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D-2-AMINOPIMELIC ACID AND *TRANS*-3,4-DEHYDRO-D-2-AMINOPIMELIC ACID FROM *ASPLENIUM UNILATERALE*

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Key Word Index—*Asplenium unilaterale*; Aspleniaceae; (*R*)-2-aminoheptanedioic acid; (*E*)-(*R*)-2-amino-3-heptenedioic acid; D-amino acids; stereochemistry; chemotaxonomy.

Abstract—D-2-Aminopimelic acid and a novel amino acid, *trans*-3,4-dehydro-D-2-aminopimelic acid were isolated and characterized from *Asplenium unilaterale*. Their distribution in Aspleniaceae was also investigated.

INTRODUCTION

Of the many nonprotein amino acids known in plants, relatively few have been found in Pteridophytes. Several uncommon monoaminodicarboxylic acids were, however, reported from Aspleniaceae by Virtanen and his co-workers. Thus, 2-aminopimelic acid [1, 2] and 4-hydroxy-2-aminopimelic acid [3] were identified from *Asplenium septentrionale*, 4-methylglutamic acid, 4-methyleneglutamic acid and 4-hydroxy-4-methylglutamic acid from *Phyllitis scolopendrium* [4].

Except for 2-aminopimelic acid, their stereochemistry was later investigated. Blake and Fowden characterized their isolates from *P. scolopendrium* as (2*S*,4*R*)-methylglutamic acid and (2*S*,4*S*)-4-hydroxy-4-methylglutamic acid [5]. More recently, Meier and Sørensen reported the distribution of such acidic amino acids in nine ferns including five members of the Aspleniaceae. According to them, 4-hydroxy-4-methylglutamic acid and 4-hydroxy-2-aminopimelic acid occur often as a mixture of (2*S*,4*S*)- and (2*S*,4*R*)-forms in Aspleniaceae [6]. 2-Aminopimelic acid was isolated from *A. septentrionale* once in small amount by Berg and Virtanen and characterized only by chromatography, mp and titration [2]. Its optical rotation was not reported. The configuration of natural 2-aminopimelic acid was, therefore, unknown.

In the present work we have isolated and characterized D-2-aminopimelic acid (D-APA) from *Asplenium unilaterale* Lam. by various spectroscopic methods. From the same plant we obtained also a novel dehydro-form of the above amino acid, *trans*-3,4-dehydro-D-2-aminopimelic acid (D-Δ-APA). Both belong to D-series of amino acids which are rarely found in plants as free forms.

RESULTS AND DISCUSSION

Isolation of APA was carried out by established methods using anion exchange resin and cellulose powder. Elementary analysis of the purified crystals was in good agreement with the formula $C_7H_{13}NO_4$. Its IR spectrum was identical with that of L-APA reported by Wade *et al.* [7]. Comparison of its chromatographic behaviour on TLC, R_f in AA (automated amino acid analysis), SIMS-(see Experimental) and ^{1}H NMR-spectra with those of the authentic DL-APA was also satisfactory. The optical rotation value, $[\alpha]_D$ −19.4° was very near to that of the D-isomer, −21.0° (5 M HCl; *c* 1) reported by Wade *et al.* [7]. Therefore, we concluded that our isolate is D-2-aminopimelic acid [(*R*)-2-aminoheptanedioic acid].

Δ-APA was isolated by successive use of CC with anion exchange resin and HPLC with cation exchange resin. The ninhydrin coloration was first light yellow turning to normal violet with time, as is known for many β,γ- and γ,δ-unsaturated amino acids. The catalytic hydrogenation product could be identified as APA by SIMS and ^{1}H NMR, as well as TLC and AA. According to SIMS its MW was 173 (cf. APA: MW 175), suggesting strongly that it is a dehydro-form of APA. ^{1}H NMR showed the partial structure, >CH–CH=CH–CH_2–. Judging from its relatively high δ-value, the first methyne proton from the left was attributed to the α-proton of the amino acid. The *trans*-configuration was determined by the coupling constant (J = 15 Hz) of the olefinic protons. Position of the double bond was supported also by the result that a major product of $KMnO_4$ degradation was glycine. The negative shift of the $[\alpha]_D$-value in solutions with greater acidity, together with the optical rotation value of its hydrogenation product, i.e. APA, $[\alpha]_D$ = −16.3° indicated that it was also the D-isomer. Therefore it is *trans*-3,4-dehydro-D-2-aminopimelic acid ((*E*)-(*R*)-2-amino-3-heptenedioic acid). This amino acid has not been encountered before as a natural product.

Possible seasonal or geographical variations of free amino acids of the same species were investigated. We analysed leaves in various developmental stages on Hachijo Island and mature leaves from other three distant places. We also collected and analysed the leaves of *A. unilaterale* with apogamous life cycle which is distinguishable from the normal type by phenetic features [Murakami, N. and Iwatsuki, K., unpublished work]. So far, no qualitative differences, however, were found among them; APA and Δ-APA were detected in all samples by 2D-PC and AA.

Free amino acid patterns of the other 21 species of Aspleniaceae were studied. Δ-APA was found in *A. obliquissimum* (Hayata) Sugimoto et Kurata which is closely related to *A. unilaterale*, but not in the related *A. cheilosorum* Kunze ex Mett. nor in *A. excisum* Pr. These

species belong to the sect. *Hymenasplenium* (Hayata) K. Iwats. [8]. We also detected Δ-APA in *A. wilfordii* which is morphologically distinct. APA was detected in all four species above and several other species investigated. However, identification of the amino acid was only by 2D-PC and AA, and their configurations were not determined. More detailed studies are still needed. *A. unilaterale* seems to contain 4-hydroxy-2-aminopimelic acid (OH-APA), though its identification was based on only 2D-PC and its brown coloration with ninhydrin. Virtanen *et al.* [3] and Meier and Sørensen [6] have reported the occurrence of OH-APA in *A. nidus*, *A. septentrionale*, *A. trichomanes* and *A. bulbiferum*. Though it often exists as a mixture of two diastereomers, the configuration of each α-carbon was determined to be the L-form by L-amino acid oxidase [6].

EXPERIMENTAL

General. Cellulose powder and TLC plates used were 'Avicel' (Funakoshi Pharmaceutical Co.) and chromatographic solvents were (A) *n*-BuOH–HOAc–H_2O (63:10:27) and (B) PhOH–H_2O (25:8) in the presence of NH_3. 2D-PC was carried out with solvent A for the first descending and solvent B for the second ascending run. Chromatographic paper was Toyo No. 50. Automated amino acid analysis (AA) was performed on a Hitachi Model 835 using MCI 835-PF-KIT as a buffer system. ^{1}H NMR spectra were measured in CF_3COOD at 100 MHz with TMS as internal standard. Secondary ion mass spectra (SIMS) were determined by a Hitachi M-80A mass spectrometer. About 10 μg of the sample in 1 μl H_2O was loaded on a silver target coated with glycerol and bombarded by an 8.0 KeV Xe ion beam of low current density (*ca* 5×10^{-8} A cm^{-2}) on a 0.1 cm^2 area. The acceleration energy of the secondary ions was 3.0 KeV.

Plant. The fronds and rhizomes of *A. unilaterale* (4 kg) were collected in April 1982 on Hachijo Island, Tokyo and kept at 4° until extraction. In the same place we harvested leaves of the same species in various developmental stages; young leaves at the beginning of their expansion in April, mature leaves separately with and without sori in August and those bearing sori in November. We collected the mature leaves from Owase in Mie Pref., Tano in Miyazaki Pref. and Yaku Island in Kagoshima Pref., all in July. In Owase we collected also the leaves of *A. unilaterale* with apogamous life cycle. The other 21 species of Aspleniaceae were collected at different localities throughout Japan in June–August 1982. They were fixed in EtOH immediately after harvest and kept at −20° until use. All vouchers have been deposited in the Herbarium of Faculty of Science, The University of Tokyo (TI).

Free amino acids of various species or developmental stages. About 10 g of each sample was extracted with 80% EtOH × 4. The combined extract was subjected to DIAION SK-1B (MCI) (H^+ form, 5 ml) column. The column was eluted with 50 ml of 2 M NH_4OH, the eluate coned and analysed by 2D-PC and AA.

Isolation. The fronds and rhizomes of *A. unilaterale* were homogenized repeatedly in a mixer with 80% EtOH and filtered. The combined filtrate (65 l.) was passed through a column of Amberlite IR-120B (H^+ form, 400 ml). The resin was then washed thoroughly with 80% EtOH and H_2O, successively, and the amino acids adsorbed were eluted with 2 M NH_4OH (4 l.). The syrup obtained on conen of the eluate was applied to a Dowex 1 × 4 column (200–400 mesh, OAc^- form, 135 × 2.2 cm) and fractionation was carried out with 0.5 M HOAc (8 ml/fraction). Fractions 20–50, neutral and basic amino acids; fractions 122–201, APA and OH-APA; fractions 211–245, Δ-APA; fractions 270–400, Glu. Fractions 122–201 were combined and the concentrate was applied to a cellulose column (135 × 2.2 cm) and fractionated with solvent A, yielding pure fractions which showed a single spot of APA on TLC. Conen of them gave crystals of APA (160 mg). They were recrystallized twice from EtOH–H_2O, mp 219–220° (decomp.). $[\alpha]_D^{20} = -19.4°$ (5 M HCl; *c* 0.52). (Found: C, 47.94; H, 7.57; N, 7.96. Calc. for $C_7H_{13}NO_4$: C, 47.99, H, 7.48; N, 8.00%). IR ν_{max}^{KBr} 3050, 2970, 2940, 2880, 2750, 2650, 2545, 1675, 1645, 1590, 1515, 1445, 1530, 1355, 1320, 1290, 1255, 1215, 1185, 1135, 1080, 1055, 1035, 1010, 930, 905, 805, 770, 755, 710, 700 and 680 cm^{-1}. ^{1}H NMR (CF_3COOD): δ 1.82 (4H, *m*, H-4, H-5), 2.22 (2H, *m*, H-3), 2.58 (2H, *t*, H-6), 4.40 (1H, *t*, H-2). SIMS *m/z* (G: glycerol, MW = 92): 176 $[M+H]^+$ (100%), 268 $[M+G+H]^+$ (29%), 360 $[M+2G+H]^+$ (7%). Fractions 211–245 were combined and purified on a HPLC column of MCI GEL CK 10S (20 × 1.0 cm) using Li-citrate buffer (pH 2.85) resulted in crystalline Δ-APA (26 mg), which gave a single spot and peak on TLC and AA, respectively. Mp 172–177°. $[\alpha]_D^{20} = -70°$ (H_2O; *c* 0.3), −110° (3 M HCl; *c* 0.15). ^{1}H NMR: δ 2.65 (4H, *m*, H-5, H-6), 4.86 (1H, *d*, *J* = 8 Hz, H-2), 5.75 (1H, *dd*, *J* = 15, 8 Hz, H-3), 6.28 (1H, *dt*, *J* = 15, 5 Hz, H-4). SIMS: *m/z* 174 $[M+H]^+$ (100%), 266 $[M+G+H]^+$ (40%), 358 $[M+2G+H]^+$ (11%).

Hydrogenation. The pure sample (*ca* 10 mg) was hydrogenated over Adams' Pt catalyst at room temp. and atm pres.

Degradation. A small amount of the pure sample was dissolved in 10% H_2SO_4 and the same volume of 2% $KMnO_4$ added. After 17 hr at 4°, the mixture was treated with DIAION SK-1B (H^+ form). Amino acids were eluted with 2 M NH_4OH and analysed using TLC and AA.

Chromatographic data. R_{Ala}-values on TLC and elution times in AA are listed in Table 1.

Table 1. R_{Ala}-values and R_t

Amino acids	R_{Ala}-values: Solvent A	R_{Ala}-values: Solvent B	R_t (min) in AA
Glu	0.77	0.40	33
Pro	1.23	1.46	53
Val	2.00	1.31	81
APA	1.43	0.65	77
Δ-APA	1.20	0.64	44

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