In the Pursuit of a Better Sweetener

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Dipeptide sweeteners of the AspNHCH(R^1)COR type, where R^1 = H, Me, CH_2OH, and CO_2Me and R = OR^2 or NHR^2 with $R^2 \leq C_{10}$ atoms, i.e., alkyl, aryl, heterocyclic, bi- or tricyclic group, were synthesized to remedy the well-known shortcomings of aspartame. For the design of these sweeteners, the classic theory of Cohn–Oertly–Myers, Ariyoshi's rule of steric size difference, Newman's rule of H-6 number as well as our C_9 rule and a model of induced fitting of multiple-point attachment with the sweet receptor were employed. On the basis of our results as well as those of others, qualitative and semiquantitative structure–activity relationships were discussed according to the current concepts of polar–steric–hydrophobic factor analyses for the pursuit of an ideal high sweetness potency dipeptide sweetener. Compounds of high sweetness potency prepared in this work are all of high stabilities and indifferent to microorganisms or any known genetic diseases.

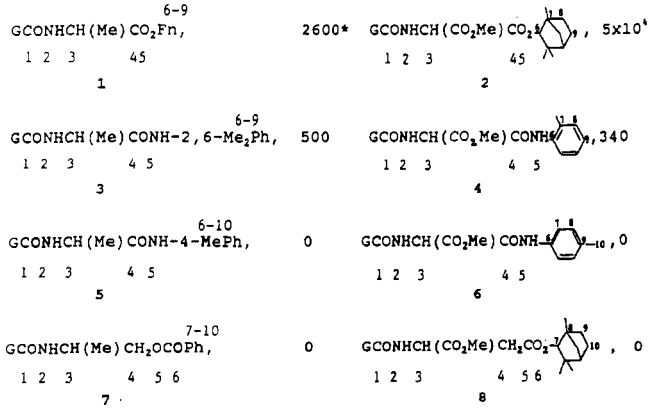
INTRODUCTION

An ideal sweetener should be of a clean sugar taste, harmless, healthful, strong in sweetness potency, versatile in its applicability, and economically profitable. Since saccharine was first synthesized in 1879, no ideal synthetic sweetener has ever appeared of the above calibre. Aspartame is a methyl ester of aspartylphenylalanine (APM for short), which is a synthetic product formally legalized by the FDA of the United States in dry uses (1981) and in soft drinks (1983). It is being sold widely as the fashion of today. However, it still has inherent drawbacks to be remedied. It is definitely unsuitable for phenylketonurics. There were also a number of unfavorable reports about its toxicity in the medical circle. For this reason the U.S. FDA approved it in 1974 and suspended it in 1975. As it is a methyl ester, its chemical stabilities toward heat, acids, and bases are limited. It can be used only under mild conditions of low temperatures at pH 3–5. The shelf life of its products is rather short. Although it has the merit of being delicious, the strength of its sweetness is merely 150–170 times that of sucrose, and thus it is still relatively expensive. As a result, its scope of acceptability and applicability is rather limited.

Early in 1974 we suggested replacing the phenylalanine moiety with glycine, alanine, or another cheap protein amino acid from which no genetic disease was found (Tseng et al., 1986). The merits of the first two amino acids are as follows: Both are of low cost and low toxicity (LD_{50} ca. 3–5 g/kg in mouse). Nonketotic hyperglycinemia appears to be a rare autosomal recessive condition, most instances of which have been sporadic. Besides, the amount of glycine that is synthesized in a subject's body is usually 10 times that taken from his food; i.e., the amount taken from a sweetener is negligibly small. D-Alanine and its L enantiomer are equivalent in nutritional value as well as caloriewise if the sweetener is at all digested and, if not, the sweetener is of no calorie value. Both alanine and aspartic acid can enter the tricarboxylic acid cycle and no genetic diseases have ever been found. Dipeptide of D-alanine is resistant to microbial degradation. Esters and amides of L-aspartylglycine and D-alanine are sweeter when made from higher alcohols and amines than are those made from lower ones in contrast to esters of L-aspartyl-L-phenylalanine. It is well-known that higher order alcohols form much more stable esters than methanol. Unlike the bitter L-phenylalanine, both glycine and D-alanine are sweet themselves and the byproducts and degradation products of their sweet dipeptide esters such β-aspartyl esters, racemized diastereomers, and diketopiperazines should be nonbitter by Ney's Q rule as their component amino acids are of low hydrophobicity, i.e., $Q < 1300$ (Ney, 1979). The contrary is true for APM; only its methyl ester is of a strong sweet taste and its higher esters are not, while its amides are bitter and so are its β-aspartyl ester and diastereomers. With these ideas in mind we have synthesized the three series of aspartyl dipeptide compounds of glycine, D-alanine, and D,L-serine except those of aminomalonic acid (Liu et al., 1982) as shown in Table I (Zeng et al., 1986).

RESULTS AND DISCUSSION

Following the tradition of Cohn–Oertly–Myers (Moncrieff, 1967), the glucophore of this type of dipeptide sweetener is taken to be its polar head moiety G = (–O_2-$CCH_2CHNH_3^+$) and the auxogluc is the rest of its nonpolar tail part A = (–CONHCHRR^1). According to Ariyoshi's rule, to be sweet, R must be larger than R^1 in size (Ariyoshi, 1976). According to our C_9 rule (Tseng and He, 1987), the maximal sweetness strength of a potential sweetener can be attained at an auxogluc chain length that is equivalent to ca. C_9, which is counted from the carbonyl carbon atom to the end of R, which then may have a chain length of R = C_6:



(All figures in this paper indicate multiples of sweetness to that of a 3% sucrose solution as a standard of 1.0 unless indicated otherwise. Fn = Fenchyl.) Of course, the explanations here are just rationales of empiricisms.

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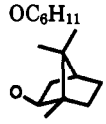
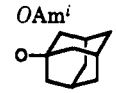
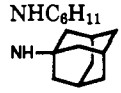
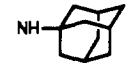
Table I. Summary of Relative Sweetness of Compounds L-AspNHCH(R^1)COR Synthesized in This Work[a] (Sucrose = 1 on Weight Basis)

R^1	R	times	R^1 (D)	R	times	R^1 (DL)	R	times
H	OMe	8	Me	OPr^i	70	CH_2OH	NH-*o*-MePh	40
H	OEt	13	Me	OBu^i	87	CH_2OH	NH-*m*-MePH	0
H	OPr	14	Me	OBu^s	45	CH_2OH	NH-*p*-MePh	0
H	OPr^i	20	Me	OBu^t	240	CH_2OH	NHPh	20
H	OBu^t	30	Me	OCHMeBu^i	30	CH_2OH	NH-*o*-MeOPh	15
H	OC_6H_{11}	16	Me	OC_6H_{11}	70	CH_2OH	NH-*o*-CO_2MePh	0
H	*O*-(+)-α-Fn[b]	0	Me		33	CO_2Me	*O*Pr^i	1–2
H	*O*-(−)-β-Fn	0	Me	*O*-(+)-α-Fn	0	CO_2Me	*O*Bu^i	310
H	*O*-(−)-α-Fn	60	Me	*O*-(−)-β-Fn	180	CO_2Me	*O*Bu^t	68
H	*O*-(+)-β-Fn	600	Me	*O*-(−)-α-Fn	600	CO_2Me	*O*Am^i	500
H	NH-*o*-MePh	0	Me	*O*-(+)-β-Fn	2600	CO_2Me		1000
H	NH-*m*-MePh	0	Me	2,6-$Me_2C_6H_9O$	400	CO_2Me		760
H	NH-*p*-MePh	0	Me	2,6-Me_2PhO	150	CO_2Me	*O*-(+)-α-Fn	1000
H	NHPh	0	Me	NHPr	72	CO_2Me	*O*-(−)-β-Fn	5000
H	NH-*o*-OMePh	0	Me	NHC_6H_{11}	100	CO_2Me	*O*-(−)-α-Fn	30000
H	Nh-*o*-CO_2MePh	0	Me		43	CO_2Me	*O*-(+)-β-Fn	50000
H	NH-*o*-ClPh	0	Me	NH-(−)-β-Fn	150	CO_2Me	NHBu^s	15
H	NH-*o*-BrPh	0	Me		50	CO_2Me		0
			Me	NHPh	38	CO_2Me	NHPh	150
			Me	NH-*o*-MePh	75	CO_2Me	NMePh	0
			Me	NH-*m*-MePh	0	CO_2Me	NH-*o*-MePh	340
			Me	NH-*p*-MePh	0	CO_2Me	NH-*m*-MePh	80
			Me	NH-*o*-MeOPh	30	CO_2Me	NH-*p*-MePh	0
			Me	NH-*o*-ClPh	60	CO_2Me	NH-*o*-OMePh	71
			Me	NH-*o*-BrPh	60	CO_2Me	NH-*o*-ClPh	22
			Me	NH-*o*-CO_2MePh	0	CO_2Me	NH-*o*-BrPh	17
			Me	NH-2,6-Me_2Ph	500	CO_2Me	NH-*o*-NO_2Ph	0

[a] With the exception of the reference of Liu et al. (1982). [b] Fn, fenchyl.

An electron-releasing substituent on the structure of R increases its sweetness strength, while an electron-withdrawing substituent decreases the strength (Tseng and He, 1987). The reverse is true for the small R^1 group. A semiquantitative relationship can be found for the electronic effects on R and R^1 separately. For a constant R, the sweetness of R^1 = CO_2Me > CO_2Et > CH_2OH > CHMeOH > Me > Et > *i*-Pr.

When R^1 = CO_2Me, R = *o*-XC_6H_4NHCO, where X = Me, 340; X = H, 150; X = OMe, 71; X = Cl, 22; X = Br, 17; X = NO_2, 0.

$$\log X_{sw} = -2.715\sigma_m^- + 2.239,\ r = 0.99,\ s = 0.09,\ n = 5$$

Judging from the above findings, the association of an auxogluc with its receptor site cannot be limited to the hydrophobic bonding alone excluding other polar interactions. This is the reason we advanced a model of induced fitting with multiple-point attachment (Tseng and Wei, 1980). However, the characteristics of a hydrophobic auxogluc are still outstanding. For example, so far as the sweetness strength of various R groups of equal carbon atoms is concerned, the sweetness order is as follows: a straight chain < a branched chain, an acyclic chain < a cyclic chain, an aromatic group < an aliphatic group; e.g., for R′ = CO_2Me, R = Am, 50; *i*-Am, 80; Hex, 70; *c*-Hex, 100; CH_2Ph, 150; CH_2-*c*-Hex, 255 (Mazur et al., 1973). Moreover, our C_9 rule can also be considered as one of the hydrophobic effects.

Adjacent branching on an acyclic or cyclic chain of R in the standard structure not only strengthens the stability of the dipeptide by Newman's rule of six (Newman, 1956) but also increases its sweetness capacity; e.g., when R^1 ≡ Me, R = CONHBu, 100; CONHCH(*i*-Pr)$_2$, 250; CONHCH(*t*-Bu)$_2$, 450; CONH–2,5-Me_2–*c*-Am, 520; CONH–2,2,5,5-Me_4–*c*-Am, 800. Branching in trans position is better than that in cis form. This effect seems to be one of space filling. It also is in line with a favorable electron-donating effect on R. Besides, an induced fit of the sweetener with its receptor is not as good as a rigid key to fit its lock for taste stimulation since a flexible R group can only trigger a weak sweet taste. The R^1 group should be kept smaller than R. For R ≡ CONHCH(*c*-Pr)$_2$, R^1 = Me, 2000; Et, 500; *i*-Pr, 100 (Brennan and Hendrick, 1982). The R^1 group should not be too small either. For R ≡ β-(+)-OFn, R^1 = CO_2Me, 5×10^4; R^1 = Me, 2.6×10^3; R^1 = H, 6×10^2, which is, however, the sweetest one ever found in the glycine series (Tseng et al., 1986).

EXPERIMENTAL PROCEDURES

Elemental analyses were done by our Analytic Laboratory. All intermediates and products were controlled by TLC on silica. Fully protected compounds were run in a mixture of PhH–MeOAc–MeOH = 10:2:2. Spots were detected by first bathing in Cl_2 gas for 5 min and then treating with an aqueous solution of KI plus starch. Unprotected compounds were developed in MeOH–$CHCl_3$-glacial acetic acid = 4:2:1 or 1-BuOH–MeOH–glacial acetic acid = 4:1:1 and were spotted by warm-air heating after being sprayed with an alcoholic solution of ninhydrin.

The products were tasted by three to six co-workers in our laboratory. Threshold values of sucrose for each one of the panel were determined with 1, 2, 3, 4, and 5%, etc. aqueous solutions

of sucrose, and an average value (ca. 3%) was then taken as a standard one for comparison by this panel. Likewise, those of unknown sweeteners for each panelist were determined with 0.006, 0.008, 0.01, 0.02, 0.04, 0.06, 0.08%, etc. aqueous solutions, and an average value was then taken to divide that of the standard. The resultant value is regarded as the multiple of sweetness capacity of the unknown.

Most of our products were prepared by classical methods of peptide synthesis, i.e., mixed anhydride, activated ester, or carbodiimide methods. We have also explored industrial methods of employing the inner anhydride of aspartic acid with the amino group protected by carbobenzoxyl chloride (z for short) or a formyl group for the more promising dipeptide sweeteners. A few examples are presented as follows.

Z-D-AlaOH. NaOH (4 N, 28 mL, 112 mM) was cooled to −5 °C with dry ice bath in a three-neck flask equipped with mechanical stirrer, thermometer, and two separatory funnels. With continuous stirring, D-AlaOH (10 g, 112 mM) was added into the flask, 4 N NaOH (33 mL, 132 mM) and CbzCl (22 mM) in two separate dropping funnels were added dropwise alternately in ca. 0.5 h, and the reaction solution was kept alkaline. The reaction continued for 2 h more. It was extracted with 2 × 20 mL of ethyl ether to remove the unreacted CbzCl. The aqueous layer was carefully acidified under cooling to pH 2 with 5 N HCl. The product was extracted with 5 × 20 mL of EtOAc, the solution of which was dried with anhydrous Na_2SO_4 and then filtered. The filtrate was evaporated under reduced pressure with a rotatory evaporator. The syrup obtained was dissolved in 10 mL of ether, petroleum ether was dropped in until slightly cloudy, and the mixture was stored in a refrigerator overnight. White crystalline product (19.24 g, 85 mM) was obtained: yield 78.4%.

Z-D-Ala-NH-2-MePh. Z-D-AlaOH (1.69 g, 7.6 mM) and dry ether (60 mL) were added into a 250-mL three-neck flask which was cooled to −5 °C with dry ice bath. Phosphorus pentachloride (0.8 g, 8.0 mM) was then added under constant stirring until it was completely dissolved. Precooled *o*-toluidine (0.8 g, 8.0 mM) and triethylamine (4 mL) in 30 mL of dry ether were added. White precipitate appeared immediately. It was stirred at −5 °C for 3 h and then filtered. The white residue was extracted repeatedly with 4 × 30 mL of hot EtOAc. The ethereal filtrate and the EtOAc extracts were combined and washed with 2 × 30 mL of distilled water, 30% aqueous $NaHCO_3$ solution, and 2 × 30 mL of distilled water again. The organic layer was dried overnight over anhydrous Na_2SO_4 which was filtered off later, and the filtrate was evaporated under reduced pressure. The white solid obtained was recrystallized with 15 mL of EtOAc and the yield (1.5 g, 4.8 mM) was 63%.

GlyO-(+)-β-Fn. Z-GlyOH (1.335 g, 6.4 mM) and (+)-β-FnOH (1.53 g, 10 mM) were refluxed in 55 mL of benzene with TsOH (0.12 g, 0.63 mM) as a catalyst for 18.5 h. It was filtered after cooling. The filtrate was washed with saturated $NaHCO_3$ (2 × 20 mL), dried with Na_2SO_4, and then filtered. The filtrate was evaporated to the crude product (2.510 g), which was dissolved in 40 mL of dried methanol. The solution was hydrogenolyzed with 250 mg of Pd black for 3.5 h. It was filtered and evaporated to a crude product, which was chromatographed with silica gel (100 g, 200–300 mesh) and eluted separately with EtOAc: petroleum = 1:4 (700 mL), 1:1 (2000 mL). The pure product was collected from the 1:1 eluent, yield 1.440 g, (75.3%): $[\alpha]^{30}_D$ −12.2° (EtOH, *c* 2.8).

L-Asp-Gly-(+)-β-OFn. Gly-(+)-β-OFn (1.240 g, 4.14 mM) and β-Bzl-N-Z-L-AspOH (2.066 g, 5.90 mM) were dissolved in CH_2Cl_2 (40 mL), into which was added DCCI (1.499 g, 5.90 mM), and the solution was kept stirring for 5 h. It was then filtered. The filtrate was washed consecutively with 2 × 20 mL of 3% $NaHCO_3$, 2 × 20 mL of 0.5 N HCl, and 3 × 20 mL of H_2O. It was dried with Na_2SO_4, filtered, and evaporated to a syrup (3.305 g), from which 3.020 g was dissolved in methanol and hydrogenolyzed with 250 mg of Pd black for 3 h. It was again filtered and evaporated to a crude product (1.930 g), from which 0.895 g was chromatographed with a mixture of EtOAc and MeOH as the eluent. A colorless pure product [0.470 g (82.1%)], was obtained: mp 125–126 °C; $[\alpha]^{26}_D$ −15.5° (HOAc:H_2O = 1:1, *c* 3.9); MS 327 (M + 1), 173, 137 (100%), 81.

Note: Some of our products are extremely hygroscopic.

CONCLUDING REMARKS

The whole series of works indeed indicate that the shape of the receptor of these dipeptide sweeteners can be depicted as a gourd-shaped pocket; the upper compartment contains the aspartic glucophoric group open to the surface, while the lower one, which is limited in its depth, accommodates the other C-end amino acid derivative (Lelj et al., 1976). Aside from the peptide amino group, the other three groups attached to the α-carbon atom of the latter amino acid must be arranged by size from large, medium, to small clockwise and, aside from the large R group, those of the other two substituents can be of an equal size but not larger than that of a chain of two carbon atoms; then the sweetness of this compound will then be weaker than those compounds with the three groups of differential sizes (Tsang et al., 1984). Therefore, our glycine series of compounds is always much less sweet than those of our alanine and other series.

Among the compounds of our glycine, D-alanine, and D,L-serine series, there are quite a number of them having sweetnesses from 1 to 20 times that of aspartame. Nevertheless, a high sweetness potency (inter alia) is not the sole requirement of an ideal sweetener. in conformity with Newman's rule of H-6 number and our C_9 rule, compounds of high sweetness potency prepared in our work are all of high stabilities and indifferent to microorganisms or any known genetic diseases (Pavlova et al., 1981).

Our work (Zeng et al., 1986) was partly covered by a patent of General Foods Co. (Zanno et al., 1986) in which no numerical data of the covered compounds were disclosed. Tagasago Co. (Nagakura et al., 1986) disclosed their similar discoveries in detail, and Dr. Nagakura sent a gracious letter to the senior author of this paper that they were inspired by the publications of our previous systematic work on dipeptide sweeteners including all four diastereomeric esters of fenchyl alcohol. We, in turn, were encouraged by the patent of Takeda Co. (Fujino et al., 1974) wherein the highly potent sweetener of the (−)-α-fenchyl and methyl diester of L-aspartyl-D,L-aminomalonic acid was first synthesized.

Supplementary Material Available: Data sheets of structural formulas of compounds synthesized including elemental analysis, melting points, and optical rotations (10 pages). Ordering information is given on any current masthead page.

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