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Interaction of α-L-Aspartyl-L-phenylalanine Methyl Ester with the Receptor Site of the Sweet Taste Bud

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Abstract: The sweetening agent, α-L-aspartyl-L-phenylalanine methyl ester, has been studied in aqueous solutions in the pH range 3.5–11.7. The combination of NMR methods and potential energy calculations gives a very accurate description of the preferred conformations in solution. The results of this analysis have been used to select a conformation as the interacting species with the receptor site of the sweet taste bud. Comparison with known sweet molecules shows the consistency of all the features of the chosen conformation with the models proposed by current theories on sweet taste. The receptor site can be described as a narrow cleft with two interacting parts, one for locking the sweet molecule and another for triggering the nerve impulse.

It has been known for a long time that, besides sugars, many apparently unrelated molecules (with a large spectrum of chemical groups and stereochemical features) can elicit a sweet taste response in man and other animals.[2] The identification of the essential features that impart the sweet taste to these molecules may lead to a satisfactory description of the geometric and chemical aspects of the receptor site and, in turn, provide a sound basis for the design of new potentially useful sweet tastants. In fact, it goes without saying that this problem is quite relevant not only for its biochemical and physicochemical aspects but also from a nutritional point of view.

A major step in the search for common features of sweet agents was made by Shallenberger and Acree[2] who recognized that all sweet compounds possess a bifunctional entity consisting of an acidic (AH) and a basic (B) moiety with a proton to B distance of about 0.3 nm.

Further insight in the nature and geometry of the receptor site was afforded by the observation that the D isomers of most bifunctional amino acids are sweet whereas the corresponding L isomers are bitter.[3] This difference can be explained with the hypothesis of a "spatial barrier" probably apolar in character, placed at about 0.3–0.4 nm from the AH–B entity of the receptor site.

An independent theory by Kier[4] postulates, on the basis of less cogent evidence, the existence of a third binding side that involves a "dispersion bonding" at the receptor. A weak point of both theories is that they are not able to explain the large differences in relative sweetness among known tastants. The complex aspects of a new class of powerful sweet agents[5] may now provide useful clues to an understanding of these differences and, in general of the factors controlling structure–activity relationship in all sweet molecules. The accidental discovery[6] that the dipeptide α-L-Asp-L-PheOMe is at least 150 times as sweet as sucrose has stimulated, during the last few years, the search for other sweetening agents of peptide nature. Many dipeptide derivatives of the type α-L-Asp-X were found to be as sweet or sweeter than α-L-Asp-L-PheOMe.[4,6-9] The X moiety can be an esterified amino acid residue stereo-

chemically akin to -PheOMe such as -TyrOMe[5] or -cyclohexylalanine OMe,[5] but also a completely different chemical grouping as an optically active amine,[7] provided it contains a side chain as bulky as the phenyl group.

More in general, the constitutional and configurational features that cannot be changed without loss of sweetness are the following: (i) The AH-B entity of α-L-Asp, (ii) two apolar groups in the same configurational relationship as $-C_6H_5$ and -COOMe in -PheOMe, and (iii) the absence of functional groups other than the AH-B entity. It should also be pointed out that all these dipeptides are larger than other sweet molecules and, being very specific in their interaction, it may be inferred that they fit the receptor site more precisely than most previously known sweet substances. However, in order to use these constitutional and configurational pieces of information, it is essential to have also detailed conformational knowledge because all the dipeptides mentioned are flexible molecules. Accordingly, we undertook a conformational study of the prototype molecule α-L-Asp-L-PheOMe (henceforth called α-APM) in solution, with the aid of both spectroscopic (NMR) and theoretical methods.

Experimental Section

Materials. Solutions were made up with 99.7% D_2O (Merck, Sharp & Dohme) and pH adjustments were made with DCl and NaOD solutions in 99.7% D_2O. α-L-Aspartyl-L-phenylalanine methyl ester[5,10] [mp 246–247 °C; $[\alpha]^{22}D$ −2.3° (c 1.1, 1 N HCl)] was prepared from *N*-benzyloxycarbonyl-β-benzyl-L-aspartyl-L-phenylalanine methyl ester[5,10] by hydrogenation at room temperature and pressure in the presence of 5% palladized charcoal in aqueous acetic acid. *N*-Benzyloxycarbonyl-β-benzyl-L-aspartyl-L-phenylalanine methyl ester was in turn synthesized starting from β-benzyl *N*-benzyloxycarbonyl-L-aspartate[10–12] and L-phenylalanine methyl ester hydrochloride[13] via dicyclohexylcarbodiimide[14,15] in the presence of triethylamine in anhydrous tetrahydrofuran.

Methods. Most NMR spectra were run on a Varian XL-100-15 spectrometer at probe temperature (~29 °C), using internal DSS for reference. Measurements of the J_{NC} coupling constant were performed on a Bruker 270-MHz instrument. Conformational energy calculations were performed with the aid of a very general Fortran V program that can treat any molecules with an arbitrary number of degrees of freedom.[16]

Results

NMR Results. The conformational information one can extract from ^1H NMR spectra of a linear peptide such as α-APM comes essentially from the coupling constants of the ethane-like fragment of the side chains and from the J_{NC} coupling constant of the backbone. Whereas the two vicinal coupling constants of the $CHCH_2$ groupings can be measured with fairly good accuracy and used in a standard manner[17] for conformational analysis, the backbone coupling constant is somewhat obscured by exchanging phenomena (at certain pH's) and by ^{14}N quadrupolar relaxation. Besides, it is not possible, in a flexible molecule, to use the Bystrov-Karplus relationship[18] directly to extract torsional angle values because the form of the equation usually allows a few values of the torsional angle to be consistent with a given J_{NC} value.[19] Accordingly, we chose to rely on ^1H NMR measurements for the side-chain conformations and to resort mainly to a priori potential energy calculations for the backbone conformation.

All resonances were easily assigned on the basis of the published data on the closely related peptides of pentagastrin.[20] The assignments were confirmed by spin-decoupling experiments and by the pH dependence of some bands. Table I summarizes all coupling constant data. The vicinal coupling constants were derived from ABX analyses of the spectra of $CHCH_2$ fragments.

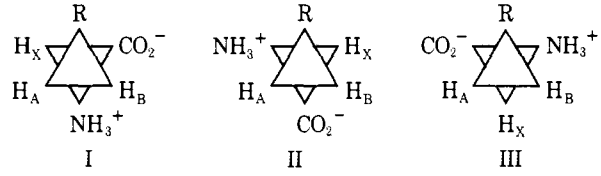
The most prominent feature of the data of Table I is the marked pH dependence of the Phe coupling constants. This behavior is well illustrated by the graphs in Figure 1 which shows the variation of Asp and Phe J_{AX}'s as a function of pH. While the influence of pH on the Asp residue can be related directly to electrostatic effects ensuing from changes in the state of ionization of the amino and carboxyl groups, the effect of pH on the Phe residue can only be indirect and points to conformational changes of the whole molecule in the pH range examined. As shall be discussed in the section of energy calculations, we thought that to reproduce such changes would be a critical test of the internal energy calculations and, in turn, might yield reliable information on the most probable backbone conformation.

Table I. Vicinal H-H Coupling Constants (Hz)[a] for α-APM at Various pH Values[b]

	Asp			Phe		
pH	J_{AB}	J_{AX}	J_{BX}	J_{AB}	J_{AX}	J_{BX}
3.5	17.2	8.2	5.0	14.4	8.8	5.6
6.2	17.2	8.3	5.1	13.8	9.1	5.9
7.0	17.0	8.5	4.6	13.9	9.7	5.9
8.5	17.0	8.5	4.9	14.0	8.2	4.8
11.7	17.0	8.1	5.1	13.8	8.2	4.8

[a] Accuracy ±0.2 Hz. [b] α-APM hydrolyzes readily at pH's higher than 12, but the NMR spectrum changes dramatically upon hydrolysis and no change of our spectra was observed after completion of measurements at pH 11.7.

The fractional populations P_I, P_{II}, and P_{III} of rotamers I, II, and III in α-amino acids in solution are routinely estimated



by means of high resolution NMR methods.[21] The simplest approach is due to Pachler[17] and we shall use it because we feel that more sophisticated approaches[22] are not really justified by the underlying theoretical assumptions.

By measuring the vicinal coupling constants J_{AX} and J_{BX} (averaged over *all* possible rotamers), it is possible to calculate fractional population using the equations

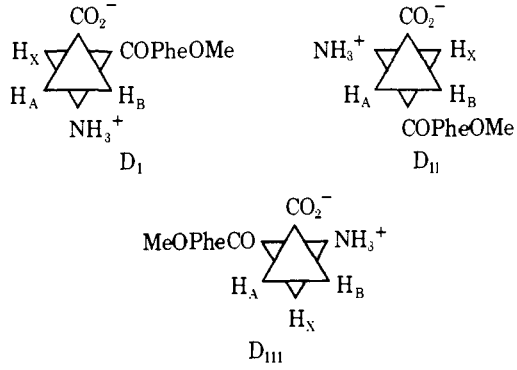
$$P_I = (J_{BX} - J_g)/J_t - J_g)$$
$$P_{II} = (J_{AX} - J_g)/(J_t - J_g)$$
$$P_{III} = 1 - P_I - P_{II}$$

and reliable values for gauche and trans vicinal coupling constants obtained from studies of model compounds. It is implicit in this approach that equal J_g values should be used for the two gauche conformations and that the J_g and J_t values are the same in all three rotamers. These approximations are rather severe and may lead to errors in the absolute values of calculated populations but are commonly regarded as quite acceptable in comparative studies where one is rather interested in following trends in populations as a function of some external parameter (pH, temperature, etc.).

Large differences in the actual values of fractional populations may also result depending on the choice of the numerical values of J_g and J_t. Again this choice is far less critical if only relative values of the populations are of interest. We decided to use the values[17] $J_g = 2.56$ and $J_t = 13.6$ Hz, mainly to make our figures directly comparable to those calculated by Feeney et al.[20] in their work on the pentagastrin peptides. (As will be discussed in the energy calculation section, other

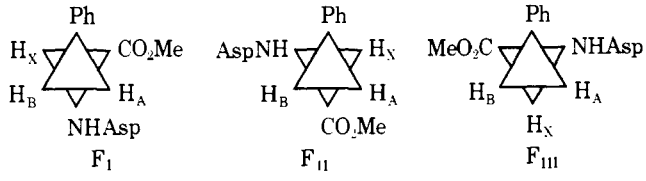
choices would not alter the goodness of our fit with calculated conformations.)

A crucial point in the estimation of fractional populations turned out to be the assignment of H_A and H_B in the two residues. It is clear that rotamers I and II can only be distinguished if one can assign H_A and H_B unambiguously. As already pointed out by Feeney et al.,[20] the assignment of $C(\beta)H_2$ protons in Asp is greatly facilitated by the observation of specific effects of pH changes in the chemical shifts of the CH_2 protons. Since we observed essentially identical effects in our compound, the assignment of H_A and H_B was taken as that of ref 20.



(The symbols D_I, D_{II}, and D_{III} and F_I, F_{II}, and F_{III} will be used from now on to indicate the rotamers, and their populations, of the Asp and Phe moieties, respectively.)

On the other hand, the assignment was reversed for the analogous protons of PheOMe, essentially on the basis of energetic considerations. Let us examine the three possible staggered rotamers for the PheOMe side chain.



A choice between rotamers I and II is not easy for free amino acids, because the $-NH_3^+$ and $-COO^-$ groups are of comparable bulkiness. In our case, however, it is clear that the grouping -NH-Asp is far bulkier than -COOMe and an assignment that leads to the condition $F_I < F_{II}$ is unlikely. Accordingly, we have chosen to reverse the assignment of H_A and H_B for PheOMe with respect to the quoted work of Feeney et al.

These elementary energetic considerations were later proven to be consistent with much more sophisticated energy calculations (vide infra). It may be noted that our assignment coincides with that of Martin and Mathur for numerous histidine and cysteine derivatives.[23]

The relative rotamer populations are reported in Table II. The J_{NC} coupling constant in the acidic pH range is 8.0 Hz. This rather high value may reflect the presence of high values of populations of conformations with angles φ in the range -60 to $-180°$.[18] Still in order to have a reasonable description of the skeleton conformation we need narrower ranges for φ and ψ and to locate them we resorted to internal conformational energy calculations.

Energy Calculations. Experimental values of coupling constants in flexible molecules can only reflect a mean Boltzmann average[19] of the coupling constants of all possible rotamers in solution. Thus, it is not straightforward to extract information on the preferred conformations and on their relative weights. Moreover, in the case of the dipeptide studied, it must be remembered that NMR data give us *independent*

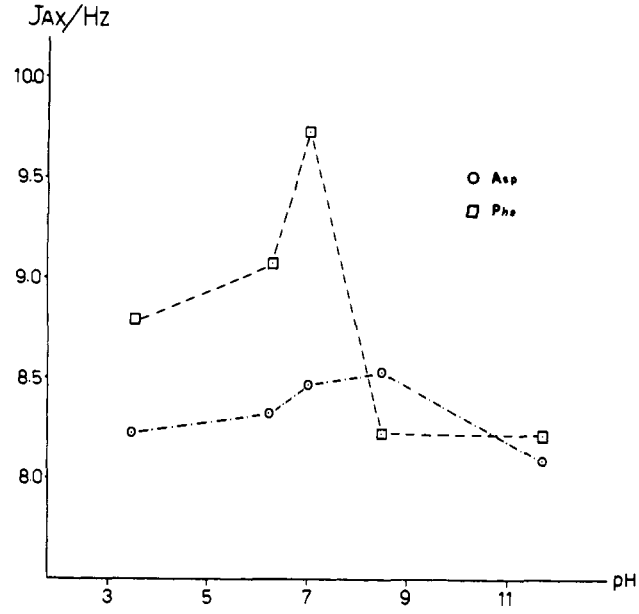


Figure 1. pH dependence of the vicinal H_A–H_X coupling constant for the side chains of two moieties of α-APM.

Table II. Rotamer Fractional Populations for α-APM Side Chains

	Asp			Phe		
pH	D_I	D_{II}	D_{III}	F_I	F_{II}	F_{III}
3.5	0.22	0.51	0.27	0.56	0.28	0.16
6.2	0.23	0.52	0.25	0.59	0.30	0.11
7.0	0.19	0.53	0.28	0.65	0.30	0.05
8.5	0.21	0.54	0.25	0.51	0.20	0.29
11.7	0.23	0.50	0.27	0.51	0.20	0.29

information on the side chains of the two residues. Accordingly, it is impossible from NMR studies alone to shed light upon conformational relations between the two amino acid moieties of the molecule and, in part, also within each moiety.

In order to gain a deeper insight into these relations we performed some internal energy calculations of the isolated molecule within the partitioned energy model (PEM).[24,25] In our calculations we assumed that the conformational energy (E_c) is mainly due to electrostatic and nonbonded terms, so that

$$E_c = \sum_{i>j} C\frac{q_i q_j}{D r_{ij}} + \sum_{i>j} \frac{A_k}{r_{ij}^{6}} - \frac{B_k}{r_{ij}^{12}}$$

where q_i and q_j are the partial charges on atoms i and j expressed in fractional electronic charges, r_{ij} is the interatomic distance between atoms i and j in nm, C is a conversion factor (equal to 139) to give E in kJ/mol when r_{ij} is in nm,[26] D is the *effective* dielectric constant,[27] and A_k and B_k are the coefficients of a Lennard-Jones potential function.

The values of q's were obtained by summing the σ contribution calculated according to the method of Del Re[28] and the π contribution calculated according to Berthod and Pullman.[29] They are shown in Figure 2 for two of the three possible ions. The values of the A_k's and the B_k's, as well as bond angles and bond distances, were those proposed by Scheraga.[30] This choice for the nonbonded atoms potential functions, among the many possibilities presented by a huge literature on the argument, was dictated both by the widespread use of Scheraga's functions and by the convenience of using, for a flexible molecule, rather soft potentials.

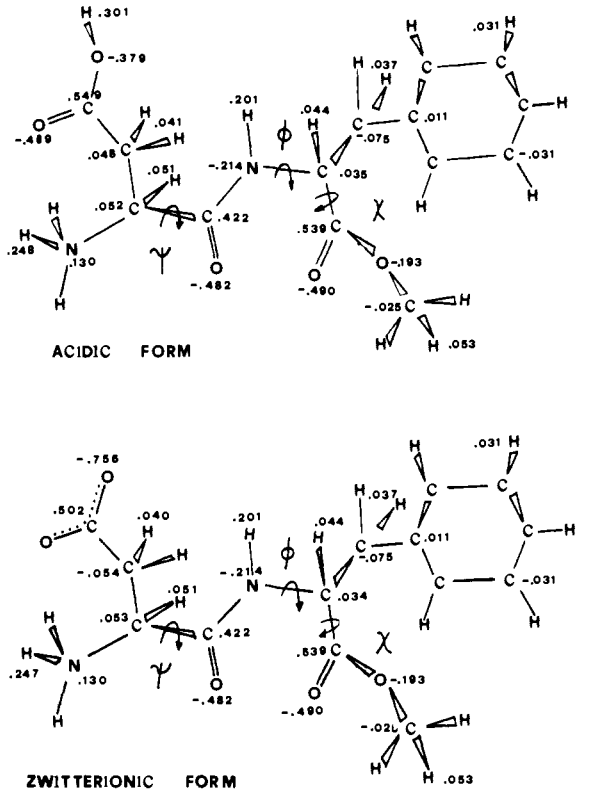


Figure 2. Schematic molecular models of α-APM with fractional electronic charges.

Owing to the high number of parameters, a complete energy calculation as a function of all internal rotation angles would have been prohibitive timewise and too costly. Thus we were forced to make reasonable assumptions about some internal degrees of freedom. That is, we assumed that (i) the conformations that determine the values of the $^3J_{CHCH_2}$ coupling constants are essentially the three staggered conformations (F_I, F_{II}, F_{III} and D_I, D_{II}, D_{III}), (ii) the phenyl group of Phe and the β-carboxyl group of Asp have always the same orientation[31] with respect to the C-C bond of the adjacent CH_2-CH groups, and (iii) the peptide bond is trans.

The dihedrals φ, ψ, and χ, indicated in Figure 2, were the only angles free to change over 360°. A typical energy map, as a function of φ and ψ, is shown in Figure 3. The interpretation of maps of this sort, in the case of flexible molecules in solution, is not as direct as for the analogous PEM calculations in the solid state or even of fairly rigid cyclic molecules in solution. This is due to the fact that it is not possible to take the deepest minima of the E_c as a guideline for stability. In fact, tumbling and related internal motions of molecules in solution may excite large internal degrees of freedom that contribute to the overall stability from an entropic point of view.

Although this problem, strictly speaking, should be solved in a quantum-statistical scheme, we tried to simplify it by means of an approximate treatment. We could think of the energy of our system as partitioned in the following manner

$$E(q,p,\alpha,\beta,\varphi,\psi) = E_0(q,p,\alpha,\beta) + E_c(q,\alpha,\beta,\varphi,\psi)$$

where q and p stand for generalized coordinates and moments, respectively, α and β are the torsional angles of the ethane-like side chains, and φ and ψ are the skeleton torsional angles. It is further assumed that E_c, the "conformational" term, is small with respect to E_0, which accounts for all remaining energetic contributions. The partition function may be written as $\exp(-F/RT)$, where $F = E - TS$ is the free energy of the system, T its temperature, R the gas constant, and S the entropy.[32] On the other hand, in a classical scheme, the partition

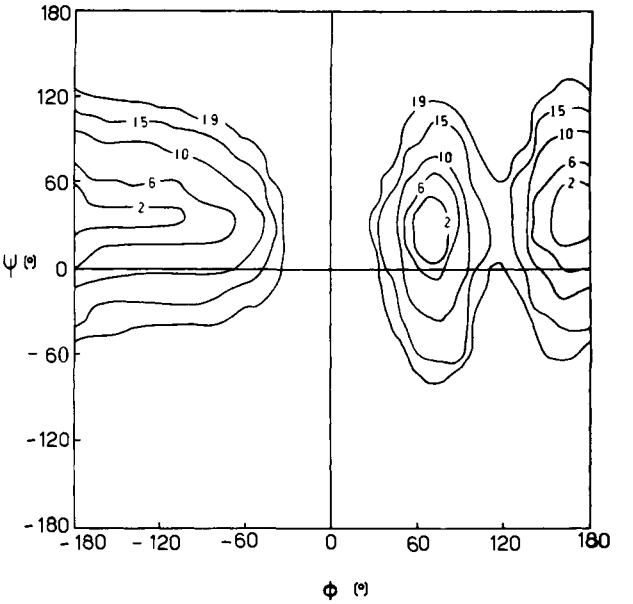


Figure 3. Typical energy map section as a function of the internal rotation angles φ and ψ for $F_I D_{II}$ (χ = 150°) in the zwitterionic form.

function, with correct counting, is

$$Z = \int e^{-[E_0(q,p,\alpha,\beta)+E_c(q,\alpha,\beta,\varphi,\psi)]/RT} d\Gamma$$

where

$$d\Gamma = dp_1 \ldots dp_N dq_1 \ldots dq_{N-4} d\alpha d\beta d\varphi d\psi$$

Let us now split the integral into 3 × 3 integrals in such a way that the middle point of the ranges of α and β are just the values (trans, gauche, and gauche') that characterize conformations F_I, F_{II}, and F_{III} and D_I, D_{II}, and D_{III}, respectively.

$$Z = \sum_{\substack{k=0,\\ n=0}}^{2} \int_{2k\pi/3}^{2(k+1)\pi/3} \int_{2n\pi/3}^{2(n+1)\pi/3} \int e^{-(E_0+E_c)/RT} d\Gamma$$

If the values of $|E_0|$ are very large in the points corresponding to the staggered trans, gauche, and gauche' conformations with respect to the other possible values of α and β (and this holds true for each of the remaining q variables), function $e^{E_0(q,p,\alpha,\beta)}$ can be replaced by a Dirac δ for each of the chosen ranges of α and β. Each of the nine integrals can thus be put in the form

$$Z_{D_L F_J} = \int e^{-E_c(q,\alpha,\beta,\varphi,\psi)/RT} \delta(\alpha - \bar{\alpha}_L)\delta(\alpha - \bar{\beta}_J) \times \delta(q - \bar{q}) d\alpha d\beta d\varphi d\psi dq$$

and reduces to

$$\int e^{-E_c(\varphi,\psi,\bar{\alpha},\bar{\beta},\bar{q})/RT} d\varphi d\psi$$

The power expansion of the integrand, neglecting terms of higher order, is

$$e^{-E_c/RT} = 1 - E_c(\varphi,\psi)/RT$$

so that

$$Z = \int (1 - E_c(\varphi,\psi)/RT) d\varphi d\psi$$

Let us now consider the equilibrium between two states of the system **1** and **2** with energies $E_0 + E_c^1$ and $E_0 + E_c^2$. The free energy difference is given by

$$-\Delta F = RT \ln \frac{\int \left(1 - \frac{E_c^1(\varphi,\psi)}{RT}\right) d\varphi d\psi}{\int \left(1 - \frac{E_c^2(\varphi,\psi)}{RT}\right) d\varphi d\psi} = RT \ln K \qquad (1)$$

Table III. Comparison of Experimental and Calculated Combined Fractional Populations for α-APM

	Acidic form		Zwitterionic form		Basic form	
	Exptl[a]	Calcd	Exptl[a]	Calcd	Exptl[a]	Calcd
F_ID_I	0.123	0.130	0.136	0.121	0.117	0.118
F_ID_{II}	0.286	0.183	0.307	0.184	0.255	0.193
F_ID_{III}	0.151	0.136	0.148	0.146	0.138	0.130
$F_{II}D_I$	0.062	0.081	0.069	0.073	0.046	0.068
$F_{II}D_{II}$	0.143	0.144	0.156	0.141	0.100	0.151
$F_{II}D_{III}$	0.076	0.081	0.075	0.088	0.054	0.078
$F_{III}D_I$	0.035	0.065	0.025	0.056	0.067	0.064
$F_{III}D_{II}$	0.082	0.106	0.057	0.113	0.145	0.130
$F_{III}D_{III}$	0.043	0.074	0.027	0.075	0.078	0.066

[a] Figures under the heading exptl are obtained from products of the corresponding values of Table II.

where K is the equilibrium constant for the process **1** ⇄ **2**.

When applied to our system these assumptions amount to saying that conformational changes modify the total energy only to a small extent, whereas the moments of inertia may be considered as approximately constant with respect to the variables φ and ψ.

Recalling assumption i we may say that E_c is calculated as a function of φ and ψ for each of the nine combinations of the three most probable staggered conformations around the C_α-C_β bonds and for the most favorable value of χ. Since $E_c(\varphi,\psi)$ is actually calculated as a grid for discrete values of φ and ψ, we may put the integrals of **1** as sums

$$\int\left(1-\frac{E_c(\varphi,\psi)}{RT}\right)d\varphi d\psi=\sum_{i,j}\left(1-\frac{E_c(\bar{\varphi},\bar{\psi})}{RT}\right)\Delta\varphi_i\Delta\psi_j \qquad (2)$$

where $\bar{\varphi},\bar{\psi}$ are the points of the grid for which the function E_c was calculated. The numbers $\Sigma_{i,j}(1-E_c/RT)_k\Delta\varphi_i\Delta\psi_j$ after normalization are then the populations of molecules containing given pairs of staggered conformations of the Asp and Phe side chains (see Table III).

As a consequence of assumption i and of the fact that NMR data give us independent information on the conformational states of the two side chains, these numbers should be compared with the pairwise products of the "experimental" populations of Table II (see Table III).

An inspection of Table III shows that the relative values of the (experimental) NMR populations are reproduced very well by the conformational PEM calculations. The quality of the fit between experimental and calculated populations can best be grasped from the histograms of Figure 4 where both series of values are plotted vs. the nine possible combinations of staggered conformations. It is clear now that a different assignment of the β-CH_2 protons of Phe (leading to a reversal of populations F_I and F_{II}) would alter the trends of the graphs in such a way as to prevent any agreement between experimental and calculated values. It is also clear that the discrepancies between *absolute* values of "experimental" and calculated populations would be affected by a different choice of J_t and J_g and/or by the use of different potential functions in the PEM calculations. Needless to say, rather than trying a posteriori of choosing different values of J_t and J_g (that could give a better agreement also for each of the nine combinations), we contented ourselves with the fact that a check with all pairs of J_t and J_g values that appeared in the literature for amino acids[21] showed identical relative trends in the nine combinations of side-chain populations. Other causes of error that may have impaired the agreement for single pairs of experimental and calculated populations are the approximations inherent in eq 2 and the fact that the charges calculated with the method of Del Re have been shown to be a little too high.[33]

Notwithstanding these possible sources of error and the numerous approximations, it is fair to say that the calculations

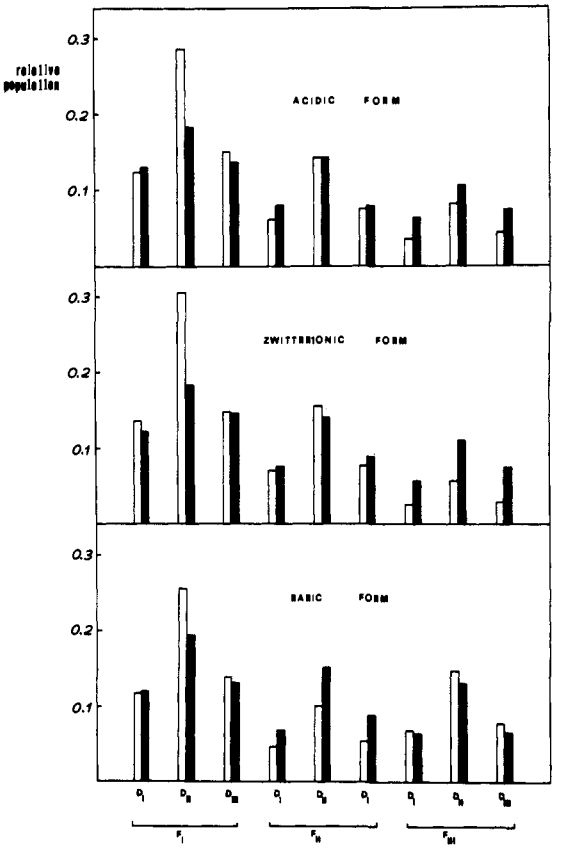


Figure 4. Histograms of the data of Table III. Solid bars refer to calculated populations.

underlying the graphs of Figure 4 give a rather accurate description of the conformational state of α-APM in solution. To the best of our knowledge ours is the first example of a detailed fit of NMR data by means of a priori conformational energy calculations for a flexible molecule.

Thus, if we take into account the fact that most of the conformations are characterized by very similar skeleton rotation angles, we can further restrict the ranges of φ and ψ with respect to the indication given by the J_{NC} coupling constant value (vide infra).

The allowed ranges are 30-0° and 120-135° for ψ and −150 to −160° (with a strong preference for −60 to −90°) for φ, respectively.[34] The ranges for φ are consistent with the experimental value of 8.0 Hz for J_{NC}. However, our main concern is not the description of the state of α-APM in solution, per se, but rather its significance with respect to the interaction with the receptor site and, with all the information furnished by NMR and by PEM calculations, we are now in the position

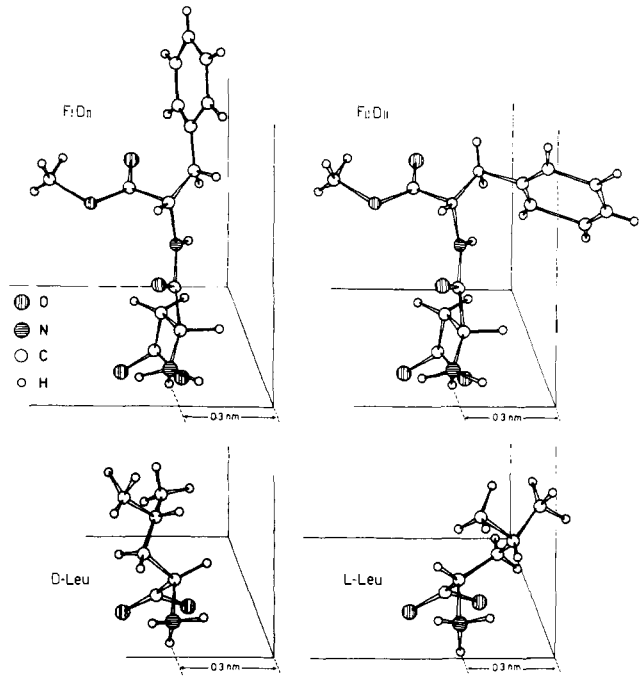


Figure 5. Comparison of the molecular models of conformations F_ID_{II} and $F_{II}D_{II}$ with the schematic models of D- and L-leucine.

Table IV. Relevant Internal Rotation Angles of the Four Most Populated Conformations of α-APM in the Zwitterionic Form

	ψ	φ	χ
F_ID_{III}	30	−150 ÷ −60	+150
F_ID_I	0	−60	+150
F_ID_{II}	30	−150 ÷ −60	+150
$F_{II}D_{II}$	30	−180 ÷ −90	+150

of discussing this problem in detail. Owing to obvious physiological considerations, only the results for the zwitterionic form will be used in this connection.

Discussion

If we focus our attention on the interaction of α-APM with the receptor site, contrary to the previous problem of conformations in solution, we need consider only one or a few conformations. Actually, even if only one conformation were able to accommodate the receptor site, we can take into account all related conformations that can be easily interconverted into it, both as a consequence of the interaction inside the receptor and because of a shift of the equilibrium in solution. A glance at Figure 4 shows that we need only consider the four most populated conformations since they account for the majority of molecules in solution. Table IV shows, for these conformations, the internal rotation angles corresponding to the deepest minima for the skeleton bonds and for the ester side chain. The least populated of the four (F_ID_I) can be immediately dismissed because a trans arrangement of $-NH_3^+$ and $-COO^-$ in the Asp moiety is not consistent with the known features of the AH-B entity of all sweet substances.[2] The remaining three conformations all have an AH-B "fork" that can interact with the receptor site but can be easily discriminated on the basis of the sterical requirements imposed by the spatial barrier that has been shown to select D and L isomers of simple amino acids.[3] In fact, we can further restrict our attention to the two most populated conformations, i.e., F_ID_{II} and $F_{II}D_{II}$, since in F_ID_{III} the AH-B entity is shielded by the Phe side chains and cannot interact freely with the corresponding entity of the

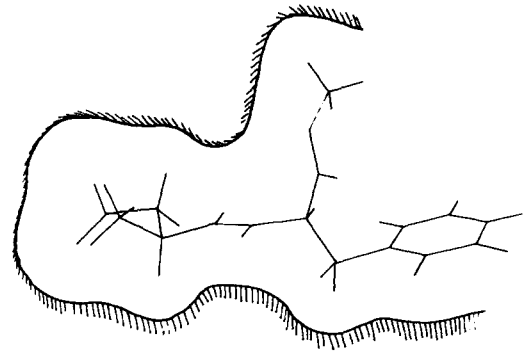


Figure 6. Possible inner section of the receptor site as limited by the van der Waals radii of conformation F_ID_{II} of α-APM.

receptor site. Figure 5 shows a comparison of the molecular models of conformations F_ID_{II} and $F_{II}D_{II}$ with the schematic models of D- and L-leucine proposed to justify the existence of the spatial barrier.[2] It is clear that only conformation F_ID_{II} can interact with the receptor site without "invading" the spatial barrier. This is the most populated conformation, according to the PEM calculations. However, even the second most populated conformation (i.e., $F_{II}D_{II}$) is identical with it in all respects but for the internal rotation angle around the $-CH-CH_2$ bond of the Phe moiety. It is not difficult to envisage that interconversion of $F_{II}D_{II}$ into F_ID_{II} may be very easy even inside the receptor site, possibly as part of the interaction mechanism. Only to have a rough indication of the easiness of this process, we have calculated the conformational conversion path between the two isolated conformations. The results show that the barrier between the two most favored conformations is quite small, of the order of 1.0 kJ/mol. We may note, on passing, that both in F_ID_{II} and in $F_{II}D_{II}$ the benzene ring is not in close proximity to any aliphatic proton. The absence of significant aromatic shielding effects in the chemical shifts of α-APM may thus be taken as a further proof of the goodness of our conformational analysis.

Conformation F_ID_{II} of α-APM can thus be considered as a very likely "substrate" for the receptor site both from a constitutional and a stereochemical point of view. It is consistent with all known requirements for sweet molecules but has also some additional interesting features that can help to improve our knowledge of the topology of the receptor site. The differences between the molecular models of slightly sweet molecules such as D-amino acids and sugars and (very sweet) α-APM are all confined to those parts of the molecules not directly involved in the stereochemistry of the AH-B entity. Accordingly we may speculate that while the common part is a necessary requirement for the interaction (possibly for locking the molecule through the AH-B entity), only the bulky apolar side chains of α-APM fit the receptor site tightly enough to elicit a strong response and are then a good (negative) replica of that part of the site that triggers the nerve impulse. A similar hypothesis has, in fact, already been put forward on the basis of the chemical formulas of α-APM and related molecules,[7,35] but we can now substantiate it with much more precise conformational data. We think it interesting to emphasize two general features characteristic of very sweet substances, emerging from our investigation. As can be appreciated from the molecular model of F_ID_{II} in Figure 5, the conformation is very flat in the plane containing the AH-B entity. Thus, it seems probable that the receptor site is not simply limited by a spatial barrier to one side of this plane, but it has rather the shape of a narrow cleft, at least in the region immediately above AH-B. This indication is consistent with the molecular models of very sweet rigid molecules such as saccharin, cyclamates, and nitrobenzenes. It is also consistent with the peculiar fact

that the only apolar amino acids that are not sweet (both as D or L isomers) are valine and isoleucine, i.e., the only two that have a methine β-carbon atom.[4] The second feature for very sweet molecules is that they must possess a bulky apolar part located above AH-B and widely spaced with respect to it. Our model indicates a distance of ~1.0 nm between the line joining A and B and the top of the benzene ring. This distance may be taken as the approximate (minimum) vertical dimension of the cleft.

As a purely speculative indication we may use the shape of α-APM, as limited by the van der Waals radii of the outer atoms, to delineate the approximate contours of the cleft. Figure 6 shows a view of conformation F_ID_{II} of α-APM along a direction parallel to the plane containing the AH-B entity and to the spatial barrier.

An indirect check of the validity of F_ID_{II} as interacting conformation is furnished by a further experimental datum that can be explained on the basis of our model. According to an observation of Mazur et al.,[7] methylation of the amide NH in molecules like α-APM destroys their ability to elicit the sweet taste completely. One obvious consequence of methylation is that the cis configuration becomes approximately isoenergetic with the trans, characteristic of unsubstituted peptide bonds. Examination of Dreiding models of *cis-N*-methyl-α-APM shows, in fact, that it is difficult to build conformations that do not invade the "spatial barrier". At any rate, if we restrict our attention to *trans-N*-methyl-α-APM, it is very interesting to observe that the energy of the conformation F_ID_{II} would be very high for *N*-methyl-α-APM because of many unfavorable contacts between the hydrogens of the methyl group and most of the atoms of the Asp moiety. On the other hand, it is clear from the shape of F_ID_{II} (see Figure 5) that substitution of the hydrogen of Phe's α-CH with a methyl group may be accomplished with only minor perturbations of the basic skeleton conformation. It is reassuring to note, in this connection, that a recent work of Mazur shows that compounds of general formula L-Asp-NH-CR(R')-COOMe (with R = $CH_3 \neq R' \neq H$) are sweet.[36]

Finally, we may remark that only hydrogen atoms of apolar groups point toward the spatial barrier in F_ID_{II} while functional groups such as C=O and NH are essentially contained in a parallel plane (see Figure 5). This observation substantiates the need of a strictly apolar nature for the side of the sweet molecule facing the spatial barrier.

Conclusions

The main conclusions of the present investigation are the following. (1) The relative weights of the most populated conformations of a flexible molecule, such as the dipeptide α-APM, can be reproduced very well by conformational internal energy calculations. (2) Combining our results with previously known features of sweet molecules, it is possible to indicate the conformation of α-APM that is most likely to interact with the receptor site of the taste bud. (3) The receptor site can be described as a narrow cleft with two interacting parts, one for locking the sweet molecule and another for triggering the nerve impulse. (4) The loss of sweet taste upon alkylation of the amide NH of very sweet dipeptides is explained in conformational terms.

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