Acknowledgment. The authors express their appreciation to Pauline Baker, William Walker, Susan Roland, Barbara Leak, David Cleland, Patricia Parker, and Gregory Strayhorn for technical assistance and to Dr. Carol Sharer of the Wellcome Development Laboratories for the preparation of **6**. The authors thank Dr. S. R. M. Bushby and Margaret Bushby for the antibacterial testing and for the potentiation studies.

Registry No. **6**, 98525-47-4; **7**, 1194-21-4; **8**, 1007-99-4; **9**, 85840-20-6; **10**, 108-53-2; **11**, 54004-20-5; **12**, 98510-35-1; **12**·HCl, 98510-49-7; **13**, 879-44-7; **14**, 55482-22-9; **15**, 2387-48-6; **16**, 86296-75-5; **17**, 78523-14-5; **18**, 98510-36-2; **19**, 98510-37-3; **20**, 98510-38-4; **21**, 33344-20-6; **22**, 33344-24-0; **23**, 98510-39-5; **24**, 98510-40-8; **25**, 98510-41-9; **26**, 98510-42-0; **27**, 98510-43-1; **28**, 98510-44-2; **29**, 98510-45-3; **30**, 98510-46-4; **31**, 85840-24-0; **32**, 98510-47-5; **33**, 98510-48-6; NH_2Ph, 62-53-3; $NH_2(CH_2)_4Ph$, 13214-66-9; $NH_2(CH_2)_5Ph$, 17734-21-3; $NH_2(CH_2)_6Ph$, 17734-20-2; $NH_2(CH_2)_3SO_2Ph$, 98510-52-2; $NH_2(CH_2)_3OPh$, 7617-76-7; methylamine, 74-89-5; 2-amino-6-(diethylamino)-4-methoxypyrimidine, 98525-48-5; 3-phenylpropylamine, 2038-57-5; 2-amino-6-(3-phenylpropyl)aminopyrimidin-4(3*H*)-one, 98510-50-0; potassium phthalimide, 1074-82-4; 3-phenoxypropyl bromide, 588-63-6; *N*-(3-phenoxypropyl)phthalimide, 83708-38-7; 3-phenoxypropylamine hydrochloride, 83708-39-8; 3-(phenylsulfonyl)-propylamino hydrochloride, 98510-51-1; 3-(phenylsulfonyl)-propionitrile, 10154-75-3; 2-amino-6-mercaptopyrimidin-4(3*H*)-one, 6973-81-5; 2-amino-6-(methylthio)pyrimidine-4(3*H*)-one, 6307-40-0; dihydropteroate synthase, 9055-61-2.

Synthesis and Biological Activities of Some Pseudo-Peptide Analogues of Tetragastrin: The Importance of the Peptide Backbone

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Pseudo-peptide analogues of the C-terminal tetrapeptide of gastrin, in which a peptide bond has been replaced by a CH_2–NH bond, i.e. (*tert*-butyloxycarbonyl)-L-tryptophyl-$\psi(CH_2$–NH)-L-leucyl-L-aspartyl-L-phenylalanine amide (**8**), (*tert*-butyloxycarbonyl)-L-tryptophyl-L-leucyl-$\psi(CH_2$–NH)-L-aspartyl-L-phenylalanine amide (**13**), (*tert*-butyloxycarbonyl)-L-tryptophyl-L-leucyl-L-aspartyl-$\psi(CH_2NH)$-L-phenylalanine amide (**20**), were synthesized. The pseudo-peptides **8** and **13** were shown to have the same affinity as (*tert*-butyloxycarbonyl)-L-tryptophyl-L-leucyl-L-aspartyl-L-phenylalanine amide (**21**) for the gastrin receptor on isolated mucosal cells. The pseudo-peptide **20** exhibited lower affinity ($IC_{50} \simeq 10^{-5}$ M). The biological activity of these pseudo-peptides was studied on acid secretion in the anesthetized rat. Compound **8** stimulated acid secretion, identically with that of **21**. Compound **13** did not exhibit any agonist activity but was able to antagonize the action of gastrin (ED_{50} = 0.3 mg/kg). Compound **20** did not show any agonist activity but was able to inhibit gastrin-induced acid secretion, with lower potency (ED_{50} = 15 mg/kg). The importance of the peptide bonds in the mode of action of gastrin is discussed, and a hypothetical approach of the mechanism of action is presented.

Gastrin, a 17 amino acid hormone isolated from hog antral mucosa,[1] plays a major role in the stimulation of gastric acid secretion. It was early recognized that, of the 17 amino acid residues of the molecule, only the C-terminal tetrapeptide sequence, Trp-Met-Asp-Phe-NH_2 (which is also found in cholecystokinin and caerulein) is required for the remarkable range of physiological effects displayed by the natural hormone.[2] The finding of the whole activity in such a small molecule offered scope for an investigation of structure–function relationships on an unprecedented scale.[3] Changes can be made in the Trp, Met, and Phe positions, giving active analogues providing evidence that these positions were concerned only with binding at the site of action. On the other hand, even small changes at the Asp position resulted in loss of activity, pointing toward a functional rather than a binding role for the aspartyl residue. However, despite the numerous studies in the gastrin area, the mechanism of action of the hormone remains unclear. We recently showed that elimination of the C-terminal phenylalanyl residue resulted in potent gastrin antagonists,[4,18,19] and we concluded that, although the phenylalanyl residue was essential for the biological activity, it was not crucial for binding to gastrin receptors. Morley reported that the side chains of the binding amino acid residues rather than the peptide backbone are important in the binding interactions and that the peptide backbone serves mainly in providing correct spacing of the side chains.[3] We recently pointed out the significance of the peptide bonds in the mode of action of gastrin.[5] In the present report, we present the synthesis and the biological activities of three pseudo-peptide analogues of the C-terminal tetrapeptide of gastrin in which an amide bond has been replaced by a CH_2–NH bond, an isosteric modification that has already been used in the series of renin inhibitors.[6] For avoiding side reactions related to methionine and because it is known that leucine can replace methionine in the gastrin family without any loss of activity, we chose to prepare analogues containing leucine instead of methionine. Binding of these pseudo-peptides to isolated gastric mucosal cells and their biological activity on acid secretion in anaesthetized rats are reported.

Chemistry. The pseudo-peptide Boc-Trp-$\psi(CH_2$-NH)-Leu-Asp(Bzl)-Phe-NH_2 (**8**) was synthesized according to Scheme I. The tripeptide Boc-Leu-Asp(Bzl)-Phe-NH_2 (**3**) was prepared stepwise starting from the C-terminal residue, with BOP as coupling reagent.[7] It was partially deblocked by trifluoroacetic acid to produce the tri-

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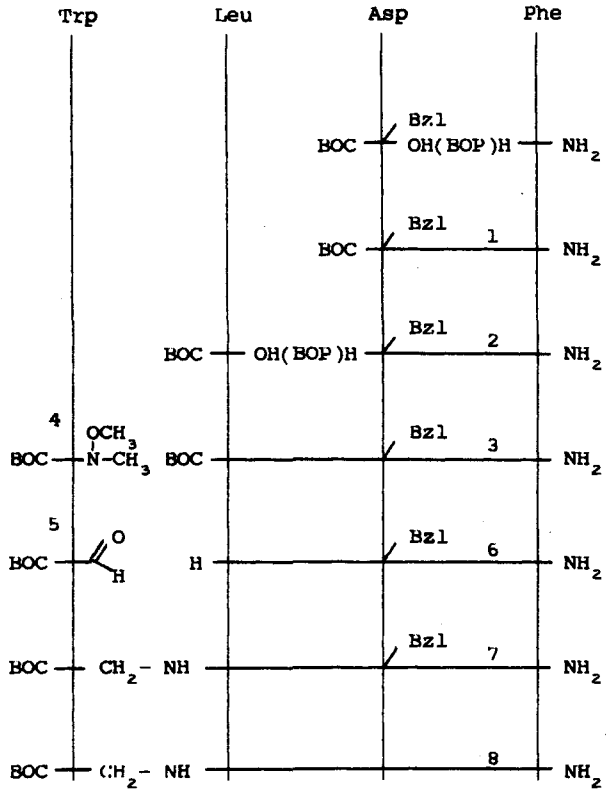
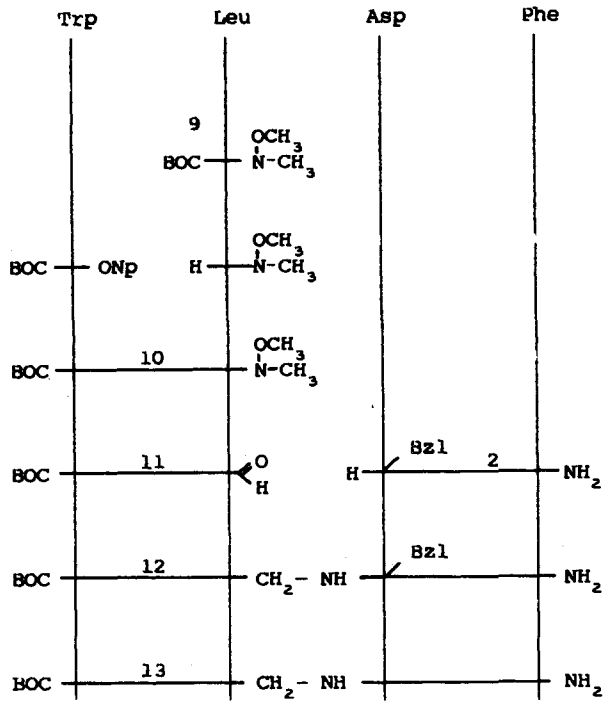
Scheme I

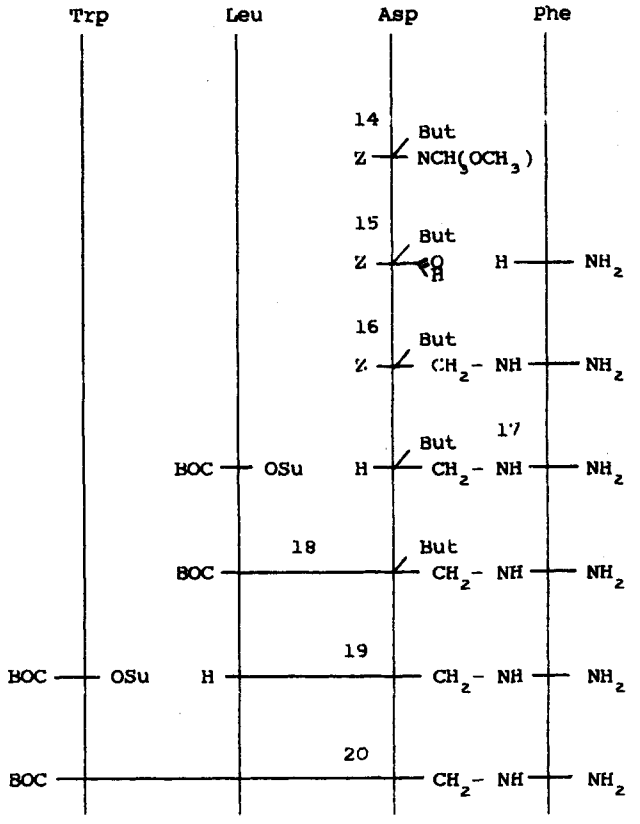


fluoroacetate salt **6**. Boc-Trp was reacted with the hydrochloride salt of *O,N*-dimethylhydroxylamine in the presence of BOP reagent or DCC/DMAP[8] to yield Boc-Trp-N(OCH_3)CH_3 (**4**). Compound **4** was converted into the corresponding aldehyde **5**, in the presence of $AlLiH_4$[9] which reacted with the trifluoroacetate salt (**6**), in a methanol–acetic acid mixture, with $NaBH_4CN$[10] as reducing reagent, to yield after purification by silica gel column chromatography Boc-Trp-ψ(CH_2–NH)-Leu-Asp-(Bzl)-Phe-NH_2 (**7**). Hydrogenolysis of compound **7**, in the presence of a 10% Pd/C catalyst yielded Boc-Trp-ψ-(CH_2–NH)-Leu-Asp-Phe-NH_2 (**8**) in pure form. The pseudo-peptide Boc-Trp-Leu-ψ(CH_2–NH)-Asp(Bzl)-Phe-NH_2 (**12**) was synthesized according to Scheme II. Boc-Leu-N(OCH_3)CH_3 (**9**) was obtained by reaction of Boc-leu with the hydrochloride salt of *O,N*-dimethylhydroxylamine in the presence of BOP or DCC/DMAP. Compound **9** was partially deprotected by trifluoroacetic acid and the resulting trifluoroacetate salt reacted with Boc-Trp-ONp to yield Boc-Trp-Leu-N(OCH_3)CH_3 **10**. Compound **10** was treated with $AlLiH_4$ to produce the corresponding aldehyde **11**, which reacted with the dipeptide H-Asp(Bzl)-Phe-NH_2 (**2**) in a methanol–acetic acid mixture with $NaBH_4CN$ as reducing reagent. After purification by silica gel column chromatography, the pseudo-peptide Boc-Trp-Leu-ψ(CH_2–NH)-Asp(Bzl)-Phe-NH_2 (**12**) was isolated in pure form and hydrogenated in the presence of a 10% Pd/C catalyst to yield Boc-Trp-Leu-ψ(CH_2–NH)-Asp-Phe-NH_2 (**13**). The synthesis of the pseudo-peptide **20**, Boc-Trp-Leu-Asp(Bzl)-ψ(CH_2–NH)-Phe-NH_2, was carried out according to Scheme III. Z-Asp(But)-OH was allowed to react with the hydrochloride salt of *O,N*-dimethyl-

Scheme II



Scheme III



hydroxylamine in the presence of BOP or DCC/DMAP to yield Z-Asp(But)-N(OCH_3)CH_3 (**14**). This compound was converted into its corresponding aldehyde, at 0 °C, with $AlLiH_4$, and coupled with phenylalanine amide to produce Z-Asp(But)-ψ(CH_2-NH)-Phe-NH_2 (**16**), which was purified by silica gel column chromatography. Partial deblocking of **16** by hydrogenolysis and coupling with Boc-Leu-OSu yielded Boc-Leu-Asp(But)-ψ(CH_2–NH)-Phe-NH_2 (**18**), which was purified by silica gel column chromatography. No significant amounts of side product coming from N-acylation at the amino function of the

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Scheme IV. Synthetic Analogues of the C-Terminal Tetrapeptide of Gastrin

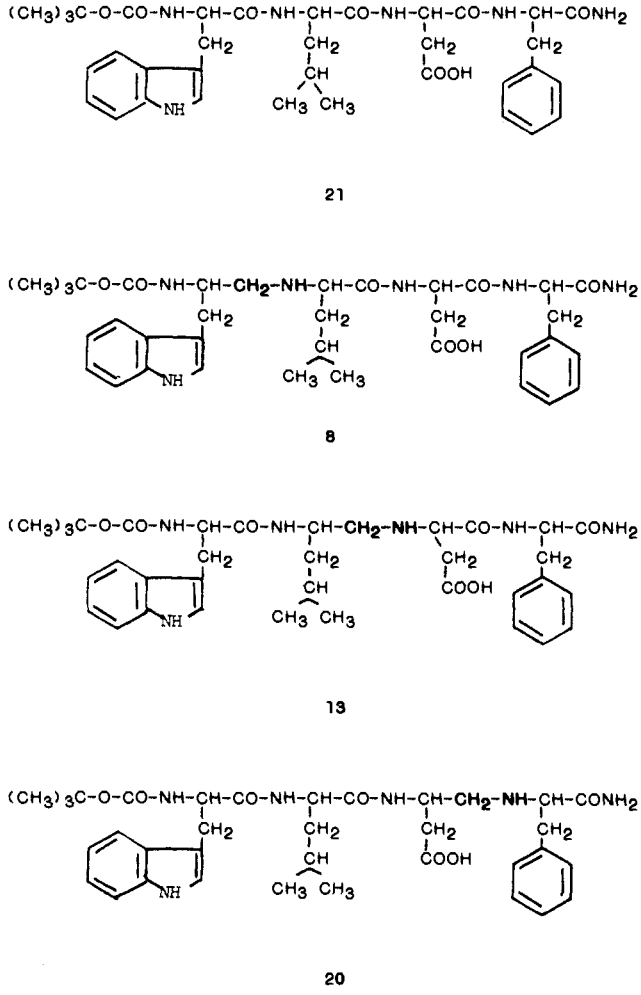

Table I. Biological Activity of Synthetic Compounds, on Gastrin-Induced Acid Secretion in the Rat in Vivo according to Ghosh and Schild and on in Vitro Inhibition of Binding of Labeled Gastrin to Isolated Mucosal Cells

compd	in vivo act.	binding: IC_{50}, M
Boc-Trp-Leu-Asp-Phe-NH_2 (**21**)	agonist	5×10^{-7}
Boc-Trp-ψ(CH_2–NH)-Leu-Asp-Phe-NH_2 (**8**)	agonist	3×10^{-7}
Boc-Trp-Leu-ψ(CH_2–NH)-Asp-Phe-NH_2 (**13**)	antag; ED_{50} = 0.3 mg/kg	2.5×10^{-7}
Boc-Trp-Leu-Asp-ψ(CH_2–NH)-Phe-NH_2 (**20**)	antag; ED_{50} = 15 mg/kg	5×10^{-6}

CH_2–NH could be detected. Therefore, the secondary amine was left unprotected. Removal of the protecting groups by trifluoroacetic acid produced the TFA salt **19**. This was allowed to react with Boc-Trp-OSu to yield Boc-Trp-Leu-Asp-ψ(CH_2–NH)-Phe-NH_2 (**20**), which was purified by silica gel column chromatography.

Biological Results and Discussion. Table I shows the results of the inhibition of [^{125}I]-(Leu^{15})-HG-17 binding to isolated gastric mucosal cells, by compounds **8**, **13**, **20**, and **21** and their biological activities. Compound **8**, Boc-Trp-ψ(CH_2-NH)-Leu-Asp-Phe-NH_2, is a very potent agonist of acid secretion, in the anaesthetized rat, according to Ghosh and Schild.[12] This compound is as potent as the corresponding peptide with the sequence, Boc-Trp-Leu-Asp-Phe-NH_2 (**21**) (Figure 1). On the inhibition of binding of [^{125}I]-(Leu^{15})-HG-17 to its receptors, compound

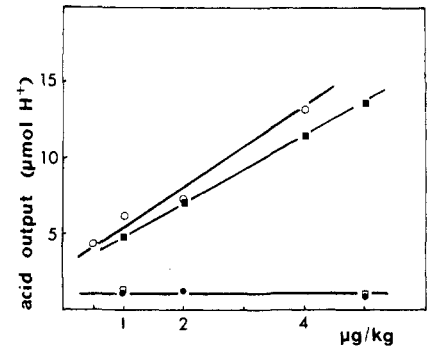

Figure 1. Gastric acid secretion in anaesthetized rat stomach (Ghosh and Schild model). Peptides were injected iv in small volumes (0.05–0.1 mL) at different doses. The pH was recorded for 40 min, and acid output was calculated by difference between acid secretion after peptide injection and basal acid secretion. The curves are representative of three separate experiments: ○, Boc-Trp-Leu-Asp-Phe-NH_2; ■, Boc-Trp-ψ(CH_2–NH)-Leu-Asp-Phe-NH_2; □, Boc-Trp-Leu-ψ(CH_2–NH)-Asp-Phe-NH_2; ●, Boc-Trp-Leu-Asp-ψ(CH_2–NH)-Phe-NH_2.

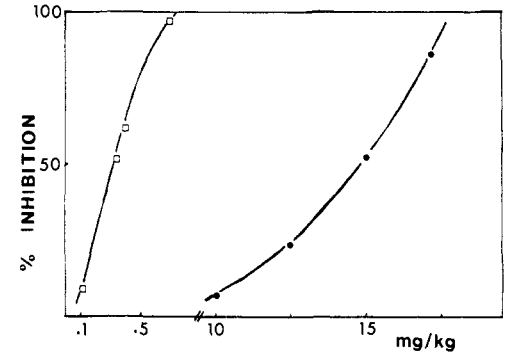

Figure 2. Inhibitory effect of the peptides on gastrin-induced acid secretion in anaesthetized rat (Ghosh and Schild model). Peptides were injected iv in small volumes, together with 80 pmol of (Leu^{15})-HG-17 (submaximal dose). The pH was recorded for 40 min, and the percent of inhibition was calculated for each dose of peptide as the ratio of acid output induced by peptide plus gastrin to acid output induced by gastrin alone. The curves are representative of three separate experiments: □, Boc-Trp-Leu-ψ(CH_2–NH)-Asp-Phe-NH_2; ●, Boc-Trp-Leu-Asp-ψ(CH_2–NH)-Phe-NH_2.

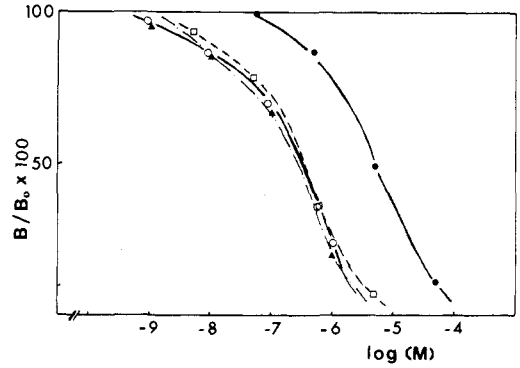

Figure 3. Inhibitory effect of peptides on gastrin binding to isolated gastric mucosal cells. Isolated cells (5×10^6 per mL) were incubated in Earle's medium with [^{125}I]-(Leu^{15})-HG-17 (20 pM) for 30 min at 37 °C, in the presence (B) or not (B_0) of various concentrations of the peptides. The curves are the mean of quadruplicate determinations: ○, Boc-Trp-Leu-Asp-Phe-NH_2; ■, Boc-Trp-ψ(CH_2–NH)-Leu-Asp-Phe-NH_2; □, Boc-Trp-Leu-ψ-(CH_2–NH)-Asp-Phe-NH_2; ●, Boc-Trp-Leu-Asp-ψ(CH_2–NH)-Phe-NH_2.

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8 showed almost the same potency as **21** (Figure 3). The pseudo-peptide **20**, Boc-Trp-Leu-Asp-ψ(CH_2–NH)-Phe-NH_2, did not show any agonist activity on acid secretion but exhibited an antagonist effect on gastrin-induced acid secretion (ED_{50} = 15 mg/kg). The compound **20** has a lower affinity to isolated gastric mucosal cells ($IC_{50} \simeq 5 \times 10^{-6}$). On the other hand, the pseudo-peptide **13**, Boc-Trp-Leu-ψ(CH_2–NH)-Asp-Phe-NH_2, which showed the same affinity as **8** to gastric mucosal cells, did not exhibit any agonist activity on acid secretion in the anaesthetized rat, even at doses as high as 10 mg/kg. However, this peptide proved to be a very potent antagonist of gastrin-stimulated acid secretion, with a half-maximal effective dose of 0.3 mg/kg (Figure 2). In the pseudo-peptide **8**, the peptide bond between Trp and Leu has been replaced by a CH_2–NH bond. This isosteric change did not seem to significantly affect the recognition of the gastrin receptor, and compound **8** stimulates acid secretion. It is as good agonist as the peptide **21** itself. The pseudo-peptide **13**, Boc-Trp-Leu-ψ(CH_2–NH)-Asp-Phe-NH_2, in which the peptide bond between Leu and Asp has been replaced by a CH_2–NH bond, showed the same affinity for gastrin receptors on isolated mucosal cells as **8**. This pseudo-peptide, however, did not exhibit any agonist activity on acid secretion but was able to antagonize the action of (Nle[11])-HG-13 on acid secretion, in the anaesthetized rat, according to Ghosh and Schild.[12] These results prompted us to postulate that, for exhibiting agonist activity on acid secretion, the bond between Leu and Asp in the C-terminal tetrapeptide of gastrin has to be a peptide bond, a cleavable bond. And an enzymatic system might exist that cleaves the C-terminal of gastrin, between Met and Asp. Compound **13**, which is able to occupy all the subsite sites of the receptor, cannot be cleaved between Leu and Asp and behaves as a potent antagonist of gastrin. Compound **20** exhibited lower affinity for the gastrin receptor and behaved as an antagonist of gastrin-induced acid secretion, with lower potency than **13**. This suggests that the peptide bond between Asp and Phe is of some importance for gastrin receptor recognition, probably by correctly positioning the aromatic ring of phenylalanine which is an important point of anchoring to the receptor. The carbonyl moiety of the peptide bond between Asp and Phe may also play some role. Its replacement by a CH_2, introducing a different electronic environment, has no good influence in this case. The importance of the carboxylic acid of the aspartyl residue was already recognized: a functional rather than a binding role was supposed for this residue. This functional role can be explained by the fact that the possible enzymatic system that cleaves the peptide bond between Leu and Asp needs the β-carboxylate of the aspartyl residue. The dipeptide that is liberated from the peptide by this enzymatic cleavage is probably responsible for the biological activity. The fact that the pseudo-peptide **20** is an antagonist, even though it can be cleaved between the Leu and Asp residues, suggested that the pseudo-dipeptide Asp-ψ(CH_2–NH)-Phe-NH_2, which is liberated after this cleavage, cannot play the same role as Asp-Phe-NH_2. The hypothesis of a cleavage between Met and Asp in the mechanism of action of gastrin may be supported by the careful examination of previously obtained results. It was reported that when in the C-terminal tetrapeptide of gastrin L-Trp was replaced by D-Trp to give Boc-D-Trp-Met-Asp-Phe-NH_2, some agonist activity was found.[14] Again, when L-Phe was replaced by D-Phe, to yield Boc-Trp-Met-Asp-D-Phe-NH_2, active compounds were obtained.[14] In these two examples, the bond between Met and Asp can be enzymatically cleaved. However, substitution of L-Met by D-Met (Boc-Trp-D-Met-Asp-Phe-NH_2), or substitution of L-Asp by D-Asp (Boc-Trp-Met-D-Asp-Phe-NH_2), which will prevent an enzymatic cleavage at the Met–Asp bond, produced inactive compounds.[14] Any change at the aspartyl residue yielded inactive derivatives as well. The N-terminal portion of the gastrin molecule does not probably participate in the chemical event but participates in the binding of the hormone to the receptor at the site of action. It has already been demonstrated that N-terminal extensions of the tetragastrin molecule by the residues of the sequence of the natural hormone[13,18] or by a β-alanine[3,19] led to compounds of better affinity and of stronger potency. This was again proved in our study: extensions at the N-terminal end by a β-alanine of the pseudo-peptides synthesized in this work led to more affine and more potent compounds. (These results will be reported separately.)

Conclusions. This study on analogues of the tetrapeptide of gastrin modified at peptide bonds points out the significance of the peptide bonds in the biological activity of gastrin derivatives and is suggestive of a mechanism of action for this hormone. An explanation about the possible role of the C-terminal aspartic acid, which is known to be essential, can now be proposed. It is clear that, in the gastrin series, the replacement of the peptide bonds between Trp and Leu, or between Leu and Asp, by a CH_2–NH bond did not significantly affect the affinity of those derivatives for isolated gastric mucosal cells. However, if the peptide bond between Trp and Leu is not crucial for the biological activity, the peptide bond between Leu and Asp is essential for the exhibiting of activity on acid secretion. The C-terminal dipeptide of gastrin is probably cleaved from the parent molecule and is responsible for the biological response. The peptide bond between Asp and Phe is of some importance for gastrin receptor recognition, and its replacement by a CH_2–NH bond leads to antagonists of the parent molecule. This result seems to point out the importance of the structural integrity of the C-terminal dipeptide of gastrin for exhibiting its activity. Gastrin, cholecystokinin, and caerulein share an identical C-terminal pentapeptide amide sequence, and it is possible that one way of action of these hormones may be accounted by this mechanism.

Experimental Section

Melting points were taken on a Buchi apparatus in open capillary tubes. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by le Service de microanalyses de l'ENSCM (Montpellier, France). Ascending TLC was performed on precoated plates of silica gel 60 F_{254} (Merck) with the following solvent systems (by volume): A, hexane/ethyl acetate (7:3); B, $CHCl_3$; C, AcOEt/hexane (1:1); D, AcOEt; E, $CHCl_3$/MeOH (97:3); F, AcOEt/py/AcOH/H_2O (80:20:3:3); G, $CHCl_3$/MeOH/AcOH (80:10:5). Peptide derivatives were located with charring reagent or ninhydrine. Column chromatographies were performed with silica gel 60, 60–229 mesh, ASTM (Merck). L amino acids and derivatives were from Bachem. All reagents and solvents were of analytical grade. BOP was recrystallized from acetone and ether. The following abbreviations were used: DMF, dimethylformamide; HOBt, 1-hydroxybenzo-

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triazole; DIEA, *N,N*-diisopropylethylamine; BOP, [(benzotriazolyl)oxy]tris(dimethylaminophosphonium hexafluorophosphate; DMAP, 4-(dimethylamino)pyridine; DCC, *N,N'*-dicyclohexylcarbodiimide. Other abbreviations used were those recommended by the IUPAC-IUB Commission.[20]

Synthesis. (*tert*-Butyloxycarbonyl)-β-benzyl-L-aspartyl-L-phenylalanine Amide (1). To a solution of L-phenylalanine amide (2.88 g, 16.9 mmol) in DMF (20 mL), were added (*tert*-butyloxycarbonyl)-β-benzyl-L-aspartic acid (4.85 g, 15 mmol) and BOP (6.63 g, 15 mmol)[7] followed by DIEA (3 mL). After the mixture was allowed to stand overnight at room temperature, the solvent was removed in vacuo and the residue dissolved in ethyl acetate (250 mL) and washed with a saturated sodium bicarbonate solution (2 × 50 mL), water (2 × 50 mL), a 10% citric acid solution (2 × 50 mL) and water (2 × 50 mL). The organic layer was dried over sodium sulfate and then concentrated in vacuo. The residue, triturated with ether, gave a white powder: yield 6.5 g (92%); R_f(A) 0.24, R_f(B) 0.61; mp 86–89 °C; $[\alpha]_D$ −22.5 (*c* 1, DMF). Anal. ($C_{25}H_{31}N_3O_6$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-leucyl-β-benzyl-L-aspartyl-L-phenylalanine Amide (3). Compound 1 (3.6 g, 7.7 mmol) was partially deprotected with trifluoroacetic acid (20 mL). After the mixture was allowed to stand at room temperature for 30 min, ether (300 mL) was added. The precipitate which formed was collected, washed several times with ether, and dried in vacuo over KOH. It was dissolved in DMF (30 mL) containing Boc-L-Leu (1.62 g, 7 mmol) and BOP (3.09 g, 7 mmol). The solution was cooled in an ice water bath, and DIEA (2.6 mL, 15 mmol) was added. After standing overnight at room temperature, the reaction mixture was treated as described for compound 1. The residue, triturated with ether, yielded a white powder: 3.78 g (93%); R_f(A) 0.18, R_f(D) 0.62; mp 135–145 °C; $[\alpha]_D$ −33 (*c* 1.1, DMF). Anal. ($C_{31}H_{42}N_4O_7$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophan *N,O*-Dimethylhydroxamate (4). Boc-L-Trp (9.12 g, 30 mmol) was dissolved in dichloromethane (300 mL) containing the hydrochloride salt of *N,O*-dimethylhydroxylamine (3.22 g, 33 mmol). DCC (6.18 g, 30 mmol) and 4-(dimethylamino)pyridine (0.3 g) were added, followed by DIEA (5.5 mL, 33 mmol). After 5 h at room temperature, the solvent was concentrated in vacuo and the reaction mixture treated as described for 1. By trituration with ether, a crystalline compound was obtained: 8.5 g (82%); R_f(C) 0.50; mp 130–132 °C; $[\alpha]_D$ −24 (*c* 1, DMF). Anal. ($C_{18}H_{25}N_3O_4$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophanal (5). Compound 4 (0.87 g, 2.5 mmol) was dissolved in tetrahydrofuran (10 mL), and the reaction mixture was cooled down to 0 °C. $AlLiH_4$ (0.24 g, 6.5 mmol) was added portionwise over a period of 15 min. After an additional 30 min, ether (100 mL) was added followed by a cold solution of 20% citric acid (100 mL). The mixture was vigorously stirred during 30 min. The organic layer was collected and the aqueous phase extracted again with ether (100 mL). The ether extracts were pooled, washed with a saturated sodium bicarbonate solution (1 × 50 mL), water, a 10% citric acid solution (1 × 50 mL), and water, and dried over sodium sulfate. The solution was concentrated in vacuo to yield a clear oil that was stored in the refrigerator: R_f(C) 0.75. Some hydroxamate 4 seemed to remain.

Trifluoroacetate Salt of L-Leucyl-β-benzyl-L-aspartyl-L-phenylalanine Amide (6). Compound 3 (0.873 g, 1.5 mmol) was treated with TFA (5 mL) during 30 min. Addition of ether (100 mL) under vigorous stirring yielded a white powder that was collected by filtration, rinsed several times with ether, and dried in vacuo over KOH.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-ψ(CH_2–NH)-L-leucyl-β-benzyl-L-aspartyl-L-phenylalanine Amide (7). The aldehyde 5 (from the reduction of 2.5 mmol of 4) was dissolved in a mixture of methanol–acetic acid (99:1; 10 mL) containing the TFA salt 6 (1.5 mmol). Sodium cyanoborohydride (0.120 g) was added portionwise during 45 min. After 1 h, no more TFA salt 6 could be detected by TLC. The reaction mixture was cooled in an ice water bath, and a saturated sodium bicarbonate solution (100 mL) was added under stirring, followed by ethyl acetate (150 mL). The organic layer was collected, washed with water (1 × 20 mL), dried over sodium sulfate, and concentrated in vacuo (*t* < 40 °C). The residue was purified by silica gel column chromatography, with ethyl acetate as eluent, to yield a pure compound that gave a white powder by trituration with ether: 0.756 g (65%); R_f(D) 0.62, R_f(F) 0.11; mp 154–160 °C; $[\alpha]_D$ −29 (*c* 1, DMF). Anal. ($C_{42}H_{54}N_6O_7$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-ψ(CH_2–NH)-L-leucyl-L-aspartyl-L-phenylalanine Amide (8). Compound 7 (0.200 g, 2.65 mmol) was dissolved in 95% ethanol (10mL) and hydrogenated in the presence of a 10% Pd/C catalyst. After 6 h, no more starting material could be detected by TLC. The catalyst was removed by filtration and the solvent concentrated in vacuo (*t* < 40 °C). The residue gave a white powder upon trituration with ether. It was collected by filtration, rinsed several times with ether, and dried in vacuo over P_2O_5: yield 0.160 g (91%); R_f(F) 0.33, R_f(G) 0.09; mp 115 °C dec; $[\alpha]_D$ −30 (*c* 0.6, DMF). Anal. ($C_{35}H_{48}N_6O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.2 μm), and lyophilized.

(*tert*-Butyloxycarbonyl)-L-leucine *N,O*-Dimethylhydroxamate (9). To a solution of Boc-Leu·H_2O (4.98 g, 20 mmol) in dichloromethane (150 mL) were added the hydrochloride salt of *N,O*-dimethylhydroxylamine (2.1 g), DIEA (3.5 mL, 20 mmol), DCC (4.12 g), and DMAP (0.2 g). After stirring 5 h at room temperature, the precipitate was removed by filtration and the solvent was removed in vacuo. The reaction mixture was treated as described for compound 4. It was purified by silica gel column chromatography with a mixture of AcOEt–hexane (1:1) as eluent, to yield a colorless oil: 4.5 g (82%); R_f(C) 0.6; $[\alpha]_D$ −7.8 (*c* 1, DMF).

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucine *N,O*-Dimethylhydroxamate (10). Compound 9 (2.74 g, 10 mmol) was treated with TFA (10 mL) during 30 min. The solvent was removed in vacuo (*t* < 40 °C) and the residue triturated several times with ether, to yield a foam that was dried in vacuo over KOH. This TFA salt was dissolved in DMF (10 mL) in the presence of Boc-Trp-ONp[15] (4.06 g, 9 mmol), HOBt[16] (1.4 g, 9 mmol), and DIEA (3.75 mL, 22 mmol). After standing overnight at room temperature, the reaction mixture was treated as described for compound 1. The residue was purified by silica gel column chromatography, with a mixture of AcOEt–hexane (1:1) as eluent: yield 3.5 g (85%); R_f(C) 0.50; mp 75–80 °C dec; $[\alpha]_D$ −22 (*c* 1, DMF). Anal. ($C_{24}H_{36}N_4O_5$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucinal (11). Compound 10 (2.3 g, 5 mmol) was dissolved in an anhydrous mixture of THF–ether (1:1; 30 mL), cooled in an ice water bath, and treated with $AlLiH_4$ (0.38 g, 10 mmol) added portionwise over a period of 45 min. After 1 h, the reaction mixture was treated as described for compound 5 to produce a colorless oil that was used rapidly for the next reaction; R_f 0.75 (AcOEt–hexane (1:1)).

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucyl-ψ(CH_2–NH)-β-benzyl-L-aspartyl-L-phenylalanine Amide (12). The aldehyde 11 (from the reduction of 5 mmol of 10) was dissolved in a mixture of methanol–acetic acid (99:1; 20 mL) containing the TFA salt 2 (2 mmol). Sodium cyanoborohydride (4 mmol) was added portionwise over a period of 45 min. After an additional 1 h, the reaction mixture was treated as described for compound 7. The residue was purified by silica gel column chromatography with a mixture of ethyl acetate–hexane (7:3) as eluent. Trituration of pure fractions with ether yielded a white powder: 0.98 g (65%); R_f(D) 0.56, R_f(E) 0.07; mp 199–201 °C; $[\alpha]_D$ −11 (*c* 2, DMF). Anal. ($C_{42}H_{54}N_6O_7$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucyl-ψ(CH_2–NH)-L-aspartyl-L-phenylalanine Amide (13). Compound 12 (0.200 g, 2.65 mmol) was dissolved in 95% ethanol (10 mL) and hydrogenated in the presence of a 10% Pd/C catalyst. After 3 h, no more starting material could be detected by TLC. The reaction mixture was treated as described for compound 8: yield 0.165 g (94%) of a white powder; R_f(F) 0.27, R_f(G) 0.09; mp 180 °C dec; $[\alpha]_D$ −20 (*c* 0.32, DMF). Anal. ($C_{35}H_{48}N_6O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.2 μm), and lyophilized.

(Benzyloxycarbonyl)-β-*tert*-butyl-L-aspartic Acid *N,O*-Dimethylhydroxamate (14). To a solution of Z-Asp(But)-OH (3.23 g, 10 mmol) in dichloromethane (100 mL) was added the hydrochloride salt of *N,O*-dimethylhydroxylamine (1.17 g, 12

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mmol), DIEA (2.06 mL, 12 mmol), DCC (2.06 g, 10 mmol), and DMAP (0.61 g). After overnight at room temperature, the reaction mixture was treated as described for compound **9** The compound crystallized in a mixture of ethyl acetate–hexane: yield 2.85 g (78%); R_f(A) 0.65, R_f(E) 0.70; mp 75–76 °C; $[\alpha]_D$ −12 (*c* 1.05, DMF). Anal. ($C_{18}H_{26}N_2O_6$) C, H, N.

(Benzyloxycarbonyl)-β-*tert*-butyl-L-aspartic Acid Aldehyde (15). To a cooled solution (0 °C) of compound 14 (4.39 g, 12 mmol) in an anhydrous mixture of THF–ether (1:1; 100 mL) was added portionwise and under vigorous stirring $AlLiH_4$, and the reaction was monitored by TLC. Approximately 1.14 g of $AlLiH_4$ was added. The reaction mixture was then treated as described for compound 11. TLC showed the presence of some remaining hydroxamate 14 and of slow-moving impurities (AcOEt–hexane (1:1)). The oily residue recovered was used immediately.

(Benzyloxycarbonyl)-β-*tert*-butyl-L-aspartyl-ψ(CH_2–NH)-L-phenylalanine Amide (16). To a freshly prepared solution of compound **15** (from the reduction of 12 mmol of **14**) in a mixture of methanol–acetic acid (99:1; 30 mL) was added phenylalanine amide (0.98 g, 6 mmol). Sodium cyanoborohydride (1.14 g) was added portionwise over a period of 30 min. After 3 h, no more phenylalanine amide could be detected by TLC. The reaction mixture was treated as described for compound **7**. The recovered residue was purified by silica gel column chromatography with ethyl acetate–hexane (7:3) as eluent. Pure fractions were pooled and the solvent removed in vacuo. Trituration of the residue with ether yielded a crystalline compound: 1.64 g (60%); R_f(A) 0.18, R_f(E) 0.25; mp 104–106 °C; $[\alpha]_D$ −15 (*c* 1, DMF). Anal. ($C_{25}H_{33}N_3O_5$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-leucyl-β-*tert*-butyl-L-aspartyl-ψ(CH_2–NH)-L-phenylalanine Amide (18). Compound **16** (1.64 g, 3.4 mmol) was hydrogenated in 95% ethanol (50 mL) containing hydrochloric acid (1 mL) in the presence of a 10% Pd/C catalyst. After 4 h, no more starting material could be detected by TLC. The catalyst was removed by filtration, and the solvent was concentrated in vacuo (t < 40 °C). The residue was triturated several times with ether and dried in vacuo over KOH. It was dissolved in DMF (10 mL), cooled in an ice water bath, and treated with Boc-Leu-OSu[11] (1.05 g, 3.2 mmol) and DIEA (0.75 mL, 5 mmol). After overnight stirring at room temperature, the reaction mixture was treated as previously described for compound **7**. The residue was purified by silica gel column chromatography with ethyl acetate as eluent. Pure fractions were pooled and the solvent was removed in vacuo (t < 40 °C). The residue was triturated with ether to yield a white powder: 0.96 g (55%); R_f(A) 0.20, R_f(E) 0.25, R_f(F) 0.83; mp 185–187 °C; $[\alpha]_D$ +9.3 (*c* 1, 2 DMF). Anal. ($C_{28}H_{46}N_4O_6$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucyl-L-aspartyl-ψ(CH_2–NH)-L-phenylalanine Amide (20). Compound **18** (0.53 g, 1 mmol) was treated with TFA (5 mL) during 1 h. Addition of anhydrous ether under stirring yielded a precipitate which was collected by filtration, rinsed several times with ether, and dried in vacuo over KOH. It was dissolved in DMF (10 mL), cooled in an ice water bath, and treated with Boc-Trp-OSu (0.36 g, 0.9 mmol) and DIEA (0.4 mL, 2.4 mmol). After 4 h of stirring at room temperature, the solvent was removed in vacuo (t < 40 °C). The residue was dissolved in ethyl acetate (100 mL) and washed with water (20 mL). The organic layer was dried over sodium sulfate and concentrated in vacuo. The oily residue was dissolved in ethyl acetate (10 mL) and treated with freshly distilled dicyclohexylamine (0.3 mL). The reaction mixture was concentrated to dryness and the residue triturated with a mixture of ethyl acetate–ether (1:1) to yield a crystalline compound, which was collected by filtration, rinsed several times with ether, and dried in vacuo. It was then dissolved in 0.5 N $KHSO_4$ (10 mL) and ethyl acetate (20 mL) and stirred during 10 min. The organic layer was discarded, washed with water (10 mL), dried over sodium sulfate, and concentrated in vacuo. Trituration of the residue with a mixture of ethyl acetate–ether yielded a white powder: 0.34 g (53%); R_f(F) 0.44, R_f(G) 0.16; mp 164–166 °C; $[\alpha]_D$ −29 (*c* 1, DMF). Anal. ($C_{35}H_{48}N_6O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.2 μm), and lyophilized.

Biological Tests. Gastric acid secretion was determined in vivo in the reperfused rat stomach according to the method of Ghosh and Schild.[12] The gastric pouch of an anaesthetized rat (urethane ip) was continuously washed at 30 °C with a propionate–succinate solution. The cumulative pH was recorded with time and used as an index of acid secretion. Synthetic (Leu[15])-human gastrin I (gift from Professor E. Wunsch, Max Plank Institut, München), compounds **8**, **13**, **20**, or **21** were dissolved in 0.9% NaCl and bolus-injected intravenously. The amount of H^+ secreted was determined by the pH difference between stimulated and basal recorded traces. The inhibitory effect of synthetic peptides was measured after simultaneous bolus injection of the compounds in water alkaline solution and of gastrin (80 pmol was usually employed). The amount of H^+ secreted in presence of various doses of the peptides was reported to the amount of H^+ secreted after gastrin alone and expressed as percent of inhibition. The mean H^+ secretion after gastrin injection was 203 ± 28 μmol of H^+/nmol of peptide (n = 17).

Binding Studies. Isolation of gastric cells was carried out by the collagenase/EDTA previously described procedure.[17] Fundic mucosa was scraped, and tissues were chopped into small cubes and then dispersed in medium A (132 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM Hepes, 0.2% glucose, 0.2% bovine serum albumin, 0.02% phenol red, pH 7.4) (gassed O_2/CO_2) containing 0.30 mg/mL collagenase. After 15-min incubation at 37 °C, tissue fragments were allowed to settle and the medium was discarded. The fragments were washed in Ca^{2+}-free medium A containing 2 mM EDTA and then incubated in the same medium for 10 min. The fragments were transferred to medium A containing fresh 0.30 mg/mL collagenase and incubated for 15 min at 37 °C with continuous gassing (O_2/CO_2). The cell suspension was centrifuged for 15 min at 200*g* and then washed twice with medium A. This procedure gave about 5 × 10^7 cells/g of wet mucosa with 95% viability (trypan blue exclusion). The mixed population contained 45% parietal cells. Specific gastrin binding was determined by incubation in medium B (Earle's balanced salt medium without bicarbonate and containing 10 mM Hepes and 0.2% BSA, pH 7.4) of 20 pM labeled (Leu[15])-HG-17 (≈40 000 cpm/mL) for 30 min at 37 °C with 5 × 10^6 cells/mL ± various concentrations of peptides or unlabeled (Nle[11])-HG-13. Nonsaturable binding was determined as the amount of radioactivity associated with cells in presence of 1 × 10^{-6} M of unlabeled (Nle[11])-HG-13.

Registry No. 1, 60058-69-7; **2**·TFA, 60058-91-5; **3**, 97530-02-4; **4**, 97530-05-7; **5**, 82689-14-3; **6**·TFA, 97530-04-6; **7**, 97530-06-8; **8**, 97530-00-2; **9**, 87694-50-6; **10**, 97530-07-9; 11, 97530-08-0; **12**, 97530-09-1; **13**, 97530-01-3; **14**, 98482-69-0; **15**, 98482-70-3; **16**, 98482-71-4; **17**·HCl, 98482-72-5; 18, 98482-73-6; **19**, 98482-75-8; **20**, 98482-76-9; Boc-Asp(Bzl)OH, 98482-77-0; H-Phe-NH_2, 5241-58-7; Boc-Leu-OH, 13139-15-6; Boc-Trp-OH, 13139-14-5; CH_3-$NHOCH_3$·HCl, 6638-79-5; CH_3NHOCH_3·TFA, 98482-78-1; Boc-Trp-ONp, 15160-31-3; Z-Asp(Bu-*t*)-OH, 5545-52-8; Boc-Leu-OSu, 3392-09-4; Boc-Trp-OSu, 3392-11-8.