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Note

On the Sweetness of *N*-(Trifluoroacetyl)aspartame

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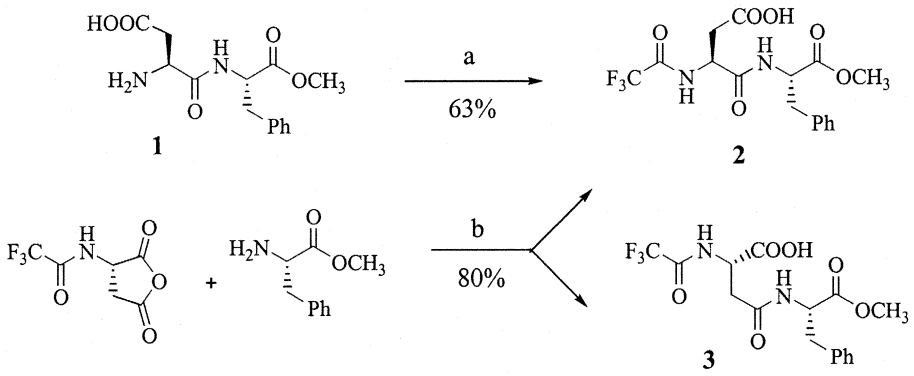
A panel of tasters has found that the *N*-trifluoroacetyl derivative of aspartame is five times less sweet than the parent compound, contrary to the tenet in the literature, but consistent with sweet receptor models which require this nitrogen to exist in protonated form.

Key words: artificial sweetener; aspartame derivative; sweetness test

Since the discovery of the edulcorant properties of aspartame (**1**) 30 years ago,[1] well over a thousand dipeptide derivatives have been prepared in order to understand the structural features required for sweetness.[2] Aspartame is generally accepted as being about 200 times sweeter than sucrose; while considerable alteration of the phenylalanine ester part can be tolerated, most modifications to the aspartate moiety result in unsweet compounds. Notably, derivatizing the *N*-terminal amine function usually leads to a loss of sweetness, which has contributed to the belief that the free α-amino-β-carboxylate system of aspartame is necessary for sweet properties. Indeed, most models for the receptor interaction of aspartame derivatives make account for this zwitterionic system (the AH/B model).[3] In some recently-described derivatives of aspartame, an *N*-terminal substituent itself bears a potent AH/B system;[4] however, the only simple *N*-terminal substituents which allow retention of the sweetness of aspartame are alkyl groups, since this derivatization permits the nitrogen to remain in its charged form.[5] It is curious, then, that *N*-(trifluoroacetyl)aspartame (TFA-aspartame; **2**) should have been reported some time ago as having "very little difference in sweetness" when compared to aspartame (which the authors found to be around 150 times sweeter than sucrose).[6] Receptor models do not (or cannot) account for this; nevertheless, this observation has gone unquestioned and other sweetener analogues containing a TFA-Asp moiety have even been devised and tested for sweetness on this premise.[7] In the course of recent work on conformationally restricted Asp-peptides,[8] we had occasion to reassess the taste properties of **2**.

In our hands, the method of synthesis indicated in the original report[6] on **2**, *i.e.* condensation of *N*-trifluoroacetyl-L-aspartic acid anhydride with L-phenylalanine methyl ester,[9] proved unsatisfactory. We found that mixtures of the required α-amide derivative **2** and the isomeric β-(*N*-trifluoroacetyl-L-Asp)-L-Phe-OMe **3** were formed (Scheme 1). In fact, this observation is not surprising, since complete control of the regioselectivity of nucleophilic attack on *N*-trifluoroacetyl-L-aspartic acid anhydride is not always guaranteed.[10] Since recrystallization of mixtures of **2** and **3** failed to give a pure sample of the former, we prepared it instead by an unequivocal synthesis involving trifluoroacetylation of aspartame (Scheme 1).



Scheme 1. Reagents and Conditions.
a) $(CF_3CO)_2O$, $NaHCO_3$, acetone, rt, 1 h; b) THF, rt, 24 h: typically, a 2:1 ratio of **3**:**2** is obtained.

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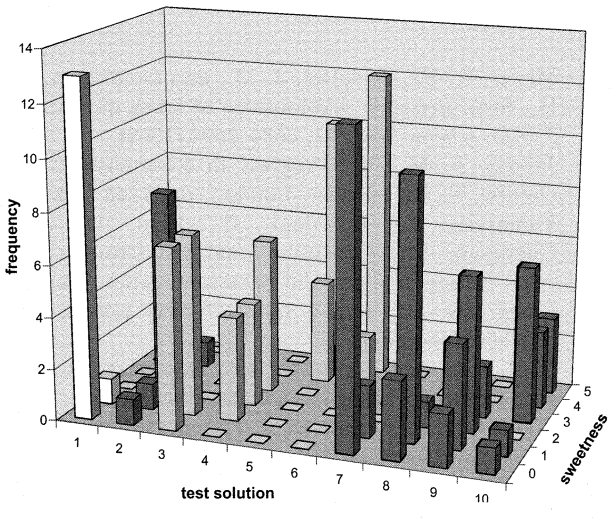

Fig. 1. Results of Sweetness Test.

Test solutions were as follows: Solution 1, pure mineral water; Solution 2, standard 2% sucrose (58.4 mM); Solutions 3–6, aspartame (0.06 mM, 0.29 mM, 1.45 mM, 2.90 mM, respectively); Solutions 7–10, TFA-aspartame (0.04 mM, 0.22 mM, 1.10 mM, 2.20 mM, respectively). Sweetness response varied from 0 (not sweet) to 5 (very sweet).

Solutions of varying concentrations of **2** in water were tested, along with reference solutions of aspartame and sucrose, by a panel of tasters in double-blind experiments. Results are presented in Fig. 1. Accurate responses were obtained for the reference solutions, with aspartame being evaluated as 200 times sweeter than sucrose. In contrast, solutions of TFA-aspartame produced distinctly lower responses: at high concentrations (millimolar), the results had a wider dispersion, perhaps due to the accompanying bitter taste, but this compound was clearly less sweet than aspartame. We estimate a sweetness factor of about 40 (compared with sucrose), which is only one-fifth of the value for aspartame.

The maximum solubility of **2** in water at 20°C was 2.20 mM, and this solution had a pH of 3.1 (which compares with pH values of 2.6 and 4.6 for 2 mM solutions of aspartic acid and aspartame, respectively), which may explain its bitterness at higher concentrations. We verified the stability of solutions of **2**, since degradation might have produced free aspartame in solution, giving an artificially high sweetness response. Unbuffered solutions were stable at room temperature for at least several days, confirming that the sweetness response was genuinely that of **2**. However, the lability of the *N*-trifluoroacetyl group was apparent in the ageing of aqueous solutions buffered at pH 7.0 (50 mM phosphate): up to 20% free aspartame appeared over a period of several days, as detected by HPLC analysis, accompanied by other components probably derived from the subsequent degradation of aspartame under these conditions.[11,12]

Nonetheless, we verified that pH differences had no effect on the appreciation of sweetness. The taste panel detected no difference in sweetness between a freshly prepared 1.5 mM solution of TFA-aspartame and the same solution adjusted to pH 7. Indeed, the same result was observed for a 0.3 mM aspartame solution. These test concentrations were chosen to match the sweetness of 2% sucrose solution and confirmed as such.

In conclusion, our observations indicate that, contrary to a previous suggestion, the elaboration of the *N*-terminal of aspartame as a trifluoroacetamide provokes a diminution in the sweetness properties; this phenomenon is entirely consistent with the requirement that the terminal nitrogen should exist in protonated form, prevalent in current models for the sweetness receptor.

Experimental

Melting point data were recorded with a Reichert microscope apparatus and are uncorrected. Infrared spectra were recorded with a Perkin Elmer PE 881 spectrophotometer. NMR spectra were recorded with Bruker AC-400 instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. For the latter nucleus, observed phasing in *J*-modulation experiments is indicated as up (u) or down (d). Mass spectra were recorded with a HP MS-Engine 5989B spectrometer. Optical rotations were measured with a Jasco DIP digital polarimeter.

N - Trifluoroacetyl - L - aspartyl - L - phenylalanine methyl ester (**2**). A solution of trifluoroacetic anhydride (1.20 ml, 8.5 mmol) in acetone (14 ml) was added to a stirred suspension of aspartame (Aldrich; 2.06 g, 7.0 mmol) and $NaHCO_3$ (1.56 g, 18.6 mmol) in acetone (28 ml) at 0°C. The reaction was completed by adding further portions of trifluoroacetic anhydride (0.30 and 0.50 ml) after 10 and 20 min. All aspartame has been solubilized by this time. After 1 h the solvent was evaporated and the residue partitioned between saturated aqueous $NaHCO_3$ solution and EtOAc. The aqueous layer was extracted twice with EtOAc, the combined organic layers were washed once with brine and dried with anhydrous $MgSO_4$. After evaporation of the solvent, the residue was filtered through a short column of silica gel, using 3:1 cyclohexane-EtOAc as eluent. The white solid obtained by evaporation of the filtrate was recrystallized from cyclohexane-EtOAc to yield the title compound as a white microcrystalline solid (1.70 g, 63 %): mp 168–169°C (cyclohexane-EtOAc); $[\alpha]_D^{25}$ −31.6° (*c* 0.93, EtOH); IR ν_{max} (KBr) cm^{-1}: 3290 (s, br), 3090 (m), 3030 (w), 2960 (w), 1740 (s), 1720 (s), 1700 (s), 1670 (s), 1555 (s), 1440 (w), 1410 (m), 1360 (m), 1305 (m), 1280 (m), 1220 (s), 1185 (s), 980 (w), 910 (w), 880 (w), 750 (w), 725 (w), 700 (m); ^{1}H-NMR δ_H (DMSO-d_6): 2.65–2.78 (2H, m, CH_2), 2.97 (1H,

dd, $J=13.7$ Hz, $J=9.0$ Hz, CH_2), 3.06 (1H, dd, $J=13.8$ Hz, $J=5.7$ Hz, CH_2), 3.62 (3H, s, OCH_3), 4.47 (1H, m, CH), 4.72 (1H, m, CH), 7.25–7.36 (5H, m, Ar-H), 8.63 (1H, d, $J=7.3$ Hz, NH), 9.64 (1H, d, $J=7.7$ Hz, NH), 12.54 (1H, s, br., COOH); ^{13}C-NMR δ_C (DMSO-d_6): 35.40 (u), 36.31 (u), 49.72 (d), 51.90 (d), 53.92 (d), 115.78 (u, q, $J=291.2$ Hz), 126.61 (d), 128.26 (d), 129.06 (d), 136.99 (u), 156.04 (u, q, J = 37.1 Hz), 169.44 (u), 171.20 (u), 171.68 (u). CIMS (CH_4) m/z: 391 (MH^+, 100), 373 (20), 359 (25), 331 (35), 162 (13). Anal. Found: C, 49.22; H, 4.35; N, 7.17. Calcd. for $C_{16}H_{17}F_3N_2O_6$: C, 49.24; H, 4.39; N, 7.18.

Sweetness Test. The following solutions of sucrose, aspartame, and TFA-aspartame were prepared freshly at room temperature in still mineral water (Volvic) having a low salt content (109 mg dry residue at 180°C): Solution 1, pure mineral water; Solution 2, standard 2% sucrose (58.4 mM); Solutions 3–6, aspartame (0.06 mM, 0.29 mM, 1.45 mM, 2.90 mM, respectively); Solutions 7–10, TFA-aspartame (0.04 mM, 0.22 mM, 1.10 mM, 2.20 mM, respectively). Solution 10 had the highest concentration of TFA-aspartame which could be achieved at room temperature. The solutions were assessed within 2 hours of their preparation, in double blind experiments in a randomized order, by 14 volunteers who rated the taste from 0 (not sweet) to 5 (very sweet). Responses are presented pictorially in Fig. 1.

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