were successively added and evaporated from the residue to give the trifluoroacetate salt of 12 (100% yield spectrophotometrically) as a colorless gum which was homogeneous by cellulose TLC in *n*-BuOH-AcOH-H_2O (4:1:1), R_f 0.51 (R_f 0.74 for 10), and 2-propanol-15% NH_4OH (7:3), R_f 0.50 (R_f 0.89 for 10), and on paper electrophoresis (Table I). Anal. ($C_{13}H_{17}F_3N_6O_5 \cdot 0.5C_2H_5OH \cdot 1.5H_2O$) C, H, N.

5′(*S*)-(Aminomethyl)-5′-deoxy-5′-L-homocystein-*S*-yl-adenosine (11b). Compound **6b** was converted to **11b** as described for the synthesis of **12**. The product was chromatographed on a C_{18} silica gel column (1.9 × 15 cm) with MeOH-H_2O (2:3) as eluent to give **11b** as a colorless gum (40 μmol) which was homogeneous by TLC and HPLC analysis (Table I): ^1H NMR (D_2O), see Table II.

Compound **6a** (117 μmol) gave **11a** (100 μmol) as a colorless gum under the above conditions; **11a** was homogeneous by TLC and HPLC analysis (Table I); ^1H NMR (D_2O), see Table II.

5′(*S*)-[(*N*-triphosphoamino)methyl]-5′-deoxy-5′-L-homocystein-*S*-yladenosine (13b). Aqueous $Na_3P_3O_9 \cdot 6H_2O$ (120 μL of 0.6 M) was added to **11b** (39 μmol) and the solution was adjusted to pH 9.5–10 with 1 M NaOH (5 μL). Aliquots (7 × 5 μL) of 1 M NaOH were added periodically over 120 h to maintain the pH at 9.5–10. Aqueous $Et_3NH \cdot HCO_3$ (1 mL of 0.5 M) was added, and the solution was applied to a C_{18} silica gel column (0.8 × 15 cm) preequilibrated with 0.5 M $Et_3NH \cdot HCO_3$ (45 mL) at 4 °C. The column was eluted at 4 °C with 0.5 M $Et_3NH \cdot HCO_3$ and then with 2%, 5%, and 10% MeOH, respectively, in 0.5 M $Et_3NH \cdot HCO_3$ (30 mL each) by applying a positive pressure of N_2 and collecting 2–3-mL fractions. Fractions were analyzed by HPLC in 0.1 M K_2HPO_4–0.025 M Bu_4NHSO_4 (pH 7.5) with a linear gradient of 0–30% MeOH over 30 min. $Na_3P_3O_9$ eluted in the first fractions. Fractions containing **13b** (10 μmol) were evaporated in vacuo, and to the residue were successively added EtOH (2 × 5 mL) and MeOH (5 mL) and evaporated. The residue was dissolved in MeOH (1 mL), and 1 M NaI in MeOH (130 μL) was added and the tetrasodium salt was isolated as previously described[5] to give **13b** (4 mg, 4.8 μmol) as a white powder, UV_{max} (pH 8.5) 260 nm, homogeneous by TLC, HPLC (Table I), PEI-cellulose TLC in 1.2 M LiCl containing 0.05 M $NaHCO_3$ (R_f 0.60; ATP, 0.60), and paper chromatography in *n*-PrOH-NH_4OH-H_2O (55:10:35) (R_f 0.30; ATP, 0.40). Anal. ($C_{15}H_{22}N_7SP_3O_{14}Na_4 \cdot 4H_2O \cdot 0.5CH_3OH$) C, H, N.

Compound 13a, prepared from **11a** in 32% yield by the above method, was indistinguishable from **13b** in the systems of Table I and in the PEI-cellulose and the *n*-PrOH-NH_4OH systems above.

5′-[(*N*-Triphosphoamino)methyl]-5′-deoxyadenosine (14). To the trifluoroacetate salt of **12** (0.17 mmol) was added 0.6 M $Na_3P_3O_9 \cdot 6H_2O$ (670 μL). The solution was adjusted to pH 9.0 (with 0.1 M NaOH) and was maintained at pH 9.0 by additions of 0.1 M NaOH. After 4 h HPLC (2% MeOH in 0.5 M $Et_3NH \cdot HCO_3$) showed 77% conversion of **12** to **14** and minor amounts of several other UV-absorbing products. Compound **14** was obtained as its triethylammonium salt by column chromatography over C_{18} silica gel and converted to its sodium salt (0.06 mmol) by the methods used with **13b**: UV_{max} (pH 8.5) 260 nm. Other physical properties are given in Table I.

Enzyme Studies. M-2 and M-T preparations were obtained as described previously[27] except that the isolation and concentration of M-2 (final volume 0.14 mL/g of kidney extracted) and M-T (final volume 0.27 mL/g of Novikoff ascitic hepatoma cells extracted) were completed within 1 day.

Enzyme assays were conducted for 10 min at 37 °C in a final volume of 0.1 mL containing 150 mM KCl–15 mM $MgCl_2$–5 mM dithiothreitol–50 mM Tris·HCl, pH 8.2.[7] Each mixture was made up in duplicate. L-[*methyl*-^{14}C]Methionine (New England Nuclear Co., 54 Ci/mol) and MgATP were included at the levels specified below and in Table III, footnote *a*. A working enzyme solution was prepared freshly each day by 10-fold dilution of a stock solution.[27] Reactions were started by addition of 10 μL of working enzyme solution [(9.5–10.5) × 10^{-6} units of activity; 1 unit gives a V_{max} with 2 mM ATP of 1 μmol of product per min] and terminated by addition of 10 μL of 4 N $HClO_4$–10 mM L-methionine after immersing the solution in an ice bath. Each suspension was centrifuged and 50 μL of supernatant was applied to a 2.3-cm disk of phosphocellulose paper. Disks were washed as described,[28] then immersed in a toluene solution of phosphors, and counted in a Packard liquid scintillation spectrometer (Model 2425). Controls were provided by incubations carried out in the absence of ATP. Reaction velocities were linear for at least 30 min and were proportional to the amount of enzyme added at the levels of enzyme activity employed.

Inhibition studies were made with six to eight levels of MgATP or L-methionine in the range (0.5–4.0) × K_M for each of two inhibitor levels that were in the range (1–10) × K_i and for control mixtures lacking inhibitor. Inhibitors were dissolved in the above pH 8.2 buffer solution prior to testing. Inhibition constants (K_i values) were obtained to within ±15% from replots of inhibitor concentrations vs. slopes or intercepts on the vertical axis of double-reciprocal plots of velocity vs. substrate level. All of the latter plots were linear, as were the replots.

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Antisecretory Activity of Human, Dog, and Rat Metabolites of Fenoctimine

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Fenoctimine (**1a**), a nonanticholinergic inhibitor of gastric acid secretion in dogs and rats, was evaluated as a gastric antisecretory agent in humans. In humans it exhibited weak antisecretory activity and caused anticholinergic-like side effects such as dry mouth and nasal passages. Studies of the metabolic fate of fenoctimine in humans, dogs, and rats provided structures of the resultant metabolites. These were synthesized and tested for antisecretory and anticholinergic activity. The human metabolites were all less active than fenoctimine as antisecretory agents, and some displayed significant anticholinergic activity. These results suggest that the unexpectedly weak effect of fenoctimine as a gastric antisecretory agent in humans, as well as anticholinergic effects, may be due to its extensive metabolism, which is different from that seen in dog and rat.

Fenoctimine (**1a**) is active as a gastric antisecretory agent in animals[1-3] by an undetermined mechanism of action. In vitro studies on guinea pig ileum and atria[1] indicated that it was not a histamine H_2 antagonist, while its failure

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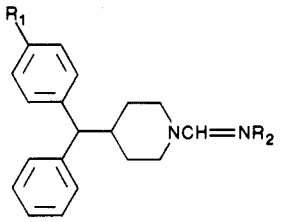
Table I. 4-(Diphenylmethyl)-1-[(imino)methyl]piperidines



compd	R_1	R_2	formula	mp, °C	recrystn solv	yield, %	anal.
1a	H	$(CH_2)_7CH_3$					
1b	H	$(CH_2)_6C(O)CH_3$	$C_{27}H_{36}N_2O\cdot H_2SO_4\cdot 0.7H_2O$	98.5–103	acetone–ether	43	C, H, N, H_2O
1c	H	$(CH_2)_6CH(OH)CH_3$	$C_{27}H_{38}N_2O\cdot C_{10}H_8SO_3\cdot 0.25H_2O$[a]	129.5–132.5	methyl ethyl ketone	20	C, H, N, H_2O
1d	OH	$(CH_2)_7CH_3$	$C_{27}H_{38}N_2O\cdot C_{10}H_8SO_3$	177.5–180.5	acetone–ether	35	C, H, N
1e	H	$(CH_2)_7CH_2OH$	$C_{27}H_{38}N_2O\cdot C_2H_2O_4\cdot 0.3H_2O$	131 (sinter) 135–137	2-propanol–ether	45	C, H, N, H_2O
1f	H	$(CH_2)_5CH_2OH$	$C_{25}H_{34}N_2O\cdot C_2H_2O_4\cdot 0.1H_2O$	137–139	methanol–ether	35	C, H, N, H_2O
1g	H	$(CH_2)_7CO_2Zn$	$C_{27}H_{35}N_2O_2\cdot 0.5Zn\cdot HCl\cdot 0.6H_2O$	82 dec	methylene chloride–ether	4	C, H, N, H_2O, Zn[b]
6	H	$(CH_2)_6\overline{CO(CH_2)_2O}CH_3$	$C_{29}H_{40}N_2O_2\cdot C_4H_4O_4\cdot 0.7H_2O$[c]	143 (sinter) 145.5–148.5	2-propanol	46	C, H, N, H_2O
7	H	$(CH_2)_6C(NOH)CH_3$	$C_{27}H_{37}N_3O\cdot C_{10}H_8SO_3\cdot 0.2H_2O$	123 (sinter) 125–128	acetone	26	C, H, N, H_2O
9	CH_3O	$(CH_2)_7CH_3$	$C_{28}H_{40}N_2O\cdot C_4H_4O_4\cdot 0.75H_2O$	154.5–156	2-propanol	27	C, H, N, H_2O
11a	H	$(CH_2)_7CO_2CH_3$	$C_{28}H_{38}N_2O_2\cdot C_4H_4O_4\cdot 0.5H_2O$	113 (sinter) 121–124	methanol–ether	29	C, H, N, H_2O

[a] $C_{10}H_8SO_3$ represents 2-naphthalenesulfonic acid. [b] Zn: calcd, 6.54; found, 5.69. [c] $C_4H_4O_4$ represents fumaric acid.

to displace [^{3}H]QNB from the acetylcholine receptor precluded its activity as an anticholinergic agent.[2] Also, **1a** inhibited ($H^+ + K^+$)-ATPase and ($Na^+ + K^+$)-ATPase, but apparently due to a drug-induced perturbation of the microsomal membrane rather than a direct interaction with these enzymes.[4]

Compared to cimetidine, as an inhibitor of gastric acid secretion, **1a** was 2.5–3 times more potent in the rat and 3–4 times more potent in the dog and in both species had a far longer duration of action.[3] Its relative ineffectiveness as an antagonist of the cholinergic agent bethanechol, in the dog, was further evidence of its lack of anticholinergic activity.[3] Because of these characteristics and its lack of toxicity in animals, fenoctimine was evaluated clinically as a gastric antisecretory agent.

In humans, fenoctimine at high doses exhibited surprisingly weak gastric antisecretory activity against food, pentagastrin, and histamine stimulation and little or no activity at low doses.[5,6] Additionally, subjects complained of dry nose and mouth at high doses, effects that suggest anticholinergic activity. These incongruities between animal studies and clinical experience may be explained by species differences in metabolism. Although fenoctimine was not anticholinergic in animals, it is possible that an anticholinergic metabolite was being formed in humans.

Studies of the metabolic fate of fenoctimine in rats, dogs, and humans showed that it was well-absorbed, underwent extensive biotransformation, and was rapidly excreted.[7] The metabolites of fenoctimine, compounds **1b–g** (see Table I) and **5**,[2] were isolated from rat liver homogenates and rats, dogs, and humans.[8] The metabolites varied among different species, with the exception of **5**, which was common to all species.[7] This paper describes the synthesis and biological activities of these metabolites and their analogues **6**, **7**, **9**, and **11a**. On the basis of metabolic data and biological activity of the metabolites, an explanation is proposed for the weak gastric antisecretory activity and apparent anticholinergic effects of fenoctimine observed in humans.

Chemistry

The fenoctimine metabolites **1b–g** and their analogues **6**, **7**, **9**, and **11a** were synthesized as shown in Schemes I–III. Metabolite **5** was prepared as described previously.[2]

Treatment of 5-bromophthalimidopentane (**2**)[9] with sodium–ethyl acetoacetate, following the procedure of Delpierre et al.,[10] gave keto ester **3** (Scheme I). Decarboxylation of this material to the ketone, followed by protection as the ethylene ketal and subsequent removal of the phthalimide group with hydrazine, afforded amino ketal **4**. The iminium chloride derived from formamide **5**[2] and phosgene was treated with **4** to give amidine **6**,[11] which was hydrolyzed to ketone **1b** by the method of Poos et al.[12] Reduction of **1b** with sodium borohydride gave alcohol **1c**. The hydroxyimino derivative **7** was prepared from **1b** and hydroxylamine.

Amidine **9**[13] was prepared from formamide **8**,[10] the

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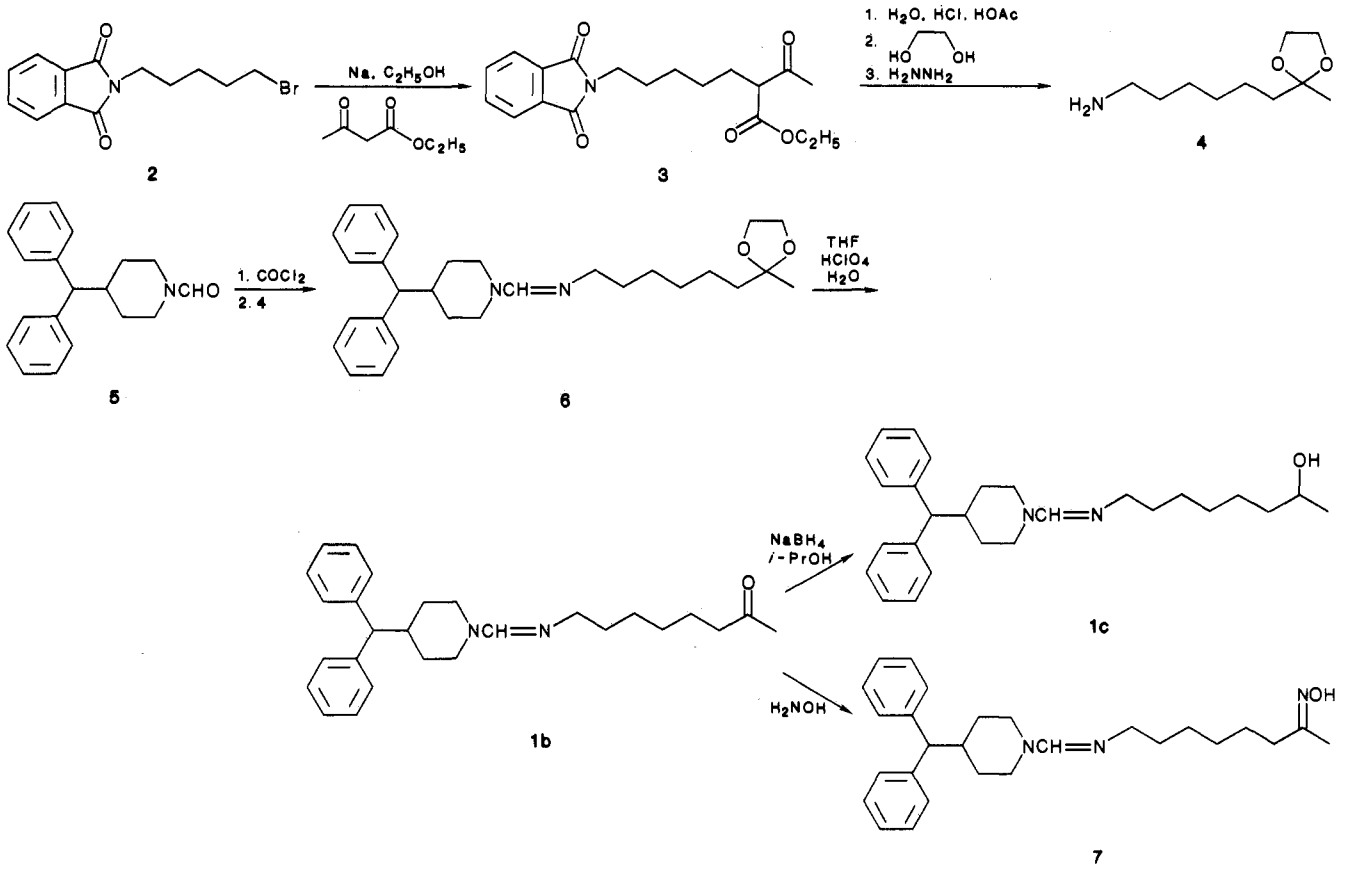
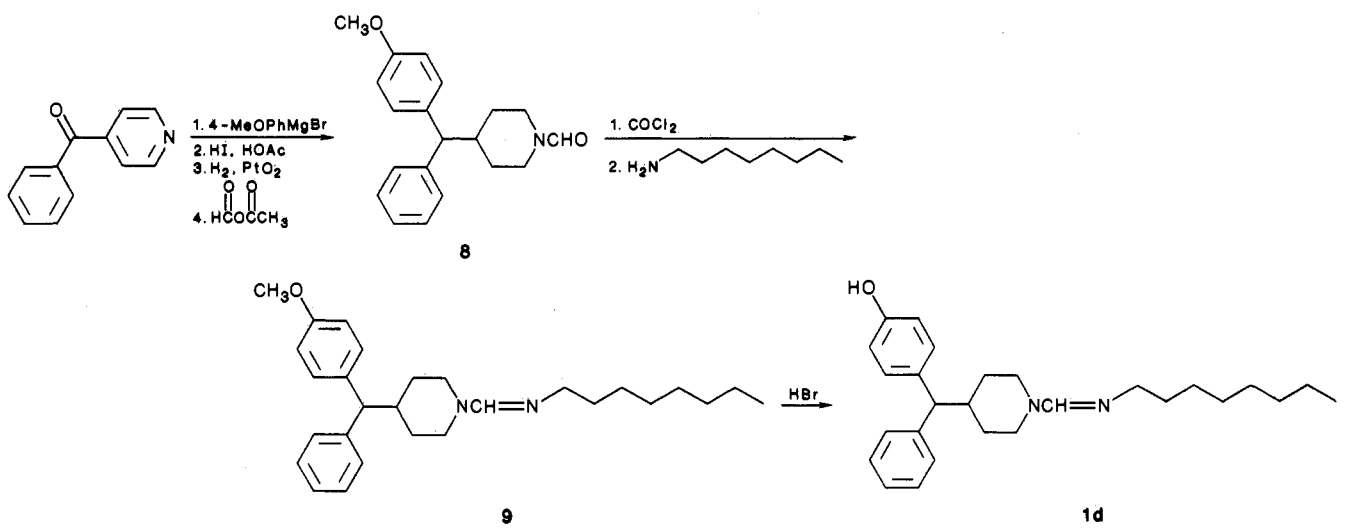
Scheme I



Scheme II



product of a four-step sequence (Scheme II). 4-Benzoylpyridine was treated with (4-methoxyphenyl)magnesium bromide; the resulting carbinol was dehydroxylated with hydriodic acid–acetic acid; the obtained pyridine was reduced catalytically; and the corresponding piperidine was treated with formic acetic anhydride to give **8**. Metabolite **1d** was obtained by treating **9** with refluxing hydrobromic acid.

Metabolites **1e** and **1f** were prepared by lithium aluminum hydride reduction of amidino esters **11a** and **11b** (Scheme III). The syntheses of **11a** and **11b** involved esterification[14] of the appropriate amino acids **10**, followed by treatment with **5** in the presence of phosgene.

The preparation of metabolite **1g** required that the acid functionality of the starting amino acid be protected with a group that could be removed in neutral or acidic media, since **1a** and its analogues are susceptible to basic hydrolysis. Accordingly, the 2,2,2-trichloroethyl ester of amino acid **10b** was prepared and converted to amidine **11c**. Deprotection of **11c** using Zn–acetic acid afforded acid **1g**.[15]

Results and Discussion

Compounds **1a–g**, **6**, **7**, **9**, and **11a** were evaluated in the acute gastric fistula rat for gastric antisecretory activity

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Scheme III

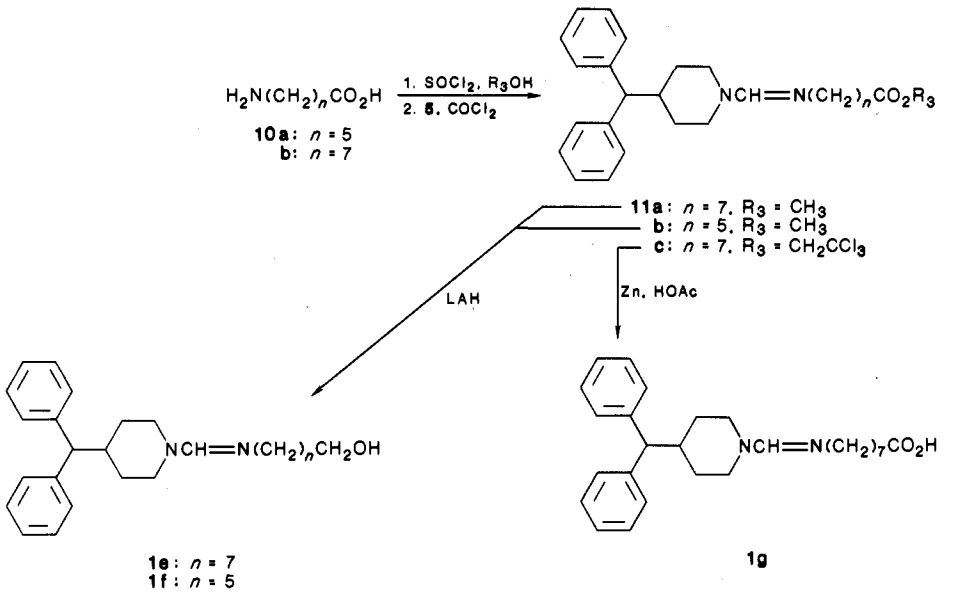

Table II. Antisecretory and Anticholinergic Activity of Fenoctimine and Its Metabolites[a]

compd	origin of metabolite	AGF:[b] ED_{50} (rat), mg/kg po	AGF:[b] ED_{50} (rat), mg/kg ip	AGF:[b] ED_{50} (rat), mg/kg iv	guinea pig ileum vs. acetylcholine IC_{50}, μg/mL
1a		3.9 (3.4–4.5)	0.6 (0.3–0.9)	1.1 (0.18–2.49)	1.8 (1.67–1.95)
1b	dog	10.5 (6.6–14.5)*	1.5 (1.2–1.9)		2.2 (1.8–2.7)
1c	rat liver homogenate	8.9 (5.4–12.4)			1.7 (0.99–4.31)
1d	dog	>40	3.1 (1.2–5.2)		0.96 (0.68–1.34)
1e	human	8.5 (6.7–10.5)*	0.44 (0.04–0.95)	9.7 (6.5–19)*	0.29 (0.22–0.37)
1f	human	11.2 (8.4–17.4)	2.0 (1.7–2.3)*	4.5 (3.0–8.1)*	0.26 (0.22–0.31)
1g	human		—		1.2 (0.98–1.47)
5	human rat dog		inactive at 40		21.9 (20.5–23.6)
6		4.8 (4.2–5.5)*	0.98 (0.5–1.6)		1.96 (1.76–2.17)
7		12.5 (9.5–16.0)	8.8 (6.0–14.5)*		4.1 (2.4–6.4)
9		15.7 (12.6–20.0)	0.63 (0.22–0.99)*		1.9 (1.5–2.3)
11a		10.1 (3.1–18.3)*		4.7 (3.2–8.1)*	0.3 (0.21–0.40)
cimetidine		7.6 (5.7–10.8)			
atropine		1.1 (0.7–2.9)			0.0038 (0.0032–0.0041)

[a] 95% confidence limits are given in parentheses. Parentheses marked with asterisk indicates 90% confidence limits. [b] AGF = acute gastric fistula.

and in guinea pig ileum, with an acetylcholine challenge, for anticholinergic activity. These methods are described in the Experimental Section, and the results are shown in Table II. For comparative purposes, data for the reference drugs atropine and cimetidine are included.

Fenoctimine was metabolized extensively in humans, rats, and dogs.[8] A major metabolic pathway in common for all these species involved hydrolysis of the imino bond of **1a** to give formamide **5**, which had no gastric antisecretory or anticholinergic activity. On the other hand, oxidative pathways seemed to be species specific. Thus, human metabolites **1e**, **1f**, and **1g** arose from oxidation of the aliphatic terminus, and dog metabolites **1d** and **1b** arose from aromatic oxidation and oxidation of the 7-position of the octylimino chain. The human oxidative metabolites **1e**, **1f**, and **1g** (tested as the methyl ester **11a** because of solubility problems), by intravenous administration, were 4–10 times less active antisecretory agents in dogs[17] than fenoctimine. However, the IC_{50}'s of **1e** and **1f** to block the effects of acetylcholine in the guinea pig ileum test indicated significant anticholinergic activity relative to fenoctimine. The dog oxidative metabolites **1b** and **1d** were not anticholinergic, but they retained gastric antisecretory activity.

In order to establish that the in vivo activity seen with fenoctimine was not solely the result of active metabolites, the inherent antisecretory activity of **1a** was tested. It inhibited secretion from isolated rat,[1] dog, and guinea pig[16] parietal cells stimulated by db-cAMP, carbachol, histamine, and gastrin.

Analogues **6**, **7**, and **9** exhibited antisecretory activity in rats when given by ip administration, with **6** and **9** being roughly equipotent with **1a** while **7** was 14 times less potent than **1a**. Orally, **7** and **9** were weakly active and **1c** was moderately active whereas **6** was about as potent as **1a**. Interestingly, **6** was more potent than ketone **1b**, both po and ip, suggesting that it was not easily hydrolyzed in vivo in the rat. None of the analogues was active in vitro as an anticholinergic agent.

Thus, the weak effect of fenoctimine in humans on gastric acid secretion may be due to the extensive metabolism of **1a** to **1e–g** and **5**, while the drying effects that occurred at high doses may be attributed to sufficient plasma concentration of metabolites such as **1e** and **1f** with anticholinergic properties. Conversely, fenoctimine was converted in dogs and rats to nonanticholinergic metabolites.

Experimental Section

Chemistry. All melting points are uncorrected and were taken

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on a Thomas-Hoover Uni-Melt melting point apparatus in capillary melting point tubes. The ^1H NMR spectra were obtained on a 90-MHz Perkin-Elmer R-32 NMR spectrometer with Me_4Si as an internal standard. IR spectra were taken on a Perkin-Elmer 552 infrared spectrophotometer. GC analyses were performed on a Perkin-Elmer PE-900 instrument equipped with a 1.82 m × 2 mm glass Chromosorb Q column with 3% SE-30 as the liquid phase. All GC runs were carried out at 90–280 °C at a rate of 16 deg/min. The spectral data for each compound supported the assigned structure, and all elemental and Karl-Fischer analyses were within 0.4% of the calculated values.

2-Methyl-1,3-dioxolane-2-hexanamine (4). According to the method of Delpierre et al.,[10] ethyl acetoacetate (133.9 g, 1.014 mol) was added to a solution of sodium ethoxide in ethanol, prepared from 23.69 g (1.03 mol) of sodium and 800 mL of absolute ethanol. The resulting solution was pumped, under N_2, into 308.0 g (1.04 mol) of *N*-(5-bromopentyl)phthalimide (**2**)[9] with stirring. This mixture was refluxed for 3.5 h, cooled, diluted with 1500 mL of H_2O, and neutralized with 1 N HCl. The ethanol was removed on a rotary evaporator, and the aqueous residue was extracted three times with 400 mL of diethyl ether. The ether extracts were combined, dried over anhydrous Na_2SO_4, and filtered, and the filtrate was evaporated to afford 350 g of orange oil **3**.

This material was refluxed for 3 h in a solution of 1000 mL of glacial acetic acid, 1000 mL of concentrated HCl, and 1000 mL of H_2O, after which the solvents were evaporated. The residue was dissolved in CH_2Cl_2 and made basic with 3 N NaOH. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to give 283 g of brown oil.

A solution of 141.45 g of this material, 58 mL of ethylene glycol, 5.80 g of *p*-toluenesulfonic acid, and 290 mL of benzene was refluxed overnight, cooled, and made basic with 3 N NaOH. The organic layer was separated, dried, filtered, and evaporated, affording 121 g of orange-brown oil.

A solution of this oil in 365 of mL of 95% ethanol was treated with 2.2 mL of 85% hydrazine and refluxed for 4 h. After standing overnight at 25 °C, the reaction mixture was filtered and the filtrate evaporated to a residue, which was slurried in 300 mL of H_2O and made basic with 3 N NaOH. Extraction of this mixture with CH_2Cl_2, separation of the organic layer, drying, and evaporation afforded 75.0 g of brown oil, which was distilled to give 26.0 g (13%) of **4** as a clear oil, 98% pure by GC (retention time 6.07 min); the remaining 2% spread over nine peaks: bp 80–83 °C (0.10 mm); ^1H NMR ($CDCl_3$) δ 3.92 (s, 4, OCH_2CH_2O), 2.85–2.35 (m, 2, NCH_2), 1.80–1.25 [m, 10, $(CH_2)_5COOC$], 1.15 (s, 3, CH_3).

4-(Diphenylmethyl)-1-[[[6-(2-methyl-1,3-dioxolan-2-yl)hexyl]imino]methyl]piperidine (6). Phosgene was passed through a solution of 37.0 g (0.133 mol) of **5**[2] in 165 mL of CH_2Cl_2 until gas evolution ceased. The CH_2Cl_2 and excess phosgene were evaporated on a rotary evaporator, and the residue was dissolved in 165 mL of CH_2Cl_2. To this solution were added 25.0 g (0.134 mol) of **4** and 52.5 g of anhydrous K_2CO_3 followed by 22.5 mL of triethylamine. The resulting mixture was stirred at 25 °C for 4 h and filtered and the filtrate neutralized with 3 N NaOH. The organic layer was separated, dried, filtered, and evaporated to give 67.0 g of orange oil, which was eluted with ether through an alumina column (105 mm × 6-cm diameter) to afford 27.65 g (46%) of **6** as a clear oil, 99% pure by GC (retention time 19.56 min). This oil (3.75 g) was dissolved in 2-propanol and treated with fumaric acid (0.98 g). The resulting solution was cooled, affording a white solid, which was collected and recrystallized three times with use of charcoal (Darco-G) to give the fumarate salt of **6** as a white solid: yield 1.15 g (46%); mp (143 °C, sinter) 145.5–148.5 °C.

4-(Diphenylmethyl)-1-[[(7-oxooctyl)imino]methyl]piperidine (1b). A solution of **6** (11.03 g, 0.025 mol), 24 mL of 70% $HClO_4$, 24 mL of H_2O, and 100 mL of THF was allowed to stand for 3 h at 25 °C, after which the THF was evaporated and CH_2Cl_2 was added to the residue. This mixture was basified with 3 N NaOH, and the organic layer was separated, dried, and evaporated. The resulting oil was passed through neutral alumina, using $CHCl_3$ as eluant, to give 4.42 g (43%) of **1b** as a clear oil, 98% pure by GC (retention time 16.43 min). A solution of this material (2.0 g, 0.005 mol) in 4.0 mL of acetone was treated with concentrated H_2SO_4 (0.26 mL). Ether was added, causing a white solid to precipitate, which was recrystallized from acetone–ether, affording the sulfate salt of **1b**: yield 1.41 g (43%); mp 98.5–103 °C; IR (KBr) ν_{max} 1692 cm^{-1} (C=O); ^1H NMR (Me_2SO-d_6) δ 2.38 (t, J = 7.0 Hz, 2, CH_2C=O), 2.04 (s, 3, CH_3C=O).

4-(Diphenylmethyl)-1-[[(7-hydroxyoctyl)imino]methyl]piperidine (1c). A solution of **6** (5.00 g, 0.012 mol) in 5 mL of 2-propanol was added slowly to a stirred mixture of $NaBH_4$ (0.25 g, 0.0065 mol) and 25 mL of 2-propanol. The resulting mixture was stirred for 1.5 h at 25 °C and then treated slowly with 20 mL of H_2O. Extraction with CH_2Cl_2 and separation of the organic layer followed by drying and evaporation afforded 4.75 g of oil containing 55% of **1c** by GC (retention time 16.41 min). This material was dissolved in acetone and treated with 2-naphthalenesulfonic acid hydrate (1.70 g), which gave a white solid on cooling. Recrystallization of this material from acetone–ethyl acetate and then methyl ethyl ketone afforded the 2-naphthalenesulfonic acid salt of **1c**: yield 1.75 g (20%); mp 129.5–132.5 °C; ^1H NMR (Me_2SO-d_6) δ 4.27 (d, J = 5 Hz, 1, *H*COH), 1.04 (d, J = 5 Hz, 3, CH_3).

Zinc 8-[[[4-(Diphenylmethyl)-1-piperidinyl]methylene]amino]octanoate Hydrochloride Hydrate (1g). According to the method of Waksman et al.,[14] thionyl chloride (4.08 g, 0.034 mol) was added dropwise at 0 °C to a stirred mixture of 8-aminooctanoic acid (5.0 g, 0.032 mol) and 2,2,2-trichloroethanol (47.65 g, 0.32 mol). This mixture was refluxed for 1 h, cooled, and treated with ether. The 2,2,2-trichloroethyl ester of aminooctanoic acid, which precipitated (2.80 g), was collected by filtration and used without further purification.

This material (2.80 g, 0.0097 mol) was added to a solution of **5** (2.42 g, 0.0087 mol) in 35 mL of CH_2Cl_2, which had been treated with phosgene as in example **6**, to give crude amidino ester **11c** (3.23 g) as an oil.

A mixture of **11c** (3.23 g, 0.0058 mol) and 33 g of zinc dust was refluxed for 3.5 h, cooled, and filtered. The filtrate was evaporated and the residue dissolved in CH_2Cl_2. Five percent $NaHCO_3$ solution was added with stirring until the pH of the aqueous layer was ca. 7. The CH_2Cl_2 layer was separated, dried, filtered, and evaporated to give a solid residue, which was dissolved in a minimum amount of CH_2Cl_2. Ether was added, causing **1g** to precipitate as a solid (0.10 g): IR (KBr) ν_{max} 1600 cm^{-1} (carboxylate anion stretching).

Methyl 8-[[[4-(Diphenylmethyl)-1-piperidinyl]methylene]amino]octanoate (11a). This compound was prepared as described for **6**, by using **5** (1.33 g, 0.0048 mol) and methyl aminooctanoate hydrochloride (1.0 g, 0.0048 mol), prepared by the method of Waksman et al.[14] The fumarate salt of **11a** was prepared by adding fumaric acid (0.5 g) to a methanol solution of 1.90 g of **11a** followed by the addition of ether, which caused precipitation of a white solid. This material was crystallized once from methanol–ether: yield 0.88 g (29%); mp (113 °C, sinter) 121–124 °C; ^1H NMR ($CDCl_3$) δ 7.95 (s, 1, CH=N), 3.66 (s, 3, CH_3).

Methyl 6-[[[4-(Diphenylmethyl)-1-piperidinyl]methylene]amino]hexanoate (11b). Compound **11b** was prepared as an oil, by using the method for **11a**.

4-(Diphenylmethyl)-1-[[(8-hydroxyoctyl)imino]methyl]piperidine (1e). Ester **11a** (3.0 g, 0.0069 mol) in 5 mL of dry THF was added to a slurry of LAH (0.53 g, 0.014 mol) at such a rate that reflux was maintained. The resulting mixture was refluxed for 3 h at 25 °C, cooled in ice, and treated sequentially with 0.5 mL of H_2O, 1.5 mL of 3 N NaOH, and 0.5 mL of H_2O. Anhydrous $MgSO_4$ was added, and the mixture was filtered and evaporated to give **1e** as an oil (2.30 g, 83%). A solution of **1e** (1.87 g), oxalic acid dihydrate (0.58 g), and 2-propanol was treated with ether until slightly cloudy and then cooled. A white solid precipitated, which was collected and recrystallized from 2-propanol–ether to give the oxalate salt of **1e**: yield 1.55 g (45%); mp (131 °C, sinter) 135–137 °C; IR (KBr) ν_{max} 3228 cm^{-1} (OH); ^1H NMR (Me_2SO-d_6) δ 8.07 (s, 1, CH=N).

4-(Diphenylmethyl)-1-[[(6-hydroxyhexyl)imino]methyl]piperidine (1f). According to the method for preparation of **1e**, **11b** (5.0 g, 0.012 mol) and LAH (0.98 g, 0.026 mol) gave 3.75 g (83%) of **1f** as an oil. The oxalate salt of **1f** was prepared as described for **1e** by using methanol–ether (Table I).

4-[(4-Methoxyphenyl)phenylmethyl]-1-[(octylimino)methyl]piperidine (9).[13] 4-[(4-Methoxyphenyl)phenyl-

methyl]piperidine[13] (20.0 g, 0.071 mol) was slowly added to a mixture of 7.04 mL of formic acid and 14.0 mL of acetic anhydride at 5 °C. The resulting solution was stirred at 25 °C overnight, dissolved in 35 mL of $CHCl_3$, and neutralized with saturated $NaHCO_3$ solution. The organic layer was separated, dried over anhydrous K_2CO_3, filtered, and evaporated to give **8** as a yellow oil (22.4 g).

A mixture of **8** (9.48 g, 0.031 mol) and dimethyl sulfate (3.87 g, 0.031 mol) was heated on a steam bath for 3 h under anhydrous conditions. The reaction mixture was cooled, dissolved in 40 mL of CH_2Cl_2, and treated with 15 mL of 3 N NaOH with stirring. The organic layer was separated, dried, filtered, and evaporated to give **9** (10.86 g) as an orange oil. This material and fumaric acid (3.0 g) were dissolved in 2-propanol and cooled. A white solid precipitated, which was collected and recrystallized from 2-propanol, affording the fumarate salt of **9**: yield 4.73 g (27%); mp 154.5–156 °C; ^1H NMR (Me_2SO-d_6) δ 7.98 (s, 1, CH=N), 6.84 (d, J = 9 Hz, 2).

4-[(4-Hydroxyphenyl)phenylmethyl]-1-[(octylimino)-methyl]piperidine (1d). A mixture of **9** (1.90 g, 0.0045 mol) and 42 mL of 47–49% HBr was refluxed for 1 h and cooled, and the aqueous layer was decanted from the residual oil. This material was dissolved in CH_2Cl_2 and neutralized with saturated $NaHCO_3$ solution. The organic layer was separated, dried, filtered, and evaporated to an oil (**1d**, 1.60 g), which was dissolved in acetone, treated with 2-naphthalenesulfonic acid (0.82 g), and cooled. The resulting white solid precipitate was collected and recrystallized from acetone–ether to give the 2-naphthalenesulfonic acid salt of **1d** as a white solid: yield 0.96 g (35%); mp 177.5–180.5 °C; IR (KBr) ν_{max} 1360 cm^{-1} (C–O, phenol); ^1H NMR (Me_2SO) δ 9.13 (s, 1, PhOH), 8.03 (s, 1, CH=N), 7.10 (d, J = 8 Hz, 2, aromatic), 6.66 (d, J = Hz, 2, aromatic).

4-(Diphenylmethyl)-1-[[[7-(hydroxyimino)octyl]imino]-methyl]piperidine (7). A solution of **1b** sulfate (1:1) hydrate (10:7) (3.00 g, 0.0058 mol), hydroxylamine hydrochloride (0.47 g, 0.0069 mol), and 15 mL of 95% ethanol was stirred for 2.5 h and then concentrated. The residue was dissolved in CH_2Cl_2 and made basic with 3 N NaOH, and the organic layer was separated, dried, and evaporated to give 2.80 g of **7** as an oil. A solution of this oil in acetone was treated with 2-naphthalenesulfonic acid hydrate (1.40 g) and cooled, affording a white solid. Recrystallization of this material gave the 2-naphthalenesulfonic acid salt of **7** as a white crystalline solid: yield 0.95 (26%); mp (123 °C, sinter) 125–128 °C; IR (KBr) ν_{max} 1693 cm^{-1} (C=NO); ^1H NMR (Me_2SO-d_6) δ 10.15, 10.11 (s, 1, syn-anti NOH).

Pharmacological Methods. The ED_{50} values for gastric antisecretory activity were determined with at least five animals/dosage group and at least three dosage groups/ED_{50}.

Gastic antisecretory activity was evaluated in the acute gastric fistula rat.[17] In this preparation, drug or vehicle (0.5% methocel solution) was administered po 1 h before surgery.

Female Sprague–Dawley rats (Charles River, Inc.) weighing 120–160 g were deprived of food for at least 18 h. The rats were anesthetized with diethyl ether in an anesthesia jar, and after laparotomy, a flanged polyethylene tube was inserted into the fundal portion of the stomach. The wound was closed, and the rats were placed in a plastic cage with a slit to allow the cannula to pass through. A 10-mL collecting tube was attached, and the collection was begun. The first 30-min sample was discarded, and then two 1-h samples were collected. Each sample was centrifuged, the volume was determined, and a 1-mL aliquot was removed for titration to pH 7 using 0.01 N NaOH. Results are expressed as volume (milliliters), titratable acidity (milliequivalents per liter), and total acid output (milliequivalents of H^+). ED_{50}'s and confidence limits were calculated by the method of least-squares regression analysis and represent the dose (milligrams per kilogram) required to produce an average of 50% inhibition in total acid output vs. controls in the animals tested for a particular compound.

Anticholinergic activity of the compounds was determined in vitro by measuring their effect on the acetylcholine chloride induced contraction of guinea pig ileum.

Female guinea pigs (Hazelton-Dutchland, Denver, PA) weighing 200–500 g were sacrificed in a carbon dioxide chamber and their ileums quickly removed. The ileum was trimmed and cleaned with cold oxygenated Krebs bicarbonate solution. Strips (1–2 cm) were cut from the ileum. The segments were placed in 50-mL organ baths (35 °C) and attached to an isometric tension transducer (Narco). Tension was adjusted to 1 g after a 30–60-min period of equilibration in the Krebs bicarbonate solution. The agonist, acetylcholine chloride (Sigma), was then added in increasing concentrations until the maximum response was obtained. Compounds were tested for antagonism of the maximal dose of acetylcholine. The agonist was given 2 min after the administration of the test compound. Full concentration–response studies were carried out. ED_{50}'s and 95% confidence limits were determined by using the percent inhibition of the control acetylcholine response, with use of regression analysis and an ED_{50} program on a DEC 10 computer.

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