# Synthesis of L-Aspartyl-$\Delta^Z$-phenylalanine Methyl Ester. Dehydroaspartame

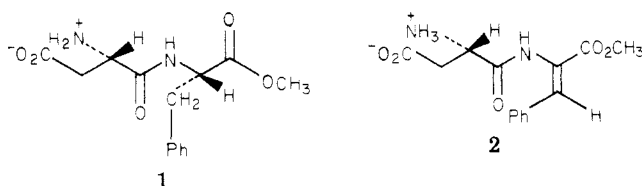
Stephen W. King and Charles H. Stammer*

*Department of Chemistry, University of Georgia, Athens, Georgia 30602*

*Received June 16, 1981*

L-Aspartyl-$\Delta^Z$-phenylalanine methyl ester has been synthesized by DDQ oxidation of the dipeptide azlactone **4**. Methanolysis of the unsaturated azlactone **5** was useful *only* when catalyzed by 4-(dimethylamino)pyridine. The dehydroaspartame was tasteless.

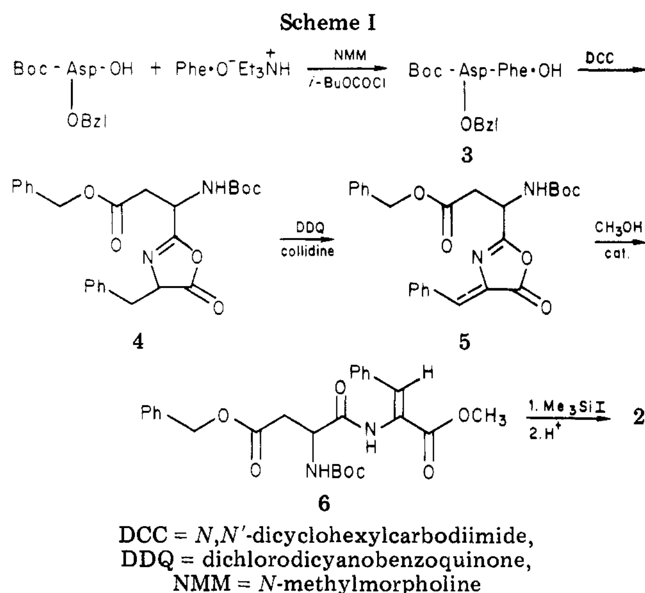
There has been a great deal of recent interest in sweet organic compounds and in the use of their diverse structures to "map" the sweetness receptor site.[1] Aspartame is the generic name given to the dipeptide L-aspartyl-L-phenylalanine methyl ester (1), which is about 190 times



sweeter than sucrose. Since the accidental discovery[1b] of its sweet taste, numerous analogues have been synthesized,[1c–i] sweetness tested, and possible conformations calculated that test the original Schallenberger[1a] hypothesis. Our interest in dehydropeptides led us to prepare the dehydro analogue **2** with the expectation that it might be more stable to enzymatic hydrolysis[2] and to diketopiperazine formation[3] due to a deactivated ester carbonyl function. Since only the L,L isomer of aspartame is sweet, whereas the L,D isomer is bitter, it was also of interest to determine whether chirality at the carboxyl terminus was a requirement for taste. This paper reports our synthesis of **2**.

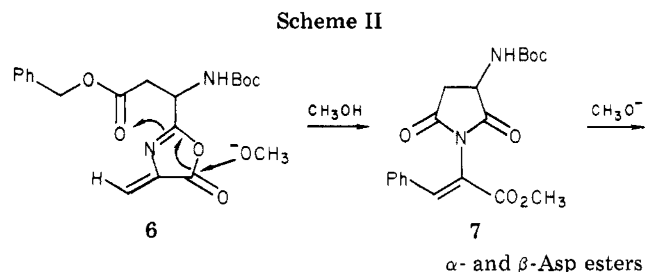
The synthesis, outlined in Scheme I, was not quite as straightforward as expected. The dipeptide **3** was prepared by the mixed anhydride method (66% yield) and then converted to the unsaturated azlactone **5** by using the DCC/DDQ procedure[4] (31% yield) without isolation of the saturated azlactone **4**. Surprisingly, the most difficult step in the reaction sequence was the methanolysis of **5** to obtain the ester **6**. Base-catalyzed ($Et_3N$, $CH_3OH$) methanolysis of **5** gave a mixture of at least three (TLC) compounds. NMR analysis of these mixtures showed loss of the benzyl ester function and the formation of *two* methyl esters ($\delta$ 3.8 and 3.7) among other products. Apparently

Scheme I



DCC = $N,N'$-dicyclohexylcarbodiimide, DDQ = dichlorodicyanobenzoquinone, NMM = $N$-methylmorpholine

Scheme II



azlactone ring opening by nucelophilic attack at the carbonyl group was causing formation[5] of the succinimide (**7**), which rapidly afforded a mixture of $\alpha$- and $\beta$-aspartyl peptide esters on methanolysis (Scheme II). In order to overcome this we attempted to deblock the dehydro azlactone **5** before methanolysis by using both liquid HF and trimethylsilyl iodide ($Me_3SiI$) but no product corresponding to **2** could be obtained from these reaction mixtures.

Methanolysis of **5** was finally accomplished, without side reactions, giving chromatographically pure **6** in 85% yield by using a molar equivalent of 4-(dimethylamino)pyridine (DMAP) as catalyst.[6] In view of difficulties often resulting from imide formation by aspartyl peptides, we took this

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(5) This is a well-known side reaction encountered during work with aspartyl peptides; cf. Bodanszky, M.; Kwei, J. Z. *Int. J. Peptide Protein Res.* **1978**, *12*, 69.

(6) A catalytic amount of DMAP works equally well in the alcoholysis of azlactones.

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opportunity to examine the effect of DMAP on Boc-Asp(Bzl)-Phe-OMe (**8**) and Boc-Asp(Bzl)-Gly-OMe (**9**). Both **6**, the dehydro compound, *and* **8** were stable to DMAP in methanol for at least *4* h, while triethylamine and diisopropylethylamine caused rapid imide formation in less than 15 min. However, the most reactive[5] Asp peptide, **9**, was converted to imide by these bases, including DMAP; thus DMAP can probably be used to advantage on Asp peptides other than those having adjacent glycine residues.

Final conversion of the blocked dipeptide (**6**) into dehydroaspartame (**2**) was accomplished by treatment of **6** with $Me_3SiI$[7] in refluxing chloroform overnight. After chromatography of the crude product on a Bio-Gel P-2 column in 0.2 N acetic acid, the pure dipeptide (**2**), $[\alpha]^5_D$ 10.1° (*c*, 1, 1 N HCl), was obtained as a hemihdyrate, mp 121–124 °C dec, in 32% yield. This material was tasteless.

Several workers[1e,f] have used NMR and potential energy calculations to develop a picture of the sweetness receptor from preferred conformations of aspartame. In the most favorable conformation of **1**, $\chi_1$ of the phenylalanine residue, which describes rotation about the $C_\alpha$–$C_\beta$ bond, is chosen as ~150° in order for the aromatic ring to remain inside the "spatial barrier" of the receptor site. In our dehydroaspartame, the *Z* configuration of the Δ-Phe residue makes a $\chi_1$ angle of 0° obligatory and forces the aromatic ring outside the spatial barrier. The fact that **2** is tasteless is therefore consistent with a current picture[1e] of the sweetness receptor and perhaps indicates that a $\Delta^E$-Phe compound would be sweet.

## Experimental Section

**Instrumentation.** All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were measured with a Perkin-Elmer Model 297 recording spectrophotometer with polystyrene as standard. Proton NMR spectra were recorded on a Varian EM-390 spectrometer with $Me_4Si$ as internal reference. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. elemental analyses were provided by Atlantic Microlab, Atlanta, GA. Thin-layer chromatography was performed on Whatman precoated silica gel plates using the following solvent systems: (I) $CHCl_3$/MeOH (4:1), (II) $CHCl_3$/EtOAc (15:1), (III) $CHCl_3$/EtOAc (5:1), (IV) *n*-BuOH/HOAc/$H_2O$ (5:4:1), (V) *n*-BuOH/HOAc/Pyr/$H_2O$ (4:1:1:2). Thin-layer plates were visualized with UV light, 1% ninhydrin/*n*-BuOH (w/v), $I_2$ vapor, and $Cl_2$/tolidine. silica gel for column chromatography was purchased from Baker Chemical Co. (60–200 mesh) unless specified otherwise.

**Materials.** *N*-(*tert*-Butoxycarbonyl)aspartic acid β-benzyl ester and L-phenylalanine were purchased from Sigma Chemical Co. and used without further purification, *N,N'*-Dicyclohexylcarbodiimide (DCC), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), *N*-methylmorpholine, iodotrimethylsilane, 4-(dimethylamino)pyridine (DMAP), and isobutyl chloroformate were purchased from Aldrich Chemical Co. and used without further purification. Collidine, triethylamine, and diisopropylethylamine were distilled and stored over CaO. Tetrahydrofuran was distilled over potassium and stored over sodium. All other solvents were used without purification unless specified.

***N*-(*tert*-Butoxycarbonyl)-β-benzyl-L-aspartyl-L-phenylalanine (3).** A solution of 6.50 g (0.02 mol) of *N*-(*tert*-butoxycarbonyl)-L-aspartic acid β-benzyl ester dissolved in ca. 50 mL of dry tetrahdyrofuran (THF) was cooled to −20 °C in a dry ice–carbon tetrachloride bath, and 2.23 g ($2.2 \times 10^{-2}$ mol) of *N*-methylmorpholine and 3.00 g ($2.2 \times 10^{-2}$ mol) of isobutyl chloroformate were added. After 20 min, a solution of 4.94 g (0.03 mol) of L-phenylalanine in 20 mL of dioxane–water (7:3) containing 4.16 mL (0.03 mol) of triethylamine was added. The mixture was stirred at room temperature overnight, 10 mL of 1 N NaOH was added, and the THF was removed in vacuo. the solution was extracted with ether (2 × 30 mL) and the aqueous extract was acidified to pH 4 with saturated citric acid. the aqueous solution was then extracted with ether (3 × 30 mL), the ether layers combined, washed with 5% citric acid (2 × 20 mL), and dried over anhydrous $Na_2SO_4$. The solution was filtered and concentrated to ca. 30 mL, and hexanes were added until cloudy. A total of 6.1 g (66%) of phenylalanine was obtained as a white crystalline solid: mp 124–125 °C; $[\alpha]^{25}_D$ 33.4° (*c*, 1, $CHCl_3$); $R_f$ (I) 0.62; IR, (KBr) 3320 (amide NH), 3200–3000 (COOH), 1690 (C=O), 1620 $cm^{-1}$ (C=O); NMR ($CDCl_3$) δ 8.8 (br s, 1 H, COOH), 7.4–7.1 (m, 10 H, ArH), 6.7 (m, 1 H, NH), 5.6 (m, 1 H, NH), 5.1 (s, 2 H, $CO_2CH_2Ph$), 4.8–4.4 (m, 2 H, α-H), 3.1–2.6 (m, 4 H, β-$CH_2$), 1.4 (s, 9 H), $(CH_3)_3C$).

Anal. Calcd for $C_{25}H_{30}O_7N_2$: C, 63.82; H, 6.38; N, 5.95. Found: C, 63.70; H, 6.44; N, 5.93.

***N*-(*tert*-Butoxycarbonyl)-β-benzyl-L-aspartyl-$\Delta^Z$-phenylalanine Azlactone (5).** To a solution of 2 g ($4.25 \times 10^{-3}$ mol) of *N*-(*tert*-butoxycarbonyl)-β-benzyl-L-aspartyl-L-phenylalanine (**3**) in 20 mL of dry THF was added 0.96 g ($4.67 \times 10^{-3}$ mol) of *N,N'*-dicyclohexylcarbodiimide (DCC) and the mixture was allowed to stand at 5 °C overnight. the dicyclohexylurea was filtered and the filtrate concentrated to give 2.1 g of the crude saturated azlactone. The IR spectrum confirmed the presence of the azlactone ring (C=O), 1830 $cm^{-1}$), and the oil was used without further purification. It was dissolved in 15 mL of dry dimethoxyethane. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.96 g, $4.25 \times 10^{-3}$ mol) and 0.56 mL ($4.25 \times 10^{-3}$ mol) of collidine was added, and the reaction mixture was stirred and protected from moisture for 24 h at room temperature. the solution was filtered and the filtrate concentrated to give a dark oil, which was chromatographed on a silica gel (60–200 mesh) column (80 g, 30 × 4 cm) with a petroleum ether/ether (5:1) eluant. The product was collected in the first 400 mL, which was kept at 5 °C to give 0.6 g (31.4%) of the unsaturated azlactone (**5**) as a white crystalline solid: mp 153–154 °C; $[\alpha]^{25}_D$ −61.6° (*c*, 1, $CHCl_3$); $R_f$ (II) 0.85; IR 1800 (oxazoline C=O), 1690 (Boc C=O), 1630 $cm^{-1}$ (C=C); NMR ($CDCl_3$) δ 8.1–8.0 (m, 2 H, ortho protons), 7.4–7.1 (m, 9 H, HC=C and ArH), 5.5 (m, 1 H, NH), 5.1 (s, 2 H, $OCH_2Ph$), 4.6 (m, 1 H, α-H), 3.1 (m, 2 H, β-$CH_2$), 1.5 (s, 9 H, $(CH_3)_3$).

Anal. Calcd for $C_{25}H_{26}O_6N_2$: C, 66.66; H, 5.77; N, 6.22. Found: C, 66.65; H, 5.87; N, 6.13.

***N*-(*tert*-Butoxycarbonyl)-β-benzyl-L-aspartyl-$\Delta^Z$-phenylalanine Methyl Ester (6).** A total of 1.3 g ($2.88 \times 10^{-3}$ mol) of the unsaturated azlactone (**5**) was suspended in 20 mL of absolute methanol. To this was added 0.35 g ($2.88 \times 10^{-3}$ mol) of 4-(dimethylamino)pyridine and the mixture was stirred at room temperature for ca. 30 min. Saturated citric acid was added to pH 4, methanol was removed in vacuo, and the mixture was extracted with ca. 30 mL of ether. The aqueous portion was extracted further with ether (2 × 15 mL), and the ether extracts were combined, washed with 5% citric acid (2 × 15 mL), saturated NaCl (1 × 15 mL), and dried over anhydrous $Na_2SO_4$. The ether was removed in vacuo to give 1.41 g (100%) of the crude ester as an amorphous solid. The ester was dissolved in ether and chromatographed on E. Merck silica gel (200 mesh, 300 g, 40 × 3 $cm^{-1}$) with ether to give 1.17 g (85%) of **6**. Crystallization from ether/petroleum ether gave 0.91 g (66%) of the desired compound **6** as a white solid: mp 55–58 °C; $[\alpha]^{25}_D$ −2.8° (*c*, 1, in $CHCl_3$): $R_f$ (III) 0.64; IR (KBr) 3300 (amide NH), 1720 (ester CO), 1670 (C=O), Boc), 1690 $cm^{-1}$ (amide C=O); NMR ($CDCl_3$) δ 7.9 (br s, 1 H, NH) 7.4–7.1 (m, 11 H, HC=C and ArH), 5.5 (m, 1 H, NH), 5.2 (s, 2 H, $OCH_2Ph$), 4.5–4.4 (m, 1 H, α-H), 3.8 (s, 3 H, $COOCH_3$), 3.0–2.8 (m, 2 H, $CH_2CO_2Bzl$), 1.5 (s, 9 H, $(CH_3)_3$).

Anal. Calcd for $C_{26}H_{30}O_7N_2$: C, 64.73; H, 6.22; N, 5.81. Found: C, 64.66; H, 6.30; N, 5.78.

**L-Aspartyl-$\Delta^Z$-phenylalanine Methyl Ester (2).** A total of 0.76 g ($1.57 \times 10^{-3}$ mol) of *N*-(*tert*-butoxycarbonyl)-L-β-benzylaspartyl-$\Delta^Z$-phenylalanine methyl ester (**6**) was dissolved in ca. 30 mL of chloroform. To this was added via syringe 0.5 mL (3.5 × $10^{-3}$ mol) of iodotrimethylsilane and the solution was refluxed under nitrogen for ca. 17 h. The chloroform was removed in vacuo, ca. 30 mL of 10% acetic acid and 30 mL of ether was added, and the solution was extracted. The aqueous portion was extracted further with ether (5 × 30 mL) until the aqueous phase was colorless. The aqueous extract was concentrated to ca. 5 mL and eluted through a Bio-Gel $P_2$ column (2 × 110 cm), using 0.2 N acetic acid as the eluant with a flow rate of ca. 2 mL/h. Fractions 119 and 120 containing pure dehydroaspartame were combined and lyophilized to give 0.150 g (32%) of **2** as a white solid. The solid was dissolved in ca. 40 mL of water and lyophilized again

to remove traces of acetic acid: mp 121–124 °C dec; $R_f$ (IV) 0.82, $R_f$ (V) 0.60; IR (KBr) 3200–3000 (COOH and NH), 1760 (ester C=O), 1660 (amide C=O), 1630 cm$^{-1}$ (C=C); NMR ($CD_3OD$) δ 7.7–7.3 (m, 6 H, HC=C and ArH), 4.3 (m, 1 H, α-H), 3.8 (s, 3 H, $COOCH_3$), 3.0–2.8 (m, 2 H, β-$CH_2$).

Anal. Calcd for $C_{14}H_{16}N_2O_5 \cdot {}^1/_2H_2O$: C, 55.75; H, 5.64; N, 9.29. Found: C, 55.76; H, 5.74; N, 9.22.

**Acknowledgment.** We gratefully acknowledge the partial financial support of this work by Research Corporation, Summer, 1979.

**Registry No. 2**, 79043-73-5; **3**, 68763-45-1; **4**, 79043-74-6; **5**, 79043-75-7; **6**, 79043-76-8; *N*-(*tert*-butoxycarbonyl)-L-aspartic acid β-benzyl ester, 7536-58-5; L-phenylalanine, 63-91-2.

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# Leucettidine, a Novel Pteridine from the Calcareous Sponge *Leucetta microraphis*[1]

John H. Cardellina II*

*Department of Chemistry, Montana State University, Bozeman, Montana 59717*

Jerrold Meinwald

*Department of Chemistry, Cornell University, Ithaca, New York 14853*

*Received June 16, 1981*

Leucettidine, 6-(1-hydroxypropyl)-3-methylpteridine-2,4(1*H*)-dione (1), has been isolated from dichloromethane and acetone extracts of *Leucetta microraphis*, a calcareous sponge from low-light zones in Bermudan waters. The structure of leucettidine was deduced by analysis of spectral data and the absolute configuration was proposed on the basis of comparison of optical rotation data of leucettidine and other pteridines of known configuration.

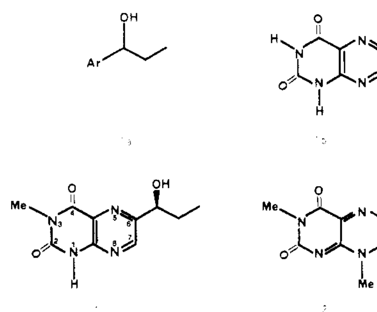
The last 15 years have witnessed a rapid growth of research interest in marine natural products. The chemistry of sponges has attracted much of this attention and work by numerous groups has yielded a plethora of novel compounds, many of which exhibit interesting pharmacological activity. Most of the research on sponges has been focused on the Demospongiae, a major class of widely distributed siliceous sponges.[2]

The class Calcispongiae (Calcarea), on the other hand, has been largely overlooked. Herein we describe leucettidine, a minor metabolite of *Leucetta microraphis*, a calcareous sponge common in Bermudan waters. Leucettidine, a levorotatory, amorphous solid, was isolated from dichloromethane and acetone extracts of the freeze-dried sponge by a combination of adsorption chromatography and gel filtration. We have assigned structure 1 to this metabolite on the basis of the evidence discussed below.

## Discussion

The one-proton singlet at δ 8.76 in the $^1$H NMR spectrum of leucettidine is indicative of a heteroaromatic ring, while the long wavelength and relatively low molar extinction coefficients of the ultraviolet absorption maxima suggest a bicyclic heteroaromatic chromophore. High-resolution mass spectrometry supported this conclusion, revealing a molecular ion of formula $C_{10}H_{12}N_4O_3$, consistent with seven sites of unsaturation. There was very little fragmentation of the molecular ion or the base peak, which further supported the basic skeletal assignment. NMR decoupling experiments led us to partial structure **1a**, incorporating a 1-(1-hydroxypropyl) group. The chemical shift of the alcohol-bearing methine, at δ 4.84, indicates that this moiety is most likely attached to the aromatic nucleus. This assumption was substantiated by observation of mass spectral α cleavage on but one side of the alcohol, giving rise to the base peak at *m/e* 207 ($M^+ - 29$). The only other nonaromatic protons in the $^1$H NMR spectrum of **1** appear as a methyl singlet at δ 3.63.

The molecular formula and the ultraviolet absorption data are best accomodated by a pteridinedione chromophore, as shown in **1b**.[3] The intense, broad carbonyl absorption at 1710 cm$^{-1}$ in the infrared spectrum of **1** supports this suggestion; a model compound, the antibiotic toxoflavin, **2**,[4] has two overlapping infrared absorptions between 1710 and 1720 cm$^{-1}$.



Further examination of, and correlation of our data with, the pteridine literature has led us to propose structure **1** for leucettidine. Placement of the methyl group on the nitrogen at position 3 of the pteridine nucleus is prompted by chemical shift data for various methyl-substituted pteridines (**3–5**)[5] and analogues (**6–7**).[6] The hydroxypropyl

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