Tetrazoles as Carboxylic Acid Surrogates in the Suosan Sweetener Series

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Received March 3, 1989, from *Chemical Products Discovery, NutraSweet R & D, 601 E. Kensington Road, Mt. Prospect, IL 60056.* Accepted for publication November 16, 1989.

Abstract □ The structure–activity relationship (SAR) in the suosan series of sweeteners has been extended to include additional replacements for the carboxyl group. Tetrazole analogues have been prepared and were found to be sweet. However, both the urea and thiourea tetrazolyl analogues exhibited reduced potency when compared with the carboxyl compounds. Because of the larger size of tetrazole compared with carboxylic acid, chain-shortened tetrazolyl analogues were prepared and found to not be sweet. Some important aspects about the requirements for promoting a sweet taste in vivo can be gleaned from these results. The importance of the degree of delocalization and the orientation of charge density in the anionic group are discussed.

Suosan (1), a urea derivative of β-alanine, is a sweet compound having a potency reported to be 700 times that of sucrose.[1] The structural simplicity of this compound combined with its relatively high potency makes it an attractive starting point in the search for a commercially viable sweetener. Analogues of 1 have been prepared in an attempt to improve the qualities of this compound. Nofre and coworkers[2] have reported that other electron withdrawing groups could be substituted for nitro (compound 2) and that thiourea analogues (compound 3) increased potency by a factor of ~4. Examination of the distance requirement between the urea and carboxyl groups through the synthesis of compounds having varying chain lengths has shown that a two-carbon chain gives optimal potency (compounds 4 and 5)[1,2] (see Table I).

We became interested in further extending the SAR of this class of compounds by exploring possible replacements for the carboxyl group. Previous work[1,2c] has shown that sulfonic acid analogues of 1 are tasteless. It is possible that this lack of taste response could be due to the large difference in acidity between sulfonic acid and carboxylic acid (pK_a ~0 versus ~4.5) or the tetrahedral shape and charge distribution of the sulfonic acid moiety. Because the tetrazole group has a pK_a which is approximately equal to that of carboxyl[3] and the charge is distributed about a planar atomic array, we wanted to examine the feasibility of incorporating it into analogues of 1. Examples exist in the literature where 5-tetrazolyl can be substituted for carboxyl with various results (i.e., maintenance, reduction, or elimination of biological activity). Butler[4] and Singh et al.[5] have reviewed the literature describing the successful substitution of tetrazole for carboxylic acid in compounds having activity in a number of physiological areas including anti-inflammatory, analgesic, antibacterial, antiallergic, and hypolipemic. However, other workers have found that tetrazole analogues of carboxylic acids have substantially lower activity or are totally inactive. For example, Crenshaw et al.[6] have demonstrated the nonequivalence of tetrazole and carboxylic acid in the antifertility area, and Almquist et al.[7] have shown that the tetrazole analogue of a potent angiotensin converting enzyme (ACE) inhibitor is much less active than the corresponding carboxylic acid. In addition, the tetrazole analogue of γ-aminobutyric acid (GABA) has been found to be inactive as a substrate for GABA transaminase. In fact, the tetrazole analogue was reported to be a specific inhibitor of the enzyme.[8] Previous research on tetrazole analogues of D-tryptophan[9] has shown these compounds are sweet. In light of the above discussion, we were interested in determining what effect tetrazole substitution might have in the suosan series of sweeteners.

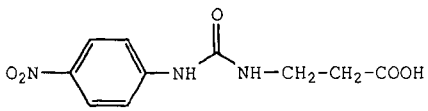

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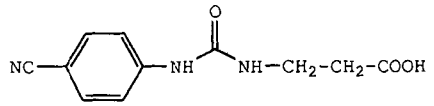
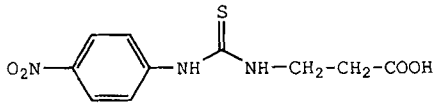
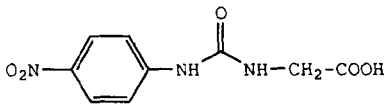
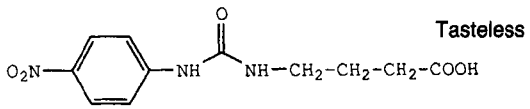
Table I—Potency of Compounds 2–5

Compound	Structure	Potency [$P_w(2)$][a]
2		450×
3		2400×
4		10×
5		Tasteless

[a] Compared with 2% sucrose.

Experimental Section

Melting points were obtained on a Thomas-Hoover Unimelt capillary apparatus and are not corrected. The IR spectra were taken as KBr pellets using either a Nicolet model 5 DXC or Perkin-Elmer model 881 spectrometer. The NMR spectra were obtained on a General Electric QE-300 spectrometer using tetramethylsilane as the internal reference. Because of the facile exchange of the tetrazolyl proton with water present in DMSO, these protons were not observed

0022-3549/90/0900-0826$01.00/0

in the NMR spectra. Microanalyses were performed by Midwest Microlab.

Method A—*Preparation of N-Cyanophenyl-N'-5-tetrazolylmethyl Urea (8)*—Aminomethyltetrazole[10] (0.50 g, 5.05 mmol) was added as a solid to a solution containing 0.73 g (5.07 mmol) of *p*-cyanophenylisocyanate in 15 mL of ethyl acetate. The heterogeneous reaction was stirred overnight at room temperature. The white solid was collected by filtration and treated with 10 mL of 1 M NaOH. The suspension was extracted with ether and the aqueous layer was filtered to remove any suspended solid. The filtrate was acidified to pH 2 with 1 M HCl giving 0.85 g (71%) of white solid: mp 186–187 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.69 (s, 1H), 7.70–7.57 (AB q, J = 7 Hz, 4H), 7.22 (t, J = 4 Hz, 1H), and 4.64 (d, J = 4 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.4, 154.7, 144.7, 133.1, 119.4, 117.6, 102.6, and 34.1; IR (KBr): 2238, 1675, 1600, 1555, 1515, 1420, 1325, and 1250 cm^{-1}. An analytical sample was obtained by recrystallizing a portion of this material from absolute ethanol.

Anal.—Calc. for (M + H) $C_{10}H_9N_7O$, 244.0947; found, 244.0980 (HRFABMS).

Method B—*Preparation of N-Cyanophenyl-N'-5-Tetrazolylmethyl Thiourea (9)*—A solution containing 0.50 g (5.05 mmol) of aminomethyltetrazole in 5 mL of water containing 0.27 g (2.55 mmol) of sodium carbonate was added to a solution containing 0.80 g (5.00 mmol) of *p*-cyanophenylisothiocyanate in 5 mL of ethyl acetate. The resulting two-phase solution was stirred rapidly at room temperature overnight. The aqueous layer was separated and acidified to pH 2 using 1 M HCl giving a white solid which was collected by filtration. This solid was dried at 56 °C and 0.1 mm Hg overnight to give 1.0 g (77%), mp 200–201 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.69 (br s, 1H), 7.81 (s, 4H), and 5.08 (d, J = 4.5 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 180.9, 154.3, 143.6, 132.8, 121.8, 118.9, 105.3, and 37.9; IR (KBr): 2238, 1605, 1580, 1545, 1519, 1360, and 1320 cm^{-1}.

Anal.—Calc. for $C_{10}H_9N_7S$: C, 46.32; H, 3.50; N, 37.81; S, 12.37. Found: C, 46.03; H, 3.45; N, 38.08; S, 12.42.

N-Cyanophenyl-N'-2-(5-tetrazolyl)ethyl Urea (6)—Compound **6** was prepared in 7% yield by treating 1.44 g (10.0 mmol) of *p*-cyanophenylisothiocyanate with 1.13 g (10.0 mmol) of 2-aminoethyltetrazole[10] in 30 mL of acetonitrile as described in method A, mp 233–235 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 1H), 7.71–7.63 (AB q, J = 7 Hz, 4H), 6.60 (t, J = 5 Hz, 1H), 3.58 (q, J = 5 Hz, 2H), and 3.11 (t, J = 5 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.5, 154.2, 144.8, 133.1, 119.4, 117.4, 102.4, 37.2, and 24.0; IR (KBr): 2236, 1696, 1615, 1601, 1560, 1515, 1420, 1370, and 1326 cm^{-1}.

Anal.—Calc. for $C_{11}H_{11}N_7O \cdot 0.4H_2O$: C, 49.96; H, 4.50; N, 37.07. Found: C, 49.62; H, 4.25; N, 37.34.

N-Cyanophenyl-N'-2-(5-tetrazolyl)ethyl Thiourea (7)—Compound **7** was prepared in 28% yield by treating 0.80 g (5.0 mmol) of *p*-cyanophenylisothiocyanate in 5 mL of ethyl acetate with 0.57 g (5.0 mmol) of 2-aminoethyltetrazole in 5 mL of water containing 0.27 g (2.55 mmol) of sodium carbonate as described in method B, mp 165–167 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.07 (s, 1H), 8.27 (br s, 1H), 7.75 (s, 4H), 3.94 (q, J = 6 Hz, 2H), and 3.24 (t, J = 6 Hz, 2 H; ^{13}C NMR (75 MHz, DMSO-d_6): δ 180.2, 154.3, 143.8, 132.7, 121.4, 119.0, 104.8, 41.4, and 22.5; IR (KBr): 2236, 1610, 1540, 1515, 1302, and 1260 cm^{-1}.

Anal.—Calc. for $C_{11}H_{11}N_7S \cdot 0.75H_2O$: C, 46.06; H, 4.39; N, 34.18; S, 11.18. Found: C, 46.06; H, 4.34; N, 33.97; S, 11.33.

Results and Discussion

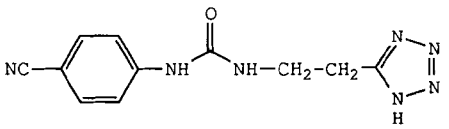
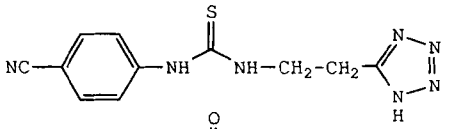
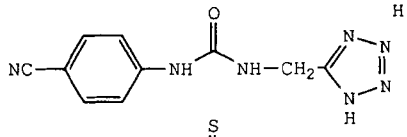
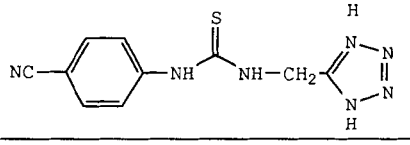
The tetrazole analogue of β-alanine can be conveniently prepared by the addition of ammonium azide to carbobenzoxy-protected aminopropionitrile[10] followed by deprotection. Heterogeneous reaction of 2-aminoethyltetrazole with *p*-cyanophenylisocyanate in ethyl acetate gave the desired urea in poor yield (method A).

An alternate procedure using the sodium salt of the tetrazole was investigated to prepare the tetrazolyl thiourea analogue (method B). The sodium salt of aminoethyltetrazole was prepared in water and added to an ethyl acetate solution of *p*-cyanophenylisothiocyanate. The resulting two-phase system was stirred rapidly at room temperature overnight. The aqueous layer was separated and acidified to pH 2 using 1 M HCl, giving a white solid that was collected by filtration. We found that the tetrazole analogues prepared were sweet, the thiourea (**7**) being significantly more potent than the urea (**6**), but the potencies were reduced by a factor of ~10 when compared with the carboxyl compounds (Table II). [Since potency is dependent on the reference sucrose concentration, it is important to include that information when reporting potency data. A standardized way to report potency data is on a weight basis (as opposed to a molar comparison with sucrose), with the reference sucrose concentration in weight percent shown in parentheses.[11]]

Various reasons may be considered to explain this difference in potency between the carboxyl compound and tetrazole analogue. As noted above, the carboxyl and tetrazole functionalities have approximately equal acidities, leading us to believe that in this respect tetrazolyl is an adequate surrogate for carboxyl. Other differences between the two groups might explain the difference in potency. One difference between the two is steric size, the tetrazolyl group containing two additional atoms. This larger size could prevent the tetrazole ring from binding as effectively at a putative sweet taste receptor,[11–14] thereby causing a decrease in potency. Since the tetrazole group has its negative charge density delocalized over all four nitrogen atoms, it was decided to move the tetrazole group closer to the urea NH. We reasoned that interaction of this chain-shortened analogue with a presumed carboxylate recognition site could improve through more facile interaction with the anionic charge on the distal nitrogen atoms. The chain-shortened urea and thiourea analogues were therefore synthesized using aminomethyl tetrazole and the procedures described previously. These compounds (**8** and **9**), however, were not sweet at 1.0 mg/mL (see Table II). The lack of sweet taste response in these analogues suggests that it is the proximal nitrogen atoms that are important in elicitation of a sweet taste response in vivo.

Some important aspects about the requirements for recognition by a putative sweet taste receptor can be gleaned from these results. We have shown the importance of the carboxyl group for sweet taste response in the suosan series. Requirements are quite specific for binding to the anionic group of a molecule. Shallenberger and Acree[13] proposed the AH-B theory of binding to explain the structural features necessary to elicit a sweet taste response. By examining structures of compounds that taste sweet, they described the presence of a proton donor group (AH) and a proton acceptor group (B)

Table II—Potency of Compounds 6–9

Compound	Structure	Potency[a]
6		$P_w(3)$ = 30
7		$P_w(3)$ = 300
8		Tasteless
9		Faintly bitter

[a] Potency is reported on a weight basis, with the reference sucrose concentration in weight percent shown in parentheses.

separated by 2.5–4.0 Å in each of the structures. They postulated these groups interact with a complementary B-AH region on a sweet receptor. Kier[14] later refined this theory to include a dispersion binding site. Our results suggest that for sweeteners requiring carboxylate recognition, the maximum size of the anionic group and also its charge distribution, must be very similar to that of a carboxyl group to provide the good receptor interaction needed to elicit a sweet taste response. Furthermore, a bidentate mode of interaction of the carboxyl group with the putative sweet receptor (possibly with an arginine guanidino group[15]) cannot be duplicated with the tetrazole group. The tetrazole ring nitrogens occupy positions where the hydrogen bonding interaction would occur between the carboxyl group of the substrate and the proposed bidentate acceptor of the putative receptor system (Figure 1). Examination of the orientation of the electron density in tetrazole reveals lobes pointing in opposite directions which does not allow any bidentate interaction with a receptor component. One set of orbitals found in the tetrazole group can interact with the putative component. This type of interaction is effective, but less efficient, and may be the cause of the reduced potency of tetrazole analogues compared with the carboxylic acid compounds.

Conclusions

Structure–activity relationships in variation of the carboxyl moiety of suosan-related sweeteners have been gained through the synthesis of tetrazolyl analogues. We determined the sweet taste response was reduced in tetrazole analogues, implying the relevance of size, electron density, and bidentate interactions of the anionic group on the phenomenon of sweet taste perception.

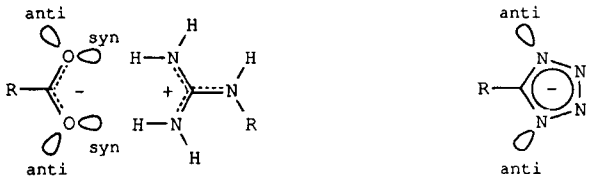


Figure 1—Proposed bidentate interaction.

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Acknowledgments

I am grateful for numerous stimulating discussions with Dr. Michael Kellogg during the course of this work.