Sweetness and Bitterness Contributions of Structural Units of Aspartame and Some Analogues

Ichizo Shinoda and Hideo Okai*

The sweetness of aspartame (H-L-Asp-L-Phe-OMe) requires the trifunctional units in the molecule, α-amino (AH, electropositive) and β-carboxyl (B, electronegative) groups of L-Asp residue and the hydrophobic side chain of L-Phe residue (X). It has left us with the question whether three sweet units need to be included in a single molecule if only the sweet taste is produced by merely fitting them to a receptor. In other words, it was imagined that a receptor of aspartame might be satisfied with plural molecules which combine with AH, B, and X components. We prepared each component of aspartame and observed whether or not the sweet taste is reproduced by the recombination of the components. During the examinations, we confirmed that the AH–X component of aspartame exhibited bitterness and the taste was changed to sweetness by the addition of the B component on the tongue. The phenomenon suggested that bitter and sweet tastes are recognized in the same taste receptor and that the receptor discriminates bitter and sweet tastes by the difference of the combination of the three units between AH–X and AH–X–B.

INTRODUCTION

In 1969, Mazur et al. discovered H-L-Asp-L-Phe-OMe to be sweet during their synthetic investigation of the gastrin C-terminal moiety (Mazur et al., 1969). They reported that this compound was 100–200 times sweeter than sucrose. It was designated "aspartame" and entered the spotlight as a new type of artificial sweetening. In many laboratories including Mazur's, numerous analogues of it have been synthesized and the taste measured to elucidate the structure–sweetness relationship of aspartame and to further develop much sweeter compounds (Ariyoshi, 1976, 1980; Ariyoshi et al., 1974; Fujino et al., 1973, 1976; Mazur et al., 1970, 1973; Miyoshi et al., 1978). Now, aspartame is being marketed for dietary purposes.

The sweet taste of aspartame may be explained by the AH,B theory proposed by Shallenberger and Acree (1967) and Shallenberger et al. (1969). In addition to this theory, the synthetic studies of aspartame to the present make it apparent that a hydrophobic group (X) also participates in the production of sweetness. Namely it is concluded that the sweet taste of aspartame is exhibited by the trifunctional units in the molecule which are composed of α-amino (AH, electropositive) and β-carboxyl (B, electronegative) groups of the L-Asp residue and the hydrophobic side chain of the L-Phe residue (X) (Fujino et al., 1976).

Taste, like other biological processes, occurs by a substance and receptor interaction. In the case of aspartame, the trifunctional units, AH–X–B, will interact to a receptor in a taste bud. Figure 1 shows the sweetness production caused by the interaction between the sweet units of aspartame and the corresponding receptor sites (A′, X′, and B′) in a taste receptor. Supposing that the sweet taste is produced by three sweet units' fitting to the receptor sites, the production of sweetness is also expected in the cases of types a–d in Figure 1, wherein type a shows the combination in which the receptor sites (A′, X′, and B′) are filled with AH–X and B components, and types b–d are other possible combinations. We then attempted to observe whether or not a sweet taste was produced by these combinations in practice. We will call this taste examination the "combination test".

As AH–X components, H-L-Ala-L(or D)-Phe-OMe (1L or 1D) and H-Gly-L(or D)-Phe-OMe (2L or 2D), missing the β-carboxyl (B) group of L-Asp residue in aspartame, were synthesized. On the contrary, succinyl-L(or D)-Phe-OMe (3L or 3D) and glutaryl-L(or D)-Phe-OMe (4L or 4D) were synthesized as X–B components. In addition, Bz-Gly-OH (Bz, benzoyl), Bz-β-Ala-OH, and Bz-ε-Aca-OH, (ε-Aca, ε-aminocaproic acid) were also synthesized as X–B components. As for other components such as AH–B, AH, B, and X, the corresponding organic compounds, which were purchased, were used.

EXPERIMENTAL SECTION

General Procedures. All the melting points are uncorrected. The thin layer chromatography was carried out on Merck silica gel G with the solvent systems: R_f^1, 1-butanol–acetic acid–pyridine–water (4:1:1:2, v/v); R_f^2, chloroform–methanol (5:1 v/v). Spots of materials possessing a free amino group on a thin layer plate were detected by spraying with ninhydrin, and those of amino group blocked materials by spraying with 25% hydrogen bromide in acetic acid and then ninhydrin. Succinyl, glutaryl, and benzoyl derivatives were detected by spraying with 10% sulfuric acid. The optical rotations were measured on an Union PM-101 polarimeter. Prior to analyses including sensory test, the compounds were dried over phosphorus pentaoxide at 66 °C (2 mmHg) (1 mmHg-133 Pa) for 4 h (see Table I).

Synthesis of Dipeptide Esters as AH–X Components. These were synthesized by the conventional methods. *tert*-Butoxycarbonyl (Boc) amino acid and amino acid methyl ester were condensed by the mixed anhydride method (Vaughan, Jr, and Osato, 1952) to yield the corresponding *tert*-butoxycarbonyl dipeptide methyl ester. It was converted to the desired dipeptide methyl ester hydrochloride by the treatment of hydrogen chloride in dioxane.

Syntheses of Succinyl- and Glutaryl-L(or D)-Phe-OMe as X–B Components. These were prepared from succinic or glutaric anhydride and H-L(or D)-Phe-OMe by the same method as described by Berse et al. (1962).

Syntheses of Benzoyl Amino Acids as X–B Components. These were prepared by the generally applicable Schotten–Bauman method. Amino acids were acylated by benzoyl chloride in an alkaline solution (Marshall and Liener, 1970).

Sensory Measurement of Threshold Value. The taste of synthesized compounds was organoleptically determined by panel evaluation employing five people. A series of aqueous solutions of decreasing concentration,

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Shitami, Saijo-cho, Higashihiroshima 724, Japan.

0021-8561/85/1433-0792$01.50/0

Table I. Yields and Analytical Data of Synthesized Compounds

compound	yield, %	mp, °C	$[\alpha]^{20}_D$, deg (c 1, MeOH)	formula	anal. found (calcd) C	H	N	R_f^1	R_f^2
Boc-L-Ala-L-Phe-OMe	90	84–85	−22	$C_{18}H_{26}O_5N_2$	61.79 (61.70)	7.45 (7.48)	8.11 (8.00)	0.95	0.79
Boc-L-Ala-D-Phe-OMe	87	96–97	−28	$C_{18}H_{26}O_5N_2$	61.50 (61.70)	7.59 (7.48)	8.13 (8.00)	0.97	0.78
Boc-Gly-L-Phe-OMe	90	oil						0.95	0.82
Boc-Gly-D-Phe-OMe	100	oil						0.95	0.85
H-L-Ala-L-Phe-OMe·HCl (1L)	96	152–154	+12	$C_{13}H_{19}O_3N_2Cl$	54.21 (54.45)	6.43 (6.68)	9.45 (9.77)	0.83	0.42
H-L-Ala-D-Phe-OMe·HCl (1D)	86	hygroscopic						0.86	0.48
H-Gly-L-Phe-OMe·HCl (2L)	87	160	+14	$C_{12}H_{17}O_3N_2Cl$	52.55 (52.85)	6.43 (6.28)	10.09 (10.27)	0.83	0.39
H-Gly-D-Phe-OMe·HCl (2D)	85	160–161	−13	$C_{12}H_{17}O_3N_2Cl$	52.61 (52.85)	6.43 (6.28)	10.48 (10.27)	0.83	0.42
succinyl-L-Phe-OMe (3L)	67	75–77	+7	$C_{13}H_{15}O_3N$	55.76 (55.90)	5.66 (5.41)	4.85 (5.02)		0.42
succinyl-D-Phe-OMe (3D)	70	74–75	−10	$C_{13}H_{15}O_3N$	55.59 (55.90)	5.34 (5.41)	5.23 (5.02)		0.45
glutaryl-L-Phe-OMe (4L)	55	73–75	+2	$C_{14}H_{17}O_3N$	57.54 (57.32)	5.89 (5.84)	5.01 (4.78)		0.54
glutaryl-D-Phe-OMe (4D)	51	73–75	−2	$C_{14}H_{17}O_3N$	57.46 (57.32)	5.64 (5.84)	4.76 (4.78)		0.46
Bz-Gly-OH	56	187		$C_9H_9O_3N$	60.46 (60.33)	5.10 (5.06)	7.80 (7.82)	0.74	
Bz-β-Ala-OH	64	118–119		$C_{10}H_{11}O_3N$	62.24 (62.16)	5.80 (5.74)	7.18 (7.25)	0.88	
Bz-ε-Aca-OH	88	79–80		$C_{13}H_{17}O_3N$	66.54 (66.36)	7.36 (7.28)	5.79 (5.95)	0.90	

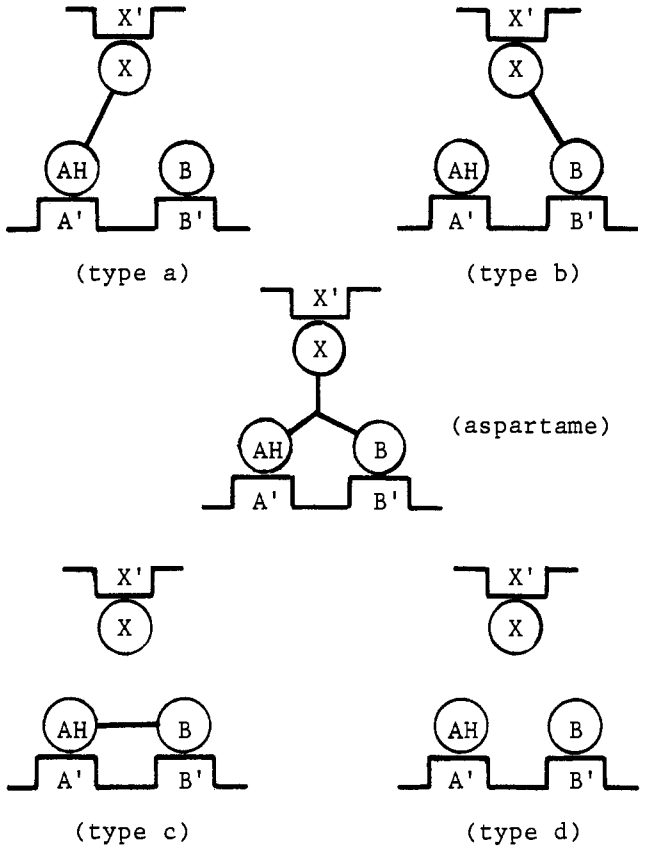

Figure 1. The production of sweetness of aspartame caused by the interaction between three sweet units (AH, X, and B) and the corresponding receptor sites (A′, X′, and B′).

each half as strong as the proceding one, were prepared. Before testing the sample, the tester's mouth was thoroughly rinsed with deionized water. The sample solution was held in the mouth for ca. 10 s and then extectorated and the threshold value (TV) was determined. The threshold values were averaged after several examinations by the panelists.

Combination Test. A 5 mM aqueous solution of each component was prepared. The concentration was equal to the threshold value of sweetness of sucrose. Before testing the sample, the tester's mouth was rinsed with deionized water. One component solution was held in the mouth for ca. 10 s and then expectorated. In that condition, another solution was then placed in the mouth immediately. A change of taste evaluation was observed at this point. This procedure was more sensitive to evaluate the change of taste than using the mixed solution of the components. Repetition of this procedure made the taste behavior even more clear. This test is a qualitative analysis, therefore, the relative potency of sweetness to the standard 5 mM sucrose solution was not quanitatively determined.

The results of both sensory measurement of threshold value and combination test are listed in Table II. As for 1L and 2L Masur et al. only reported that they were bitter (1969).

The toxicities of the samples are as follows (as far as we know): benzyl alcohol, LD_{50} orally in rats 3.1 g/kg; cyclohexylamine, LD_{50} i.p. in rats 200 mg/kg; caffeine, LD_{50} orally in rats 200 mg/kg; brucine and strychnine, MLD orally in rats 5 mg/kg; phenylthiourea, LD_{50} orally in rats 3.4 mg/kg. As to the synthetic compounds, the toxicological examinations did not carried out.

RESULTS AND DISCUSSION

First, we examined the combination of 1L which consisted of AH–X but lacked B, and acetic acid which consisted of only the B component. The former possessed bitterness, however, the taste was changed to sweetness with the addition of acetic acid. This phenomenon was also observed by the combination of acetic acid (B) and other AH–X components as shown in Table II type a. Even when a 1.5 mM solution of 1L and a 2 mM acetic acid were employed, sweetness was observed. This finding indicated that the sweet taste in the combination test was about three times stronger than that of sucrose. The use of critic acid in place of acetic acid produced the same result.

Next, another type of combination (type b, AH and X–B) was examined (Table II type b). When 3L, 3D, 4L, and 4D were used as X–B and an aqueous ammonia solution as AH, the taste did not change essentially. However, when triethylamine (Et_3N) and cyclohexylamine were used as AH, sweetness was only slightly observed in these combinations. Also, in the cases of benzoyl amino acids as X–B and Et_3N and cyclohexylamine as AH, the sour taste of X–B clearly changed to sweetness.

In type c (combination of AH–B and X), Gly, β-Ala, and γ-Abu (γ-aminobutyric acid) were used as AH–B and benzyl alcohol as X. However, sweetness was not observed in these combinations even when their concentrations were increased, as shown in Table II type c.

Finally, we examined the combination of AH, X, and B (type d). When using cyclohexylamine as AH, sweetness was observed as shown in Table II type d. Because cyclohexylamine was expected to act as both AH and X, we examined the combination test between cyclohexylamine and acetic acid. In this combination, sweetness was also observed. The same result was obtained in the combination of acetic acid and hexamethylenetetramine in place

Table II. The Results of Combination Test

combination	component[a]		sweetness
type **a**	AH–X	B	
	5 mM H-L-Ala-L-Phe-OMe (1L) (bitter TV 1.2 mM)	5 mM acetic acid	yes
		5 mM citric acid	yes
	5 mM H-L-Ala-D-Phe-OMe (1D) (bitter TV 1.4 mM)	5 mM acetic acid	yes
		5 mM citric acid	yes
	5 mM H-Gly-L-Phe-OMe (2L) (bitter TV 3.3 mM)	5 mM acetic acid	yes
		5 mM citric acid	yes
	5 mM H-Gly-D-Phe-OMe (2D) (bitter TV 3.1 mM)	5 mM acetic acid	yes
		5 mM citric acid	yes
type **b**	AH	X–B	
	5 mM aq NH_3	5 mM succinyl-L-Phe-OMe (3L)	no
	5 mM aq NH_3	5 mM succinyl-D-Phe-OMe (3D)	no
	5 mM aq NH_3	5 mM glutaryl-L-Phe-OMe (4L)	no
	5 mM aq NH_3	5 mM glutaryl-D-Phe-OMe (4D) (sour TV 3.0 mM)	no
	5 mM aq NH_3	5 mM Bz-NH-$(CH_2)_n$-COOH	no
	5 mM Et_3N	5 mM Bz-NH-$(CH_2)_n$-COOH	yes
	5 mM $NH_2C_6H_{11}$	5 mM Bz-NH-$(CH_2)_n$-COOH (n = 1, 2, 5, sour TV 3.0 mM)	yes
type **c**	AH–B	X	
	5 mM NH_2-$(CH_2)_n$-COOH	5 mM Bzl-OH	no
	20 mM NH_2-$(CH_2)_n$-COOH (n = 1, 2, 3)	10 mM Bzl-OH	no
type **d**	AH X	B	
	5 mM aq NH_3 5 mM Bzl-OH	5 mM acetic acid	no
	5 mM Et_3N 5 mM Bzl-OH	5 mM acetic acid	no
	5 mM NH_2-C_6H_{11} 5 mM Bzl-OH	5 mM acetic acid	yes
	5 mM NH_2-C_6H_{11} 5 mM Bzl-OH	5 mM citric acid	yes

[a] Individual taste of AH, X, B, and AH–B components: AH, 5 mM aq NH_3, 5 mM Et_3N, and 5 mM cyclohexylamine, is almost tasteless (undeterminable taste). X, 5 mM and 10 mM Bzl-OH, is almost tasteless (undeterminable taste). B, 5 mM acetic acid and 5 mM citric acid, is sour. AH–B, all of them (5 mM, 20 mM), is tasteless.

Table III. Tasting Behavior of Combination Test with Typical Bitter Substances as AH–X

AH-X	B	taste
(a) Sweetness Production in AH–X and B System		
0.002 mM brucine (TV 0.0008 mM)	5 mM acetic acid	bitter → sweet
0.005 mM strychnine (TV 0.003 mM)	5 mM acetic acid	bitter → sweet
0.1 mM phenylthiourea (TV 0.025 mM)	5 mM acetic acid	bitter → sweet
5 mM caffeine (TV 1.0 mM)	5 mM acetic acid	bitter → sweet
0.2 mM cyclo(-Leu-Trp-)[a] (TV 0.06 mM)	5 mM acetic acid	bitter → sweet
0.2 mM Arg-Gly-Pro-Pro-Phe-Ile-Val[b] (TV 0.05 mM)	5 mM acetic acid	bitter → sweet
AH–X	**X–B**	
(b) Depression of Bitterness by X–B Component		
2 mM caffeine	5 mM Bz-NH-$(CH_2)_5$-COOH	bitter → no
5 mM caffeine	5 mM Bz-NH-$(CH_2)_5$-COOH	bitter → weak
0.005 mM strychnine	5 mM Bz-NH-$(CH_2)_5$-COOH	bitter → no
0.05 mM strychnine	5 mM Bz-NH-$(CH_2)_5$-COOH (sour TV 3.0 mM)	bitter → weak

[a] Shiba and Numami, 1974. [b] Fukui et al., 1983.

of cyclohexylamine (a solution of 5 mM hexamethylenetetramine is tasteless). In addition, during the combination tests using cyclohexylamine, hexamethylenetetramine, and acetic acid, we found an interesting taste phenomenon. After tasting the solution of hexamethylenetetramine, bitterness was observed by next tasting the solution of cyclohexylamine, and the bitter taste was changed to sweet by the addition of acetic acid.

From the results of these examinations, we confirmed that the three sweet units are not always included in a single molecule, such as aspartame, for the production of sweetness. A series of combination tests also proved the requirement of three sweet units of aspartame for its sweetness.

The above result mentioned changing bitterness to sweetness led us to next perform the combination tests between typical bitter substances, as shown on Table III, and acetic acid. The concentration of bitter substances in the test did not exceed five times their threshold values. As shown in Table III section a, their bitter taste was changed to sweet with the addition of acetic acid (B), regardless of the chemical structure in every case. The results indicated that bitter substances are able to be regarded as AH–X components. In addition, we found that phenylthiourea taste-blind panelists responded to sweetness in the combination of phenylthiourea and acetic acid. When the concentration of phenylthiourea was increased, they described it as good tasting.

We also found an interesting taste phenomenon in the combination tests between bitter substances and the X–B component in place of acetic acid (B). The bitterness of 2 mM caffeine solution, which was not so bitter but clearly perceived as bitter, was completely eliminated by tasting a 5 mM benzoyl-ε-aminocaproic acid (Bz-ε-Aca-OH) solution (X–B) before tasting the caffeine solution. When the concentration of caffeine was 5 mM, in which a strong bitter taste was usually observed, the test resulted in decreased bitterness. The same result was obtained when using strychnine, which is one of the most bitter substances, in place of caffeine. As shown in Table III section b, in the case of 0.005 mM strychnine solution, bitterness was eliminated, and in the case of 0.05 mM strychnine

solution, bitterness was decreased by the X–B component.

The taste phenomena described in this paper produced the following assumptions. A sweet substance has trifunctional sweet units AH–X–B. Among them, AH–X corresponds to bitterness production units. Further, a taste receptor is able to recognize both sweet and bitter tastes. The receptor has three sites as described in Figure 1. Sweet and bitter tastes are easily discriminated by the difference of combination of functional units between AH–X–B and AH–X in the receptor. Therefore, bitterness (AH–X) was changed to sweetness (AH–X–B) with the addition of B on the tongue, and bitterness (AH–X) was eliminated by X–B. In addition, the result in which tasteless hexamethylenetetramine participated in both sweetness and bitterness production by the suitable combination mentioned in type d supports our assumption.

One of the authors, Okai, previously reported that the production of bitterness in peptides arose when bifunctional units (one of which is a basic or bulky side chain amino acid unit and the other is a hydrophobic amino acid unit) were brought within 3–5 Å (Okai, 1977). These units just correspond to the AH–X component of aspartame. From our point of view, aspartame is regarded as one of bitter peptides. The bitterness production in aspartame isomers (Mazur et al., 1969) conveneiently supports our identifying the sweet and bitter receptors.

Registry No. 1L, 40298-89-3; 1D, 97336-13-5; 2L, 16227-24-0; 2D, 97372-81-1; 3L, 97336-14-6; 3D, 97336-15-7; 4L, 97336-16-8; 4D, 97336-17-9; AcOH, 64-19-7; $(CO_2H)CH_2C(OH)(CO_2H)CH_2(CO_2H)$, 77-92-9; NH_3, 7664-41-7; NEt_3, 121-44-8; $BzNHCH_2CO_2H$, 495-69-2; $BzNH(CH_2)_2CO_2H$, 3440-28-6; $BzNH(CH_2)CO_2H$, 956-09-2; HGlyH, 56-40-6; β-HAlaH, 107-95-9; $CO_2HCH_2CH_2CH_2NH_2$, 56-12-2; $PhCH_2OH$, 100-51-6; hexamethylenetetraamine, 100-97-0; cyclohexylamine, 108-91-8; brucine, 357-57-3; phenylthiourea, 103-85-5; caffeine, 58-08-2; strychnine, 57-24-9; benzoyl-(*E*)-aminocaproic acid, 956-09-2; aspartame, 22839-47-0.

LITERATURE CITED

Ariyoshi, Y. *Agric. Biol. Chem.* **1976**, *40*, 983.
Ariyoshi, Y. *Agric. Biol. Chem.* **1980**, *44*, 943.
Ariyoshi, Y.; Yasuda, N.; Yamatani, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 326.
Berse, C.; Piché, L.; Lachance, L.; Laflamme, G. *J. Org. Chem.* **1962**, *27*, 3489.
Fujino, M.; Wakimasu, M.; Mano, M.; Tanaka, K.; Nakajima, N.; Aoki, H. *Chem. Pharm. Bull.* **1976**, *24*, 2112.
Fujino, M.; Wakimasu, M.; Tanaka, K.; Aoki, H., Nakajima, N. *Naturwissenchaften* **1973**, *60*, 351.
Fukui, H.; Kanehisa, H.; Ishibashi, N.; Miyake, I.; Okai, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 766.
Marshall, D. L.; Liener, I. E. *J. Org. Chem.* **1970**, *35*, 867.
Mazur, R. H.; Goldkamp, A. H.; James, A. D.; Schlatter, J. M. *J. Med. Chem.* **1970**, *13*, 1217.
Mazur, R. H.; Peuter, J. A.; Swiatek, K. A.; Schlatter, J. M. *J. Med. Chem.* **1973**, *16*, 1284.
Mazur, R. H.; Schlatter, J. M.; Goldkamp, A. H. *J. Am. Chem.* **1969**, *91*, 2684.
Miyoshi, M.; Nunami, K.; Sugano, H.; Fujii, T. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1433.
Okai, H. In "Peptide Chemistry 1976"; Nakajima, T., Ed.; Protein Research Foundation: Osaka, Japan, 1977; p 139.
Shallenberger, R. S.; Acree, T. E. *Nature (London)* **1967**, *216*, 480.
Shallenberger, R. S.; Acree, T. E.; Lee, C. Y. *Nature (London)* **1969**, *221*, 555.
Shiba, T.; Nunami, K. *Tetrahedron Lett.* **1974**, 509.
Vaughan, Jr., J. R.; Osato, R. L. *J. Am. Chem. Soc.* **1952**, *74*, 676.

Received for review January 24, 1985. Accepted June 7, 1985.

Volatile Flavor Components of Babaco Fruit (*Carica pentagona*, Heilborn)

G. John Shaw,* John M. Allen, and Frans R. Visser

The volatile flavor components of babaco fruit were isolated by low temperature high vacuum steam distillation and diethyl ether extraction. Analysis of the concentrate by capillary gas chromatography and gas chromatography–mass spectrometry led to the identification of 32 components. Preliminary sensory evaluation experiments suggest that ethyl butanoate and ethyl hexanoate are important contributors to the unique volatile flavor in this fruit.

INTRODUCTION

Carica pentagona Heilborn, better known as "babaco", is a hybrid between "mountain papaya" (*Carica pubescens* Lenné and Koch) and *Carica stipulata* Badillo. The plant was introduced into New Zealand from Ecuador where it thrives in high mountainous regions at 2000 and 3000 meters (Endt, 1981). Before 1973 babaco was only grown as a fresh crop in Ecuador (Bollard, 1981) and only for the domestic market. However since that time commercial interest in the New Zealand grown fruit has resulted in a rapid growth in domestic production for local and international markets. Presently about 70% of production is consumed as fresh fruit with another 15% exported and the balance processed into canned fruit products (Endt, 1984).

The large (0.5–1.0 kg), virtually seedless fruit have a pleasant "fruity" flavor quite distinct from that found in *Carica pubescens* Lenné and Koch. To date there have been no reports characterizing the volatile flavor components of fresh babaco fruit. This paper describes the isolation and characterization of babaco aroma and highlights those components thought to be important contributors to babaco flavor quality.

EXPERIMENTAL SECTION

Chemical Standards. Methyl acetate was prepared by reaction of acetic acid with diazomethane. Esters, (a) ethyl butanoate, ethyl crotonate, ethyl hexanoate, ethyl benzoate, (b) *n*-butyl butanoate, *n*-butyl hexanoate, (c) *n*-hexyl butanoate, *n*-hexyl hexanoate, (d) *n*-heptyl butanoate, and

Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.

0021-8561/85/1433-0795$01.50/0