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단 신

구조-맛 관계의 연구 (1). 디하이드로 칼콘 분자모델의 정량적 분석*

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Studies on the Structure-Taste Relationships (1). Quantitative Analysis of the Molecular Model of Dihydrochalcone

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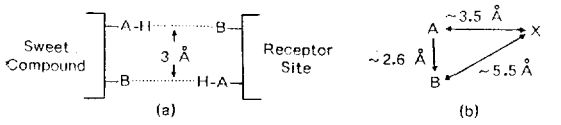
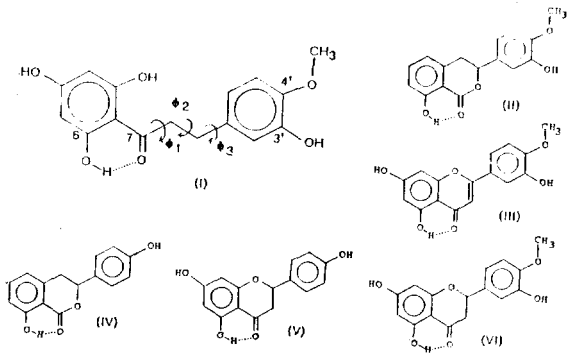
The identification of the common features of many apparently unrelated sweet tastants may lead to a description of geometric and chemical natures of the sweet receptor site and, in turn, provide a basis for the design of new useful sweeteners.[1] Schallenberger proposed that an A-H/B bifunctional group (a) is an essential glucophore for a sweet taste sensation where A and B are electronegative atoms separated by 2.5~4.0 Å.[2] Kier later postulated the existence of a third binding site (b) that involves a dispersion bonding at the receptor.[3] Both postulations are drawn in Scheme 1, where X in (b) is a third binding site. Since the discovery[4] of neohesperidin dihydrochalcone (DHC), a potent nonnutritive sweetener, hundreds of derivatives of hesperetin DHC (I), as shown in Scheme 2, have been synthesized[5] in order to improve taste of the DHC sweeteners which are slow to develop and show a lingering licorice-like aftertaste. In the course of the studies to correlate the structure with the taste property, DuBois *et al.* noticed the very similar taste characteristics between DHC and phyllodulcin (PD; II). PD possesses an intense, clean sweetness with the same problems of slow onset and lingering aftertaste.[6] They assumed that PD exists in a bent conformation where the phenyl ring is substituted in the pseudoaxial position.[7] This assumption solely depended on the notion that the conformation of PD should be markedly different from that of flavone (III) since the rigid, extended flavones are always either tasteless or bitter. DuBois *et al.*, furthermore, proposed



Scheme 1.



Scheme 2.

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that DHC and PD have similar conformations and therefore the "bent" conformation be the active form of the DHC molecule in elicitation of sweet taste since it is superimposable to that of PD. They assigned the portion involving O3′-H and O4′ to be an A-H/B unit and the acyclic O6-H···O7 portion, which is always connected by an intramolecular hydrogen bond, to be the X site of the Kier's glucophore. Through manipulation of the skeletal Drieding models, they concluded that the bent form of DHC possesses the spatial arrangement of the Kier's system.

Any hypothesis concerning the stereochemical characteristics derived by examining the molecular model may involve serious incorrect features, especially when the molecule has many freely rotatable single bonds. An obvious way to predict the stable conformations of the molecule is to calculate the potential energy as a function of all internal rotation angles. However, this may sometimes be practically prohibitive for the molecules with many single bonds. As a handy alternative, therefore, we developed a computer program ROTOR with which stereochemical validity of any molecular models and spatial relationships between the functional groups can be evaluated quantitatively and automatically. This can be regarded as an enhanced version that ultimately gives a Ramachandran plot.

Since a molecule can be described uniquely by the three different internal coordinates, *i.e.*, bond distances, bond angles and torsion angles of which the first two can be obtained from the accurate structural data, manipulation of the molecular models can be represented numerically by varying the torsion angles along the specific bonds. In ROTOR, the internal coordinates are transformed into Cartesian atomic coordinates following the algorithm due to Thompson[8] and all of the relevant interatomic distances for each of the specific conformations are calculated to test whether they are within the prespecified contact limits or not. In the current version, these calculations are performed for up to three variable torsion angles in a single run so that two dimensional steric maps can be obtained automatically. The resulting steric map consists of either zero which indicates that the conformation is sterically reasonable, or non-zero numbers which indicate the presence of critical steric hindrances identifying the pair of atoms in the shortest contact together with its distance. In order to obtain a printer output in a square form, two torsion angles are incremented *ca.* 9 and 10 degrees along the horizontal and vertical directions of the output. The increments along the third direction can be set arbitrarily. Options are also provided to calculate any specific interatomic distances and to examine the intramolecular hydrogen bonding geometry for each of the allowed conformations. The initial values for the contact limits between pairs of atoms (C-C; 3.0, C-O & O-O; 2.7, C-H & O-H; 2.2, H-H; 1.9Å) are from the work of Ramachandran.[9] Since the resulting steric map heavily depends on the choice of contact limits, provision is made to adjust these values independently for the three different kinds of contacts, that is, 1,4-torsional contacts, contacts involving the hydrogen atoms and the remaining non-bonded contacts in order to dissipate the possible inaccuracies in the input internal coordinates. ROTOR was written in FORTRAN for an IBM PC compatible and runs fairly fast when using an Intel 8087 coprocessor.

The hesperetin DHC molecule, a representative for the DHC sweeteners, was examined using ROTOR by rotating three Sorsion angles (see (I)). The initial internal coordinates are from the crystal structure of phlorizin[10] which

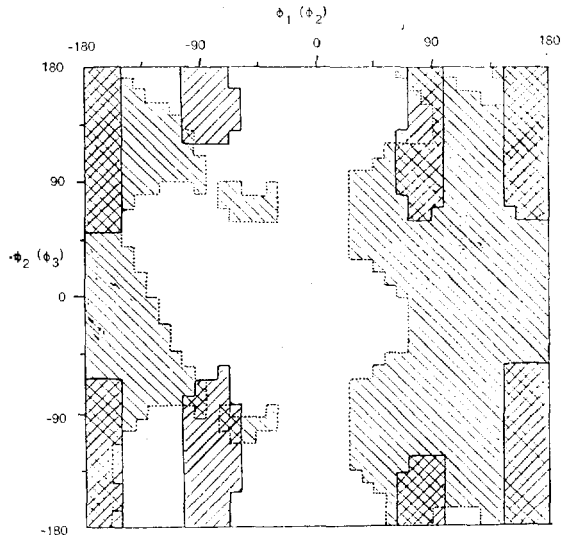

Fig. 1. Steric maps of the DHC molecule. Two sections of $\phi_3 = 0°$ (solid lines) and $\phi_1 = 90°$ (dotted lines and the labels of the axis in parentheses) are superimposed to show the most and one! of the least restricted maps. The allowed regions are cross hatched. Since the limit distances in the text are lowered by 0.2 Å for the 1,4 contacts land 0.1 Å for the others and additional 0.1 Å for the contacts involving the hydrogen atoms, the allowed regions can be regarded as widest ones as possible.

is the only structure reported to date that contains the DHC moiety. The DHC moiety in phlorizin assumes a fully extended and planar conformation. The 3'-hydroxyl and 4'-methoxy groups were generated using the standard dimensions and the latter being placed in the plane of the phenyl ring as observed in many compounds containing the methoxy group.[12]

Calculations were performed for 51,460(40×36×36) grid points. A representative steric map is given in *Fig.* 1. Qualitatively, the most restricted angle is ϕ_1 and the most allowed one is ϕ_3. The allowed conformation which is most similar to that of PD having the idealized values of $\phi_1 \fallingdotseq 30$, $\phi_2 \fallingdotseq 60$ and $\phi_3 \fallingdotseq 30°$, occurs near the values of 75, 79 and 67°, respectively. The distances between the functional groups are listed in *Table* 1 (see (B)). These are completely different from the measured values by DuBois

Table 1. Spatial separation (Å) between the functional groups in the various models and the related crystal structures

	(A)	(B)	(C)	(D)	(E)	(F)	(G)
O3'-O4'	2.8	2.7	2.7	2.7			
O6-O7	2.6	2.5	2.5	2.5	2.44	2.61	2.65
O3'-O6	3.9	8.1	4.5	9.4-9.9			
O3'-O7	3.8	6.0	4.2	7.3-7.5			
O4'-O6	6.4	8.1	6.8	10.5-10.7	10.69	10.48	10.68
O4'-O7	6.4	6.5	5.6	8.2-8.7	8.32	8.37	8.78

(A) DuBois model. (B) Most similar conformation to PD. (C) Conformation with shortest contacts ($\phi_1 \fallingdotseq -90$, $\phi_2 \fallingdotseq -51$, $\phi_3 \fallingdotseq 67°$). (D) Most populated conformations. (E) Crystal structure of phlorizin. (F) Crystal structure of hydrangenol. (G) Crystal structure of naringenin.

et al. There exist structures with short distances close to the measured values but there are still significant differences in the distances and these forms are far from being similar to the bent form of PD (see(C) in *Table* 1). Furthermore, these forms barely avoid the critical steric hindrances. In short, among the allowed conformations, we could not find the bent form similar to that of PD with the suggested spatial arrangement between the functional groups. It seems, therefore, that DuBois *et al.* probably measured the distances with the model that is totally impossible due to steric hindrances. This study shows the inherent inaccuracy involved in manipulation of the molecular models even if it is done most carefully.

Although the population of the extended, either fully or partially, forms is much larger than that of the bent forms, the sterically allowable regions are so wide, as expected, that it is meaningless to identify the favorable conformations. However, the following structural evidences lead us to suggest that the extended form of DHC is the active conformation. It was shown that hydrangenol (IV), an analog of PD, assumes an extended conformation with the phenyl ring in the pseudoequatorial posi-

tion.[12] Furthermore, naringenin (V), which is a tasteless analog of hesperetin (VI), the only sweet flavanone, has the same conformation.[13] In fact, it has been observed without exceptions that the heteroatom ring in either flavanone or isocoumarin is in the sofa form and the phenyl ring is substituted in the pseudoequatorial position regardless of its absolute configuration.[13,14] Therefore, the stable conformation for PD seems to be more likely extended. In this extended conformation, O6 and O4′ are separated by *ca.* 10Å. This longer separation between the functional groups can also be achieved for so many different conformations of DHC as listed in *Table* 1. It is quite interesting to note that the extensive studies on the conformation of aspartame also suggests a separation of *ca.* 10 Å between the two functionally critical portions excluding the presence of the Kier's A-H/B/X entity with shorter spatial arrangement.[15] In this study, the receptor site was envisioned as a narrow cleft with two interacting parts, one for locking the sweet molecule and another for trigerring the nerve impulse. The same explanation for taste sensation of DHC and PD seems quite possible although its detail may be different. The adjacent hydroxyl and methoxy groups (O3′ and O4′) seem to be one of the essential functional entities as can be seen in the taste differences between naringenin and hesperetin and other derivatives.[16] The reason why the flavones are not sweet can be explained by the assumption that the structure itself is too rigid to undergo the induced conformational change to fit the local environment of the receptor site to elicite the sweet sensation. For the medium-sized sweeteners, a reasonable spatial separation between the functional groups and flexibility in the molecule approaching the receptor site seem to be required to trigger the sensation.

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