Food Chemistry **48** (1993) 267–270

Molecular mechanisms of sweet taste: IV. Sucrononic acid and a related derivative

Tetsuo Suami

Department of Chemistry, College of Science and Engineering, Meisei University, Hino, Tokyo 191, Japan

&

Leslie Hough

Department of Chemistry, King's College London, University of London, Campden Hill, London, UK, W8 7AH

(Received 14 October 1992; accepted 9 November 1992)

The interactions of sucrononic acid and *N*-[*N*-(*S*)-α-methylbenzylamino-(3,5-dichlorophenylimino)methyl]glycine with a helical proteinaceous receptor have been studied by computer graphics, and eight possible binding sites in sucrononic acid and seven sites in the latter sweetener revealed. The excellent fits between the stimuli and receptor molecules thus account for the exceptionally high intensity of these novel sweeteners, suggesting a model for future synthetic studies.

INTRODUCTION

The essential molecular feature of sweet tasting organic compounds has been recognised as a bifunctional entity of AH_s and B_s (Shallenberger & Acree, 1967, 1969) where the AH_s is a proton-donating group, such as NH_3^+, COOH, OH, NH and NH_2, and the B_s is a proton accepting group, such as COO^-, NH_2, C=O, NO_2 and CN. The existence of a third component, a hydrophobic site (X_s) in a sweet molecule, plays a dominant role in the intensification of the sensation (Kier, 1972). The required distance between AH_s and B_s is small (about 3·0 Å,) and the X_s site is usually located *c.* 3·5 and 5·5 Å apart from AH_s and B_s, respectively (Shallenberger & Acree, 1967; Kier, 1972). The glucophoric $AH_s/B_s/X_s$ triad interacts with a complimentary $AH_r/B_r/X_r$ system on the receptor to form two hydrogen bonds: $AH_s \ldots B_r$ and $B_s \ldots AH_r$, and a dispersion bond: $X_s \ldots X_r$ to complete a three-point coupling between a sweet compound and the receptor, thus initiating a sweet sensation (Suami & Hough, 1992).

The arrangement of the glucophoric $AH_s/B_s/X_s$ components of sweet chiral compounds is stereospecific, and to achieve the necessary interaction with the receptor, a clockwise arrangement of the $AH_s/B_s/X_s$ triad, when viewed from the receptor site, is required to allow the completion of the three-point coupling with a reciprocal $AH_r/B_r/X_r$ function on the chiral receptor (Hough & Khan, 1989; James *et al.*, 1989). In fact, molecular modellings of interactions between sweet compounds and a proteinaceous receptor helix have revealed that the clockwise arrangement of the glucophoric $AH_s/B_s/X_s$ triad can account for the perception of sweet taste in the D-amino acids (Suami & Hough, 1991), sugars (Suami & Hough, 1992) and the dipeptide methyl ester sweetener, aspartame (Suami & Hough, 1993). Our stereochemical studies on the interaction between a stimulus molecule and the receptor have now been extended to sucrononic acid, *N*-[*N*-cyclononylamino-(4-cyanophenylimino)methyl]glycine, which is the sweetest compound reported to date, with a sweetness that is 200 000 times that of sucrose, and *N*-[*N*-(*S*)-α-methylbenzylamino-(3,5-dichlorophenylimino)methyl]-glycine, with a sweetness that is 120 000 times that of sucrose (Nofre *et al.*, 1987). In addition to the normal $AH_s/B_s/X_s$ triad, a fourth binding site (D) was proposed for these two compounds by Tinti *et al.*, (1982), as a second proton-accepting component, by the suitable location of groups such as CN, NO_2 and Cl in the molecule. They assigned the NH(–phenylene), COO^-, cyclononyl and CN groups as AH_s, B_s, X_s and D, respectively, in the sucrononic acid molecule (Tinti & Nofre, 1991) (Fig. 1). Furthermore, four other interacting sites: *Y*, *X*H, E_1 and E_2, have been proposed as minor binding sites in these intensely sweet molecules, where the *Y*, E_1 and E_2 components are proton accepting groups, such as C=O or halogen atoms, and the *X*H is a proton-donating group, such as NH or OH.

Food Chemistry 0308-8146/93/$06.00 © 1993 Elsevier Science Publishers Ltd, England. Printed in Great Britain

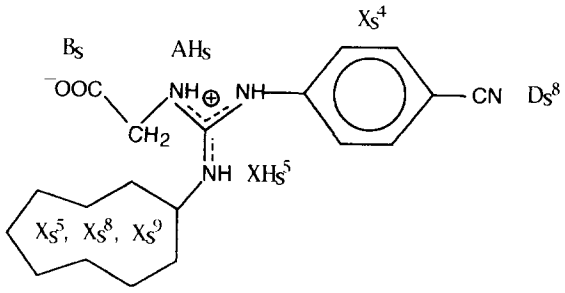

Fig. 1. Molecular formula of sucrononic acid.

RESULTS AND DISCUSSION

When we studied the interaction between sucrononic acid and the proteinaceous receptor helix (Suami & Hough, 1991) with computer graphics, using the conformation of sucrononic acid described by Tinti & Nofre (1991), a good fit was apparent with the following eight interaction or binding sites. However, the AH_s(NH–methylene) and $B_s(COO^-)$ were *c.* 3·0 Å apart, the normal distance, whilst the AH_s(NH–phenyl) and $B_s(COO^-)$ were wider (*c.* 3·4 Å); hence, the NH(–methylene) group has been assigned to the AH_s component and used in the present studies.

By constraining distances of two intermolecular hydrogen bonds: $AH_s(NH)\ldots B_r(C{=}O)$ and $B_s(COO^-)\ldots AH_r(NH_3^+)$, to be 2·90 (NH ... O) (Dean, 1987), favourable interactions between the receptor and each of sucrononic acid and *N*-[*N*-(*S*)-α-methylbenzylamino-(3,5-dichlorophenylimino)methyl] glycine were generated with energy minimisations by the molecular mechanics program CHARMm in QUANTA system (Brooks *et al.*, 1983). When the bifunctional entities, AH_s and B_s were connected with the respective B_r and AH_r components of the *N*-terminus of receptor by the two intermolecular hydrogen bonds, the bulky and hydrophobic cyclononyl group (X_s) then made significant interactions with hydrophobic sites of three side chains on the fifth (X_r^5), eighth (X_r^8) and ninth (X_r^9) amino acid residues, simultaneously. The cyano component (D_s) also made good contact with the side chain of the eighth amino acid residue (X_r^8). If this side chain has a proton donating activity or a dipole moment, the result will be a strong interaction between the D_s component and the X_r^8. Furthermore, the phenylene group was alongside the hydrophobic site situated on the fourth amino acid residue (X_r^4), so another dispersion bond is likely to be formed between them (X_s^4† ... X_r^4). The NH group that is adjacent to the cyclononyl moiety faced close to the X_r^5 side chain; with a proton-accepting ability or a dipole moment, as well as its primary hydrophobic character, this side chain would create yet another interaction (XH_s^5 ... X_r^5). Significantly, the dissymmetric arrangements of the four glucophoric $AH_s/B_s/X_s$ (with X_s^4, X_s^5, X_s^8 and X_s^9) tripartites are all clockwise orientations.

† The superscription indicates an orderly number of the amino acid residue of the receptor, counting from the *N*-terminus, which has a contact with the X_s component of the sweeteners.

The remaining NH group, that is adjacent to the phenylene group, and the CH_2 group, each project away from the receptor and, therefore, are not involved in any contact with the receptor. Thus, our computer graphic study, also supported by molecular modelling with Corey–Pauling–Koltun (CPK) molecular models, reveals that the sucrononic acid molecule has a maximum of eight possible interaction or binding sites, as shown in Fig. 1 and Plate 1, thus accounting for its intense sweetness. The additional five interaction sites: X_s^4(phenylene), X_s^8 and X_s^9(cyclononyl), D_s^8(CN) and XH_s^5(NH–cyclononyl) that we have noted, bind the sucrononic acid molecule tightly to the receptor, in support of the primary glucophoric AH_s(NH–methylene)/$B_s(COO^-)/X_s^5$(cyclononyl) tripartite system. The intimate coupling between the sucrononic acid molecule and the receptor, i.e. with all of the functional groups of the sucrononic acid, except the NH(–phenylene) and CH_2 groups, clearly plays an important role, the concerted interactions producing an intense sweetness sensation.

Analogous interactions of the potent sweetener *N*-[*N*-(*S*)-α-methylbenzylamino-(3,5-dichlorophenylimino)-methyl]glycine (Fig. 2) with the proteinaceous receptor have been studied. Four binding sites with the receptor: AH(NH–phenyl)/$B(COO^-)/X$(CHMePh)/D(Cl), were assigned by Tinti & Nofre (1991), but we have designated the NH(–methylene) group to the AH_s component for the same reason as described above for sucrononic acid. When the AH_s(NH–methylene) and B_s (COO^-) sites were connected with the B_r and AH_r components of the receptor, respectively, a good fit was again obtained with a normal distance of 3·0 Å between the AH_s and B_s, and the following seven interaction sites were significant. The CH_3 and C_6H_5 groups made close contacts with the X_r^5 and X_r^8, respectively, to give rise to two dispersion bonds, while the two chlorine substituents were alongside the X_r^4 and X_r^8 sites, thus giving rise to two dipole–dipole attractions (Cl ... X_r^4 and Cl ... X_r^8), if these side chains contain polar groups. The NH group (XH_s^5), vicinal to the α-methylbenzyl group, made contact with the X_r^5 site and it is likely to create yet another interaction, analogous to that suggested above for sucrononic acid. The remaining NH($-C_6H_3Cl_2$) and CH_2 groups projected away from the receptor; consequently they are not involved in any contact with the receptor. The steric arrangements of the two glucophoric $AH_s/B_s/X_s$ (with X_s^5 and

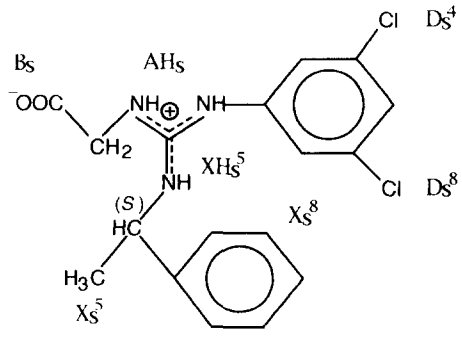

Fig. 2. Molecular formula of *N*-[*N*-(*S*)-α-methylbenzylamino-(3,5-dichlorophenylimino)methyl]glycine.

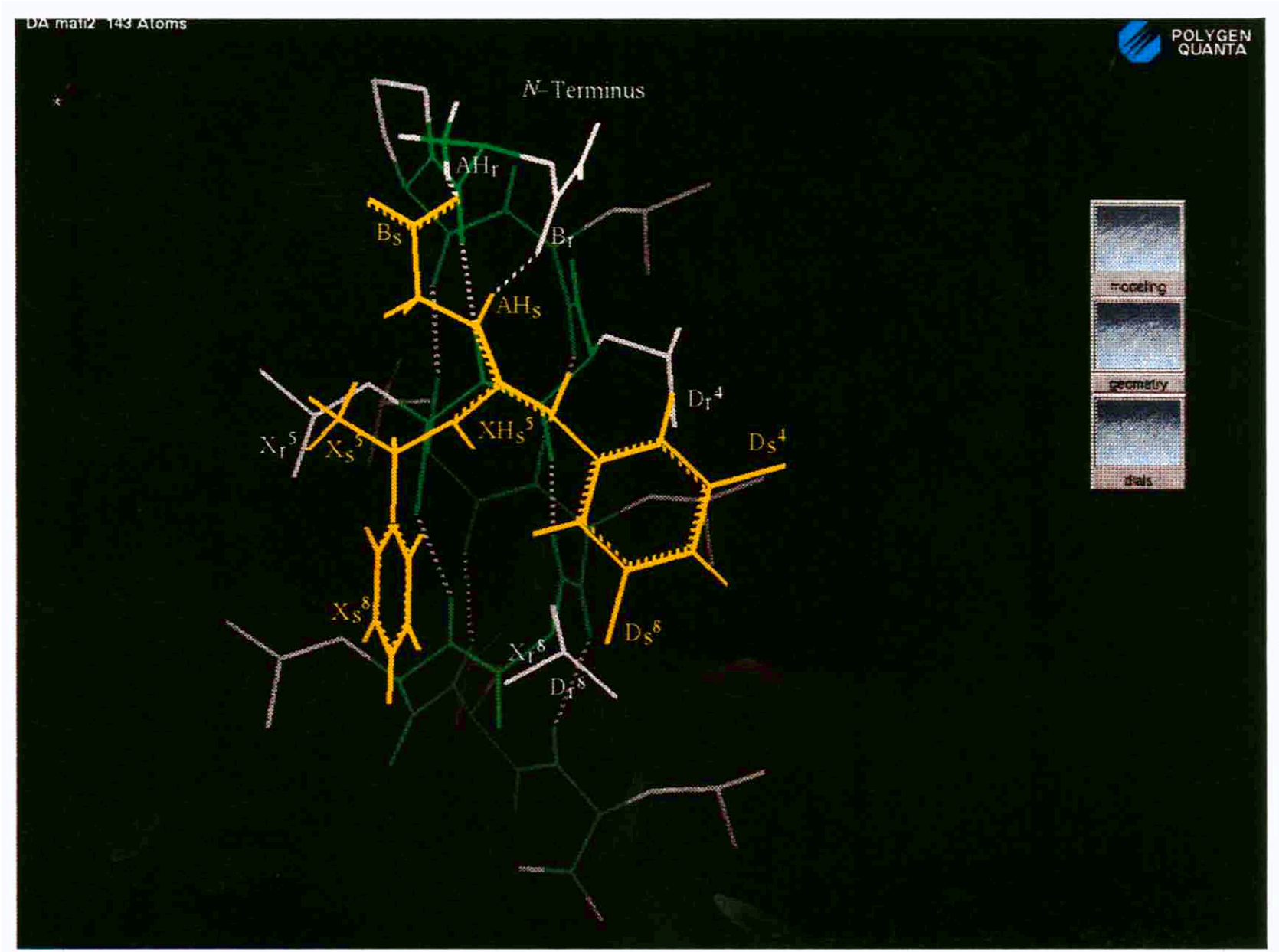

Plate 1. Interactions between sucrononic acid (yellow coloured) and the model of helical receptor protein. The α-Helix of the receptor is illustrated in green colour, the side chains of the protein (such as an isobutyl group) by the solid white lines, the hydrogen bondings by the white dotted lines, and the delocalisation of π-electrons by the yellow dotted lines.

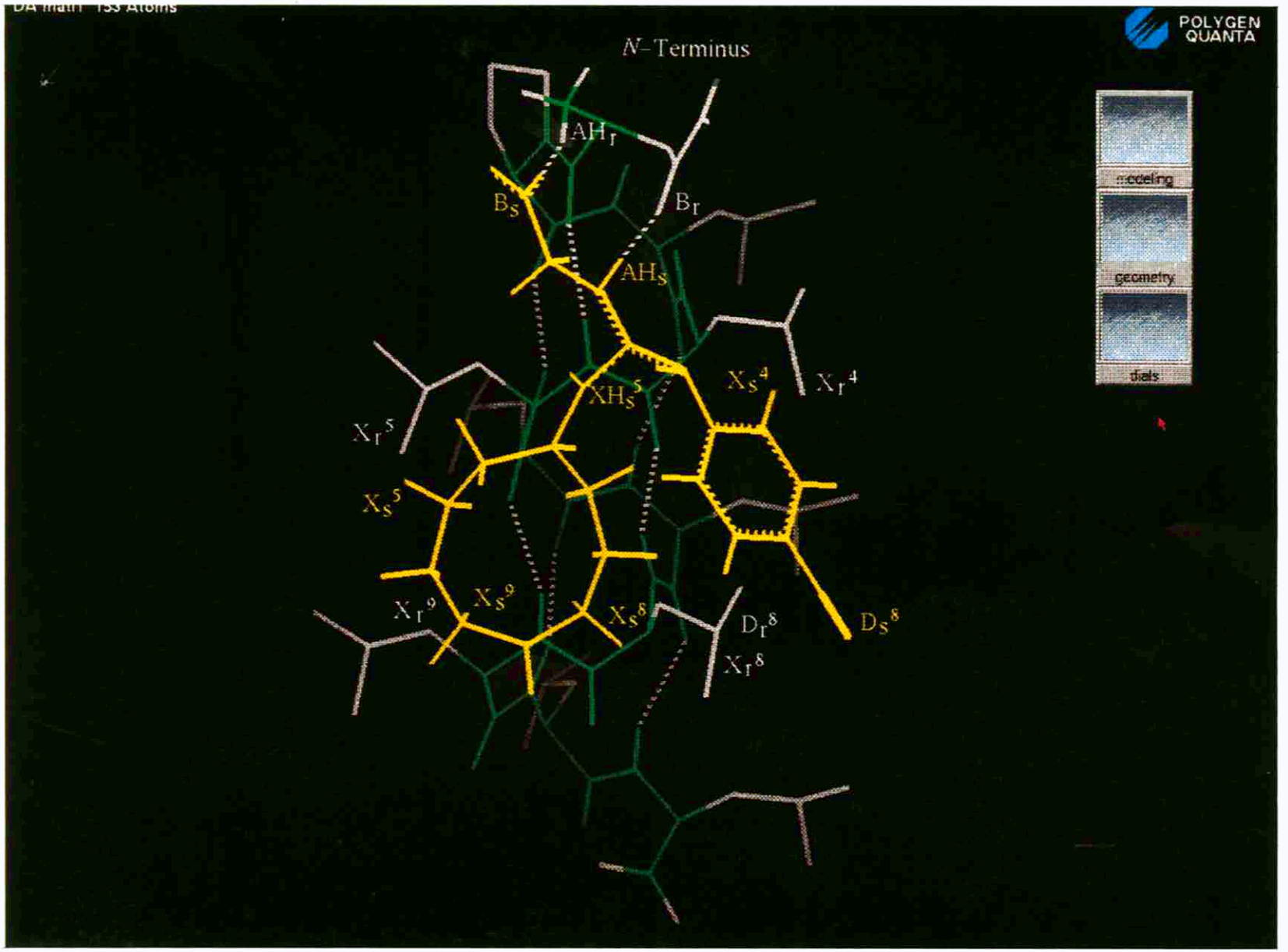

Plate 2. Interactions between *N*-[*N*-(*S*)-α-methylbenzylamino-(3,5-dichlorophenylimino)methyl]glycine (yellow coloured) and the mode of helical receptor protein. Colour illustrations are same as Plate 1.

X_s^8) triads are in the expected clockwise configurations. Therefore, there are seven possible interaction or binding sites in the molecule (Fig. 2 and Plate 2), which form the two intermolecular hydrogen bonds, two dispersion bonds and three dipole–dipole interactions (or hydrogen bonds) with the receptor. These multiple interactions account for strong binding of the stimulus molecule to the receptor, resulting in the perception of the intense sweetness sensation.

Tinti & Nofre concluded that the potency of a highly intense sweetener depends on the number of binding sites actively involved in the interaction with the receptor and on the effectiveness of each individual interaction. However, it is not necessary to have all the eight interaction sites, proposed by them, in the molecule to exhibit a sweet response, a lower number of sites often being sufficient to initiate a sweet taste (Tinti & Nofre, 1991). We have demonstrated that the sweetness potency in a sugar, such as glucopyranose, fructopyranose and sucrose, depends on the number of hydrophobic interaction sites of the sugar molecule involved in the interaction with the receptor (Suami & Hough, 1992). The analogous, but more extensive, results observed in the present studies of sucrononic acid (Fig. 1) and a related dichloro derivative (Fig. 2) confirm that the sweetness potency is dependent not only on the hydrophobic interactions, but also on the dipole–dipole attractions (or alternatively hydrogen bonds), as well as on a pair of the primary intermolecular hydrogen bonds. Therefore, the sweetness potency of a sweet compound is principally dependent upon the sum total of the strength of the various binding forces between the stimulus molecule and the receptor, and so, ultimately, it will be determined by how tightly a sweet molecule binds to the receptor. The synthesis of high intensity sweetness should in future take advantage of this model to assemble a range of groups that are suitably oriented to achieve maximum interaction and binding to the receptor, and eliminate the serendipity factor in these studies.

Approximate values of distances between the binding sites in sucrononic acid and *N*-[*N*-(*S*)-*α*-methylbenzylamino-(3,5-dichlorophenylimino)methyl]glycine have been determined by the computer analysis and are listed in Tables 1 and 2.

The distances were determined by measuring an interval between two sites, for AH_s and XH_s by the centre of the H atom of the NH group, for B_s by a middle point between the centres of two O atoms of the COO^- group, for D_s by the centre of the N or Cl atom of the CN or Cl group, and X_s was determined by the centre of the appropriate attaching point of the cyclononyl group with the receptor and by the centre of the benzene ring.

Table 1. Distances (in Å) between binding sites in sucrononic acid

	AH_s	B_s	$X_s^4(C_6H_4)$	X_s^5(c–*n*)[a]	X_s^8(c–*n*)	X_s^9(c–*n*)	D_s^8(CN)
XH_s^5	2·8	4·8	4·4	4·2	5·9	5·8	8·3
D_s^8	9·4	12·2	5·6	10·1	5·0	8·5	
X_s^9	8·6	10·0	8·3	3·3	3·5		
X_s^8	8·3	10·6	6·4	5·8			
X_s^5	6·7	7·3	8·1				
X_s^4	4·3	7·4					
B_s	3·1						

[a] (c—*n*) indicates (cyclononyl).

Table 2. Distances (in Å) between binding sites of *N*-[*N*-(*S*)-*α*-methylbenzlamino-(3,5-dichlorophenylimino)methyl]glycine

	AH_s	B_s	$X_s^5(CH_3)$	X_s^8(benzyl)	D_s^4(Cl)	D_s^8(Cl)
XH_s^5	3·4	5·7	3·7	5·2	6·7	5·1
D_s^8	8·0	10·5	7·7	5·7	5·4	
D_s^4	7·2	9·8	10·3	10·1		
X_s^8	8·3	10·1	4·4			
X_s^5	4·9	6·3				
B_s	2·9					

CONCLUSION

Interactions between each of the two intensely sweet compounds: sucrononic acid and *N*-[*N*-(*S*)-*α*-methylbenzylamino-(3,5-dichlorophenylimino)methyl]glycine, and a proteinaceous receptor helix have been examined by computer graphics. This study reveals that sucrononic acid has eight interaction sites: AH_s(NH–methylene), $B_s(COO^-)$, $X_s^4(C_6H_4)$, X_s^5, X_s^8 and X_s^9(cyclononyl), D_s^8(CN) and XH_s^5 (NH–cyclononyl), by completing four dispersion bonds, two dipole–dipole attractions (or hydrogen bonds), as well as the two principal intermolecular hydrogen bonds. The configurations of all four glucophoric $AH_s/B_s/X_s$ (with X_s^4, X_s^8 and X_s^9) triads are clockwise, thus satisfying the necessary steric requirement for sweetness. The eight binding sites of sucrononic acid combine in a concerted manner to produce the extreme sweetness sensation observed in its stimulation of the receptor protein.

The related derivative, *N*-[*N*-(*S*)-*α*-methylbenzylamino-(3,5-dichlorophenylimino)methyl]glycine showed seven binding sites: AH_s(NH–methylene), $B_s(COO^-)$, $X_s^5(CH_3)$, $X_s^8(C_6H_5)$, D_s^4(Cl), D_s^8(Cl), XH_s^5(NH–*α*-methylbenzyl), thus establishing the two primary intermolecular hydrogen bonds, two dispersion bonds and three dipole–dipole attractions (or hydrogen bonds) in its interaction with the receptor, which requires the clockwise configurations observed for the two glycophoric $AH_s/B_s/X_s$ (with X_s^5 and X_s^8) triads. The potency of a sweet compound is principally dependent on a sum total of the strength of binding forces between the stimulus compound and its receptor. The present studies provide a good model for a molecular design of intensely sweet sweeteners in future synthetic studies.

ACKNOWLEDGEMENTS

The authors with to express their thanks to Associate Professor Tomoya Machinami, Meisei University, for his faithful assistance, Dr Yasuo Takeuchi and Miss

Norie Ichihara, Pharmaceutical Research Center, Meiji Seika Kaisha Ltd, for their computer graphics, and Miss Hiromi Kanda and Mr Takayuki Aoki for their technical assistance.

REFERENCES

Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S. & Karplus, M. J. (1983). CHARMm: A program for macromolecular energy, minimization and dynamics calculations. *Comput. Chem.*, **4**, 187–217; QUANTA version 3.0 and CHARMM 21 supplied by Polygen Corp., Waltham, MA.

Dean, P. M. (1987). *Molecular foundations of drug-receptor interaction.* Cambridge University Press, Cambridge, England, p. 95.

Hough, L. & Khan, R. (1989). Enhancement of the sweetness of sucrose by conversion into chlorodeoxy derivatives. In *Progress in Sweeteners*, ed. T. H. Grenby. Elsevier Applied Science, London/New York, pp. 97–120.

James, C. J., Hough, L. & Khan, R. (1989). Sucrose and its derivatives. In *Progress in the Chemistry of Organic Natural Products*, ed. W. Herz, H. Grisebach, G. W. Kirby and Ch. Tamm. Springer-Verlag, Wien, Vol. 55, pp. 117–84.

Kier, L. B. (1972). A molecular theory of sweet taste. *J. Pharm Sci.*, **61**, 1394–7.

Nofre, C., Tinti, J. M. & Ouar Chatzopoulos, F. (1987). Agents édulcorants dérivés des acides *n*-phényl-guanidino-acétique et *n*-phénylethanamidinoacétique et compositions contenant de tels agents édulcorants. Eur. Pat. Appl. 0 241 395.

Shallenberger, R. S. & Acree, T. E. (1967). Molecular theory of sweet taste. *Nature*, **216**, 480–2.

Shallenberger, R. S. & Acree, T. E. (1969). Molecular structure and sweet taste. *J. Agric. Food Chem.*, **17**, 701–3.

Suami, T. & Hough, L. (1991). Molecular mechanisms of sweet taste. 1: Sweet and non-sweet tasting amino acids. *J. Carbohyd. Chem.*, **10**, 851–60.

Suami, T. & Hough, L. (1992). Molecular mechanisms of sweet taste. 2: Glucopyranose, fructopyranose and sucrose. *J. Carbohyd. Chem.*, **11**, 953–67.

Suami, T. & Hough, L. (1993). Molecular mechanisms of sweet taste. 3: Aspartame and its non-sweet isomers. *Food Chem.*, **46**, 235–8.

Tinti, J. M., Nofre, C. & Peytavi, A. M. (1982). Interaction of suosan with the sweet taste receptor. *Z. Lebensm.-Unters Forsch.*, **175**, 266–8.

Tinti, J. M. & Nofre, C. (1991). Why does a sweetener taste sweet? A novel model. In *Sweeteners, Discovery, Molecular Design, and Chemoreception*, ed. D. E. Walters, F. T. Orthoefer & G. DuBois. American Chemical Society, Washington, DC. *ACS Symp. Series*, **450**, 206–13.