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VII. 7 Metabolism of Auxin and the Related Compounds in Plant Roots

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Exogenously supplied auxin, indole-3-acetic acid (IAA) is known to be metabolized to indole-3-aldehyde, indole-3-acetyl-aspartic acid and 1-(indole-3-acetyl)-β-glucose in plants or esterified to IAA-myo-inositol in Zea seeds.[1)]

During the study of ^{14}C-labeled IAA translocation in Vicia seedings, we isolated two IAA metabolites (designated as compounds A and B) from the roots.[2)] Compound A was identified as 3-(O-β-glucosyl)-2-indolone-3-acetylaspartic acid (unpublished). Compound B was also a dioxindole-3-acetic acid conjugate but its chemical structure has not yet been fully clarified because of its lesser content in the plant.

Compound A and B are unique auxin metabolites since they are formed through IAA oxidation not accompanied with decarboxylation. The known process of IAA oxidation catalyzed by peroxidase always involves decarboxylation.[1)] The dioxindole-3-acetic acid conjugates are the major products of exogenously supplied ^{14}C-IAA in Vicia roots.[2)] However, their physiological roles have not yet been elucidated. In the present report, we tried to separate and detect the two metabolites by a high pressure liquid chromatography, which might much simplify the determination procedure of native dioxindole-3-acetic acid conjugates in plants.

Dark grown 4-day-old broad bean (Vicia faba L. cv. Chukyo) seedlings were cut into seven parts: apical 1 cm epicotyl tips, remaining epicotyls, cotyledons, hypocotyls and basal one-third of roots in length, middle part of roots, and apical one-third of roots. About 10 g fresh weight of each part was extracted by ethanol and fractionated as reported previously[2)], and adsorbed to DEAE Cellulose column (4 × 3 cm). Adsorbed substances were eluted with 50 ml of 500 mM sodium acetate, deionized by Amberlite IR-120 (H^+-form) and concentrated to about 2 ml. Aliquot of the samples was injected into a Waters High Performance Liquid Chromatograph (Model ALC/GPC 204) with a Waters μBondapak C_{18} column (10 × 0.8 cm) in Model RCM-100 Radial Compression Separation System, developed with 0.01 % acetic acid at 3 ml/min and detected by UV absorbance at 254 nm. Rerention volume of purified compunds A and B was 20.4 and 30.0 ml, respectively (Fig. 1-a). It was possible to detect 0.1 nmol of these compounds. Fig. 1-b is the elution profile of the basal extract, showing the peak of compound A but not compound B.

Compound A was previously isolated from the roots of Vicia seedlings,[2)] but it was found in all parts of the seedlings (Table 1). The content of compound A was prominant in cotyledons, 18.7 nmol/cotyledon, but its concentration was conspicuous in apical epicotyls and basal roots, 15.6 and 12.2 nmol/g fresh weight, respectively, where cell divisions were proceeding actively. An average

content of compound A in the plant was 8.6 nmol/g fresh weight. Liquid chromatography technique serves as a simplified separation of compound A, but a further pre-fractionation of samples is necessary for the determination of compound B.

References

1) Schneider E. A. and Wightman F., Ann. Rev. Plant Physiol. 25 (1974) 487.
2) Tsurumi S. and Wada S., Plant & Cell Physiol. 22 (1981) in press.

Table 1. Content of compound A in 4-day-old Vicia seedlings.

	nmol/g fr wt	nmol/plant
apical epicotyls	15.6	1.4
basal epicotyls	7.8	3.7
cotyledons	8.4	18.7
basal roots	12.2	3.0
middle roots	5.9	0.6
apical roots	4.8	0.3

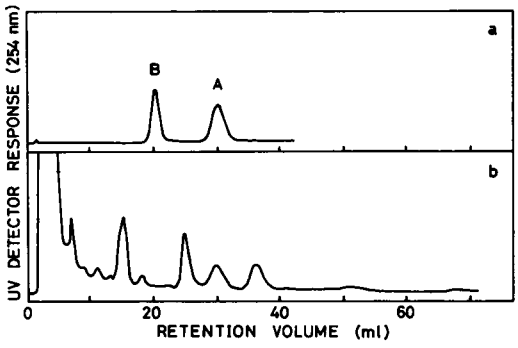

Fig. 1. Elution profiles of purified compound A (a-A), compound B (b-B) and basal root extract (b) from μBondapak C_{18} column with 0.01 % acetic acid.