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ISOASPARAGINE FROM *CHARA CORALLINA*

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Key Word Index—*Chara corallina*; Characeae; alga; amino acid amide; isoasparagine.

Abstract—Isoasparagine was isolated for the first time as a natural product from *Chara corallina*. It was characterized by elementary analysis, the optical rotation value and the ^{1}H NMR spectrum, as well as analyses of the hydrolysis products. Identification was confirmed by comparison with a synthetic sample.

INTRODUCTION

The large internodal cells of Characeae are excellent materials for studying water and ionic relationships like membrane excitation and osmoregulation [1] and cytoplasmic streaming [2]. During a study on osmoregulation, we analysed the free amino acids of *Chara corallina* and detected an unidentified ninhydrin-positive substance. It was eluted between valine and cystine from an automatic amino acid analyser and located near asparagine on 2D-TLC, but gave a normal violet color with ninhydrin.

RESULTS AND DISCUSSION

The amino acid was isolated with cellulose CC from the free amino acid fraction obtained by treatment of the ethanol extract with anion exchange resin. Elementary analysis agreed well with the formula $C_4H_6N_2O_3 \cdot H_2O$. On mild acid hydrolysis, equimolar amounts of aspartic acid and ammonia were produced. The ^{1}H NMR spectrum showed proton signals of one methylene and one methyne group. In contrast to asparagine, the ninhydrin coloration on TLC was normal violet. These results strongly suggested that the amino acid was isoasparagine (3-aminosuccinamide), $CO_2HCH_2CH(NH_2)CONH_2$. For definite identification, we compared it directly with synthetic L-isoasparagine. Both samples behaved similarly in the automatic amino acid analyser and moved a little faster than asparagine on TLC in three solvents [3]. Ninhydrin coloration and the IR and ^{1}H NMR spectra were identical. The optical rotation value showed that the isolate was of the L-form.

Amino acid analysis revealed that isoasparagine accounted for *ca* 20% of the total free amino acids in *Chara corallina*. We have also found it in some other characean species and will report elsewhere on its distribution. Isoasparagine can be synthesized by many methods, which are based on the original report of Bergmann and Zervas [4]. However, this is the first report of the natural occurrence of this α-amide of aspartic acid.

EXPERIMENTAL

General. Chromatographic solvents were *n*-BuOH–HOAc–H_2O (63:10:27) (solvent A), PhOH–H_2O (25:8) (B) and pyridine–*iso*-AmOH–H_2O (35:30:30) (C). TLC and cellulose powder for CC were products of Funakoshi Pharmaceutical Co. ^{1}H NMR spectra were measured in CF_3COOD at 100 MHz with TMS as the int. standard. Amino acids were analysed with an automatic amino acid analyser (ATTO, MLC-703).

Plant. Chara corallina Willdenow var. *corallina* was cultured in pots containing tap water and soil extract under 8 hr light–16 hr dark at 20°. This plant was originally supplied by Prof. K. Oda of the Fukushima University in 1975 and thereafter cultured in our laboratory. The voucher have been deposited in the Herbarium, The University of Tokyo (TI) and Makino Herbarium, Tokyo Metropolitan University (MAK).

Isolation. Whole plants (1.9 kg) were harvested, washed with tap water, blotted with tissue paper and stored at −20° in a plastic bottle for one week. The plant was then frozen in liq. N_2 and extraction was carried out in a mixer with EtOH, followed by filtration with glass fibre filter paper (Whatman Gf/D) under suction. The residue was homogenized and filtered × 5. The combined extract (8 l.) was passed through an Amberlite IR-120B column (50 × 3 cm). Amino acids were eluted with 2 N NH_4OH (3.5 l.) and the eluate was concd to a syrup. This syrup was dissolved in a small amount of 0.05 N HOAc (*ca* 20 ml) and applied to a column of Dowex 1 × 4 (200–400 mesh, OAc^- form, 90 × 2.5 cm) and fractionated with 0.05 N HOAc. Relevant fractions were combined and applied again to a cellulose column (133 × 2.6 cm) and eluted with solvent (A). The fractions containing the amino acid were taken up in H_2O. Evaporation yielded crude crystals (68.5 mg). The pure sample which was obtained by recrystallization from EtOH–H_2O × 3 showed a single spot on TLC and a peak in the elution profile from the automatic amino acid analyser. Mp *ca* 190° (decomp.) $[\alpha]_D^{23} = +17.0°$ (0.1 N HCl, *c*1) lit. $[\alpha]_D^{18} = +15.5°$ (0.1 N HCl) [4], $[\alpha]_D^{20} = +14.9°$ (0.1 N HCl, *c*1.55) [5]. (Found: C, 31.75; H, 6.93; N, 18.71. Calc. for $C_4H_8N_2O_3 \cdot H_2O$: C, 32.00; H, 6.71; N, 18.66%). ^{1}H NMR: δ 3.38 (1H, *d*, J = 6 Hz, H-3), 4.76 (1H, *t*, J = 6 Hz, H-2).

Synthesis. Isoasparagine was synthesized according to ref. [3]. Mp *ca* 190° (decomp.). $[\alpha]_D^{23} = +10.5$ (0.1 N HCl, *c*1). (Found: C, 31.85; H, 6.94; N, 18.39%.) ^{1}H NMR: δ 3.36 (1H, *d*, J = 6 Hz, H-3), 4.5 (1H, *t*, J = 6 Hz, H-2.)

Hydrolysis. A small amount of the isolate was heated in 1 N HCl at 100° for 1 hr. The products were then analysed with an automatic amino acid analyser. Hydrolysis was complete and equimolar amounts of Asp and NH_3 were detected.

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FAILURE TO DETECT GLUCOSINOLATES IN *PLANTAGO* SPECIES

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Key Word Index—*Plantago major*; Plantaginaceae; glucosinolates; isothiocyanates; chemotaxonomy.

Abstract—Seeds and green parts of *Plantago* species have been examined for a supposed content of glucosinolates. Sensitive methods of quantitative glucosinolate analysis have been used but no glucosinolates could be detected. The significance of this investigation is briefly discussed in relation to chemotaxonomy and previously reported occurrence of isothiocyanates in extracts from *Plantago* species.

INTRODUCTION

Glucosinolates are well-known natural products co-occurring with myrosinases (thioglucoside glucohydrolase, EC 3.2.3.1) in all of the hitherto investigated plants belonging to Brassicaceae, Capparaceae and Resedaceae (ref. [1] and refs. cited therein). A restricted number of other plant families have been shown to contain glucosinolates mostly on the basis of degradation products thereof [2]. Furthermore, glucosinolates have been used in chemotaxonomy as compounds especially characteristic for the order Capparales [3].

Plantaginaceae is systematically remote from families in the order Capparales but, nevertheless, the possibilities of occurrence of glucosinolates in *Plantago* species have been discussed [3]. Plantaginaceae is either placed in its own order Plantaginales or incorporated into Tubiflorae, near the Scrophulariaceae.

An unidentified species of *Plantago* (plantain) has been reported to give a juice containing 4-methylsulfinylbut-3-enyl isothiocyanate (sulforaphene) [4]. The finding used as evidence for glucosinolates in *Plantago* has been questioned [2], but recently another report on products of autolysis from glucosinolates incorporated 8-week-old plants of *Plantago major* L. as the source of 5-vinyloxazolidine-2-thione (5 μg/g) and isopropyl isothiocyanate (1 μg/g) [5]. Such products of autolysis are traditionally used as evidence for presence of corresponding glucosinolates and myrosinases in the plants [1, 5]. Thus, the question concerning glucosinolates in Plantaginaceae is raised again.

The present work comprises investigations of *Plantago* species for their possible content of genuine glucosinolates. Recently developed sensitive methods of analysis based on techniques involving isolation of the intact glucosinolates have been used [1].

RESULTS AND DISCUSSION

Botanically well described *Plantago* species [6] were examined: green parts of *P. major* L. subsp. *major*, *P. major* subsp. *pleiosperma* Pilg. (rhamnose-type), *P. media* L., *P. lanceolata* L., *P. rugelii* Decne. and seeds of *P. major* L. subsp. *major*, *P. major* subsp. *pleiosperma* Pilg. (rhamnose-type), *P. major* subsp. *pleiosperma* Pilg. (glucose-type).

Extractions and isolation of fractions in which glucosinolates might be expected were performed by established methods including ion-exchange chromatography [7]. Careful analysis using HPLC [8], PC, HVE [1] and quantitative determination of glucose released by myrosinase treatment revealed no trace of glucosinolates whereas several other anions including carboxylates were present.

Freeze dried leaves of *P. major* subsp. *major* (20 g) and *P. major* subsp. *pleiosperma* (rhamnose-type) (8.7 g) afforded 14 mg and 42 mg, respectively, after prep. HVE at pH 1.9 of the fractions from the Ecteola columns [7].