Synthesis of Aldoxime Derivatives and Taste Evaluation, Part II

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Summary

The sweet taste evaluation of peptide aldoximes was investigated. Many derivatives were synthesized by introducing aldoxime group into the carboxyl terminal of amino acids or peptides. N-substituted hydrophobic amino acid or N-substituted aminoacyl-glycine were used as the materials to replace the terpenoid backbone of perillartine. On sensory evaluation, some of these derivatives exhibited sweetness. The maximum sweetness was found in compounds with Z-aminoacyl aldoximes. Sweetness is subjective and dependent upon a number of factors such as steric hindrance of N-protecting groups, solubility in water, concentration of sweetener and other interfering ingredients in the product.

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Key words : sweetener, Perillartine, peptide, aldoxime.

Introduction

Sweeteners are one of the most popular physiologically active substances in our foods. Many artificial sweeteners were developed to conquer the disadvantages of sucrose, such as caries of the teeth, obesity and diabetes. Since the discovery of Aspartame (Asp-Phe-OMe), a lot of investigations about sweeteners have been done[1] to establish molecular requirements for the sweetness, and many modifications about the structures were given by many workers.[2)-5)] Up to date, AH-B-X triangle suggested by Kier[6] is most likely to conform with sweetness exhibitions. High potency sweeteners have been developed by the considerations about these functional group's locations.[6)-9)] According to their theory, the three functional groups, AH, B and X are necessary to be located on the proper positions for exhibiting sweetness. Here, AH site is the electro-

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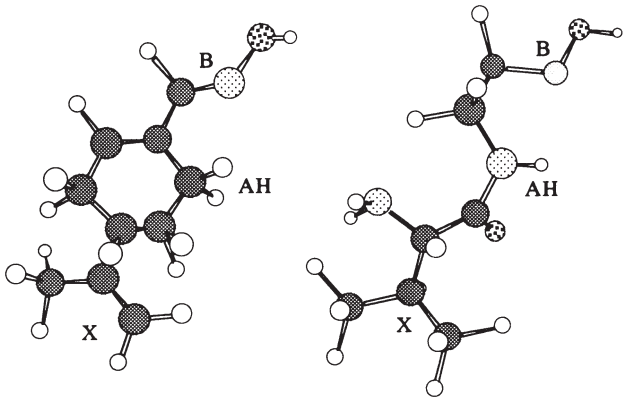

Fig. 1. Structural similarity between Perillartine (left) and Valinyl-glycinyl-aldoxime (right).
Suggested AH-B-X systems on Perillartine and ideal disposition on Valinyl-glycinyl-aldoxime are illustrated.
AH : Proton donor, B : Proton acceptor and X : Dispersion site.

positive, B is electro-negative and X is the neutral hydrophobic dispersion site. Perillartine is one of the artificial sweeteners derived from Perilla aldehyde, which is a high potent sweetener estimated to be 2000 times as sweet as sucrose. Nevertheless, due to a poor solubility in water and some toxicity, Perillartine is not popular as a sweetening agent. Recently, we reported the taste evaluation of the Schiff's base compounds which have similar structures with Perillartine[10]. It was suggested that the interaction between Schiff's base and corresponding active site on taste receptor was prevented by the presence of some steric hindrance of aromatic rings, and the products were less sweet. With these results, we tried to synthesize many aldoxime derivatives which have no ring on both sides of C = N bond. The similarity between Perillartine and Valinyl-Glycinyl-aldoxime are expected as shown in Fig. 1. Therefore, the possibility of the replacing terpenoid backbone in Perillartine with amino acids were investigated. As isopropenyl group in Perillartine, may play an important role as X site[11], amino acids such as valine, isoleucine and leucine having hydrophobic side chain moiety were used in the present study.

Materials and Methods

All the melting points are uncorrected. The thin layer chromatography (TLC) was carried out on Merck Silica Layer 60 with the two solvent systems, Rf_1 ; Chloroform : Methanol : Acetic acid (85 : 15 : 3 by vol.), Rf_2 ; Chloroform : Methanol : Ethyl acetate (95 : 5 : 3 by vol.). Spots of products were detected with ninhydrin after spraying with 25% HBr/Acetic acid. The purity of each product was confirmed by HPLC analysis using

TOSOH SC8010 HPLC system, IR, ^{1}H NMR and ^{13}C NMR analysis.

Sensory test. The taste of all aldoximes was evaluated by four panelists according to the literature[12].

Preparation of aldoxime derivatives. Amino acids and reagents were purchased from Wako pure chemical industries, Ltd. and Peptide institute, Inc. N-terminal of amino acids was protected by benzyloxycarbonyl group (Z-) or t-butoxycarbonyl group (Boc-). Introducing glycine into C-terminal was carried out by using disuccinimidylcarbonate (DSC). Half reduction of carboxyl group was achieved *via* pyrazoride[13),14)], which was prepared by the condensation of 3,5-dimethylpyrazol (3,5DMP) with the corresponding carboxyl terminal using carbodiimidazol (CDI) and water-soluble-carbodiimide (WSCD). Then pyrazoride was reduced by lithium aluminium hydride ($LiAlH_4$) and crude N-substituted aminoacyl aldehydes were obtained. Finally, aldehyde was treated with hydroxylamine, and pure aldoximes were obtained after recrystallization. The typical synthetic methods were shown as follows.

Synthesis of Boc-Valinyl-Glycinyl-aldoxime.

(a) Boc-Valinyl-Glycine (I). Boc-valine was prepared by the reaction with di-t-butyl dicarbonate. (Yield 85%). To the acetonitrile solution (20ml) of Boc-valine (20mmol, 4.35g), pyridine (22mmol, 1.74g) and DSC (20mmol, 5.12g) were added, and the reaction mixture was stirred over night at room temperature. After evaporation in vacuo, the residue was dissolved in ethyl acetate and washed with 4% $NaHCO_3$ solution, 10% citric acid solution and saturated NaCl solution. After the removal of solvent in vacuo, the residue was dissolved in 50% acetonitrile solution (20ml). To the solution, glycine

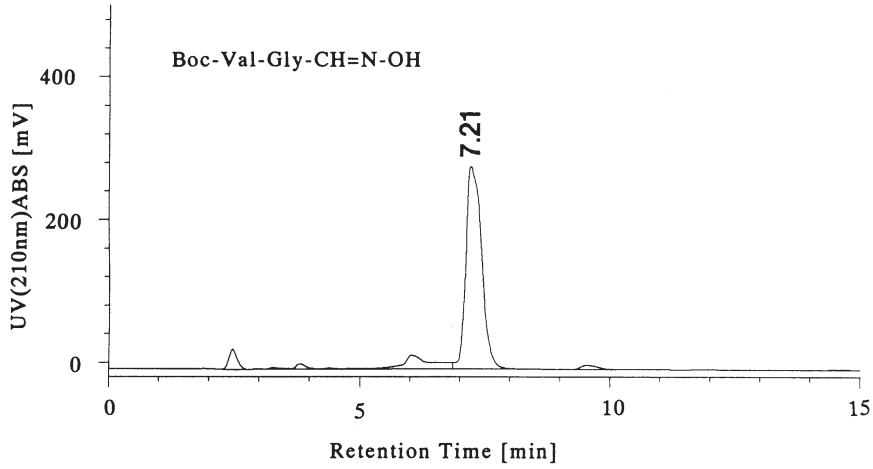

Fig. 2. HPLC analysis of Aldoxime derivatives.
Elute : 30% Acetonitrile /0.7mM Phosphate buffer(pH2.7) Column : TOSOH TSK-gel ODS 120A 4.6mm × 150mm Pump : 1.0ml/min, 40°C

Table 1. The yields and analysis data of the synthsized compounds and their intermediates.

compounds	Yield g	Yield Rate %	Rate of flow system	Rate	Melt point ℃	HPLC anal.*5 R.T/min	Area%	Status
Z-Ala-3.5DMP	2.50	41.5	Rf2	0.78	124–125	N.A.*3		White powder
Z-Val-3.5DMP	2.18	66.2	Rf2	0.86	56– 57	N.A.		White powder
Z-Leu-3.5DMP	2.22	32.3	Rf2	0.83	83– 84	N.A.		White powder
Z-Ile-3.5DMP	4.51	65.7	Rf2	0.84	oil	N.A.		Colorless oil
Z-Phe-3.5DMP	2.57	68.1	Rf2	0.74	132–133	N.A.		White powder
Z-Gly-3.5DMP	1.55	54.0	Rf2	0.72	50– 52	N.A.		White powder
Z-Val-Gly-3.5DMP	2.74	70.9	Rf2	0.72	184–185	N.A.		White powder
Z-Leu-Gly-3.5DMP	2.16	53.9	Rf2	0.84	73– 75	N.A.		White powder
Z-Ile-Gly-3.5DMP	2.66	66.4	Rf2	0.68	184–185	N.A.		White powder
Boc-Val-Gly-3.5DMP	1.43	40.6	Rf2	0.65	142–143	N.A.		White powder
Boc-Leu-Gly-3.5DMP	1.11	30.3	Rf2	0.64	106–109	N.A.		White powder
Boc-Ile-Gly-3.5DMP	1.31	35.7	Rf2	0.60	135–137	N.A.		White powder
Z-Ala-aldoxime	21.0 mg	–	Rf1*1	0.32/0.43	nm*2	9.47	80.0	White powder
Z-Val-aldoxime	66.0 mg	–	Rf1	0.28/0.40	nm	7.57	3.8*4	White powder
Z-Leu-aldoxime	29.0 mg	–	Rf1	0.34/0.46	oil	7.14	89.1	Colorless oil
Z-Ile-aldoxime	18.0 mg	–	Rf1	0.22/0.46	nm	4.37	85.2	White powder
Z-Phe-aldoxime	29.0 mg	–	Rf1	0.25/0.49	nm	6.59	89.7	White powder
Z-Gly-aldoxime	40.0 mg	–	Rf1	0.18/0.24	nm	7.12	27.7*4	White powder
Z-Val-Gly-aldoxime	17.0 mg	–	Rf1	0.20	nm	3.34	44.6	White powder
Z-Leu-Gly-aldoxime	25.0 mg	–	Rf1	0.20	oil	6.49	73.4	Colorless oil
Z-Ile-Gly-aldoxime	17.0 mg	–	Rf1	0.22	nm	4.31	86.8	White powder
Boc-Val-Gly-aldoxime	32.0 mg	–	Rf1	0.14	nm	7.21	92.0	Yellow powder
Boc-Leu-Gly-aldoxime	27.0 mg	–	Rf1	0.20	oil	11.41	67.4	Colorless oil
Boc-Ile-Gly-aldoxime	72.0 mg	–	Rf1	0.20	nm	10.25	64.7	White powder

*1 : These aldoximes contains some aminoacyl alcohol. *2 : Not measured.
*3 : The purity of these intermediates were confirmed by TLC. *4 : Principals were occupied by alcohol.
*5 : HPLC analysis conditions, Detection : UV210nm : Column/TOSOH TSKgel-ODS120A 40℃ : Mobile phase 30% Acetonitrile/Phosphate Buffer (pH2.7). @1ml/min

(20mmol, 1.50g) and triethylamine (22mmol, 2.23g) were added and the mixture was stirred over night. The solvent was evaporated in vacuo, and the residue was dissolved in ethyl acetate. The solution was washed with 10% citric acid solution and water, then extracted with 4% $NaHCO_3$ solution. The alkaline solution was washed with ethyl acetate, and acidified by the addition of sufficient 55% citric acid (pH 3). The acidified solution was extracted with ethyl acetate and the upper layer was washed with saturated NaCl solution. After drying over Na_2SO_4, the solvent was evaporated. The oily residue was triturated with ether. (Yield 46.5%, white powder).

(b) Boc-Valinyl-Glycinyl-3,5-DMP (II). (I) (10mmol, 2.74g) and 3,5DMP (10mmol, 0.96g) were dissolved in methylene chloride (50ml). Then, triethylamine (11mmol,1.11g) and WSCD. HCl (10mmol, 0.96g) were added to the solution at 0℃. The mixture was stirred for 1 hour at 0℃ and continued over night at room temperature. After washing the reaction mixture with 10% citric acid solution, saturated $NaHCO_3$ solution and saturated NaCl solution, the solution was dried over Na_2SO_4. After the evaporation,the residue was

crystallized in ethyl acetate/n-hexane. (Yield 40.6%, white powder, Rf_2 0.65, m.p. 142-143°C).

(c) Boc-Valinyl-Glycinyl-aldoxime (Ⅲ). The solutions of (Ⅱ) (2mmol, 0.70g) and $LiAlH_4$ (3mmol, 0.11g) in dry tetrahydrofuran (100ml) was stirred for 15 minutes at −20°C under argon. Immediately the mixture was acidified with cooled HCl (pH 3) and the mixture was filtrated. The filtrate was evaporated in vacuo. The residue was dissolved in ether, and washed with 10% citric acid solution followed by saturated NaCl solution. After drying over Na_2SO_4 and evaporation, oily crude aldehyde was obtained. To the methanol solution of residue (1ml), hydroxylamine•HCl (3mmol, 0.21g) and NaOH (3mmol, 0.12g) in aqueous solution (2ml) were added. After stirring for one hour, the reaction mixture was evaporated, and residue was dissolved in a small amount of 2mmol NaOH solution. After washing with ether, saturation with CO_2 resulted in a precipitate. Yield 0.032g, Rf_2 0.14, colorless oil. HPLC analysis (Fig. 2).

IR ν max (KBr)cm^{-1} : 1370, 1390cm^{-1} (t-butyl), 1680 cm^{-1} (-C = N-OH) : : NMR δ H($CDCl_3$) : 1.4(9H, s), 7.1(1H, d)/Boc- ; 0.9(6H, d), 2.2(1H, m), 4.1(1H, m), 5.5(1H,d)/valine ; 4.0(2H, m,)/glycine ; 10.0(1H, s)/aldoxime. : : ^{13}C: (28.2:C1, 77.0:C4, 155.3:C4) / Boc-; (17.2:C1, 19.0:C1, 31.0:C3, 60.2:C3, 171.0:C4)/valine; (41.0:C2)/glycine, (176.0:C3)/aldoxime.

All analytical data are summarized in Table 1.

Results and discussion

Taste profile of Z and Boc aminoacyl-glycinyl-aldoxime.

Results of sensory evaluation of Z and Boc-aminoacyl-glycinyl-aldoxime are presented in Table 2. Z-substituted analogues exhibited sweet taste, and the intensity was estimated to be a little less than sucrose. These N-substituted aminoacyl aldoxime had poor solubility in water. Therefore, evaluation of the taste potency could not be achieved

Table 2. Results of sensory evaluation with N-substituted aminoacyl-glycinyl-aldoximes

Compounds	Solubility in water Five steps*1	Taste kinds*2	Taste potency*3 R.Suc
Boc-Val-Gly-aldoxime	**	Bitter***No*	-
Boc-Leu-Gly-aldoxime	*	Bitter***No*	-
Boc-Ile-Gly-aldoxime	*	Bitter*No***	-
Z-Val-Gly-aldoxime	**	Sweet***No*	<1
Z-Leu-Gly-aldoxime	*	Sweet**No*Bitter*	<1
Z-Ile-Gly-aldoxime	*	Sweet***No*	<1

*1 : The solubility of analogues in water are displayed in five steps (*****Rich, *poor).

2 : '' is the score of the individual panelist's (Within four panelists).

*3 : Taste potency could not be evaluated.

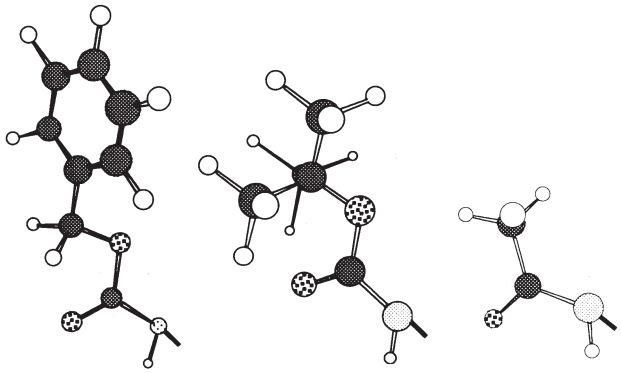

Fig. 3. Difference of spatial extension occupied by amino-protecting groups. Benzyloxycarbonyl group (Left), t-Butoxycarbonyl group (Center) and Acetyl group (Right).

correctly. As seen from Table 2, the replacement of amino acid moiety from valine to isoleucine or leucine, did not change, though similar structural backbone was provided. As Boc substituted analogues had no sweet taste, the difference in spatial extension occupied by protecting group of the N-terminal may be one of the factors to exhibit sweetness. Actually, the spatial difference among Z group, Boc group and acetyl group (as reference) are obviously understood as shown in Fig.3. It can be concluded that the poor sweetness of these derivatives is due to the steric hindrance with N-protecting groups.

Taste exhibition of Z-aminoacyl-aldoxime

With the purpose of examining the necessity of glycine for sweet taste exhibition of Z-aminoacyl-glycinyl-aldoxime in Table 2, series of Z-aminoacyl-aldoxime were synthesized. The results of sensory evaluation are summarized in Table 3. These analogues also showed weak sweetness, and sparsely soluble in water. Therefore the comparison of

Table 3. Results of sensory evaluation with N-substituted aminoacyl-aldoximes

Compounds	Solubility in water Five steps	Taste kinds	Taste potency R.Suc
Z-Gly-aldoxime*1	****	No****	–
Z-Ala-aldoxime	**	Bitter*No***	–
Z-Vla-aldoxime*1	***	Bitter*No***	–
Z-Leu-aldoxime	**	Sweet***No*	<1
Z-Ile-aldoxime	*	Sweet***No*	<1
Z-Phe-aldexime	*	No****	–

Note : Table 2 as reference.

*1 : The principal components were occupied by alcohol derivatives.

aminoacyl glycine with terpenoid backbone can hardly discuss. Nevertheless the sweet taste exhibition in a series of Z-aminoacyl aldoximes suggests that an aldoxime compound has potential to exhibit sweetness. This confirms the earlier report[4].

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アルドキシム誘導体の合成とその呈味
第２報

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アミノアシルアルドキシムやペプチドアルドキシムの甘味の発現について研究した。アミノ酸やペプチドのカルボキシル基末端にアルドキシム基を導入して，多数の誘導体を合成した。特に，Ｎ保護疎水性アミノ酸，あるいはＮ保護アミノアシルーグリシンを用いて，ペリラルチンのテルペン骨格をアミノ酸等で置換した誘導体でペリラルチンのような強い甘味が発現されるかを評価した。呈味試験の結果，一連の誘導体が甘味を呈したが，特にＺ保護アミノアシルアルドキシムで砂糖と同程度の甘味を呈した。甘味の発現には，Ｎ端保護基の立体障害，水溶性，濃度，合成物における様々な要因や，呈味試験におけるパネラーの感性など様々な要因が重要であり，このような要因がアルドキシムにおける甘味発現と，甘味強度の変化に影響を及ぼしたと考えられた。
キーワード：甘味料，ペリラルチン，ペプチド，アルドキシム