Investigations of (4-Cyanophenyl)ureas of Sweet Amino Acids as Potential Sweeteners

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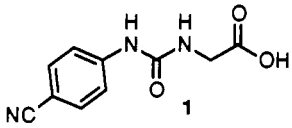
(4-Cyanophenyl)carbamoylglycine has a sweetness potency of 30 times sucrose and 20-fold higher than that of glycine itself. We sought to determine whether the potentiation of the sweetness potency of glycine by the introduction of the ureido functionality could be extended to the other sweet amino acids.

INTRODUCTION

Many amino acids are known to have a sweet taste (Berg, 1953; Solms, 1969; Schiffman et al., 1981). The simplest amino acid, glycine, derives its name from this characteristic (Greek, γλυκυσ, *glykys*, sweet). Schallenberger and co-workers (Schallenberger et al., 1969) proposed a model to account for the observation that glycine, D- and L-alanine, and many of the D-amino acids are sweet, while most L-amino acids are not (Figure 1a). Sweetness potencies for the sweet amino acids range from 1.5 (glycine, alanine) to 35 times sucrose (D-tryptophan).

Tinti et al. (1981) have reported that *N*-[(4-cyanophenyl)carbamoyl]glycine (1) has a sweetness potency of 30 times sucrose, 20-fold higher than that of glycine.



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If the urea protons of 1 have substantial partial positive charge, it would be reasonable to suppose that the ureidoglycine 1 may bind to the same receptor site as glycine. We carried out calculations of the electronic charge distribution of 1 and glycine using the INDO/S method (Ridley and Zerner, 1973) and found that the urea protons of 1 have positive charge comparable to the amino group protons of zwitterionic glycine (average partial atomic charge, +0.21 vs +0.24). Thus, 1 may occupy the site as illustrated in Figure 1b, and the arylurea functionality could induce higher potency by interaction with additional binding site(s) as shown in Figure 1b. Our interest was to determine whether the potentiation of the sweetness potency of glycine by introduction of a ureido moiety could be extended to other sweet amino acids. We tested this hypothesis by preparing (cyanophenyl)urea derivatives of the sweet amino acids listed in Table I.

The strategy to synthesize the ureas is illustrated in Scheme I. The carbamoylation of the methyl esters proceeded without difficulty. Hydrolysis of the methyl esters provided the desired ureas in good yields. The yields of the reactions were not optimized.

RESULTS AND DISCUSSION

Addition of the cyanophenylureido moiety did not enhance the potency of any of the sweet amino acids (Table I). In fact, all were either tasteless or bitter. L-Phenylalanine urea was also synthesized and tasted to determine whether the urea series has a different stereochemical requirement than do the D-amino acids; it too produced a bitter urea derivative.

The lack of sweet taste in this series may indicate that 1 and glycine do not act at the same receptor site. Alternatively, the receptor site for amino acids and 1 may

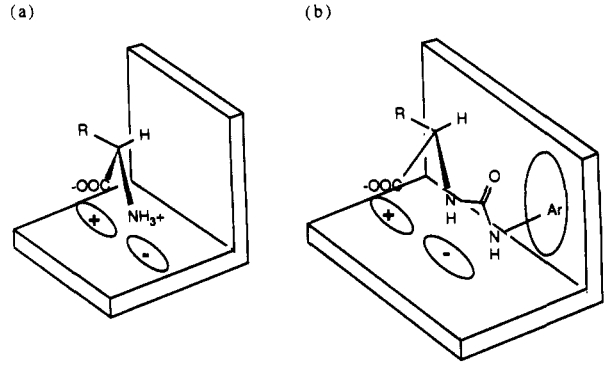


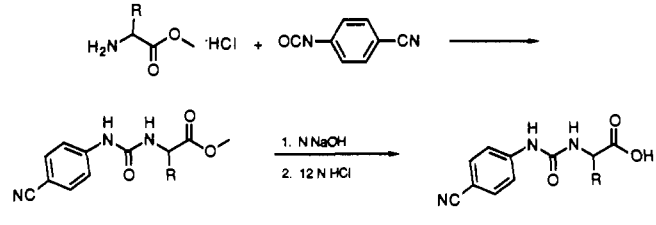
Figure 1. Schallenberger model of the sweet receptor with glycine (a) and (4-cyanophenyl)carbamoylglycine (1) (b).

Table I. Tastes of Amino Acids and Their (*p*-Cyanophenyl)carbamoyl Derivatives[a]

amino acid	taste[b]	urea	taste
glycine	sweet, Pw(0.45) = 1.5	1	sweet, Pw(2) = 30[c]
D-tyrosine	sweet, Pw(1.65) = 5.5	3	tasteless
D-leucine	sweet, Pw(1.3) = 4.3	5	bitter
D-phenylalanine	sweet, Pw(2.2) = 7.3	7	tasteless
L-phenylalanine	bitter	13	bitter
L-alanine	sweet, Pw(0.54) = 1.8	9	bitter
D-tryptophan	sweet, Pw(10.5) = 35	11	tasteless

[a] Sweetness potencies are expressed on a weight basis relative to a specified concentration of sucrose. For example, Pw(0.45) = 1.5 indicates a potency of 1.5 times sucrose relative to a 0.45% sucrose solution. [b] Data from Solms (1969). [c] Data from Tinti et al. (1981).

Scheme I. Synthesis of (4-Cyanophenyl)ureas of Amino Acids



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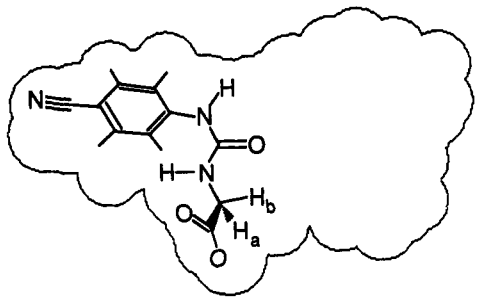

Figure 2. Modified Schallenberger sweet receptor model (Culberson and Walters, 1991) with (4-cyanophenyl)carbamoylglycine (1).

have more stringent steric requirements than the Schallenberger model suggests. We have developed a three-dimensional receptor model to account for the structure–activity relationships of high-potency dipeptides, guanidineacetic acids, and arylurea dipeptides (Culberson and Walters, 1991). The present series of compounds serves to further define the steric requirements of this model in the region near the carboxylate group. Figure 2 shows the outline of our model, with compound 1 in a low-energy conformation fit into the proposed binding site. This compound satisfies the requirements for binding (carboxylate group; aryl–NH group in the region of positive electrostatic potential; electron-deficient aromatic ring). Arylurea derivatives of D-amino acids would have side-chain substitution at the position occupied by H_a; our model suggests that this region is sterically very demanding, consistent with results published by Mazur et al. (1969). L-Amino acids (side chain in place of H_b) could not adopt the required conformation because the side chain would be sterically too close to the carbonyl oxygen.

EXPERIMENTAL PROCEDURES

Molecular modeling was carried out by using the MacroModel program (Mohamadi et al., 1990). INDO/S calculations (Ridley and Zerner, 1973) were carried out on conformations that were optimized to local (not necessarily global) minima. All compounds were shown to be ≥95% pure and were tested for safety in an Ames mutagenicity test (Ames et al., 1975) and a mouse acute limit test (Auletta, 1988) prior to taste evaluation. Taste qualities were evaluated in aqueous solution at a concentration of 10.0 mg/mL.

Methyl *N*-[(4-Cyanophenyl)carbamoyl]-D-tyrosinate (2). To a stirring solution of 1.58 g (11 mmol) of 4-cyanophenyl isocyanate in 100 mL of pyridine was added 2.32 g (10 mmol) of methyl tyrosinate hydrochloride and 0.06 g (0.5 mmol) of (dimethylamino)pyridine (DMAP). After the mixture had stirred for 16 h, the solvent was removed under water aspirator vacuum. The traces of pyridine were removed from the product by azeotroping two times with toluene. The product was purified on a flash column in 5% EtOH/dichloromethane (Still et al., 1978). The yield of product was 1.39 g (41%). The product was used immediately in the next step. ^{1}H NMR (DMSO-d_6) δ 9.38 (s, 1 H), 9.32 (s, 1 H), 7.66 (d, 2 H, J = 8 Hz), 7.52 (d, 2 H, J = 8 Hz), 6.97 (d, 2 H, J = 7 Hz), 6.69 (d, 2 H, J = 7 Hz), 6.60 (d, 1 H, J = 6 Hz), 4.47 (m, 1 H), 3.64 (s, 3 H), 3.01 (dd, 1 H, J = 5, 13 Hz), 2.90 (dd, 1 H, J = 7, 13 Hz); ^{13}C NMR (DMSO-d_6) δ 174.5, 157.3, 145.8, 134.4, 131.4, 128.1, 120.5, 118.6, 116.3, 104.0, 55.0, 54.9, 37.6.

***N*-[(4-Cyanophenyl)carbamoyl]-D-tyrosine (3).** To a stirring solution of 1.38 g (4.1 mmol) of 2 in 6 mL of MeOH was added 6.2 mL of 1 N NaOH. After 30 min, the reaction was extracted with 1 × 25 mL of EtOAc. The aqueous layer was adjusted to pH 3 with 12 N HCl. The product oiled out of solution. The aqueous layer was extracted with 1 × 25 mL of EtOAc. The organic layer was dried over Na_2SO_4 (anhydrous), filtered, and stripped; 1.01 g (77%) of product was recovered. An analytical sample was dried at 78 °C under high vacuum. Anal. Calcd for $C_{17}H_{15}N_3O_4 \cdot 0.3H_2O$: C, 61.74; H, 4.75; N, 12.71. Found: C, 62.07; H, 4.71; N, 12.30. ^{1}H NMR (DOAc) δ 7.58 (d, 2 H, J = 8 Hz), 7.52 (d, 2 H, J = 8 Hz), 7.08 (d, 2 H, J = 9 Hz), 6.79 (d, 2 H, J = 9 Hz), 4.82 (t, 1 H, J = 6 Hz), 3.18 (dd, 1 H, J = 6, 13 Hz), 3.07 (dd, 1 H, J = 7, 13 Hz); ^{13}C NMR (DOAc) δ 176.6, 155.9, 144.0, 133.7, 131.0, 127.8, 119.5, 119.0, 115.9, 54.5, 37.1.

Methyl *N*-[(4-Cyanophenyl)carbamoyl]-D-leucinate (4). Under the same conditions described for 2, 2.51 g (87%) of 4 was recovered by starting with methyl D-leucinate hydrochloride. The product was a white foam. Anal. Calcd for $C_{15}H_{19}N_3O_3$: C, 61.88; H, 6.65; N, 14.43. Found: C, 61.77; H, 6.68; N, 14.24. ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1 H), 7.43 (d, 2 H, J = 8 Hz), 7.41 (d, 2 H, J = 8 Hz), 6.14 (d, 1 H, J = 9 Hz), 4.50 (m, 1 H), 3.78 (s, 3 H), 1.5–1.8 (m, 3 H), 0.96 (d, 3 H, J = 3 Hz), 0.94 (d, 3 H, J = 3 Hz); ^{13}C NMR ($CDCl_3$) δ 175.2, 154.9, 143.6, 133.1, 119.4, 118.5, 105.0, 52.6, 51.8, 41.2, 24.9, 22.8, 21.8.

***N*-[(4-Cyanophenyl)carbamoyl]-D-leucine (5).** Urea 5 was synthesized from 4 in the manner described for 3. After recrystallization from MeOH, 1.90 g (88%) of 5 was isolated. Anal. Calcd for $C_{14}H_{17}N_3O_3$: C, 61.08; H, 6.22; N, 15.26. Found: C, 60.98; H, 6.36; N, 15.11. ^{1}H NMR (DMSO-d_6) δ 7.62 (d, 2 H, J = 8 Hz), 7.54 (d, 2 H, J = 8 Hz), 6.61 (d, 1 H, J = 9 Hz), 4.18 (q, 1 H, J = 8 Hz), 1.65 (m, 1 H), 1.53 (m, 1 H), 0.88 (t, 6 H, J = 6 Hz); ^{13}C NMR (DMSO-d_6) δ 175.1, 154.9, 145.2, 133.7, 119.9, 118.1, 103.3, 51.4, 41.4, 25.0, 23.4, 22.2.

Methyl *N*-[(4-Cyanophenyl)carbamoyl]-D-phenylalaninate (6). Urea 6 was synthesized under the same conditions described for 2 by starting with methyl D-phenylalaninate hydrochloride. A yield of 3.03 g (94%) was isolated. Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.95; H, 5.29; N, 13.08. ^{1}H NMR ($CDCl_3$) δ 8.81 (br s, 1 H), 7.50 (m, 4 H), 7.2–7.3 (m, 3 H), 7.13 (br d, 2 H), 6.41 (d, 1 H, J = 7 Hz), 4.84 (m, 1 H), 3.73 (s, 3 H), 3.14 (d, 2 H, J = 6 Hz).

***N*-[(4-Cyanophenyl)carbamoyl]-D-phenylalanine (7).** Compound 7 was prepared from 6 in the same manner as 3 to give a quantitative yield. Upon recrystallization from EtOAc–hexanes, 1.38 g (62%) of product was recovered. Anal. Calcd for $C_{17}H_{15}N_3O_3 \cdot 0.25H_2O$: C, 65.06; H, 4.98; N, 13.39. Found: C, 65.43; H, 5.01; N, 13.40. ^{1}H NMR (DOAc) δ 7.5–7.6 (m, 4 H), 7.2–7.3 (m, 5 H), 4.63 (m, 1 H), 3.20 (dd, 1 H, J = 7, 15 Hz), 3.08 (dd, 1 H, J = 9, 15 Hz); ^{13}C NMR (DOAc) δ 173.9, 154.9, 144.7, 137.4, 133.6, 129.9, 128.8, 127.2, 119.7, 118.2, 104.1, 54.1, 37.8.

Methyl *N*-[(4-Cyanophenyl)carbamoyl]-L-alaninate (8). Urea 8 was synthesized by starting from methyl L-alaninate hydrochloride under the same conditions described for 2. After chromatography, 1.89 g (77%) of 8, which was a white solid, was isolated. Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.16; H, 5.21; N, 17.01. ^{1}H NMR ($CDCl_3$) δ 8.87 (br s, 1 H), 7.52 (d, 2 H, J = 9 Hz), 7.50 (d, 2 H, J = 9 Hz), 6.52 (d, 1 H, J = 8 Hz), 4.51 (p, 1 H, J = 8 Hz), 3.76 (s, 3 H), 1.42 (d, 3 H, J = 8 Hz).

***N*-[(4-Cyanophenyl)carbamoyl]-L-alanine (9).** Under the same conditions described for 3, compound 9 was prepared from 8 and recrystallized from EtOAc–hexanes to yield 0.89 g (53%). Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.68; H, 4.82; N, 17.65. ^{1}H NMR (DMSO-d_6) δ 7.72 (d, 2 H, J = 8 Hz), 7.61 (d, 2 H, J = 8 Hz), 4.26 (q, 1 H, J = 8 Hz), 1.38 (d, 3 H, J = 8 Hz).

Methyl *N*-[(4-Cyanophenyl)carbamoyl]-D-tryptophanate (10). Urea 10 was synthesized from methyl D-tryptophanate hydrochloride under the same conditions described for 2. After chromatography, 2.90 g (80%) of a white foam was recovered. Anal. Calcd for $C_{20}H_{18}N_4O_3 \cdot 0.25H_2O$: C, 65.47; H, 5.08; N, 15.27. Found: C, 65.29; H, 5.15; N, 14.94. ^{1}H NMR ($CDCl_3$) 8.23 (br s, 1 H), 6.9–7.5 (m, 10 H), 5.72 (d, 1 H, J = 7 Hz), 4.86 (m, 1 H), 3.75 (s, 3 H), 3.38 (dd, 1 H, J = 6, 15 Hz), 3.15 (dd, 1 H, J = 7, 15 Hz).

***N*-[(4-Cyanophenyl)carbamoyl]-D-tryptophan (11).** Under the same conditions described for 3, compound 11 was isolated in a yield of 2.15 g (80%). Anal. Calcd for $C_{19}H_{16}N_4O_3 \cdot 0.25H_2O$: C, 64.67; H, 4.71; N, 15.87. Found: C, 64.38; H, 4.67; N, 15.92. ^{1}H NMR (DMSO-d_6) δ 10.8 (s, 1 H), 9.3 (s, 1 H), 6.5–8.8 (m, 9 H), 4.48 (br s, 1 H), 3.38 (br s, 1 H), 3.20 (dd, 1 H, J = 6, 16 Hz), 3.11 (dd, 1 H, J = 9, 16 Hz).

Methyl *N*-[(4-Cyanophenyl)carbamoyl]-L-phenylalaninate (12). Urea 12 was synthesized in the same manner as 2.

A yield of 2.36 g (73%) of **12** was recovered. Anal. Calcd for $C_{18}H_{17}N_3O_3 \cdot 0.1H_2O$: C, 66.49; H, 5.33; N, 12.92. Found: C, 66.34; H, 5.34; N, 12.85. ^1H NMR ($CDCl_3$) 7.1–7.5 (m, 9 H), 5.78 (d, 1 H, J = 9 Hz), 4.82 (m, 1 H), 3.80 (s, 3 H), 3.11 (dd, 1 H, J = 6, 14 Hz), 2.92 (dd, 1 H, J = 7, 14 Hz).

***N*-[(4-Cyanophenyl)carbamoyl]-L-phenylalanine (13).** Under the same conditions as described for **3**, 2.27 g (96%) of urea **13** was isolated. Anal. Calcd for $C_{17}H_{15}N_3O_3 \cdot 0.7H_2O$: C, 63.43; H, 5.13; N, 13.05. Found: C, 63.09; H, 4.71; N, 12.96. ^1H NMR (DMSO-d_6) δ 7.63 (d, 2 H, J = 8 Hz), 7.53 (d, 2 H, J = 8 Hz), 7.24 (m, 5 H), 6.72 (d, 1 H, J = 9 Hz), 4.40 (dd, 1 H, J = 7, 9 Hz), 3.09 (dd, 1 H, J = 7, 17 Hz), 2.97 (dd, 1 H, J = 9, 17 Hz).

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