for 15 min. Then 232 mg (1.26 mmoles) of *n*-BuI was added in 1 portion and the blue color was immediately discharged. The resulting soln was stirred in a Dry Ice–acetone bath for 30 min, then was removed from the bath and stirred as the NH_3 was allowed to evap slowly overnight. The residue was dissolved in 10 ml of H_2O, the soln adjusted to pH 8.0 with HCl, and then extd with $CHCl_3$ (3 × 10 ml). The dried $CHCl_3$ exts were concd *in vacuo* and the residual oil was triturated with petr ether (30–60°) to give a solid material. Crystn from EtOH gave 95 mg (32%) of yellow crystals; mp 87–88.5°, λ_{max} 260 nm. *Anal.* ($C_{14}H_{23}N_5S$) C, H, N.

9-[*S*-(3-Aminopropyl)-5-thiopentyl]adenine · 2HCl (16). The reaction between **13** (221 mg, 1 mmole) and **7b** (342 mg, 1.26 mmoles) was carried out with Na–NH_3 exactly as described for the prepn of **14**. The oily residue (**15**) thus obtained was treated with 40% HBr–HOAc overnight at ambient temp to cleave the carbobenzyloxy blocking group. The reaction soln was poured into 50 ml of Et_2O to give a very hygroscopic HBr salt, which was dissolved in EtOH, and treated with charcoal. The decolorized filtrate was treated with an equal vol of a satd soln of picric acid in EtOH, to ppt 194 mg of a yellow solid. Recrystn from EtOH gave an analytical sample; mp 176–180° dec with preliminary softening. *Anal.* ($C_{25}H_{28}N_{13}O_{14}S$) C, H, N. The dipicrate could be converted to the dihydrochloride by chromatog of an EtOH soln on Dowex (X-8, Cl^- form), followed by concn of the effluent *in vacuo*; λ_{max} 260 nm; mp 174° dec.

***S*-(Cyclopentylmethyl)homocysteine (18).** To a deep-blue soln derived from 2.05 g (9.1 mmoles) of **5** and 400 mg (17.4 mg-atoms) of Na in 35 ml of liq NH_3, was added 2.05 g (12.5 mmoles) of cyclopentylmethyl bromide.[24] The blue color disappeared immediately, and the mixt was worked up as described for **9b**. The dried white solid weighed 2.22 g, and was crystd from 95% EtOH to give an analytical sample; mp 187–188° dec, with preliminary darkening at 165°. *Anal.* ($C_{10}H_{20}NO_2S$) C, H, N.

Enzyme Assay. The assay method of Nikodejevic, *et al.*,[11] was used with minor modifications; namely, the concn of SAM was 6×10^{-4} *M* (contg 10^{-2} μCi of [*methyl*-^{14}C]SAM), and pH was maintd at 7.9 with 0.1 *M* Tris buffer. The specific activity of the enzyme prepns employed ranged from 2.8×10^{-3} to 5.6×10^{-2} μmole of product/mg of protein per min. Protein concn was detd by uv absorbance at 280 and 260 nm.[29]

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Conformation of Gastrin Tetrapeptide

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The conformation of the amino acid residues comprising gastrin tetrapeptide have been predicted using semiempirical molecular orbital theory. The conformation of gastrin tetrapeptide has been predicted by a direct assembly of these residues in their preferred conformations. The implication of this predicted structure is discussed in terms of the biological activity.

Gastrin is a heptadecapeptide, released by the gastric mucosa, stimulating gastric acid secretion, gastric motility, and pancreatic secretion.[1-3] Studies to date support the contention that gastrin does not act at sites receptive to histamine.[1] There appears to be a strong interdependence between gastrin and acetylcholine; the hypothesis that gastrin stimulates the release of ACh[4] receives considerable support.[5]

The amino acid sequence of gastrin has been defined as two natural forms of a heptadecapeptide: gastrin-II, with a sulfated tyrosyl residue, and gastrin-I, unsulfated.[6] Tracy and Gregory discovered that the entire range of physiological activity of gastrin could be demonstrated by the C-terminal tetrapeptide sequence Trp-Met-Asp-Phe-NH_2.[7] This intriguing observation prompted Morley to synthesize a large number of analogs of this tetrapeptide in an effort

to deduce relationships between structure and biological activity.[8,9]

The results of these studies implicate the Asp carboxylate as being a functional moiety with the Trp, Met, and Phe side-chain moieties serving as binding features. The latter three residues are susceptible to replacement, with the retention of some activity, if the analogs do not materially alter the polarity of the side chain or the conformation of the backbone structure.

The logical sequel to these studies is to attempt to define the 3-dimensional relationship between the functional and binding features, in other words, the conformation of the gastrin tetrapeptide. Hopefully, this would contribute sufficient information so that insight into receptor interaction and the rational design of antagonists might result.

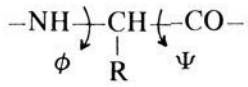
The contention has been made and supported that the conformation of a polypeptide results primarily from the short-range interaction of an amino acid side chain with the backbone of the same residue.[10] The corollary is that the conformation of the backbone of a particular residue is essentially the same regardless of the nature of the side chains on adjacent residues.[10] If this is valid (at least for small polypeptides), then it might be possible to determine the conformation of individual amino acid residues comprising a polypeptide and then to assemble these into the correct order to obtain a rough approximation of the polypeptide conformation.

A number of investigators have employed this rationale using all-valence electron molecular orbital theory to predict the conformation of model compounds simulating amino acid residues in a midchain arrangement. Hoffmann and Imamura published results of extended Hückel theory (EHT) molecular orbital (MO) calculations of the conformation of two amino acid residue models.[11] Their results were within the range of experimentally determined conformations that could be assumed by these amino acids. At the same time, Kier and George published the results of an EHT-MO study on the conformations of four amino acid residue models, wherein again there was encouraging agreement between available experimental conformations of these residues and prediction.[12] This approach has been used in subsequent studies in several laboratories, with results generally agreeing with experimental conformations.[13-16]

In view of the agreement with experiment achieved by our predictions,[12,14] we continued the calculations on residues needed to complete the amino acid complement of the nonapeptide bradykinin.[17] These residues, in their predicted preferred conformations, were then assembled into the bradykinin sequence and a complete conformation for the polypeptide was predicted.[17] Although no experimental information on the conformation of bradykinin existed at that time, we were encouraged to note that our prediction revealed no close side chain interactions. Results of recent studies employing ORD and CD on the conformation of bradykinin indicate that our prediction is consistent with these preliminary findings.[18-20] These studies reveal a disordered chain or random coil conformation. There is no evidence of interaction between the phenyl groups. There is no fixed relationship between terminal residues, which is predicted from our calculation of considerable conformational freedom in the glycine residue.

Methods. For the calcns on the residues of gastrin tetrapeptide, it was necessary to consider only model compounds for N-terminal tryptophan, methionine, and aspartic acid. We have previously reported calcns on the C-terminal phenylalanine amide model compound.[12] For the N-terminal tryptophan residue model we performed calcns on the N-protonated tryptophanamide. For the methionine residue model compd, we used *N*-acetylmethionylamide, and for the aspartic acid residue model, *N*-acetylaspartylamide with the side-chain CO_2H ionized.

EHT[21] and the parameters proposed by Hoffmann and used by us in numerous studies[22] were employed to make these calcns. We adopted bond lengths and angles used previously for amino acid residues,[12,14,17] and have assumed that the *N*-Ac Me group prefers a conformation with a Me H eclipsing C=O. The calcns sought a minimum energy as a function of rotation of the C-N bond (ϕ angle), the C-CO bond (Ψ angle), and the side-chain conformation.



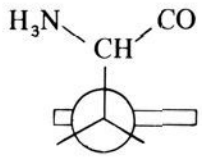
The ϕ, Ψ angle convention is that proposed by Edsall.[23] Calcns were refined to angular increments of 30° near minimal regions to insure prediction of the minimum-energy conformation.

Results

The calculations on the methionine residue model revealed a preference for $\phi = 0°$, and $\Psi = 30°$, with a secondary preference for $\Psi = 120°$. The side chain was found to lie trans to the methine hydrogen, with each group trans to the next.

The aspartic acid residue model preferred a conformation with the angles $\phi = 120°$ (and a secondary preference at $\phi = 60°$) and $\Psi = 0°$. The C_β-C_γ bond of the side chain was found to be trans to the methine hydrogen.

The angle predicted for the tryptophan residue model was $\Psi = 0°$ with the side chain trans to the C**H** and the plane of the indole ring related to the backbone atoms as



These residues, in their calculated preferred conformations, were assembled into the gastrin tetrapeptide sequence. Using the methionine $\Psi = 30°$ angle, the predicted conformation appears as in Figure 1.

The backbone of the molecule is roughly planar, describing about a 120° arc, and the two terminal N atoms are about 13 Å apart. The two aromatic groups lie on one side of this arc with their centers about 11 Å apart. The methionine and aspartate side chains are on the other side of this

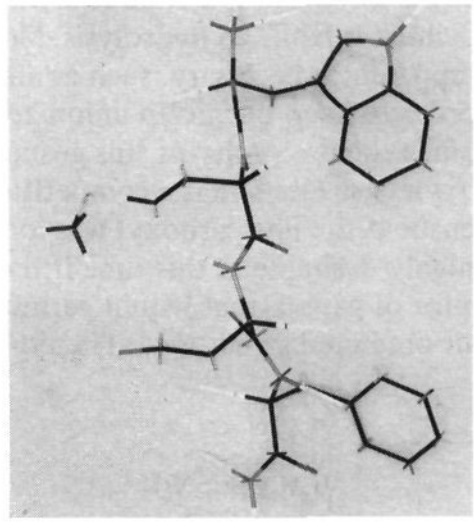


Figure 1. Gastrin (methionine ($\Psi = 30°$)).

arc, roughly parallel, and about 5 Å apart. Secondary conformations occur with Asp $\phi = 60°$, $\Psi = 0°$, Met $\phi = 0°$, $\Psi = 30°$ ($\Delta E = 0.4$ kcal), and Asp $\phi = 120°$, $\Psi = 0°$, Met $\phi = 0°$, $\Psi = 120°$ ($\Delta E = 0.75$ kcal).

Discussion

At the present time, we have no way of judging the validity of these predictions. It is encouraging to note however, that the assemblage of the residues, predicted independently, results in no close interactions of side chains.

It is interesting to reflect on our predicted structures in light of available structure-activity studies. Morley's extensive analysis of gastrin tetrapeptide analogs provides ample information for consideration.[9]

From a comparison of Morley's analogs derived from acylation of the tryptophan-free NH_2 and our predicted structure, it is understandable why a large number of analogs were found to be equipotent or more active than gastrin is in stimulating acid secretion. Assuming that the Asp carboxylate moiety is functionally active as Morley concludes, the remote position of the tryptophan amino group relative to the carboxylate permits tryptophan replacement or modification with minimum influence on activity. Thus, neither the charge nor the physical presence of the tryptophan quaternary ammonium group is necessary for activity.

Morley established that the tryptophan side chain makes a contribution that can be shared by analogs with considerable structural variation.[9] These results and our predicted models indicate that the 5-membered part of the indole ring may be involved in a bond. It is conceivable from a consideration of the predicted structures why the Trp *N*α-Me analog might be inactive. The steric influence on the position of the side chain and the Ψ angle would be considerable, which would result in the probable assumption of an unfavorable conformation.

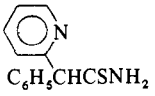
From the variations permitted at the Me position and our predicted conformation, we would judge that a more distal part of the side chain is involved in bonding, probably a dispersion force, to the receptor. Thus, the last 3 atoms in this side chain are possibly making contact with the receptor.

The Asp carboxylate is essential and will tolerate no functional group or positional variation.[9] Our predicted conformation puts this moiety in a freely accessible part of the molecule, sufficiently removed from nearby atoms so that its reactivity would not be impaired by steric interference.

Considerable variation is permitted at the site of the Phe residue.[9] The variation pattern indicates that the β carbon, and the 1, 2, and 6 positions of the Ph ring are what might be involved in a dispersion bond at the receptor.

Finally, a free CO_2H on the Phe residue is not permitted nor is an ester, which is labile to hydrolysis. More stable derivatives are apparently necessary, such as amides or hydrazides in order to keep the group unionized. This is not surprising since the proximity of this group to the aspartate carboxylate suggests that a competition for a binding site might ensue if the Phe carboxyl was ionized.

It would be highly desirable at this time if there was known a specific inhibitor of gastrin that would permit some comparison with our predicted structure. 2-Phenyl-2-(2-pyridyl)-



thioacetamide or "antigastrin" was originally thought to be a specific antagonist of gastrin.[24,25] More recent studies suggest however, that this compound is not specific in its action in antagonizing gastrin.[26] Comparison of this compound with our postulated structures reveals no obvious relations between the two that would contribute to competitive inhibition. Similarly, comparisons with histamine in its predicted conformations[27] or with ACh[28] reveal no congruent patterns.

The value in a structural prediction, such as we have made here, lies in the use to which it may be put in the form of a rationale for the design of mimicking or competitive inhibitory agents. Obviously, the latter group of compounds would have significant therapeutic impact.

In our opinion, a reasonable approach to the design of gastrin competitive inhibitors based on our predictions would involve the duplication of the Asp carboxylate group and either or both of the two flanking features involved in binding, namely, features simulating the active parts of the Me and Ph side chains. This would involve the conception of a relatively small molecule to mimic these features in the relationships predicted.

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