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N^5-(4-Methoxyphenyl)methyl-L-glutamine in xylem sap from squash root

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Abstract

A new amino acid derivative, N^5-(4-methoxyphenyl)methyl-L-glutamine was found in the xylem sap from squash root. The structure was deduced from spectroscopic data and identified by chemical synthesis.

Keywords: *Cucurbita maxima* Duchesne × *C. moschata* Duchesne; Cucurbitaceae; Xylem sap from squash root; Amino acid derivative; N^5-(4-Methoxyphenyl)methyl-L-glutamine

1. Introduction

Various organic compounds produced in the root cells of the higher plants transport through xylem vessels to the aerial parts and many important activities of the aerial organs rely on the supply of inorganic and organic compounds from the xylem sap. Previously, one of us (Satoh) found proteins and carbohydrates in xylem sap from squash root (Satoh, Iizuka, Kikuchi, Nakamura, & Fujii, 1992). Subsequently, we isolated a new amino acid derivative **1** based on its biological activity towards adventitious root formation of cucumber hypocotyls in shoot cuttings cultures. In the present work, we describe the structure of the amino acid derivative as N^5-(4-methoxyphenyl)methyl-L-glutamine (**1a**).

2. Results and discussion

The ^{1}H NMR spectrum of **1** in D_2O is shown in Fig. 1(A). The absence of signals underneath the water region was confirmed by the WEFT technique. The major signals are marked with '*', which show relative intensities in integer multiples and several other minor signals are also present. The presence of a (4-methoxyphenyl)methyl group, probably attached to a hetero atom, is indicated from the NMR signals of δ 6.91, 7.20, 3.75 and 4.24, the UV absorptions (222 and 274 nm) and the fragment ion m/z 121 formed from m/z 267 in the linked-scan ESI$^+$ mass spectrum. The other signals (δ 2.06, 2.35, 2.39 and 3.67) in the ^{1}H NMR spectrum indicate the presence of a partial structure, Z–CH_2–CH_2–CH–Y, where Y is an electron-withdrawing group and Z must be an unsaturated group because the geminal coupling constant ($J = 14.8$ Hz) is slightly larger than the typical methylene group.

The water soluble nature of **1** and the facile formation of $(M+H)^+$ and $(M-H)^-$ ion in the ESI mass spectra suggested that **1** could be a glutamic acid or glutamine (or isoglutamine) derivative, as represented by Z–CH_2–CH_2–CH–Y. The amino acid analysis of **1** (hydrolysis in 6 M HCl at 110°C) showed that only glutamic acid was detected. The high resolution mass spectrum gave $(M+H)^+$ indicative of a glutamine (or isoglutamine) derivative of the molecular formula $C_{13}H_{18}N_2O_4$. The presence of a carboxylic acid was

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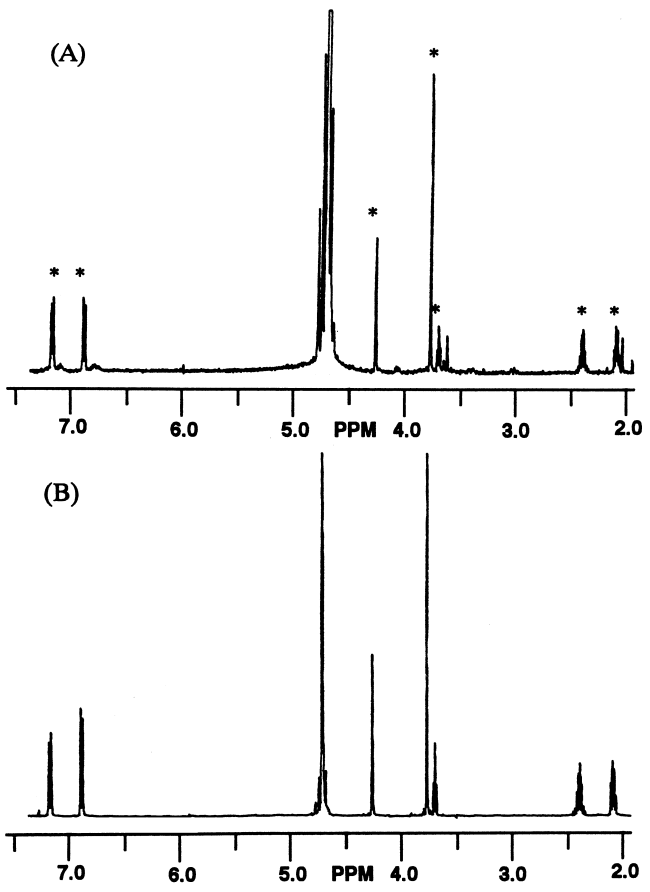


Fig. 1. ^{1}H NMR spectra of **1** (A) and **1a** (B) in D_2O.

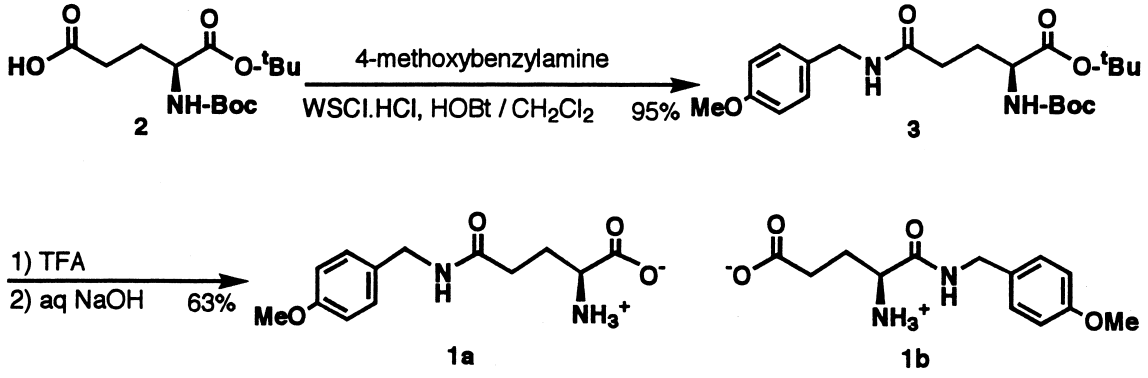
further indicated by the facts that **1** was adsorbed on the anion-exchange resin and could be eluted out by hydrochloric acid. These results established **1** as the *N*-(4-methoxyphenyl)methyl derivative of glutamine (**1a**) or isoglutamine (**1b**). The structure was finally confirmed by chemical synthesis as shown in Scheme 1.

N^2-(*t*-Butoxycarbonyl)-L-glutamic acid 1-*t*-butyl ester (**2**) was prepared from L-glutamic acid 5-benzyl ester according to the literature (Olsen, Ramasamy, & Emery, 1984). The carboxyl group in **2** was condensed with 4-methoxybenzylamine in the presence of the water soluble carbodiimide (WSCI·HCl) (Sheehan, Preston, & Cruickshank, 1965) to give an N^5-(4-methoxyphenyl)methyl-N^2-(*t*-butoxycarbonyl)-L-glutamine 1-*t*-butyl ester (**3**). Removal of both protecting groups with trifluoroacetic acid followed by neutralization gave N^5-(4-methoxyphenyl)methyl-L-glutamine (**1a**) as a white solid. The ^{1}H NMR spectrum of **1a** (Fig. 1B) was completely coincident with that of the major component of **1**. The UV spectrum of **1a** differed from **1** in the absence of 358 nm (weak and broad); this absorption is probably due to minor component(s) present in **1**.

The synthetic **1a** contained 5.1% of D-isomer as revealed by GC–MS analysis. By comparison with **1a**, the absolute configuration of N^5-(4-methoxyphenyl)-methylglutamine in **1** was determined as L.

The analogous compound N^5-(4-hydoxyphenyl)-methyl-L-glutamine has been isolated from *Fagopyrum esculentum* Moench (Koyama, Tsujizaka, & Sakumura, 1973), *Sinapsis alba* L. (Larsen, Olsen, Pedersen, Sorensen, 1964) and *Sinapsis arvensis* L. (Larsen et al., 1964), but N^5-(4-methoxyphenyl)methyl-L-glutamine is a new compound. Synthetic **1a** inhibited both adventitious root formation from hypocotyl of cucumber and development of 1st leaves of cucumber in the culture of shoot cuttings (5 mM in water) but showed no effects at a 0.5 mM concentration. Thus, the major inhibitory factor in the *n*-butanol fraction may be due to the minor component(s) present in **1**.

The role of N^5-(4-methoxyphenyl)methyl-L-glutamine in nature and the structure of the minor component(s) are now under investigation. The details on their biological activity will be reported elsewhere.



Scheme 1. Synthesis of **1a**.

3. Experimental

3.1. General

M.p.'s uncorrected. HPLC: ODS-80Tm (4.6 × 150 mm, Tosoh, Tokyo) and ODS-120T (4.6 × 250 mm, Tosoh) columns with acetonitrile–H_2O gradient (0 to 60%). GC–MS: Chirasil-L-Val column (0.25 mm × 25 m, Chrompak), helium (1.5 ml min^{-1}) as the carrier gas, programmed from 60°C (1 min), up to 120°C (10°C min^{-1}) and then to 180°C (5°C min^{-1}).

3.2. Plant material and isolation

Seeds of *Cucurbita maxima* Duchesne × *C. moschata* Duchesne cv. Shintosa-ichigou and seeds of *Cucumis sativus* cv. Shimoshirazu-jibai were obtained from Sakata Seed Co. (Kanagawa, Japan). The squash plants were grown in the field for 2–3 months (May to August).

Xylem sap (2 l) collected from squash root was mixed with four volumes of EtOH and the ppt was removed. The filtrate was concentrated and partitioned between water and *n*-butanol. The ppt and water fraction accelerated the adventitious root formation of cucumber in the culture of shoot cuttings, but the *n*-butanol fraction inhibited this growth. After concentrating the *n*-butanol fraction, the residues were purified by HPLC to give 0.2 mg of **1** as a white solid.

1: UV $\lambda_{max}^{H_2O}$ nm: 222 (strong), 274 (medium), 358 (weak and broad). ^1H NMR (D_2O, 500 MHz; only the signals designated with '*'): δ 2.06 (dt, 2H, $J=6.2$, 7.9 Hz, H-3), 2.35 (dt, 1H, $J=14.8$, 7.9 Hz, H-4a), 2.39 (dt, 1H, $J=14.8$, 7.9 Hz, H-4b), 3.67 (t, 1H, $J=6.2$ Hz, H-2), 3.75 (s, 3H, OCH_3), 4.24 (s, 2H, CH_2–N), 6.91 (d, 2H, $J=8.7$ Hz, H–Ar), 7.20 (d, 2H, $J=8.7$ Hz, H–Ar). ESI^+ (electrospray ionization) mass: m/z 267 $(M+H)^+$, 289 $(M+Na)^+$; m/z 121 by linked scan from m/z 267. ESI^- mass: m/z 265 $(M-H)^-$.

High resolution ESI^+ mass: found: m/z 267.138. calcd for $C_{13}H_{19}N_2O_4$ (M + H): m/z 267.142.

3.3. N^2-(t-Butoxycarbonyl)-L-glutamic acid 1-t-butyl ester (2)

N^2-(*t*-Butoxycarbonyl)-L-glutamic acid 1-*t*-butyl ester (**2**) was prepared from L-glutamic acid 5-benzyl ester according to the known procedure (Olsen et al., 1984) in 55% overall yield.

2: m.p. 112–114°C (from pentane-ether); $[\alpha]_D^{22}$ −29° (MeOH; *c* 0.11) (lit. Olsen et al., 1984: m.p. 102–105°C; $[\alpha]_D^{25}$ −26.5° (MeOH; *c* 1); lit. Tomasz, 1971: m.p. 110–114°C; $[\alpha]_D^{25}$ −30.2° (MeOH; *c* 1)); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.43 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu), 1.90 (m, 1H, H-4a), 2.15 (m, 1H, H-4b), 2.42 (m, 2H, H-3), 4.20 (m, 1H, H-2), 5.15 (m, 1H, H-N).

3.4. N-(4-Methoxyphenyl)methyl-N^2-(t-butoxycarbonyl)-L-glutamine 1-t-butyl ester (3)

To a cold soln of **2** (850 mg, 3 mmol), HOBt (1-hydroxybenzotriazole, 405 mg, 3 mmol) and 4-methoxybenzylamine (0.39 ml, 3 mmol) in CH_2Cl_2 (5 ml), was added a suspension of WSCI·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 580 mg, 3 mmol) in CH_2Cl_2 (3 ml). The mixture was stirred at room temp. for 1 h EtOAc (50 ml) and H_2O (20 ml) were added and the organic layer was separated. The organic layer was washed successively with H_2O, 0.5 M HCl, a satd $NaHCO_3$ soln, H_2O (2 times) and saline. After drying over Na_2SO_4, the solvent was evaporated to give almost pure crystalline **3** (1.20 g, 95%).

3: m.p. 98–100°C (triturated with pentane); $[\alpha]_D^{22}$ −21° (MeOH; *c* 0.10); ^1H NMR ($CDCl_3$, 200 MHz): δ 1.41 (s, 9H, *t*-Bu), 1.44 (s, 9H, *t*-Bu), 1.90 (m, 1H, H-4a), 2.15 (m, 1H, H-4b), 2.23 (m, 2H, H-3), 3.78 (s, 3H, OCH_3), 4.15 (m, 1H, H-2), 4.35 (d, 2H, $J=6$ Hz, CH_2–N), 5.20 (m, 1H, H–N), 6.37 (m, 1H, H–N), 7.05 (d, 2H, $J=9$ Hz, H–Ar), 7.20 (d, 2H, $J=9$ Hz, H–Ar).

Found: C, 62.45; H, 8.25; N, 6.59%. Calcd. for $C_{22}H_{34}N_2O_6$: C, 62.54; H, 8.11; N, 6.63%.

3.5. N^5-(4-Methoxyphenyl)methyl-L-glutamine (1a)

TFA (0.5 ml) was added to **3** (200 mg) and the mixture was stirred at room temp for 1 h. After evaporating the excess TFA under vacuum, residues were dissolved in H_2O (1 ml). A 0.5 M NaOH soln was added until pH 7, when ppt appeared. The ppt was collected by filtration and washed with cold H_2O followed by MeOH and dried in a desiccator to give **1a** (80 mg, 63%) as a white solid.

1a: m.p. 214–215°C (dec); $[\alpha]_D^{22}$ +23° (1 M HCl; *c* 0.10); $\lambda_{max}^{H_2O}$ nm (log ε): 224 (4.1), 273 (3.2).

Found: C, 58.52; H, 6.96; N, 10.37%. Calcd for $C_{13}H_{18}N_2O_4$: C, 58.63; H, 6.81; N, 10.52%.

3.6. GC–MS analysis

1a (ca. 0.2 mg) was stirred in 5% HCl/MeOH (0.4 ml) at room temp. for 3 h. After evaporating the reagents under vacuum the residue was treated with trifluoroacetic anhydride (0.2 ml) for 30 min. Evaporating the reagents gave an analytical sample, which was dissolved in acetone (1 ml). TIC (total ion chromatogram: $m/z=100$ to 400) of the solution was measured. The two peaks (31 and 32 min) showed a similar mass spectrum and were

identified as D- and L-isomers, respectively. The ratio of D- and L-isomer in **1a** was calculated from the area of m/z 245 (M–OMe) to be 5.1:94.9.

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