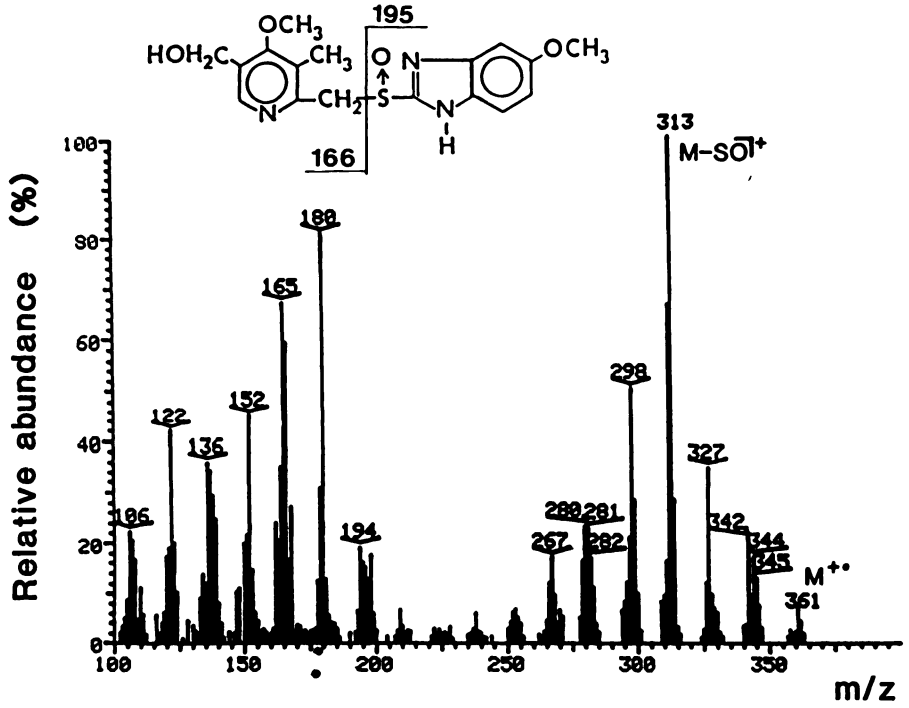


FIG. 5. *Direct probe electron impact mass spectrum of M1 (hydroxyomeprazole).*

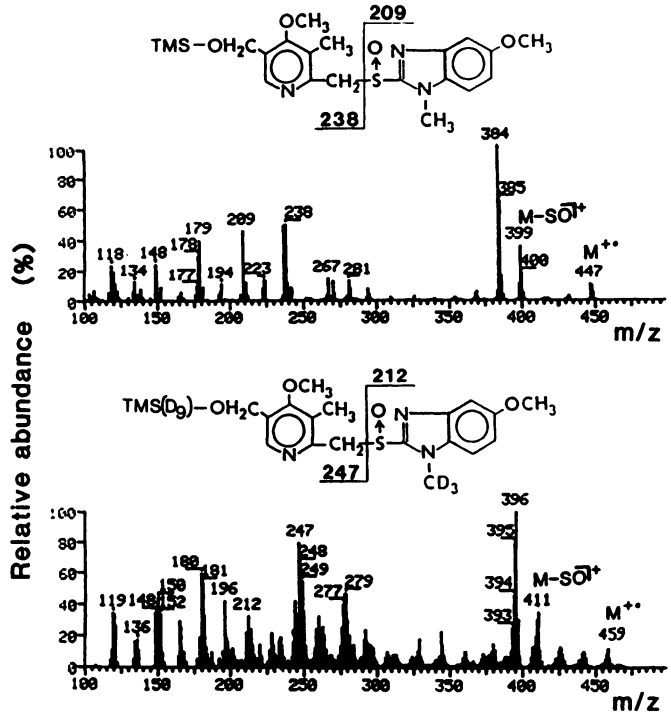


FIG. 6. *The electron impact mass spectrum of M1 (hydroxyomeprazole) after derivatization with diazomethane followed by silylation.*

TMS indicates a trimethylsilyl group. The *lower panel* shows the electron impact mass spectrum of the corresponding derivative using deuterated reagents.

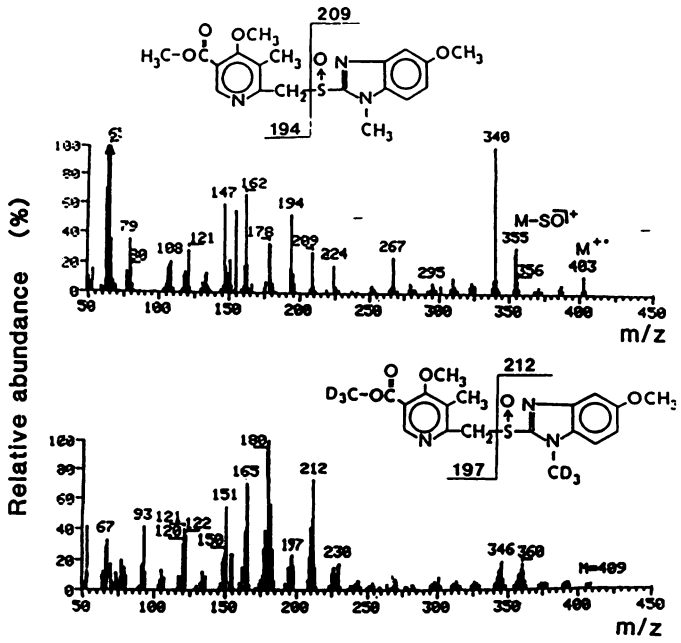


FIG. 7. *Electron impact mass spectra of M2 (omeprazole acid) after methylation with diazomethane (upper panel) and deuterated diazomethane (lower panel).*

In the ^{1}H NMR spectrum of M2 (Fig. 3, *lower panel*), all chemical shifts and the splitting pattern of M2 were essentially identical to those observed for the unchanged drug, apart from the missing singlet at 2.1 ppm corresponding to a pyridine methyl group. More important, the $-CH_2OH$ signal at 4.74 ppm in the ^{1}H NMR spectrum of M1 was not present in the spectrum of M2, suggesting a metabolic reaction at this position. The shift was the same for H_2O and the $-CH_2SO-$ protons (fig. 3). When the probe temperature was raised from 24°C to 33°C the water signal shifted and the $-CH_2SO-$ protons were detected as a quartet.

Direct probe mass spectrometry of underivatized M2 failed to detect the expected molecular ion at *m/z* 375 and the ion with the highest mass was *m/z* 208. Methylation with diazomethane gave a more stable product with *m/z* 403 as the molecular ion (fig. 7, *upper panel*). An M − 48 ion at *m/z* 355 indicated the presence of a sulfoxide group. The proposed structures of two diagnostic ions at *m/z* 194 and 209 are given in fig. 7, each representing one part of the molecule. Both ions were increased by 3 mass units after derivatization with deuterated diazomethane (fig. 7, *lower panel*), which implied that one methyl group had been added to each ring system in the molecule. Analogous to the corresponding experiment with M1, *N*-methylation of the benzimidazole moiety occurred, yielding ions at *m/z* 209 and 212, respectively. Unlike M1, however, the pyridine moiety was methylated, as apparent from the ions at *m/z* 194 and 197. The fragment originating from the intact pyridine ring in omeprazole, at *m/z* 150 (fig. 4), was increased by 44 amu in the mass spectrum of M2. It was concluded that a carboxylic acid was attached to the pyridine because this functional group reacts smoothly with diazomethane. Furthermore, these results were consistent with the detected molecular ion of methylated M2 at *m/z* 403 and the mass spectrum of the reference.

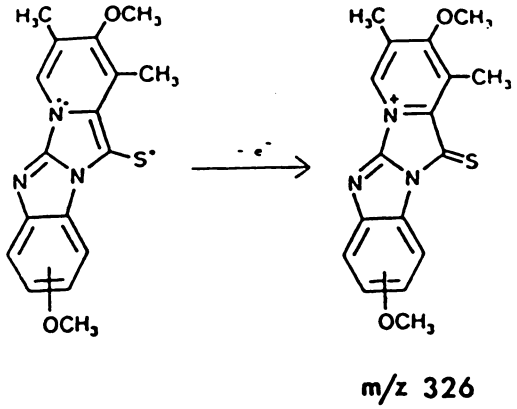
The UV spectra of the metabolite and the synthetic omeprazole acid were virtually identical, with an absorbance maximum at 302 nm.

Discussion

A previously reported study on renal excretion of radioactivity over 96 hr after oral administration of 20 mg of [^{14}C]omeprazole to healthy subjects indicated a mean recovery of about 80% of the given dose (21), which is in good agreement with the results given in table 1. Feces was not collected in the present investigation. However, fecal excretion has been found to account for approximately 20% of the dose in humans (21). Short intervals for urine collection in five subjects provided less diluted samples for metabolite identification. Due to both rapid absorption of omeprazole and excretion of metabolites, the analyzed 0–2-hr urine represented almost half of the administered dose.

Omeprazole was given as a suspension in sodium bicarbonate solution. The drug is unstable under acidic conditions (11), with a half-life of about 2 min at a pH approaching the acidity of gastric juice. Consequently, the drug has to be given as a buffered suspension and urine must be collected at pH > 7 to avoid the potential of acid decomposition of excreted metabolites during sample handling. Acid-catalyzed formation of the sulfenamide and sulfenic acid analogues of omeprazole has been reported (10) and reduction of the sulfoxide group to the corresponding sulfide occurs in biological samples at pH < 7 (15).

In the isolation procedure for the two metabolites (fig. 2), their acid lability had to be considered. The hydroxy metabolite has two calculated dissociation constants, at 3.2 (protonated pyridine nitrogen) and 8.7 (benzimidazole NH group), suggesting pH 7 as an appropriate compromise for extraction of M1. Due to the low distribution ratio (*D*) of M1 into methylene chloride at pH 7 (*D* = 2.5, equal phase volumes) (20), repeated extractions with excess



SCHEME 1.

solvent were required to recover the metabolite quantitatively. Omeprazole acid (M2) was purified by different LC methods because the metabolite exists in ionized form at pH > 7, making enrichment by extraction less suitable. Both metabolites were found to be extremely sensitive to acid-catalyzed decomposition during analysis. During NMR measurements in deuterated chloroform, M1 was rapidly degraded due to the continuous formation of small amounts of DCl, in spite of the fact that the solvent had been treated with solid potassium carbonate. This problem was circumvented by collecting the eluate from the semipreparative LC column in methanol saturated with gaseous NH_3 and by using CD_3OD as the NMR solvent.

During MS analysis, the relative abundance of the fragment ions varied considerably, depending on the chemical background, temperature programming of the direct chemical ionization wire and ion focusing. The interesting M − 19 ion was often the prominent ion at the end of the direct probe measurement (*e.g.* *m/z* 326 for omeprazole; fig. 4), which was indicative of a decomposition reaction. The tentative structures of the ion derived from omeprazole and its radical precursor are shown in scheme 1. This radical formed from omeprazole is highly resonance stabilized and might be formed during MS analysis by a mechanism analogous to that described by Rackur (22) or *via* the sulfenamide or sulfenic acid (10).

Electron impact conditions might induce positive charge on one nitrogen in the benzimidazole system, which favors the intramolecular attack of the pyridine nitrogen on the 2-carbon atom of the benzimidazole, with formation of a spiro intermediate (10). Chemical derivatization of the metabolites before direct probe MS enhanced their stability and provided additional structural information. Methylation of the imidazole ring with diazomethane as the first derivatization step generated a compound suitable for MS analysis. If silylation was performed before alkylation, the formed derivatives were not sufficiently stable for electron impact measurements. Consequently, the two-step derivatization procedure, alkylation then silylation, was used, which is likely to react with most of the functional groups encountered in drug metabolism.

The inhibitory effect of omeprazole on aminopyrine uptake in isolated gastric glands has been reported (23), where the IC_{50} value for the drug of 0.48 μmol/liter represents the concentration of the inhibitor needed to lower the uptake of aminopyrine to 50% of the histamine-stimulated control level. The two identified metabolites exhibited an IC_{50} of 33 ± 4.7 (N = 3; M1) and > 100 μM (N = 3; M2), respectively. These results might indicate that the two metabolites are too polar to penetrate the lipid cell membrane of the parietal cell. Due to the low pK_a of the pyridine nitrogen (calculated to be 3.2 for M1), the metabolites are weaker bases than omeprazole and they should accumulate in the parietal cell to a lesser extent. Consequently, the contribution of M1 and M2 to the pharmacological response to treatment with omeprazole in humans will, most likely, be insignificant.

Hydroxyomeprazole, isolated as the corresponding sulfide, is a significant urinary metabolite in animal species (16). The possible isomer of synthetic M1 with the hydroxymethylene group in position 3 of the pyridine ring was not available. However, all spectroscopic data on M1 and authentic 5-hydroxyomeprazole were virtually identical. Furthermore, if the conceivable influence of steric hindrance on metabolic reactions is taken into account, it is most likely to assign the 5-methylpyridine group of omeprazole as the major site of hydroxylation in humans. Quantification by direct injection on reverse phase LC (fig. 1) and extraction (fig. 2) showed that M1 accounted for about 25% of the excreted amount (~12% of given dose) within 2 hr after administration.

As in dogs and rats (16), hydroxyomeprazole was further oxidized to M2 in humans and the omeprazole acid corresponded to about 23% (fig. 1) in pooled urine. Attempts to identify the more polar fraction eluting from the LC column with R_T of 11–14 min (fig. 1) were unsuccessful. Different HPLC systems demonstrated the presence of several metabolites in this fraction. Interestingly, the sulfone of omeprazole, which has been identified in plasma as a major metabolite in humans (24), was not present in urine and apparently undergoes further metabolism before elimination. Glucuronidation of omeprazole metabolites is important, particularly in the dog (16). Due to the different analytical approach in the present study, with the main emphasis on avoiding acidic decomposition, no attempts were made to hydrolyze potential conjugates. LC combined with MS might be an alternative method for the direct analysis of unextractable urine metabolites of omeprazole in humans.

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