Molecular Design of Sweet Tasting Compounds Based on 3β-Amino-3β-deoxy-18β-glycyrrhetinic Acid: Amido Functionality Eliciting Tremendous Sweetness

So Ijichi and Seizo Tamagaki
Department of Applied & Bioapplied Chemistry, Graduate School of Engineering, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka, 558-8585

(Received November 9, 2004; CL-041339)

A series of 18β-glycyrrhetinic acid (GA)-based, sweet tasting compounds has been prepared, where anionic NHCO-$(CH_2)_nCO_2K$ side chains are attached to the 3β-C position of the GA skeleton through an amide linkage. As compared with the corresponding ester-linking compounds, these amido-linking ones, except that of $n = 0$, elicit 13 to 25 times increase in sweetness. The sweetness enhancement is due probably to the proton donation capability of the amido NH group which would render the binding of these compounds to the taste bud cell receptor advantageous.

There have been known a huge number of natural or synthetic sweet substances with low molecular weight: sugar such as D-glucose and sucrose; almost D-amino acids; several L-amino acids such as histidine, leucine and tryptophan, chloroform, nitrobenzene, benzimidazole, cyclamate, aspartame, saccharin etc.[1]

Meanwhile, nature provides comparatively large organic molecules exhibiting sweet taste,[2] such as stevioside and potassium mono- and diglucronides of 18β-glycyrrhetinic acid (GA) (abbreviated as MGGR[3] and GK2,[4] respectively). GK2, the traditional herbal medicine glycyrrhizin, has been recognized as a surface-active, triterpenoid saponin with potent anti-inflammatory and anti-allergic activities widely used even today. Recently, we have demonstrated that sweetnesses are elicited for all the compounds that possess a proton-donation and a proton-acception site at each terminus of the hydrophobic GA scaffold; these distal polar binding sites are separating each other at a distance of 13 Å.[5] Therefore, we have inferred that there exist at least two different kinds of receptive sites in a sweetness-sensing protein receptor that would bind a tastant molecule through attractive forces such as hydrogen-bonding and electrostatic interactions: Most possibly, the one is acting as a proton accepting entity (B) like $-COO^-$ or $-NH_2$, while the other as a proton donating entity (AH) like –COOH or $-NH_3^+$ on the protein surface.

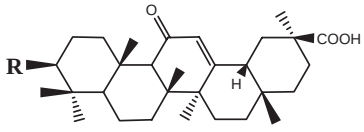
However, in spite of much effort to develop highly sweet GA-based compounds there have been found no sweet compounds superior to MGGR, which has been reported to exhibit 900-fold sweetness over sucrose.[3] Incidentally, GK2 is 150 times sweeter than sucrose. Thus, it is natural that our primary aim is in creating a new sweetness-enhancing site in GA-derived compounds and obtaining a sweetener exceeding MGGR. Potential significance of such GA-derived sweet compounds shows promise for their practical utilization in food and pharmaceutical industries.

In this work, by using 3β-amino-substituted GA (3β-amino-GA) as the scaffold[6] we have prepared a series of GA-derived anionic carboxylates with a different chain length through an amido linkage. The chemical structures of the compounds thus prepared are presented in Table 1.[7] As anticipated from the characteristic features of the structures, all the compounds are considerably surface-active. The utilization of an amido-linkage has two advantages over the ester-linkage: (1) the 3β-aminoGA can be easily prepared in an excellent yield from GA according to a conventional synthetic route; namely, the oxidation of GA to the corresponding 3-oxoGA followed by the reaction with hydroxylamine to give the 3-iminoGA and then its reductive cleavage with $NaBH_3CN$ and $TiCl_3$. The 3β-aminoGA thus obtained is modified with acid dichlorides or anhydrides to afford the desired amido derivatives and (2) the amido bond is far more stable than the ester bond toward acid- or alkaline-hydrolysis.

Thus, our major concern in this study is to know whether or not anionic 3β-amidoGA derivatives so formed exhibit favored sweetness. Generally, determination of sweetness was made for sample solutions at 25 °C with a human sensory panel in the following way: A solution of a known concentration of about 4 mM of a compound was diluted to a desired concentration with distilled water. The concentration at which the taste was closest to that of 2 or 4% (W/V) sucrose solution was determined by tasting the sample solutions of different concentrations. The results thus obtained are included in Table 1 together with the previous ones for the corresponding ester derivatives, for comparison.[8]

It is obvious from Table 1 that there is a remarkable difference in sweetness extent between these two series of GA-derivatives; the amido derivatives are much sweeter than the corresponding ester derivatives, indicative of the significance of the amido-linking in sweetness enhancement. Furthermore, the increasing number of spacer methylenes from $n = 1$ to $n = 3$ decreases the sweetness in a similar manner for both series, implying that the unfavorable entropic loss for the complexation di-

Table 1. Relative sweetness of GA derived carboxylates



	R	Sweetness[a]	
		X = NH[b]	X = O[c]
a	KO_2CCOX	60	150
b	KO_2CCH_2COX	1200	90
c	$KO_2C(CH_2)_2COX$	750	30
d	$KO_2C(CH_2)_3COX$	400	20
e	$KO_2CCH{=}CHCOX$ (cis)	60	200
	$KO_2CCH{=}CHCOX$ (trans)	—	10

[a]Sweetness relative to 2 or 4% sucrose. [b]This work. [c]Previous data.

minished sweetness. Previously, Shallenberger and coworkers proposed that the presence of complementary, bifunctional AH/B entities is the structural feature common to sweet compounds, where A and B are electronegative atoms separated by a distance of greater than 2.5 Å and less than 4 Å.[9] This AH/B molecular theory has often been employed as a guide for understanding and designing the structures of sweet substances. We can safely say, therefore, that those above compounds also possess one pair of an amido (AH) and a carboxylate (B) group with a separation of 2.5 to 4 Å. However, in contrast to the ester series, the amide series shows the least sweetness with the shortest side chain ($n = 0$), suggesting that, as inferred from the CPK molecular models, an intramolecular attraction between the carboxylate anion and the amido NH dipole at a distance of less than 2.5 Å disadvantages the sweetener-receptor complexation. The low sweetness of amido compound **e** is due to the same reason.

The above conclusion concerning the amido compounds may not be inconsistent with Shallenberger's model. However, the action of the hydrophobic GA skeleton is critical for the GA-related compounds to retain their sweetness, because potassium mono(cyclohexylaminocarbonyl)malonate, which involves the same AH/B functionality in the molecule, had no sweet taste. There is no doubt, therefore, that the interaction category of GA sweetners differs greatly from that of the Shallenberger–Acree–Kier three-points interaction model,[10] in which a third hydrophobic site plays only an auxiliary role rather than the major role in sweetness keeping operation. Further detailed information is required to sophisticate the mechanism.

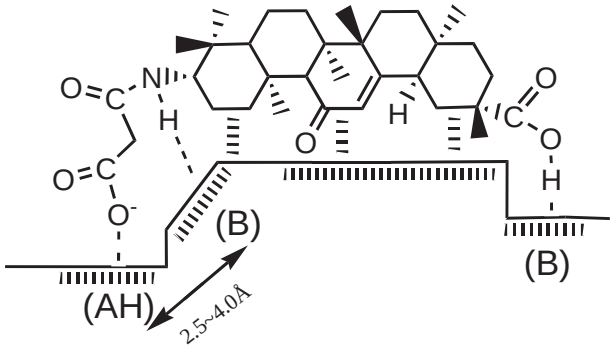


Figure 1. Possible sweet taste sensation model.

In conclusion, amido-for-ester substitution in sweet tasting anionic GA derivatives led to remarkably enhanced sweetness, which not only allowed us to implicate the possible molecular process of the sweetness expression, but also to infer the existence of a new AH locus in the sweetness-sensation protein. The study will serve basic information for developments and future discoveries of new sweet substances.

References and Notes

1 A. van der Heijden, "Structure of Sweeteners: An Overview," in "Sweet-Taste Chemoreception," ed. by J. A. Kanters and G. G. Birch, Elsevier Applied Science Publishers Baking (1993); "Aji to nioi no Bunsi-ninshiki," ed. by Nihonkagakukai Kihan Kagaku Sosetu, No. 40, Gakkai Shuppan Center, Tokyo (1999), Chap. 2, pp 35–49.

2 T. Kuramoto, Y. Ito, M. Oda, Y. Tamura, and S. Kitahara, *Biosci. Biotechnol. Biochem.*, **58**, 455 (1994); T. Kuramoto, Y. Tamura, N. Ohtaka, S. Doi, N. Nakamura, and O. Tanaka, *Biosci. Biotechnol. Biochem.*, **58**, 554 (1994); K. Mizutani, T. Kambara, T. Ikeda, O. Tanaka, M. Takasaki, and T. Konoshima, "Towards Natural Medicine Research in the 21st Century," ed. by H. Ageta, N. Aimi, Y. Ebizuka, T. Fujita, and G. Honda, Elsevier, Amsterdam (1998), p 225.

3 R. Nakamura, H. Amino, and T. Takamoto (Ajinomoto Co. LTD.), Japan. Kokai, 1999-11-131; MGGR was tasted as its NH_3-EtOH solution.

4 Maruzen Pharmaceuticals Co. LTD, *Dipotassium Glycyrrhizinate*, March (1994).

5 S. Tamagaki, M. Koide, M. Takahashi, T. Mizushima, J. Ukawa, and W. Tagaki, *J. Chem. Soc., Perkin Trans. 2*, **1996**, 1257.

6 Preparation of 3β-amino-3β-deoxy-18β-glycyrrhetinic acid (3β-aminoGA): The reaction of 3-oxo-18β-glycyrrhetinic acid with hydroxylamine was performed in THF at 50 °C for 24 h, followed by SiO_2-column-chromatogrphic purification using $CHCl_3$–MeOH, providing 3-oxo-18β-glycyrrhetinic acid (3-hydroxyiminoGA) in 94% yield; mp 238 °C; ^{1}H NMR (DMSO-d_6) δ: 0.77, 1.00, 1.04, 1.07, 1.10, 1.17, 1.41 (each 3H, each s, C–C<u>H</u>$_3$), 5.42 (1H, s, C12–H); MS Calcd for $C_{30}H_{46}NO_4$ ([M + H]$^+$) 484.3, Found 484.3. 3-HydroxyiminoGA thus obtained was treated with $NaBH_3CN$ and $TiCl_3$ according to the literature procedure: Chao-mei Ma, N. Nakamura and M. Hattori, *Chem. Pharm. Bull.*, **48**, 1681 (2000); The reaction mixture was acidified to pH 2 and then subjected to HW-60 gel-column chromatography and then HPLC on an ODS column using MeOH solvent, and was found to contain two isomers with a ratio of 5:1, where the major product was able to be assigned as 3β-isomer, since the $NaBH_3CN$ reductive amination of 3β-oxoGA will provide a thermodynamically stable 3β-isomer as the major product: cf. C. H. Brieskorn and H. Eschelbach, *Arch. Pharm. (Weinheim)*, **312**, 752 (1979). Finally. HPLC isolation gave 3β-aminoGA·HCl in 35% yield: ^{1}H NMR (CD_3OD) δ: 0.75, 0.84, 0.89, 0.91, 1.09, 1.17, 1.40 (each 3H, each s, C–C<u>H</u>$_3$), 2.90 (1H, m, C<u>H</u>–NH), 5.60 (1H, s, C12–H); MS Calcd for $C_{34}H_{51}NO_6$ ([M − HCl]$^+$) 470.3, Found 470.3.

7 The preparation method for amido compounds **a** and **b** was as follows: The GA-amine (100 mg, 0.2 mmol) was reacted with oxyalyl and malonyl chlorides (3.4 mmol) in the presence of Et_3N (1 mmol) in dry THF at −20 °C. Acidification with aq HCl, removal of the solvent, and then purification by gel-column chromatography using MeOH on an HW-60 column afforded **a** and **b**, isolated as potassium salts; (**a**): yield 48 mg (42%); ^{1}H NMR (CD_3OD) δ: 0.90, 1.09, 1.16, 1.28, 1.30, 1.43, 1.58 (each 3H, each s, C–C<u>H</u>$_3$), 3.60 (1H, m, C<u>H</u>–NH), 5.60 (1H, s, C12–H); IR (cm^{-1}) 1732 (COOH), 1651 (C=C–C=O) in KCl; MS Calcd for $C_{32}H_{46}NO_6$ ([M − K]$^-$) 540.3, Found 540.3. (**b**): yield 27 mg (28%); ^{1}H NMR (CD_3OD) δ: 0.90, 1.16, 128, 1.30, 1.32, 1.43, 1.59 (each 3H, each s, C–C<u>H</u>$_3$), 2.15 (1H, s, CCOC<u>H</u>C), 5.62 (1H, s, C12–H); IR (cm^{-1}) 1724 (COOH), 1659 (C=C–C=O), 1551 (CO–NH–CH) in KCl; MS Calcd for $C_{33}H_{50}NO_6$ ([M − K + 2H]$^+$) 556.3, Found 556.3. The preparation method for amido compounds **c**, **d**, and **e** was as follows: The amine (100 mg, 0.2 mmol) was reacted with succinic, glutaric, and maleic anhydrides (0.4 mmol) in dry THF at room temperature. Usual treatment of the reaction mixture, purification by the gel-column chromatography, and the conversion to the potassium salts gave the desired products **c**, **d**, and **e**; (**c**): yield 38 mg (31%); ^{1}H NMR (CD_3OD) δ: 0.75, 0.82, 0.89, 1.09, 1.16, 1.19, 1.39 (each 3H, each s, C–C<u>H</u>$_3$), 2.49 (2H, m, KOOC–C<u>H</u>$_2$), 5.71 (1H, s, C12–H); IR (cm^{-1}) 1701 (COOH), 1655 (C=C–C=O), 1551 (CO–NH–CH) in KCl; MS Calcd for $C_{34}H_{50}NO_6$ ([M − K]$^-$) 568.3, Found 568.3. (**d**): yield 27 mg (21%); ^{1}H NMR (CD_3OD) δ: 0.80, 0.84, 0.98, 1.13, 1.21, 1.25, 1.36 (each 3H, each s, C–C<u>H</u>$_3$), 2.07 (2H, m, CH$_2$–C<u>H</u>$_2$–CH$_2$), 2.77 (4H, m, C<u>H</u>$_2$–CH$_2$–C<u>H</u>$_2$), 4.46 (1H, m, CH–OH), 5.41 (1H, s, C12–H); IR (cm^{-1}) 1741 (COOH), 1650 (C=C–C=O), 1558 (CO–NH–CH) in KCl; MS Calcd for $C_{35}H_{53}NO_6$ ([M − K]$^-$) 582.3, Found 582.3. (**e**): yield 100 mg (83%); ^{1}H NMR (CD_3OD) δ: 0.79, 0.82, 0.90, 1.15, 1.16, 1.19, 1.37 (each 3H, each s, C–C<u>H</u>$_3$), 3.60 (4H, m, C<u>H</u>$_2$–O–C<u>H</u>$_2$), 5.81 (1H, s, C12–H); IR (cm^{-1}) 1638 (C=C–C=O), 1555 (CO–NH–CH) in KCl; MS Calcd for $C_{34}H_{47}KNO_6$ ([M − K]$^-$) 604.3, Found 604.3.

8 T. Toyoshima, M. Fujiwara, and S. Tamagaki, *J. Oleo Sci.*, **51**, 313 (2002).

9 R. S. Shallenberger, T. E. Acree, and W. E. Guild, *J. Food Sci.*, **30**, 560 (1965); R. S. Shallenberger and T. E. Acree, *Nature*, **216**, 480 (1967); R. S. Shallenberger and T. E. Acree, *J. Agric. Food Chem.*, **17**, 701 (1969); T. Machinami, The Optimization of Sweet Taste Quality Meeting, September 27–28, Viareggio, Italy, (1999) p 8.

10 L. B. Kier, *J. Pharm. Sci.*, **61**, 1394 (1972).

Published on the web (Advance View) February 12, 2005; DOI 10.1246/cl.2005.356