Metabolites of Ebrotidine, a New H_2-Receptor Antagonist, in Human Urine

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Abstract □ Ebrotidine is a new H_2-receptor antagonist which exhibits a remarkable ability for gastric mucosal protection. A preliminary metabolic pathway for this compound was proposed and the hypothetic metabolites were synthesized. The presence of ebrotidine and its metabolites ebrotidine *S*-oxide and 4-bromobenzenesulfonamide in human urine has been confirmed by HPLC separation and spectroscopic characterization of the collected fractions by FT-IR and ^1H NMR. Ebrotidine *S,S*-dioxide has been identified by HPLC using diode-array detection.

Introduction

H_2-receptor antagonists are chemical substances, characterized as being weak bases with varying lipophilicity, which are stable in solution in slightly acid medium. Generally they are whitish powders, occasionally polymorphic in crystalline form, with a very bitter taste in solution.

Studies on these inhibitors of gastric acid secretion have confirmed that their major effect is to reduce the capacity of parietal stomach cells to secrete HCl by inhibiting their ability to respond to histamine as a stimulant, which acts on receptors at the cell surface.[1]

More widely used H_2-receptor antagonists are ranitidine, cimetidine, and famotidine.[2] Ebrotidine is a new H_2-receptor antagonist synthesized by Centro de Investigación Grupo Ferrer (Barcelona, Spain) with antisecretory activity comparable to that of ranitidine.[3] It has recently been proved that ebrotidine, in addition to its antisecretory activity, protects gastric mucosal against ethanol-induced injury in rats[3,4] and humans.[5] This antisecretory and gastroprotective agent is also a potent inhibitor of protease and lipase enzymes elaborated by *Helicobacter pylori*, which exert a mucolytic activity toward protein and lipid constituents of the gastric mucus layer.[6,7]

Structurally, ebrotidine contains an *N*-sulfonylformamidine group instead of the *N*-cyanoguanidine group of cimetidine and the 2-nitroethenediamine group of ranitidine, while the imidazole ring of cimetidine is substituted by guanidinothiazole.

In view of the occurrence of drug-related adverse reactions and their possible relationship to accumulation of derivatives by susceptible individuals, it is important to establish in detail the metabolic profile of ebrotidine in healthy adults. All the H_2-antagonists that contain a thioether in the linking bridge are subjected to oxidative metabolism at the sulfur of that moiety; the *S*-oxide of cimetidine, ranitidine, famotidine, and oxmetidine have been identified in human urine.[8-11] Metabolites obtained by oxidation of other groups (ranitidine), hydrolysis (cimetidine), and demethylation (ranitidine) have also been described.[8,9]

Taking into account the above mentioned metabolic reactions and the compounds obtained by alkaline hydrolysis in vitro, the preliminary metabolic pathway for ebrotidine showed in Figure 1 was proposed. These compounds were synthesized and their presence in human urine after oral administration of ebrotidine was investigated by HPLC. Characterization of the main metabolites was carried out by HPLC using diode-array detection and semipreparative HPLC followed by FT-IR and ^1H NMR of the collected fractions. The presence of ebrotidine, ebrotidine S-oxide, ebrotidine *S,S*-dioxide, and 4-bromobenzenesulfonamide in human urine after oral ebrotidine administration has been confirmed.

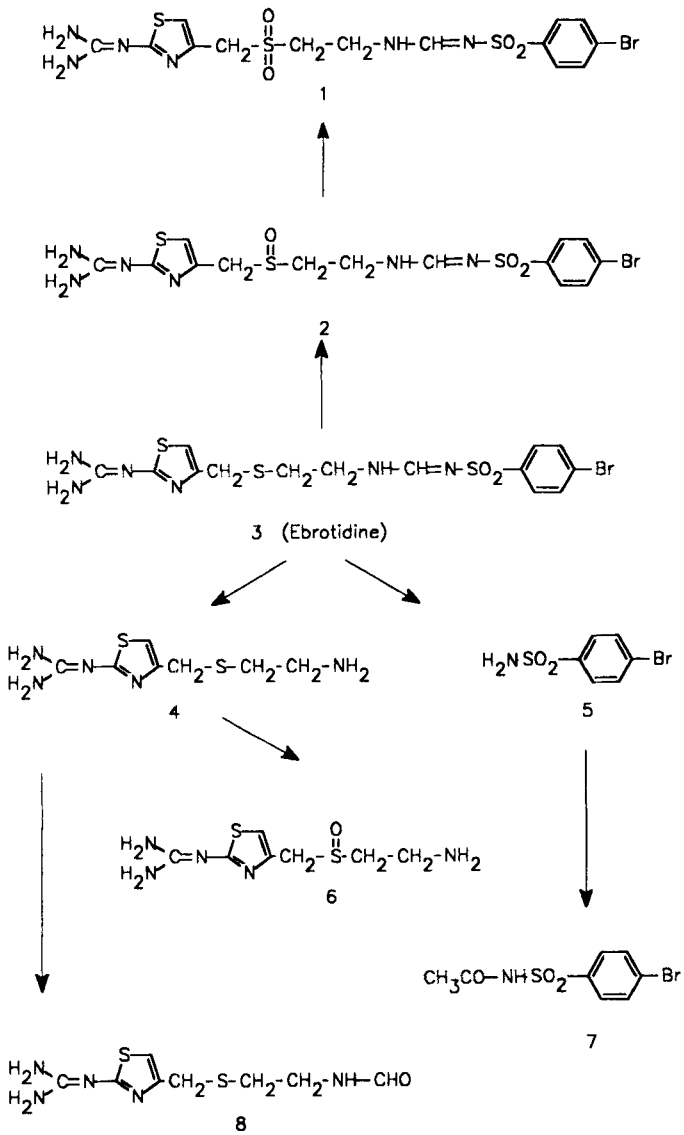

Figure 1—Metabolic pathway proposed for ebrotidine.

Experimental Section

Chemicals—All the reagents used were analytical grade and supplied by Merck (Darmstadt, Germany). Solvents for HPLC were HPLC grade (Merck, Darmstadt, Germany), and water for HPLC was purified using a Milli-Q plus system (Millipore) and filtered through a 0.45-μm membrane filter (Millipore). All of the HPLC solvents were degassed ultrasonically for 15 min before use. Monosodium phosphate and

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disodium phosphate used for the pH 7.5 buffer were of analytical grade (Merck, Darmstadt, Germany).

The synthetic precursor 4-bromobenzenesulfonyl chloride (98%) was purchased from Lancaster and 4-bromobenzenesulfonyl formimidate was prepared according to the method proposed by Anglada et al.[12]

Reference Compounds—All reference compounds, the structures of which are given in Figure 1, were synthesized by Centro de Investigación Grupo Ferrer (Barcelona, Spain) with >99% chemical purity, confirmed by elemental analysis and HPLC.

2-[4-[[(2-Aminoethyl)thio]methyl]-2-thiazolyl]guanidine (4)—The dihydrochloride of this compound was prepared by alkylation of cysteamine with 4-(chloromethyl)-2-guanidinethiazole according to the method proposed by Gitman et al.[13] The following analytical data were obtained: mp >250 °C; IR ν_{max} (KBr) 3295 (NH_2), 1682 (C=N), 1622 (C=N), 1226 (CH_2S) cm^{-1}; ^1H NMR (DMSO) δ = 2.76 (t, J = 7.0, 3H, SCH_2CH_2), 2.98 (m, 3H, $CH_2CH_2NH_3^+$), 3.82 (s, 2H, thiazole-CH_2), 7.27 (s, 1H, thiazole), 8.30 (br, 3H, NH_3^+), 8.42 (br, 5H, guanidine-HCl).

***N*-[2-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl]thio]ethyl]formamide (8)**—The hydrochloride salt was synthesized as follows: 2-[4-[[(2-aminoethyl)thio]methyl]-2-thiazolyl]guanidine dihydrochloride (10 g; 32.9 mmol) and formamide (100 mL) were heated at 75 °C for 22 h. The excess formamide was removed by distillation at reduced pressure. The residue was crystallized twice in ethanol (60 mL) to give 7.0 g of the product (yield 72%). The following analytical data were obtained: mp 142–144 °C; IR ν_{max} (KBr) 3272 (NH), 1683 (C=N), 1634 (C=O), 1600 (C=N), 1533 (C–N) cm^{-1}; ^1H NMR (DMSO) δ = 2.56 (t, J = 6.7, 2H, SCH_2CH_2), 3.24–3.30 (m, 2H, CH_2CH_2NH), 3.77 (s, 2H, thiazole-CH_2), 7.15 (s, 1H, thiazole), 8.03 (s, 1H, NHCHO), 8.37 (s, 4H, guanidine).

2-[4-[[(2-Aminoethyl)thio]methyl]-2-thiazolyl]guanidine *S*-Oxide (6)—The dihydrochloride of this compound was prepared as follows: to a suspension of 2-[4-[[(2-aminoethyl)thio]methyl-2-thiazolyl]-guanidine dihydrochloride (3.04 g; 10 mmol) in methanol (20 mL) was added a solution of selenium dioxide (1.1 g; 10 mmol) and hydrogen peroxide (10 mmoL) in methanol (5 mL) dropwise. The reaction mixture was stirred for 2 h, filtered, washed with methanol (10 mL), and dried under reduced pressure to give 1.65 g of the product (yield 51%) as white powder. The following analytical data were obtained: mp 245–246 °C; IR ν_{max} (KBr) 3303 (NH_2), 1608 (C=N), 1011 (S=O) cm^{-1}; ^1H NMR (DMSO) δ = 3.03–3.20 (m, 2H, $SOCH_2CH_2$), 3.21 (br, 2H, CH_2-NH), 4.22 (d, J = 13.2, 1H, thiazole-CH_A), 4.34 (d, J = 13.2, 1H, thiazole-CH_B), 7.32 (s, 1H, thiazole), 8.44 (br, 8H, NH_3^+ and guanidine-HCl).

Ebrotidine, *N*-[(4-bromophenyl)sulfonyl]-*N'*-[2[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]ethyl]formamidine (3) was synthesized as follows: 2-[4-[[(2-aminoethyl)thio]methyl]-2-thiazolyl]guanidine dihydrochloride (3.04 g; 10 mmol) was suspended in methanol (10 mL) and treated with a 1.66 M solution of potassium hydrochloride in methanol (12 mL). The mixture was stirred for 1 h and filtered and 4-bromobenzenesulfonyl formimidate (2.92 g; 10 mmol) was added to the filtrate. The reaction mixture was stirred for 2 h at room temperature and was allowed to crystallize to give 2.82 g of the product (yield 59%). The following analytical data were obtained: mp 145–147 °C; IR ν_{max} (KBr) 3445 (NH_2), 3349 (NH_2), 1651 (C=N), 1605 (C=N), 1537 (Ph), 1329 (SO_2), 1146 (SO_2) cm^{-1}; ^1H NMR (DMSO) δ = 2.61 (t, J = 7.1, 2H, SCH_2CH_2), 3.45–3.48 (m, 2H, CH_2CH_2NH), 3.57 (s, 2H, thiazole-CH_2S), 6.42 (s, 1H, thiazole), 6.90 (br, 4H, guanidine), 7.71–7.77 (AB system, J = 8.8, 4H, ArH), 8.20 (s, 1H, CH=N), 9.09 (br, 1H, CH=NH).

4-Bromobenzenesulfonamide (5) was prepared as follows: 4-bromobenzenesulfonyl chloride (14.67 g; 57.6 mmol) was suspended in 25% ammonium hydroxide (43 mL). The suspension was stirred for 16 h and then filtered, washed with water, and dried under reduced pressure to give 11.9 g of the product (yield 88%) as a white powder. The following analytical data were obtained: mp 163–166 °C; IR ν_{max} (KBr) 3327 (NH_2), 3238 (NH_2), 1575 (Ph), 1325 (SO_2), 1172 (SO_2) cm^{-1}; ^1H NMR (DMSO) δ = 7.52 (br, 2H, SO_2NH_2), 7.80–7.81 (AB system, J = 8.8, 4H, Ar-H).

***N*-[(4-Bromophenyl)sulfonyl]acetamide (7)** was synthesized as follows: a solution of 4-bromobenzenesulfonamide (4.71 g; 20 mmol) in acetic anhydride (20 mL) and 98% sulfuric acid (1 mL) was stirred for 2 h at room temperature. To the mixture reaction was added 1:1 water/ice (150 g) and the solid obtained was filtered, washed with water (100 mL), and dried under reduced pressure to give 5.2 g of the product (yield 93%) as white powder. The following analytical data were obtained: mp 199–201 °C; IR ν_{max} (KBr) 1694 (CO), 1574 (Ph), 1353 (SO_2), 1163 (SO_2) cm^{-1}; ^1H NMR (DMSO) δ = 1.94 (s, 3H, CH_3), 7.84 (s, 4H, Ar-H), 12.20 (br, 1H, NH).

***N*-[(4-Bromophenyl)sulfonyl]-*N'*-[2-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]ethyl]formamidine *S*-oxide (2)** was synthesized by oxidation of ebrotidine with selenium dioxide as follows: to a solution of *N*-[(4-bromophenyl)sulfonyl]-*N'*-[2-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]ethyl]formamidine (4.77 g; 10 mmol) in methanol (20 mL) was added a solution of selenium dioxide (1.1 g; 10 mmol) and hydrogen peroxide (10 mmol) in methanol (5 mL) dropwise. The reaction mixture was stirred for 2 h, filtered, washed with methanol (10 mL), and dried under reduced pressure to give 2.05 g of the product (yield 41%) as white-yellow powder. The following analytical data were obtained: mp 197.2–198.8 °C; IR ν_{max} (KBr) 3406 (NH_2), 3331 (NH_2), 1652 (C=N), 1612 (C=N), 1550 (Ph), 1314 (SO_2), 1009 (S=O) cm^{-1}; ^1H NMR (DMSO) δ = 2.89 (dt, J = 13.2 and 6.61; 1H, $SOCH_ACH_2$), 3.03 (dt, J = 13.2 and 6.6; 1H, $SOCH_BCH_2$), 3.65 (br, 2H, CH_2NH), 3.97 (d, J = 13.1, 1H, thiazole-CH_A), 4.09 (d, J = 13.1, 1H, thiazole-CH_B), 6.61 (s, 1H, thiazole), 6.89 (br, 4H, guanidine), 7.70–7.77 (AB system, J = 8.8, 4H, Ar-H), 8.19 (s, 1H, CH=N), 9.20 (br, 1H, NHCH).

***N*-[(4-Bromophenyl)sulfonyl]-*N'*-[2-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]ethyl]formamidine *S,S*-dioxide (1)** was synthesized by oxidation of ebrotidine with hydrogen peroxide as follows: to a solution of *N*-[(4-bromophenyl)sulfonyl]-*N'*-[2-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]ethyl]formamidine (4.77 g; 10 mmol) in methanol (20 mL) was added a solution of 30% hydrogen peroxide (4.5 mL; 40 mmol) dropwise. The reaction mixture was stirred for 2 h, filtered, washed with methanol (10 mL), and dried under reduced pressure to give 2.92 g of the product (yield 57%) as white-yellow powder. The following analytical data were obtained: mp 132–134 °C; IR ν_{max} (KBr) 3489 (NH_2), 3380 (NH_2), 1606 (C=N), 1540 (Ph), 1302 (SO_2), 1149 (SO_2) cm^{-1}; ^1H NMR (DMSO) δ = 3.36 (t, J = 6.9, 2H, SCH_2CH_2), 3.67 (br, 2H, CH_2NH), 4.43 (s, 2H, thiazole-CH_2S), 6.73 (s, 1H, thiazole), 6.88 (br, 4H, guanidine), 7.69–7.78 (AB system, J = 8.7, 4H, ArH), 8.19 (s, 1H, CH=N), 9.09 (br, 1H, CH=NH).

Standard solutions of 0.5 mg/mL of these compounds in acetonitrile and acetonitrile–dimethyl sulfoxide were prepared. More dilute solutions were obtained from these standards.

Instrumentation—Melting points were taken in a Büchi Model 535 melting point apparatus. FT-IR spectra were performed using a Perkin-Elmer Model 1710 infrared Fourier transform spectrometer coupled to a Perkin-Elmer Model 3710 data station. ^1H NMR spectra in deuterated dimethyl sulfoxide (DMSO-d_6) were obtained on a Varian Model Gemini 300 NMR spectrometer (300 MHz) for drug standards and on a Varian Model Unity 500 (500 MHz) for the collected fractions.

High-performance liquid chromatography was conducted using a Merck-Hitachi Model L-6200 gradient pump and a Kontron Model 735-LC variable-wavelength detector set at 235 nm. The system was fitted with a Reodyne 7125 manual injector, and the chromatograms were recorded with a Waters Model 740 integrator. Diode-array spectra were taken using a Merck-Hitachi Model L-3000 photo-diode-array detector and recorded with a Merck-Hitachi Model D-2500 chromatointegrator.

Chromatographic Procedures—The analytical chromatographic system used was a C-18 10 μm μBondapack column (30 × 0.39 cm) (Waters, Millipore) eluted with 0.01 M Na_2HPO_4/NaH_2PO_4 pH 7.5: acetonitrile at a flow rate of 0.8 mL/min, using the following gradient: 85:15 held for 10 min, linear program to 65:35 in 20 min, and then held for 10 min. The chromatogram of the eight reference compounds is shown in Figure 2.

The semipreparative chromatographic system used was a C-18 10 μm μBondapack column (30 × 0.78 cm) eluted with the same mobile phase as before at a flow rate of 2.5 mL/min with the following gradient: 100:0 for 15 min and then a linear program to 70:30 in 60 min held for 45 min. After injection of 100 μL of urine extract, fractions corresponding to each peak were collected and analyzed by FT-IR. To obtain enough sample for the ^1H NMR analysis, 10 injections of 100 μL of urine extract were needed.

Human Drug Administration and Urine Collection—Ebrotidine tablets (800 mg each) were orally administered to 10 healthy male subjects in a single dose. Urine samples corresponding to the first 12 h after the administration were collected, pooled, and immediately frozen to −40 °C until analysis.

Analysis of Urine Sample—A 1300-mL sample of urine, alkalinized to pH 9 with 1 N sodium hydroxide, was placed in a separatory funnel and extracted twice with equal volumes of dichloromethane:2-propanol (9:1) using a mechanical shaker (Ika) for 30 min. The organic layer was evaporated to dryness using a rotary evaporator at 40 °C. The resulting

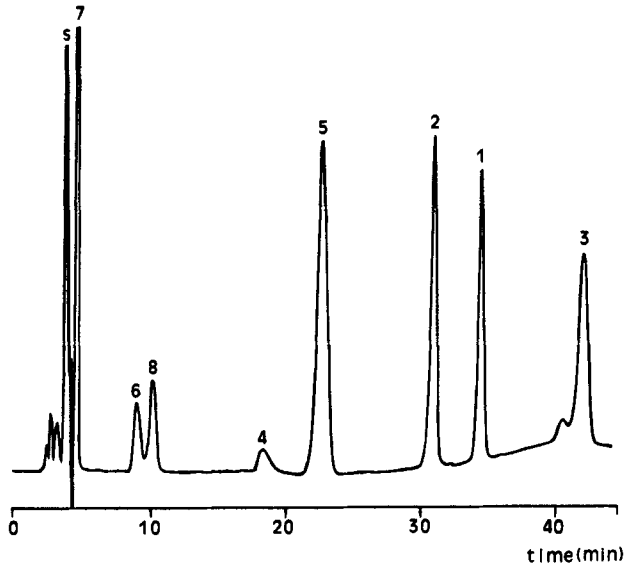

Figure 2—Chromatogram of the standards. Injected amount: 500 ng of each compound. Sensitivity: 0.4 AUFS, S: solvent.

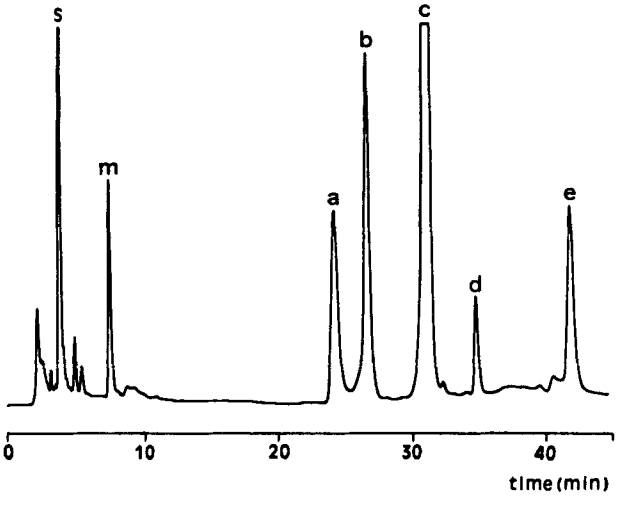

Figure 3—Chromatogram of the urine extract. Injected volume: 5 μL. Sensitivity: 0.4 AUFS,S: solvent, m: matrix peak.

residue was then dissolved in dimethyl sulfoxide:acetonitrile (10:90) and injected into the HPLC system.

The fractions collected from the semipreparative HPLC were concentrated for subsequent FT-IR and ^{1}H NMR analysis. Acetonitrile was eliminated by evaporation and the resulting aqueous solution, alkalinized to pH 9 with 0.1 N NaOH, was extracted with five volumes of dichloromethane:2-propanol (9:1). The organic solvent was evaporated to dryness and the residue was dissolved in 1 mL of DMSO-d_6 and analyzed by ^{1}H NMR or mixed with KBr for the FT-IR measurements.

Results and Discussion

The chromatogram of the urine extract is shown in Figure 3. Peaks a, c, and d were identified as 4-bromobenzenesulfonamide (5), ebrotidine *S*-oxide (2), and ebrotidine *S,S*-dioxide (1), respectively. The capacity factors *k* and also the UV spectra of the metabolites agree with those of the standards. Metabolite b could not be assigned by retention time to any of the reference compounds studied and peak e was identified as ebrotidine.

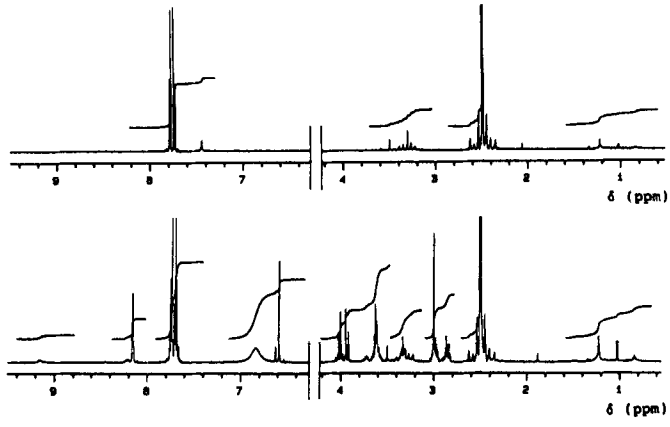

Figure 4—500-MHz ^{1}H NMR spectra of metabolites a (top) and c (bottom).

In order to confirm the presence in the urine sample of the compounds and also ascertain the chemical structure of the unknown metabolite b, present in the urine extract in relatively high amounts, semipreparative HPLC was carried out. Fractions corresponding to peaks a–c and e were collected, extracted, and submitted to FT-IR and ^{1}H NMR analysis. The FT-IR results obtained confirm the presence of the aforementioned compounds.

^{1}H NMR spectra of the peaks a and c are given in Figure 4. The ^{1}H NMR chemical shifts for peak a agree with those of 4-bromobenzenesulfonamide. Comparison of the spectra of peak c and ebrotidine *S*-oxide allow identification of this compound, taking into account that signals between 3.2 and 3.4, 2.4 and 2.5, and 1 and 1.2 ppm can be attributed to artifacts of the separation and concentration procedure. The presence of ebrotidine was also confirmed, but metabolite b could not be identified by this technique although the ^{1}H NMR spectra shows a para-substituted phenyl moiety; additional work to actualize the identification of this metabolite is being carried out.

References and Notes

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