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NEW ASPARTAME-LIKE SWEETENERS CONTAINING L-(αMe)Phe

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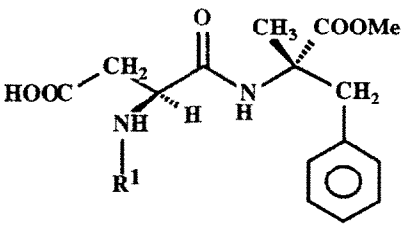
Abstract: The [L-(αMe) Phe]^2-analogue of aspartame was synthesized and analyzed by X-ray diffraction. This compound is as sweet as aspartame itself but far more stable at pH=4. Several N-protected analogues were synthesized. The N-formylcarbamoyl [L-(αMe Phe)]^2-aspartame analogue is also sweet. The compounds fit well within the sweet perception model as developed by Temussi, Toniolo and coworkers.

Aspartame (α-L-aspartyl-L-phenylalanine methylester) is a low calorie dipeptide sweetener produced on a large scale (several thousand of tons/year). Our interest in artificial sweeteners dates back to the early seventies, when Boesten et al.[1] developed a technically and economically attractive synthetic route to aspartame. Recently, we published our results obtained in developing a more stable aspartame derivative with respect to temperature and pH[2]. Elsewhere, others have elaborated on the synthesis of new artificial sweeteners[3]. Of current interest is the development of new low calorie, high intensity sweeteners and the study of structural characteristics required to exhibit sweet perception.

At DSM a general approach to the synthesis of α-alkylated α-amino acids has been developed[4]. This research showed that these amino acids are not easily esterified and/or amidated. In order to overcome the drawback of aspartame, i.e. low stability at higher pH values and higher temperatures, we started a programme aimed at the development of new stable low calorie sweeteners. In this article we describe the synthesis and X-ray structure determination of the new artificial sweeteners 1 and 2 (fig. 1). The α-L-aspartyl-D,L(α-methyl)phenylalanine methylester diastereomeric mixture was already known as being 5 times as sweet as sucrose[5]. To the authors' knowledge the preparation of neither the optically pure analogue nor the N-formyl, N-carbamoyl and N-formylcarbamoyl derivatives has been previously reported. Compound 1 was prepared from Z-L-Asp(OtBu)-OH and H-L-(αMe)Phe-OMe. Z-L-Asp(OtBu)-OH was prepared according to standard literature procedures[6a]. H-L-(αMePhe)OMe·HCl[6b] was prepared using the $SOCl_2$/MeOH method, for quantitative yields the addition of fresh $SOCl_2$ being required [4a]. The coupling procedure was performed in THF using N-methylmorpholine and *iso*-butylchloroformate (yield 70%)[7]. Quantitative deprotection was achieved by treatment with trifluoroacetic acid and subsequent hydrogenation (5% Pd/C)[8]. The product was crystallized and subjected to X-ray diffraction analysis (fig. 2).



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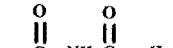
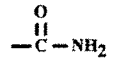
2 R^1 = 

3 R^1 = 

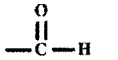
4 R^1 = 

Figure 1

Starting from the L-(αMe)Phe aspartame analogue 1, the N-carbamoyl derivative 3 was prepared using potassium isocyanate (yield 66%). From 3, using the mixed anhydride method ($HCOOH/Ac_2O$), we obtained the N-formylcarbamoyl derivative 2[9]. From 1 using the foregoing formylation method we obtained compound 4.

The sweetness of all these compounds was tested. The N-formyl- 4 and N-carbamoyl- 3 derivatives were bitter and tasteless, respectively, while compounds 1, the L-(αMe)Phe analogue of aspartame, and 2, the N-formylcarbamoyl derivative, were as sweet as aspartame itself (± 200 times as sweet as sucrose). Noteworthy was the dramatic difference in sweetness pattern of H-L-Asp-L-(αMe)Phe-OMe compared to H-L-Asp-D-(αMe)Phe-OMe. The latter compound has a bitter taste.

It is often stated that the zwitterionic structure of the aspartic acid moiety is a prerequisite for a sweet taste (AH, B-X system [3a]). This, however, leaves one to explain why amongst others the N-trifluoroacetyl aspartame[10] and N-formylcarbamoyl aspartame 2[2] are sweet. From this study it can be concluded that the C^{α}-hydrogen of the phenylalanine residue is not essential for sweetness. We have also obtained indications that the acidity of the NH proton of aspartic acid is critical for sweetness. In our case the imide NH of compounds 2 has a pK_a of about 10 and is therefore acidic enough to interact with the basic site (B) of the receptor.

The stability at pH=4 was tested for compound 1 and compared to the stability of aspartame under the same conditions. A 0.1% (w/w) solution of compound 1 was stirred for 21 hours at 90°C in citric acid (pH=4). The procedure was repeated with aspartame. The new artificial sweetener is two times ($t_{½}$ 14.0, 6.8 h respectively) more stable than aspartame under these conditions [11]. At higher pH values the stability of compound 1 drops dramatically and at pH=8 its stability is less than that of aspartame, possibly because diketopiperazine formation of compound 1 is facilitated due to conformational restriction.

In conclusion we have prepared two new artificial sweeteners (compounds 1 and 2). The pK_a value of a NH group at the N-terminus of the molecule is very important for sweet perception. The stability pattern of compound 1 is better at pH=4 and at elevated temperature compared to that of aspartame. The X-ray diffraction analysis shows that compound 1 fits only with slight modifications with the sweet perception model, as developed by Temussi, Toniolo and coworkers [12] (fig. 3). In addition we are investigating the X-ray structures of the N-blocked derivatives. The work on other new artificial sweeteners is currently in progress in our laboratory.

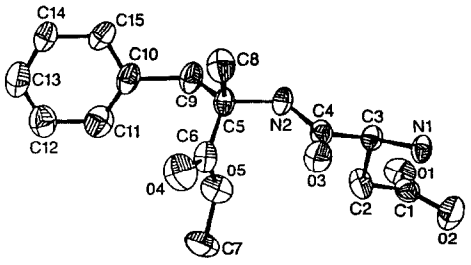


Fig. 2 X-ray diffraction structure of the [L-(αMe)Phe]^2-analogue of aspartame

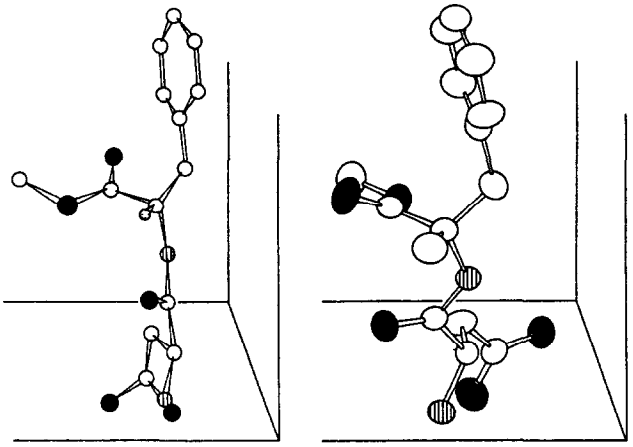


Fig. 3 Model of the receptor-site
left: aspartame (from 'H NMR study and energy calculations)
right: [L-(αMe)Phe]^2-analogue (from X-ray analysis)

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7. To a cooled, (-10°C) stirred solution of 32.3 g Z-L-Asp(OtBu)-OH in 400 ml THF, 11 ml N-methylmorpholine and 14,3 ml *iso*butylchloroformate were added. After 30 minutes stirring a solution of 22.85 g H-L-(αMe)Phe-OMe·HCl in 11 ml N-methylmorpholine and 400 ml chloroform was added. The reaction mixture was warmed to room temperature and stirred overnight, evaporated until dry and dissolved in ethyl acetate. The organic layer was washed with water, citric acid (0.5M), water, 5% $NaHCO_3$ and water. After drying over $MgSO_4$ and evaporation of the solvent the product was crystallized from diethylether/n-hexane. Yield 36.1 g white powder (m.p. 85-86°C) $[\alpha]_D^{20}$ = -40.1° (c = 0.5, methanol).

8. A quantity of 44.2 g Z-L-Asp(OtBu)-L-(αMe)Phe-OMe was dissolved, while being stirred for 2 hr at 25°C, in 500 ml of trifluoroacetic acid. The solvent was evaporated *in vacuo* and the product was crystallized from ethyl acetate and n-hexane (yield quantitative; m.p. 138-140°C). A solution of 30.8 g of Z-L-Asp-L-(αMe)Phe-OMe in methanol was catalytically hydrogenated using 3.0 g 5% Pd/C. After 1 hr. stirring at room temperature the catalyst was filtered and the solvent evaporated *in vacuo*. The product was crystallized from a mixture of methanol and diethylether (1).
$C_{15}H_{20}N_2O_5 \cdot H_2O$, m.p. 265-266°C; $[\alpha]^{25}$ = -35.6° (c = 0.5, methanol); TLC (silicagel F_{254}) R_f=0.65 (1-butanol-acetic acid-water 60:20:20); IR (1 x 10^{-2}M DMSO): 1736, 1712 (shoulder), 1672, 1627, 1551 cm^{-1} (carbonyl region).
^{1}H NMR (2.94 mg/0.4 ml DMSO): δ 8.55 (1 H, s, (αMe)Phe NH); 7.30-7.07 (5 H, m, (αMe)Phe aromatic); 3.60 (1 H, m, Asp αCH); 3.58 (3 H, s, OCH_3); 3.17 and 3.03 (2 H, m, αMePhe βCH_2); 2.33 (2 H, m, Asp βCH_2); 1.28 (3 H, s, (αMe)Phe βCH_3). Amino acid analysis: Asp 0.95; (αMe)Phe 1.05.
Elemental analysis: $C_{15}H_{20}N_2O_5 \cdot 2H_2O$; Calculated: 52.3; H 7.0; N 8.1. Found: 54.7; H 6.7; N 8.3.
X-ray diffraction data of 1: dihydrate (Philips PW 1100 four-circle diffractometer). $C_{15}H_{20}N_2O_5 \cdot 2H_2O$ crystallizes from a methanol solution in the orthorhombic system. Space group $P2_12_12_1$ with a=38.664 (1), b=7.225 (1) and c=6.173 (1) A°; V=1724.4 (3), $A°^3$; Z=4; D_c=1.26 g cm^{-3}. The final conventional R factor for 1907 reflections considered observed, $F \geq 6\sigma(F)$, was 0.038; Rw=0.043 with $w=1/(\sigma^2/F/ + 0.0006/F/^2]$; S=1.38; μ=0.61 cm^{-1}; F(000)=696.0; max. and min. heights = ± 0.16 · $A°^{-3}$.

9. In 30 ml H_2O 1.3 g potassium isocyanate and 4.0 g dipeptide 1 were dissolved at 40°C. This solution was stirred at room temperature overnight, extracted twice with ethyl acetate and concentrated (evaporation of half the amount of water). At 70°C the pH is adjusted to 2.0-2.5 using 2N HCl. After cooling to 50°C crystallization of the product started. The product was recrystallized from water to afford 3.0 g (66% yield) of pure product 3, m.p.: 118-120°C, $[\alpha]_D^{20}$ = -26.0° (c=1, methanol).
^{1}H NMR (DMSO): δ 1.25 (3 H, s, (αMe)Phe βCH_3) 2.55 (2 H, m, diastereotopic, Asp βCH_2); 3.1 (2 H, m, diastereotopic, (αMe)Phe βCH_2) 3.6 (3 H, s, OCH_3); 4.4 (1 H, m, Asp αCH); 5.2 (2 H, s, carbamoyl NH_2); 6.3 (1 H, d, Asp NH); 7.1-7.25 (5 H, m, (αMe)Phe aromatic), 7.95 (1 H, s, (αMe)Phe NH), 12.35 (broad 1 H, s, CO_2H). ^{13}C NMR ($CDCl_3$); σ 173.6, 172.3, 171.4, 158.1 (4 x CO), 136.1, 130.6, 128.2, 126.9 (4 aromatic C), 59.2, 52.1, 50.0, 41.3, 37.0, 22.3 (6 aliphatic C).

3.51 g of N-carbamoyl-(αMe)Phe aspartame 3 was dissolved in 200 ml of formic acid. Then, 7 ml of acetic acid anhydride was slowly added with the temperature being maintained at 5-15°C using an ice bath. After stirring for 1 hour the ice bath was removed and the reaction was stirred at room temperature overnight. Water was added and evaporated *in vacuo* (purity of the product is ± 85%). Recrystallization from chloroform gave pure product 2, m.p.: 79-81° (decomp.), $[\alpha]_D^{20}$ = -27.1° (c=1, methanol).
^{1}H NMR (DMSO); δ 1.25 (3 H, s, (αMe)Phe βCH_3); 2.55 (2 H, m, diastereotopic, Asp βCH_2); 3.1 (2 H, m, diastereotopic, (αMe)Phe βCH_2), 3.56 (3 H, s, OCH_3), 4.52 (1 H, m, Asp αCH); 7.1-7.25 (5 H, m, (αMe)Phe aromatic), 7.95 (broad 1 H, s, Asp NH), 8.56 (broad 1 H, s, formyl), 8.85 (broad 1 H, d, carbamoyl). ^{13}C NMR ($CDCl_3$); δ 174.8, 173.6, 171.8, 163.3, 153.7 (5 x CO); 136.0, 130.7, 128.0, 126.8 (4 aromatic C), 59.1, 52.0, 51.0, 41.5, 31.1, 22.2 (6 aliphatic C).

Compound 4:
^{1}H NMR ($CDCl_3$); δ 1.6 (3 H, s, (αMe)Phe βCH3); 2.8 (2 H, m, diastereotopic, Asp βCH_2); 3.25 (2 H, m, diastereotopic, (αMe)Phe βCH_2); 3.7 (3 H, s, OCH_3); 4.85 (1 H, m, Asp αCH); 7.0-7.25 (5 H, m, (αMe)Phe aromatic); 7.30 (1 H, s, (αMe)Phe NH); 7.55 (broad 1 H, d, Asp NH); 8.05 (1 H, s, formyl); 10.35 (broad 1 H, s, CO_2H).

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