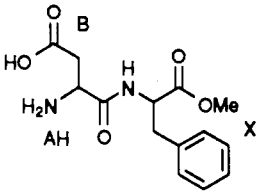
Structural Correlation between Some Amides and a Taste Receptor Model

Masahiro Tamura, Ichizo Shinoda, Hideo Okai,* and Charles H. Stammer*

Several α-aspartic acid amides and ω-amino acid amides were prepared to investigate their structural relationships to a model of the taste receptor. All of the α-aspartic acid amides produced a sweet taste and all of the ω-amino acid amides produced a bitter taste even though some of them had chains expected to be too long to produce either taste. There is apparently a strong affinity between certain functional groups of a sweet or bitter compound and special "affinity sites" on the taste receptor responsible for the kind of taste produced.

Aspartame (1), the well-known peptide sweetener, is the L-aspartyl-L-phenylalanine methyl ester and has a sucrose-like taste 150–200 times that of sucrose. Since the



1

discovery of aspartame, numerous research groups have tried to synthesize more potent peptides but very few compounds have been discovered with both the sucrose-like taste and a greater sweetness potency. Research in this area has lead to the development of some important information about the "sweetness receptor". An electronegative function (CO_2^-) designated B, a positive group (NH_3^+) called AH, and a neutral hydrophobic function labeled X appear to be necessary on the sweet molecule. Analogues of aspartame in which the aspartic acid moiety was replaced by other amino acids were found to be bitter (Mazur et al., 1969). Many other research groups attempted to replace the aspartic acid moiety of aspartame and found that only aminomalonic acid could replace aspartic acid with retention of the sweet taste (Fujino et al., 1973, 1976). Because of those results, it is now believed that the three functional groups, AH, B, and X, are required *at the proper positions* on the receptor to produce a sweet taste (Figure 1). When the hydrophobic group is varied, a greater or lesser sweetness potency is produced and its distance from the other important groups must be within somewhat more variable limits.

Recently, we reported the synthesis and taste of *N*-L-aspartyl-1-aminocyclopropanecarboxylic acid esters (Asp-Acc-O-*n*-Pr; Mapelli et al., 1987). In that structure, the aspartic acid moiety carries the AH and B functional groups and the ester at the peptide C-terminus is the hydrophobic (X) functional group. All of the esters prepared were sweet except the benzyl ester (Asp-Acc-OBzl, 2), which was tasteless. This result can be rationalized as in Figure 2, since Asp-Acc-OBzl is apparently too long to fit the receptor sites corresponding to AH, B, and X. Thus, there appears to be a limit to the chain length of aspartic acid sweeteners.

In this paper, we will discuss the structural relationship between the A′, B′, X′ model of the sweet taste receptor and the taste of compounds in which the aspartic β-carboxyl group B (Table I), the electropositive group AH, and the hydrophobic function X are missing from the above AH, B, X system (see Tables I and III).

Department of Chemistry, School of Chemical Sciences, University of Georgia, Athens, Georgia 30602 (M.T., C.H.S.), and Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Higashi-hiroshima, Hiroshima 724, Japan (I.S., H.O.).

Table I. Aspartic Acid Amides

3	H-Asp-$NHCH_2CH_2CH_2CH_3$
4	H-Asp-$NHCH_2CH_2CH_2CH_2CH_3$
5	H-Asp-$NHCH_2CH_2CH_2CH_2CH_2CH_3$
6	H-Asp-$NHCH_2CH_2CH_2CH_2CH_2CH_2CH_3$
7	H-Asp-$NHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
8	H-Asp-$NHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
9	H-Asp-$NHCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
10	H-Asp-$NHCH_2C_6H_5$
11	H-Asp-$NHCH_2CH_2C_6H_5$
12	H-Asp-$NHCH_2CH_2CH_2C_6H_5$
13	H-Asp-$NHCH_2CH_2CH_2CH_2C_6H_5$

Table II. ω-Amino Acid Amides

14	$NH_2CH_2CONHC_6H_5$
15	$NH_2CH_2CH_2CONHC_6H_5$
16	$NH_2CH_2CH_2CH_2CONHC_6H_5$
17	$NH_2CH_2CH_2CH_2CH_2CONHC_6H_5$
18	$NH_2CH_2CH_2CH_2CH_2CH_2CONHC_6H_5$
19	$NH_2CH_2CONHC_6H_{11}$
20	$NH_2CH_2CH_2CONHC_6H_{11}$
21	$NH_2CH_2CH_2CH_2CONHC_6H_{11}$
22	$NH_2CH_2CH_2CH_2CH_2CONHC_6H_{11}$
23	$NH_2CH_2CH_2CH_2CH_2CH_2CONHC_6H_{11}$

Table III. *N*-Benzoyl-ω-amino Acids

24	$C_6H_5CONHCH_2COOH$
25	$C_6H_5CONHCH_2CH_2COOH$
26	$C_6H_5CONHCH_2CH_2CH_2COOH$
27	$C_6H_5CONHCH_2CH_2CH_2CH_2COOH$
28	$C_6H_5CONHCH_2CH_2CH_2CH_2CH_2COOH$

MATERIALS AND METHODS

Materials. *N-tert*-Butoxycarbonyl-L-aspartic acid *tert*-butyl ester was purchased from Bachem Biochemical Co. All amines used in the aspartic acid amides were purchased from Aldrich Chemical Co. ω-Amino acids, cyclohexylamine, and aniline were purchased from Nakarai Chemical Co. Ltd. Ethyl chloroformate and 4-methylmorpholine were purchased from Aldrich and Nakarai. All solvents were used without further purification or drying except THF, which was distilled from potassium metal.

Methods. (*1*) *Sensory Tests.* The tastes of the amides were organoleptically evaluated by a panel of four people. A series of solutions of decreasing concentration was prepared in which each solution was half as strong as the preceding one. Before the sample was tasted, the mouth was thoroughly rinsed with deionized water. The sample size, usually 2–3 mL, was held in the mouth for about 10 s and then expectorated. Each sample was evaluated to determine which concentration of sweetener was isosweet with a 2% sucrose solution.

In the case of bitter compounds, the threshold value of the sample was determined. The bitterness intensity was determined by dividing the threshold value of the sample into that of caffeine

0021-8561/89/1437-0737$01.50/0

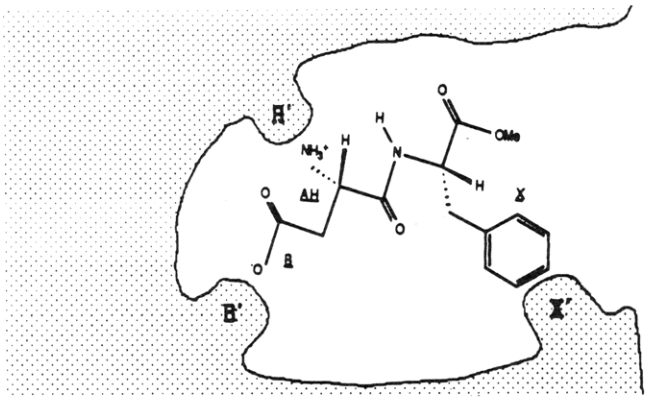


Figure 1. Receptor site holding aspartame: A′, the receptor site corresponding to the electropositive group AH; B′, the receptor site corresponding to the electronegative group B; X′, the receptor site corresponding to hydrophobic group X. All three functional groups are able to reach all other corresponding receptor site groups A′, B′, and X′.

Table IV. Analytical Data of Aspartic Acid Amides

compd	yield,[a] %	yield,[b] %	mp, °C dec	$[\alpha]_D$, deg (c 2, MeOH)	formula
3	82	78	195–196	−2	$C_8H_{16}N_2O_3 \cdot H_2O$
4	84	77	196–197	−1	$C_9H_{18}N_2O_3 \cdot H_2O$
5	80	76	199–203	−3	$C_{10}H_{20}N_2O_3$
6	85	74	193–195	−2	$C_{11}H_{22}N_2O_3$
7	82	77	191–193	−2	$C_{12}H_{24}N_2O_3 \cdot H_2O$
8	80	75	193–195	+4[c]	$C_{13}H_{26}N_2O_3 \cdot H_2O$
9	82	74	189–191	+4[c]	$C_{14}H_{28}N_2O_3 \cdot {}^1/_{10}H_2O$
10	80	78	188–191	+5[c]	$C_{11}H_{14}N_2O_3 \cdot {}^1/_3H_2O$
11	85	76	204–206	−16[d]	$C_{12}H_{16}N_2O_3$
12	81	77	187–188	−1	$C_{13}H_{18}N_2O_3$
13	80	75	194–195	+4[c]	$C_{14}H_{20}N_2O_3 \cdot {}^3/_{10}H_2O$

[a] Yields of the *N-tert*-butoxycarbonyl-L-aspartic acid *tert*-butyl ester amides. [b] Yields of the zwitterions. [c] Optical rotation was measured as trifluoroacetate. [d] Optical rotation was measured in MeOH/TFA (1:1, v/v).

(1 mM concentration). For example, if the sample has a threshold value of 2 mM, the bitter taste potency of this sample is 0.5.

(2) *Synthesis of Amides.* (*a*) *General Procedures.* Melting points are uncorrected. Optical rotations were measured in a 0.1-dm tube with a Union Model PM-101 polarimeter. All evaporations were conducted in vacuo. Samples for elemental analysis were previously dried at 60 °C for 6 h over phophorus pentoxide in vacuo, and the analyses were done by Atlantic Microlab, Atlanta, GA.

(*b*) *Preparation of Aspartic Acid Amides.* To an ice–salt-cooled solution of *N-tert*-butoxycarbonyl-L-aspartic acid *tert*-butyl ester (2.573 g, 10 mmol) and 4-methylmorpholine (1.1 mL, 10 mmol) in THF (50 mL) was added ethyl chloroformate (1.0 mL, 10 mmol). The mixture was stirred at −15 °C for 15 min, and a solution of the amine (10 mmol) in $CHCl_3$ (10 mL) was added. The mixture was stirred at −15 °C for 30 min and then at room temperature overnight. After evaporation, the resulting residue was dissolved in ethyl acetate, washed with 4% citric acid, 4% $NaHCO_3$, and water, dried over anhydrous Na_2SO_4, and evaporated. The resulting oily residue was dissolved in CH_2Cl_2 (30 mL), and TFA (30 mL) was added at 0 °C. The solution was allowed to stand at room temperature for 1.5 h and evaporated; ether was added to precipitate a white powder that was filtered and dissolved in a minimum volume of water, and the pH was brought up to 5.0 and 2 N NH_4OH. The precipitate was collected and recrystallized from hot water. See Table IV for physical constants.

(*c*) *Preparation of ω-Amino Acid Amides.* To an ice–salt-cooled solution of *N-tert*-butoxycarbonyl-ω-amino acid (10 mmol) and 4-methylmorpholine (1.1 mL, 10 mmol) in THF (50 mL) was added ethyl chloroformate (1.0 mL, 10 mmol). The mixture was stirred at −15 °C for 15 min, and a solution of aniline or cyclohexylamine (10 mmol) in $CHCl_3$ (10 mL) was added. The reaction mixture was stirred at a −15 °C for 30 min and then at room temperature overnight and was evaporated. The residue was dissolved in ethyl acetate, washed with 4% citric acid, 4% $NaHCO_3$, and water, dried over anhydrous Na_2SO_4, and evaporated. The residue was dissolved in dioxane (20 mL), and 4.0 M HCl/dioxane (5 mL) was added. The solution was allowed to stand at room temperature for 4 h and evaporated. The oily residue was solidified by adding ether, and the solid was crystallized from methanol/ether. See Table V for physical constants.

(*d*) *Preparation of N-Benzoyl-ω-amino Acids.* To a solution of ω-amino acid (10 mmol) and $NaHCO_3$ (1.51 g, 18 mmol) in water (50 mL) was added benzoyl chloride (6 mmol). The mixture was stirred at room temperature for 3 h; second portions of $NaHCO_3$ (0.50 g, 6 mmol) and benzoyl chloride (6 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was washed with ether, acidified with 6 M HCl (pH 2), and extracted with ethyl acetate (2 × 100 mL). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The product was solidified by the addition of hexanes and crystallized from ethyl acetate/hexanes. See Table VI for physical constants.

Table V. Analytical Data of ω-Amino Acid Amides

compd	yield,[a] %	yield,[b] %	mp, °C	formula
14	84	93	200	$C_8H_{11}N_2OCl$
15	83	95	199–200	$C_9H_{13}N_2OCl$
16	85	93	142–144	$C_{10}H_{15}N_2OCl$
17	87	94	169–170	$C_{11}H_{17}N_2OCl$
18	86	92	172–173	$C_{12}H_{19}N_2OCl$
19	83	93	201–205	$C_8H_{17}N_2OCl$
20	85	91	185–186	$C_9H_{19}N_2OCl$
21	84	94	114–115	$C_{10}H_{21}N_2OCl$
22	82	92	190–192	$C_{11}H_{23}N_2OCl$
23	84	92	142–144	$C_{12}H_{25}N_2OCl$

[a] Yields of the fully protected amides. [b] Yields of deprotected amides.

Table VI. Analytical Data of *N*-Benzoyl-ω-amino Acids

compd	yield, %	mp, °C	formula
24	86	187	$C_9H_9NO_3$
25	84	118–119	$C_{10}H_{11}NO_3$
26	81	78–80	$C_{11}H_{13}NO_3$
27	80	92–93	$C_{12}H_{15}NO_3$
28	88	79–80	$C_{13}H_{17}NO_3$

Table VII. Tastes of Aspartic Acid Amides Asp-NH$(CH_2)_nCH_3$

compd	*n*	taste	sweet taste strength
sucrose		sweet	1
3	3	sweet	5
4	4	sweet	10
5	5	sweet	20
6	6	sweet	30
7	7	tasteless	
8	8	tasteless	
9	9	tasteless	

Table VIII. Tastes of Aspartic Acid Amide Hydrochlorides Asp-NH$(CH_2)_nCH_3 \cdot$HCl ($7 \le n \le 9$)

compd	*n*	taste
7[a]	7	sweet[c]/sour
8[b]	8	sweet/sour
9[b]	9	sweet/sour

[a] Tasted in 0.2% solution. [b] Tasted in saturated solutions. [c] Slightly sweet.

RESULTS AND DISCUSSION

The results of sensory testing of aspartic acid amides having a linear chain ($-(CH_2)_nCH_3$) are listed in Table VII. Sweetness potency increased as the chain length increased up to $n = 6$ and suddenly disappeared at chain lengths $n > 6$ indicating that these were too long to fit the AH′, B′, and X′ affinity sites. Compounds **7**–**9** were not very soluble in water so their hydrochlorides were prepared and found

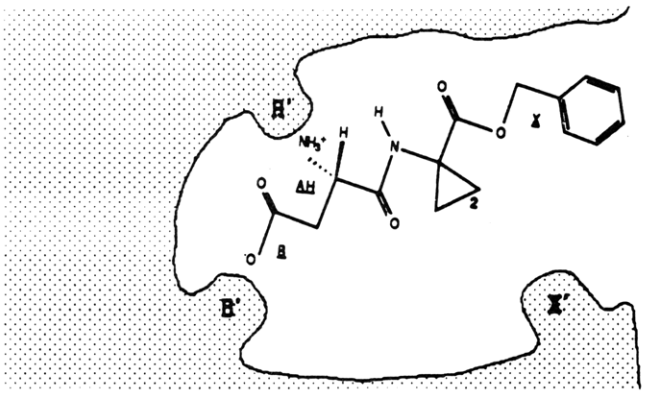


Figure 2. Receptor site holding the tasteless Asp-Acc-OBzl (**2**). The hydrophobic (X) functional group is not able to reach the X′ receptor site.

Table IX. Tastes of Aspartic Acid Amides Asp-NH$(CH_2)_n C_6H_5$

compd	n	taste	sweet taste strength
sucrose		sweet	1
10	0	sweet	15
11	1	sweet	20
12	2	sweet	40
13	3	sweet	40

to have a sweet taste masked by the expected sour flavor (Table VIII). This result indicated that even compounds in which chain lengths were longer than that of Asp-Acc-OBzl (**2**) could produce a sweet taste so long as the flexibility of these long chains can allow at least a partial fit to the receptor.

To examine the effect of an aromatic group in the X site, the aspartic acid amides (**10–13**) having a benzene ring at the end of the hydrocarbon chain were prepared. Surprisingly, these amides were more potent (Table IX) and had much greater water solubility than the straight-chain amides. Although amide **13** had approximately the same molecular length as the tasteless benzyl ester **2**, it produced a sweet taste and was 40 times more potent than sucrose. Of course, **13** is a flexible molecule while **2** is considerably more rigid. Once again, this flexibility appears to be very important with reference to the sweetness potency. That this is true is attested by the total lack of taste afforded by all four diastereomers of L-aspartyl-2,3-methanophenylalanine methyl ester (Mapelli et al., 1989). In our opinion, the lack of flexibility in the rigid methanophenylalanine residue eliminated the interaction of the benzene ring with the X′ affinity site on the receptor.

Furthermore, some early work (Mazur et al., 1970) showed that chirality in the amine moiety can be important; i.e., only one diastereomer of L-aspartyl-1-methylhexylamine was sweet. While not reported in this paper, we also prepared these compounds and found that only the isomer containing (1*R*)-1-methylhexylamine was sweet (100× sucrose) and the other was tasteless. Even though flexible, the 1*S* isomer was unable to reach the required affinity site due the configurational rigidity introduced by the chiral center.

To test further the hypothesis that all three groups, AH, B, and X, must be present in a molecule in order for it to be sweet, some ω-amino acid anilides and cyclohexyl amides were prepared in which the negative function B was missing (Table X). All of these compounds produced a bitter taste that increased in potency with chain length. These results showed that the sweet taste was easily changed to bitter by omission of the B group. These amides can bend to reach the A′ and X′ sites, and, consequently, bitter taste potency increased with increasing chain flexibility as illustrated in Figure 3 for ε-aminocaproic acid anilide (**18**), which has a more potent bitter taste than caffeine. To examine the effect of removal of the electropositive group from these molecules, some *N*-benzoyl-ω-amino acids (**24–28**) were prepared (Table XI).

Table X. Tastes of ω-Amino Acid Amides NH$(CH_2)_m$CONHC_6H_n

compd	m	n	taste	bitter taste strength
caffeine			bitter	1
14	1	5	bitter	0.12
15	2	5	bitter	0.24
16	3	5	bitter	0.50
17	4	5	bitter	0.67
18	5	5	bitter	1.25
19	1	11	bitter	0.12
20	2	11	bitter	0.19
21	3	11	bitter	0.32
22	4	11	bitter	
23	5	11	bitter	

Table XI. Tastes of *N*-Benzoyl-ω-amino Acids C_6H_5CONH$(CH_2)_n$COOH

		free acid		sodium salt
compd	n	taste	threshold value, mM	taste
24	1	sour	3.0	tasteless
25	2	sour	3.0	tasteless
26	3	sour	3.0	tasteless
27	4	sour	3.0	tasteless
28	5	sour	3.0	tasteless

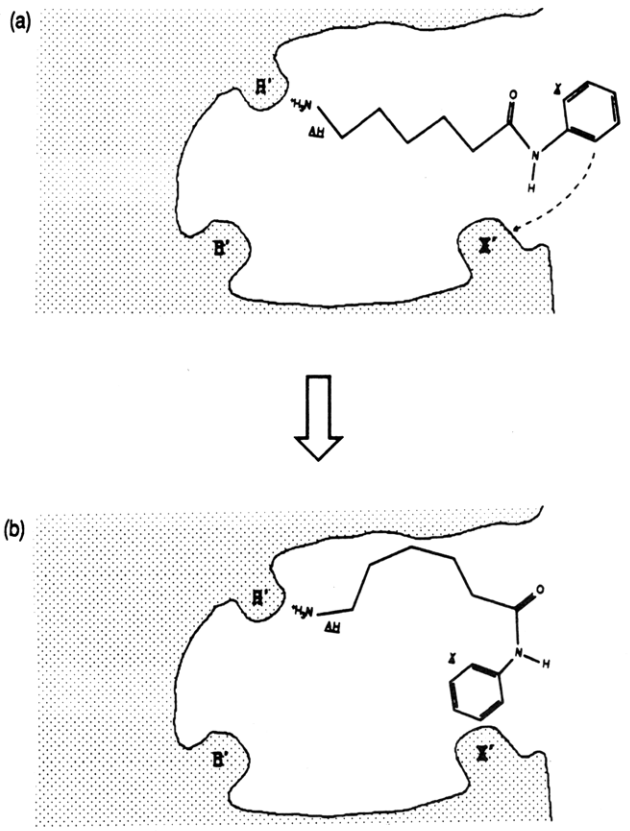


Figure 3. Taste receptor model with ε-aminocaproic acid anilide (**18**): (a) the molecule having the straight chain should not produce a strong bitter taste; (b) both AH and X functional groups reach A′ and X′ assuming a bent chain.

All of these compounds produced only a sour taste, probably due to their innate acidity, but even the sodium salts of these acids were tasteless. This result showed that omission of the AH function quickly changed the sweet to a sour taste. We have previously reported (Shinoda and

Okai, 1985) the possible similarity among the bitter, sweet, and sour taste receptors and proposed that they might be contained within the same biological structure. The results obtained in this study appear to confirm this idea.

Ariyoshi et al. (1974) proposed the possibility that more than three affinity sites might be present on the sweet receptor since they had prepared several sweet di-, tri-, and tetrapeptides indicating that hydrogen bonding with several other sites on the receptor by the amide protons might lead to production of a sweet taste (Ariyoshi et al., 1974; Ariyoshi, 1976, 1980, 1984–1986). The sweet compounds reported here, however, have only *one* amide linkage, indicating that these extra bonds are not a requirement for sweetness.

Additionally, we point out that there is some interaction between the molecular functional groups (AH, B, X) and taste receptor sites (A′, B′, X′). We explained the tastelessness of dipeptide, *N*-L-aspartyl-1-aminocyclopropanecarboxylic acid benzyl ester (**2**) by assuming that the chain length of **2** was too great to interact with all three receptor sites (see Figure 1). We also prepared several aspartic acid amides and found that they were all sweet even though some had chain lengths longer than that of **2**. This phenomenon can be explained in Figure 3. Since **13** has about the same molecular size as the tasteless dipeptide **2**, **13** should also be tasteless. However, **13** has a floppy chain and there is apparently a strong affinity between its phenyl ring (the most hydrophobic part of the molecule) and the X′ site on the receptor giving **13** a sweet taste.

LITERATURE CITED

Ariyoshi, Y. The Structure-taste Relationship of Aspartyl Dipeptide Esters. *Agric. Biol. Chem.* **1976**, *40*, 983.

Ariyoshi, Y. Synthesis of Aspartyl Tripeptide Esters in Relation to Structural Features of Sweet Peptides. *Agric. Biol. Chem.* **1980**, *44*, 943.

Ariyoshi, Y. Structure-taste Relationship of Aspartyl Tripeptide Esters. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3197.

Ariyoshi, Y. Structure-taste Relationships of Aspartyl Tetrapeptide Esters. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1727.

Ariyoshi, Y. Synthesis of Aspartyl Pentapeptide Esters in Relation to Structural Features of Sweet Peptides. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1027.

Ariyoshi, Y.; Yasuda, N.; Yamatani, T. The Structure-Taste Relationships of the Dipeptide Esters Composed of L-Aspartic Acid and β-Hydroxy Amino Acids. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 326.

Fujino, M.; Wakimasu, M.; Tanaka, K.; Aoki, H.; Nakajima, N. L-Aspartyl-Aminomalonic Acid Diesters. *Naturwissenschaften* **1973**, *60*, 351.

Fujino, M.; Wakimasu, M.; Tanaka, K.; Nakajima, N.; Aoki, H. Structure-Taste Relationships of L-Aspartyl-Aminomalonic Acid Diesters. *Chem. Pharm. Bull.* **1976**, *24*, 2112.

Mapelli, C.; Newton, M. G.; Ringold, C. E.; Stammer, C. H. Cyclopropane Amino Acid Ester Dipeptide Sweeteners. *Int. J. Peptide Protein Res.* **1987**, *30*, 498.

Mapelli, C.; Stammer, C. H.; Lok, S.; Goodman, M. The Synthesis, Taste Properties and Conformational Analysis of Four Stereoisomeric Cyclopropane Analogs of Aspartame. *Int. J. Peptide Protein Res.* **1989**, in press.

Mazur, R. H.; Schlatter, J. M.; Goldkamp, G. H. Structure-Taste Relationships of Some Dipeptides. *J. Am. Chem. Soc.* **1969**, *91*, 2684.

Mazur, R. H.; Goldkamp, A. H.; James, P. A.; Schlatter, J. M. Structure-Taste Relationships of Aspartic Acid Amides. *J. Med. Chem.* **1970**, *13*, 1217.

Shinoda, I.; Okai, H. Sweetness and Bitterness Contributions of Structural Units of Aspartame and Some Analogues. *J. Agric. Food Chem.* **1985**, *33*, 792.

Received for review July 5, 1988. Revised manuscript received October 26, 1988. Accepted December 8, 1988.

Volatile Aroma Components of *Curcuma amada* Roxb.

Alapati Srinivasa Rao, Bandaru Rajanikanth, and Ramachandran Seshadri*

The aroma concentrate of fresh rhizomes of *Curcuma amada* Roxb. was prepared by an efficient simultaneous steam distillation/solvent extraction. The qualitative analysis of these volatile aroma components was performed by using a gas chromatography/mass spectroscopy system aided by the computer library search. This led to the identification of 61 unreported compounds out of 68 compounds for which mass spectra have been recorded. Identification of the components has been confirmed by their Kovats retention indices. The aroma concentrate of mango ginger was comprised of a mixture of character impact compounds of both raw mango and turmeric.

Curcuma amada Roxb., popularly known as mango ginger, belongs to the family Zingiberaceae. The rhizomes of mango ginger are cultivated mostly in India and Malaysia where it is known as *Curcuma mangga* valet. The vegetative characteristics are similar to those of *Curcuma longa*. The rhizomes of mango ginger resemble those of true ginger and have mangolike flavor, but they have no pungency like ginger. In view of the exotic aroma it is commonly used in culinary preparations especially pickles, chutneys, etc. The rhizomes of mango ginger also find application therapeutically as a carminative and stomachic as well as for its topical use over contusions and sprains.

The chemistry and technology of *C. amada* have been comprehensively reviewed as part of a review on turmeric (Govindarajan, 1980). Recently the botany, agronomy, technology, and chemical composition of mango ginger have also been reviewed (Shankaracharya, 1982). Ocimene has been reported as the major constituent of mango ginger besides linalool, linalyl acetate, and safrole (Dutt and Tayal, 1941). Curcumene, a sesquiterpene hydrocarbon present in turmeric, has also been reported as a major aroma component in mango ginger (Jain and Mishra, 1964; Ahuja and Nigam, 1971). The essential oil content of mango ginger has been determined by distillation-extraction and low-temperature high-vacuum distillation methods as 0.3% and 0.2%, respectively (Gholap and Bandyopadhyay, 1984). Car-3-ene and *cis*-ocimene, which are

Organic Chemistry Section, Food Chemistry Department, Central Food Technological Research Institute, Mysore 570 013, India.

0021-8561/89/1437-0740$01.50/0