[Chem. Pharm. Bull. 33(11)4856—4864(1985)]

Conformational Analysis of a Peptide Segment of Gastrin in Comparison with an Antigastric Benzothiazocine

SHUICHI MIYAMOTO* and MASAFUMI YOSHIMOTO

Chemical Research Laboratories, Sankyo Co., Ltd., 2–58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo 140, Japan

(Received January 21, 1985)

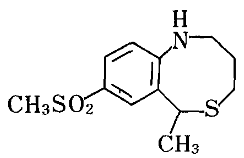
An examination of structural similarities between gastrins (3—5) and antigastric 5,1-benzothiazocines (2) suggested the presence of common functional groups and atoms, *i.e.*, a benzene ring, a nonbasic nitrogen and a sulfur atom. A working hypothesis presuming these to be essential binding moieties is presented.

A molecular mechanics calculation study of Ac–Trp–Met–NHMe (**14**) as a model peptide bearing the receptor binding sites was carried out in an attempt to find a stereochemical correlation with a representative 5,1-benzothiazocine, RS-2039 (**1**), a derivative of which had been structurally elucidated by X-ray crystallographic analysis.

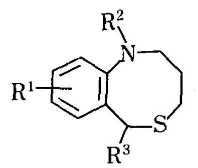
Several stable conformers of Ac–Trp–Met–NHMe were discovered to have a close approximation of the 3-dimensional array of binding sites to that of **1**. It has thus been theoretically demonstrated that gastrins and 5,1-benzothiazocines could bind with an identical receptor.

Keywords——gastrin; antigastrin; conformational analysis; molecular mechanics; ECEPP; 5,1-benzothiazocine; 3-dimensional binding site

6-Methyl-8-methylsulfonyl-1,3,4,6-tetrahydro-2*H*-5,1-benzothiazocine, RS-2039 (**1**), and structurally related derivatives (**2**)[1)] were found to have antagonistic activity against pentagastrin (**4**) in rats and dogs[2)] (Chart 1). One of the most active among the series of compounds is **1**, with an effective dose in dogs of 10 mg/kg. In view of its 2-dimensional structure, **1** is assumed to be similar to several antigastrins, notably KM-1146 (**6**),[3)] picartamide (**7**),[4)] isotiquimide (**8**),[5)] tiquinamide (**9**)[6)] and tritiozine (**10**)[7)] (Chart 2). In other words, all the compounds (**1**,**6**—**10**) have been recognized to have common functional groups (aromatic rings, sulfur atoms and nonbasic nitrogens) which are in analogous arrangements.



RS-2039 (**1**)



2

gastrin (**3**) Glp–Gly–Pro–Trp–Leu(5)–Glu–Glu–Glu–Glu–Glu(10)–Ala–Tyr(HSO$_3$)–Gly–Trp–Met(15)–Asp–Phe–NH$_2$

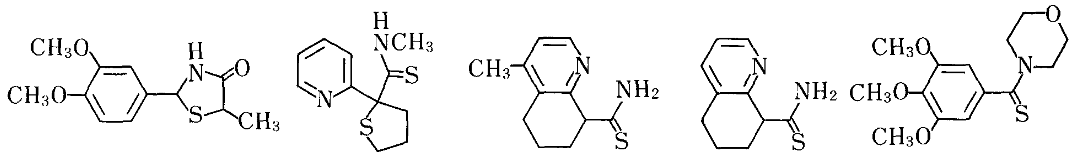
pentagastrin (**4**) Boc–β-Ala–Trp–Met–Asp–Phe(5)–NH$_2$

tetragastrin (**5**) Trp–Met–Asp–Phe–NH$_2$

Chart 1

Since the discovery by Tracy and Gregory[8)] that the entire range of physiological activities of gastrin could be exhibited by the C-terminal tetragastrin (**5**), intensive studies on the structure–activity relationships of related peptides have been carried out.[9)] Finally the Asp

NII-Electronic Library Service



KM-1146 (**6**) picartamide (**7**) isotiquimide (**8**) tiquinamide (**9**) tritiozine (**10**)

Chart 2

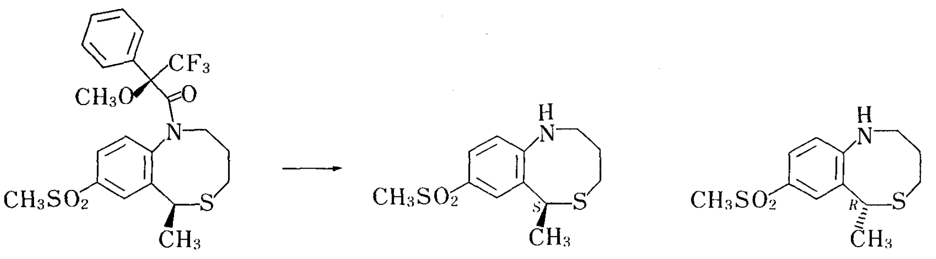
carboxy group and one of the Phe amide protons in **5** were shown to be essential for the peptide to have high activity, whereas the Trp, Met and Phe side chain moieties played a part only in the binding characteristics.

In order to explain the activities of the antigastrins (**1**,**6**—**10**), we would like to propose as a working hypothesis that **1** must have essential binding sites consisting of the common functional groups which are stereochemically in close conformity to those of **5**. In other words, we assumed that **5** and **1** bind with an identical receptor (in terms of the 3-dimensional coordinates of the essential binding sites).

An acyl derivative (**11**) of the (*S*)-isomer (**12**) of **1** has been structurally elucidated by X-ray crystallographic analysis,[10] and this structure was employed as the most stable conformer of **12** to obtain the coordinates used in the following calculations (Chart 3). The coordinates of the (*R*)-isomer (**13**) were computed as the antipode of **12**.

Considering the structural similarity of **5** to **1**, the benzene ring, the nitrogen atom and the sulfur in the latter are presumed to correspond stereochemically to the benzene ring and the nitrogen atom in the indole ring, and the sulfur atom in the methionine residue in the former. Therefore, if the essential binding sites, defined above, of **5** in an energetically stable conformation can be shown to form an array similar to that in **1**, our hypothesis will have been proved.

In the present study, sulfur atoms in **1** and **5** are taken as one of the essential binding moieties because all of the other antigastrins (**6**—**10**) mentioned above have sulfur atoms in approximately similar positions to that of **1**. Of course it might be considered that nonpolar functional groups such as $-CH_2S-$ in **1** and $-SCH_3$ in **5** have only general hydrophobic interactions with a possible nonpolar cavity in the gastric receptor.



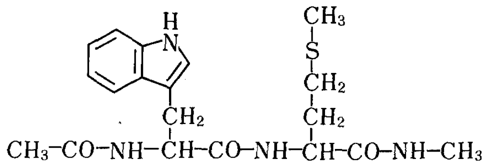
11 **12** **13**

Chart 3

Method

A conformational analysis of **5** should provide evidence for the hypothesis mentioned above. However, there are so many free-rotational bonds in the oligopeptide (**5**) that too many initial structures have to be taken into account. We thus decided to employ Ac–Trp–Met–NHMe (**14**) as a model of **5** (Charts 1 and 4). It is sufficient for our purpose to find conformers of **14** with similar coordinates for the benzene ring, the nitrogen atom and the sulfur atom as compared to those of **12** or **13**. A compound such as **14**, with many degrees of freedom, still has many stable conformations with local minima in energy. In fact, there could be stable conformers with slightly higher energy (a few kcal/mol) than that of the most stable conformer with the global minimum. Therefore, it is difficult to examine

NII-Electronic Library Service



14

Chart 4

the coordinates of all the possible conformers.

Our pragmatic method was first to calculate the lowest energy of **14** and then to examine the existence of conformers with energies close to the lowest which have an array of essential binding sites similar to that of **12** or **13**.

The calculations were performed on an IBM 4341 computer with the ECEPP program[11] combined with a program which finds a minimum of a function of several variables. The values of all bond lengths and bond angles were fixed in the calculations. The index of similarity (DS_i) between two molecules was determined from the equation below;[12] DS_i is a parameter for estimating how the distance between two specific atoms or groups in one molecule compares with that in another. Needless to say, the smaller the index is, the closer the molecules are. A simple model is shown in Fig. 1. Let us examine the similarity between molecules A and B. DS_1, DS_2 and DS_3 are calculated as shown below (Eqs. 2—4).

$$DS_i = \frac{2\,|d_{1i} - d_{2i}|}{d_{1i} + d_{2i}} \tag{1}$$

$$DS_1 = \frac{2\,|d_{11} - d_{21}|}{d_{11} + d_{21}} \tag{2}$$

$$DS_2 = \frac{2\,|d_{12} - d_{22}|}{d_{12} + d_{22}} \tag{3}$$

$$DS_3 = \frac{2\,|d_{13} - d_{23}|}{d_{13} + d_{23}} \tag{4}$$

where DS_1, DS_2 and $DS_3 < \varepsilon$, the two molecules are evaluated to be similar when ε is approximately 0.15.

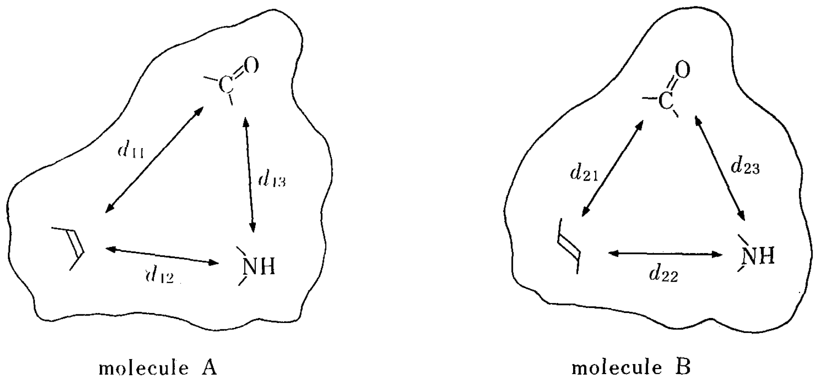


Fig. 1. A Model for Examination of the Similarity between Two Molecules

Similarity between molecules A and B is determined from Eqs. 2—4 based on the distances between three corresponding functional groups, that is, carbonyl group, double bond and nitrogen atom.

In the present case, the benzene ring was accounted for by relating every other atom of the ring with the sulfur and nitrogen. In other words d_{11}, the distance between S5 and N1 (3.68 Å), d_{12} between S5 and C6a (2.74 Å), d_{13} between S5 and C8 (4.72 Å) and d_{14} between S5 and C10 (4.73 Å) in **12** or **13** were compared with the corresponding d_{21}, d_{22}, d_{23} and d_{24}, respectively, in both X-type and Y-type arrangements; these would allow the dipeptide to be superimposed on **1**, based on an examination of molecular models (Fig. 2). The similarity index DS_i and DS_m (the mean of values DS_1—DS_4) are described by the following Eqs. 5—9.

NII-Electronic Library Service

$$DS_1 = \frac{2|3.68 - d_{21}|}{3.68 + d_{21}} \tag{5}$$

$$DS_2 = \frac{2|2.74 - d_{22}|}{2.74 + d_{22}} \tag{6}$$

$$DS_3 = \frac{2|4.72 - d_{23}|}{4.72 + d_{23}} \tag{7}$$

$$DS_4 = \frac{2|4.73 - d_{24}|}{4.73 + d_{24}} \tag{8}$$

$$DS_m = \frac{DS_1 + DS_2 + DS_3 + DS_4}{4} \tag{9}$$

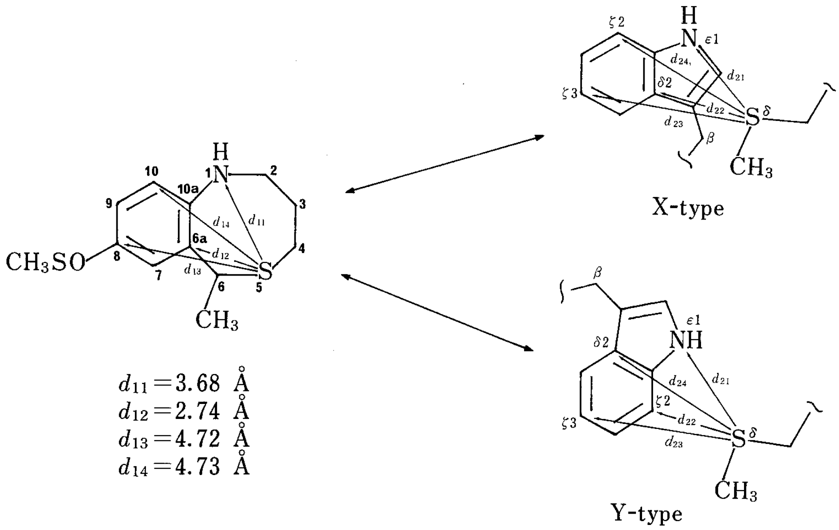


Fig. 2. Correlation of Essential Binding Sites for Estimating Similarity between **1** and **14**

In the X-type arrangement, the sulfur atom is located close to Cβ of Trp, whereas it is located at the other side of the indole ring in the Y-type. In the X-type, d_{22} (to be compared with d_{12}) refers to the distance between Sδ of Met and Cδ2 of Trp and d_{24} (to be compared with d_{14}) refers to the distance between Sδ of Met and Cζ2 of Trp. In the Y-type, however, d_{22} is assigned as the distance between Sδ and Cζ2 and d_{24} is assigned as the distance between Sδ and Cδ2.

Results and Discussion

1) Estimation of the Lowest Energy of 14

There are fifteen torsional angles that determine the conformation of **14** to be defined in Fig. 3. In the starting conformations ϕ and ψ in the main chain were presumed to be the standard values[13] in the characteristic secondary structures of polypeptides shown in Table I and $\omega_1 = \omega_2 = \omega_3 = 180°$, $\theta_1 = 180°$ and $\theta_4 = 0°$ were taken. In the side chain χ_2^1, χ_3^1, χ_3^2 and χ_3^3 were incremented systematically by 60, 180 and 300°, χ_2^2 by 30°, and χ_3^4 was set to 0 and 60°.

Initial energy calculations were performed on 15522 starting conformations as defined above. The 301 most relatively stable conformers were selected and then energetically minimized with respect to all torsional angles except for ω_1, ω_2 and ω_3. The lowest energies among the local minima are listed in Table II. The global minimum energy of **14** has been estimated to be -6.5 kcal/mol.

NII-Electronic Library Service

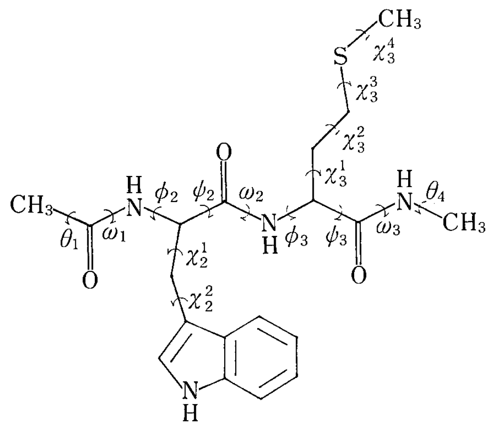


Fig. 3. Torsional Angle Labels of **14**

Table I. ϕ and ψ (°) of Ac–Trp–Met–NHMe (**14**) in Starting Conformations

		ϕ_2	ψ_2	ϕ_3	ψ_3
α-Helix		−57	−47	−57	−47
β-Sheet	Parallel	−119	113	−119	113
	Antiparallel	−139	135	−139	135
Turn	Type I	−60	−30	−90	0
	Type I′	60	30	90	0
	Type II	−60	120	80	0
	Type II′	60	−120	−80	0
	Type V	−80	80	80	−80

Table II. Lowest Energies and Torsional Angles of Ac–Trp–Met–NHMe (**14**)

		Energy (kcal/mol)	Torsional angle (°)											
			θ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	χ_2^1	χ_2^2	χ_3^1	χ_3^2	χ_3^3	χ_3^4	θ_4
1 (α-Helix)	Starting conformation	−1.2	180	−57	−47	−57	−47	60	−90	180	60	180	60	0
	Minimized conformation	−4.7	180	−71	−39	−75	−39	66	−93	−170	59	−179	60	60
2 (β-Sheet)	Starting conformation	−0.8	180	−119	113	−119	113	180	60	−60	−60	180	60	0
	Minimized conformation	−6.5	180	−81	103	−85	79	178	78	−65	−58	174	59	65
3 (Turn)	Starting conformation	5.0	180	−60	−30	−90	0	180	60	−60	180	180	60	0
	Minimized conformation	−3.8	180	−69	−49	−76	−36	175	56	−68	−176	−179	61	60

2) Searching the Stable Conformers of 14 with Similar Stereochemistry to 12 or 13 with Respect to the Essential Binding Sites

1) Starting Conformations Belonging to Energetically Favored Structures in Terms of ϕ and ψ——In the starting conformations, ψ_2 and ϕ_3 in the main chain were varied in increments of 30° in three regions as shown in Fig. 4 (25° in region A) with ω_1, ω_2 and ω_3 fixed at 180°. These regions were considered to represent energetically favored combinations of ϕ and ψ,[13] or those found frequently in proteins. The angles of θ_1, θ_4, χ_3^3 and χ_3^4 were set to 180, 0, 0 and 60°, respectively. The angles of χ_2^1, χ_2^2, χ_3^1 and χ_3^2 were each incremented by 60°. At first, the conformers with each DS (DS_1, DS_2, DS_3 and DS_4) less than 0.4 were searched for. In these conformers χ_3^3 was changed by 60°, and ϕ_2 and ψ_3 were changed in the regions shown in Fig. 4 ($\phi_2 = -60$, -90, -120 and -150°, and $\psi_3 = -45$° in region A; $\phi_2 = -65$, -95, -125 and -155°, and $\psi_3 = 115$, 145, 175° in region B; $\phi_2 = -80$, -110, -140 and -170°, and $\psi_3 = 20$, 50 and 80° in region C). Then conformers with an energy of less than 50 kcal/mol (40 kcal/mol in regions B and C) were searched for. In the resulting conformers, χ_2^1, χ_2^2, χ_3^1 and χ_3^2 were each varied by 30° to obtain the structures with DS_1—$DS_4 < 0.25$. The

NII-Electronic Library Service

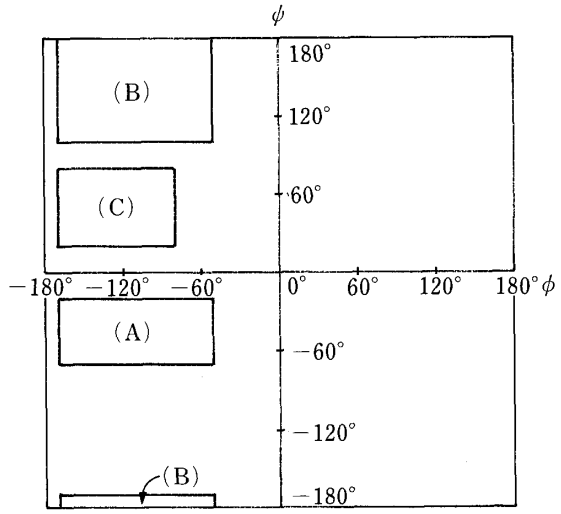


Fig. 4. Ramachandran (ϕ, ψ) Plot

TABLE III. Stable Conformers with $DS_m < 0.15$ and Energy < 0.0 kcal/mol

Con-former	Energy (kcal/mol)	DS_m [a]	Arrange-ment [b]	Torsional angle (°)											
				θ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	χ_2^1	χ_2^2	χ_3^1	χ_3^2	χ_3^3	χ_3^4	θ_4
1	−1.1	0.11	X-Type	180	−79	−20	−62	−41	65	79	−70	89	165	57	60
2	−0.5	0.11	X-Type	179	−137	31	−162	45	60	93	52	−94	175	60	60
3	−0.2	0.10	X-Type	180	−149	39	−126	98	60	−74	−81	70	84	58	60

a) Similarity index (the mean of values DS_1—DS_4). *b*) Arrangement type of **14** which correlates with **1**.

energies of the conformers obtained were calculated in order to determine those with an energy of less than 20 kcal/mol. Examinations of X-type and Y-type arrangements were carried out independently. Structures similar to **12** or **13** were automatically selected by this method.

Finlly, energy minimizations were performed to afford 172 independent stable conformers. Three conformers (Table III) with $DS_m < 0.15$ and energy < 0.0 kcal/mol were found.

2) Starting Conformations Belonging to the Turn Structures in Terms of ϕ and ψ——The angles ϕ and ψ in the main chain were given the values shown in Table IV, and those of ω_1, ω_2 and ω_3 were fixed at 180°. θ_1, θ_4, χ_3^3 and χ_3^4 were set to 180, 0, 0 and 60°, respectively, and χ_2^1, χ_2^2, χ_3^1 and χ_3^2 were each varied by 30°. Conformers with each DS_i (DS_1—DS_4) less than 0.25 were searched for. Energy calculations were done on the conformers thus obtained by changing χ_3^3 by 60° to get conformers with an energy of less than 30 kcal/mol (X-type and Y-type). Energy minimizations were carried out on these conformers to obtain 83 independent stable conformers. Six of these with $DS_m < 0.15$ and energy < 0.0 are shown in Table V.

Considering the accuracy of the calculations and the presumed environments of molecules without solvents, differences in energies of approximately 5 kcal/mol might not be considered great in such circumstances. Therefore, structures with $\Delta E = 5$ kcal/mol (difference from the lowest energy in the most stable conformer) are assumed to exist. The conformers with $DS_m < 0.15$ and $\Delta E < 6.0$ kcal/mol are summarized in Table VI. Among these, conformers 10, 11 and 12 are illustrated superimposed on **12** or **13** with respect to the essential binding sites (Fig. 5). The corresponding atoms and group discussed are found to be well fitted. These theoretical results suggest that gastrins and 5,1-benzothiazocines could bind with an identical receptor in that they have similar arrays of the essential binding moieties.

NII-Electronic Library Service

TABLE IV. ϕ and ψ (°) of Ac–Trp–Met–NHMe (**14**) in Starting Conformations with Turn Structure

Type of turn	ϕ_2	ψ_2	ϕ_3	ψ_3
I	−60	−30	−90	0
	−60	−30	−120	30
I′	60	30	60	30
	60	30	90	0
	60	30	120	−30
II	−60	120	50	30
	−60	120	80	0
	−60	120	110	−30
II′	60	−120	−50	−30
	60	−120	−80	0
	60	−120	−110	30
V	−80	80	80	−80

TABLE V. Stable Conformers with DS_m < 0.15 and Energy < 0.0 kcal/mol

Conformer	Energy (kcal/mol)	DS_m [a]	Arrangement[b]	Torsional angle (°) θ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	χ_2^1	χ_2^2	χ_3^1	χ_3^2	χ_3^3	χ_3^4	θ_4
4	−1.9	0.10	Y-Type	179	−81	−19	−138	31	69	−89	58	174	180	61	60
5	−0.4	0.10	Y-Type	179	−80	−20	−138	31	71	−89	58	173	−86	61	60
6	−0.1	0.15	X-Type	180	55	70	52	52	−177	−109	−59	82	−179	60	60
7	−3.4	0.11	X-Type	180	−80	91	50	58	−179	−100	−65	77	−179	59	60
8	−3.3	0.13	X-Type	180	−75	103	49	59	−174	82	−71	72	179	59	60
9	−0.3	0.15	X-Type	180	−80	89	50	58	−177	75	−64	120	76	57	60

a) Similarity index (the mean of values DS_1—DS_4). *b*) Arrangement type of **14** which correlates with **1**.

TABLE VI. Conformers with $DS_m \leqq 0.15$ and $\Delta E \leqq 6.0$ kcal/mol

Conformer	Energy (kcal/mol)	ΔE[a] (kcal/mol)	DS_m[b]	Arrangement[c]	Configuration[d]
10	−3.4	3.1	0.11	X-Type	*R*
11	−3.3	3.2	0.13	X-Type	*S*
12	−1.9	4.6	0.10	Y-Type	*S*
13	−1.1	5.4	0.11	X-Type	*S*
14	−0.5	6.0	0.11	X-Type	*S*

a) Difference from the global minimum energy (−6.5 kcal/mol). *b*) Similarity index (the mean of values DS_1—DS_4). *c*) Arrangement type of Ac–Trp–Met–NHMe (**14**) which correlates with RS-2039 (**1**). *d*) Configuration of **1** which is superimposed on **14**.

Gastrin plays an important role in the stimulation of gastric acid secretion. The pharmacological mode of action, however, remains to be fully elucidated. It remains uncertain[14] whether an antigastrin could be a useful therapeutic agent, but a really potent and selective antigastrin might be valuable in the treatment of peptic ulcers, because a specific antigastrin should show less adverse effects than other antisecretories such as histamine H_2-receptor antagonists and choline inhibitors. In fact, a considerable number of small molecules with antigastric activity have been found and studied. No conformational analysis, however, has been carried out to correlate nonpeptide antigastrins with gastrin. The present results may

NII-Electronic Library Service

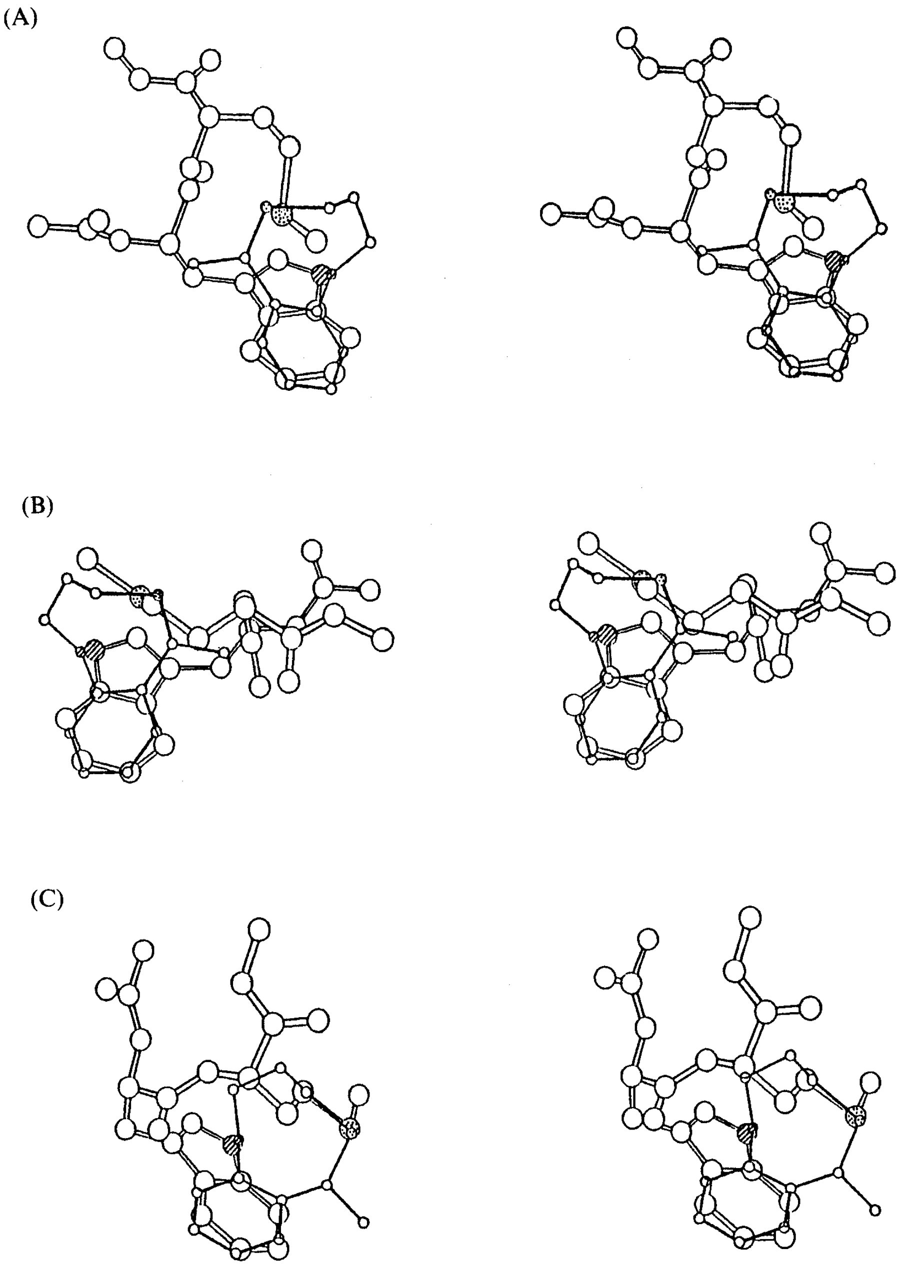


Fig. 5. Superposition of **1** (Solid Bonds) and **14** (Open Bonds) in Table VI

(A) (*R*)-isomer (**13**) and conformer 10; (B) (*S*)-isomer (**12**) and conformer 11; (C) (*S*)-isomer (**12**) and conformer 12.

Nitrogens are represented by hatched circles and sulfurs are represented by dotted circles. Methylsulfonyl groups of **1** are omitted for clarity.

suggest a rational approach to the design of gastric inhibitors.

Needless to say, conformational analysis of tetragastrin (**5**) and comparison with the crystallographic data for RS-2039 (**1**) would be preferable for developing a more sophisticated explanation of the present problems, and we are currently working in this direction.

Acknowledgment Atomic coordinates for **11** were kindly supplied by Mr. S. Sato of the Analytical and Metabolic Research Laboratories. We would also like to thank Drs. Tomita and Kobayashi, and Mr. Ryokai for discussions and encouragement.

References

1) S. Sato, K. Tomita, H. Fujita and Y. Sato, *Heterocycles*, **22**, 1045 (1984).

NII-Electronic Library Service

2) S. Kobayashi, M. Miyamoto, Y. Shimada, K. Endo, F. Asai and T. Ito, *Jpn. J. Pharmacol.*, **36**, Suppl., 89P (1984).
3) T. Onda, S. Matsuura, S. Kajiwara and R. Ito, *Oyoyakuri*, **26**, 701 (1983).
4) Y. Minaire, J. Forichon and R. Woehrle, *Lancet*, I, **1982**, 1179.
5) D. E. Beattie, G. T. Dixon, J. F. Waterfall and B. J. Alps, *Arzneim.-Forsch.*, **29**, 1564 (1979).
6) D. E. Beattie, R. Crossley, A. C. W. Curran, G. T. Dixon, D. G. Hill, A. E. Lawrence and R. G. Shepherd, *J. Med. Chem.*, **20**, 714 (1977).
7) R. Pellegrini and G. Abbondati, *Farmaco, Ed. Prat.*, **31**, 483 (1976).
8) H. J. Tracy and R. A. Gregory, *Nature* (London), **204**, 935 (1964).
9) J. S. Morley, H. J. Tracy and R. A. Gregory, *Nature* (London), **207**, 1356 (1965); J. S. Morley, *Proc. R. Soc. London, Ser. B*, **170**, 97 (1968).
10) S. Sato and C. Tamura, Unpublished data.
11) M. J. Browman, L. M. Carruthers, K. L. Kashuba, F. A. Momany, M. S. Pottle, S. P. Rosen and S. M. Rumsey, QCPE 11, 286 (1975).
12) A. Tomonaga and H. Chuman, *Kagaku No Ryoiki*, **36**, 15 (1982).
13) IUPAC-IUB, *Biochemistry*, **9**, 3471 (1970); P. N. Lewis, F. A. Momany and H. A. Scheraga, *Biochim. Biophys. Acta*, **303**, 211 (1973).
14) A. H. Soll, *J. Clin. Invest.*, **61**, 381 (1978); T. Yamada, Y. Nobuhara, A. Yamaguchi and M. Ohki, *J. Med. Chem.*, **25**, 975 (1982); M. K. Scott, H. I. Jacoby, J. E. Mills, A. C. Bonfilio and C. R. Rasmussen, *ibid.*, **26**, 535 (1983); W. A. Balhofer, A. A. Deana, C. N. Habecker, J. M. Hoffman, N. P. Gould, A. M. Pietruszkiewicz, J. D. Prugh, M. L. Torchiana, E. J. Cragoe, Jr. and R. Hirschmann, *ibid.*, **26**, 538 (1983).

NII-Electronic Library Service