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SHORT REPORTS

IDENTIFICATION OF GA_{36} IN *PSILOTUM NUDUM*

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Key Word Index—*Psilotum nudum*; Psilotaceae; pteridophyte; GA_{36}.

Abstract—GA_{36} was identified by GC/MS analysis from the sporangia-bearing fronds of *Psilotum nudum*. This is the first chemical identification of a free gibberellin in a pteridophyte.

INTRODUCTION

We have been investigating phytohormones in pteridophytes and the occurrence of cytokinins and ABA was reported in the previous papers [1, 2]. We wish to report here the identification by GC/MS analysis of GA_{36} from the pteridophyte *Psilotum nudum*.

RESULTS AND DISCUSSION

An acidic ethyl acetate fraction from sporangia-bearing fronds (250 g fr.wt) of *P. nudum* was subjected to silica gel partition column chromatography (stationary phase; 0.5 M aqueous formic acid) eluted with ethyl acetate–*n*-hexane. The GA active eluate with 40% ethyl acetate in *n*-hexane was further purified by HPLC using a Nucleosil $N(Me)_2$ column eluted with 0.05% acetic acid in methanol [3] to afford the active eluate at R_t 10–14 min. The finding that the active fraction showed GA activity on cv. Waito-C as well as cv. Tan-ginbozu in the rice seedling test, suggested that it contained a C-3 hydroxy GA as an active component [4]. Finally, we succeeded in identifying GA_{36} by GC/MS analysis of its trimethylsilyl ester trimethylsilyl ether derivative. The content of GA_{36} in *P. nudum* was estimated to be 2.34 μg/kg fr. wt.

This is not only the first chemical identification of a free gibberellin from a pteridophyte, but also the first example for the occurrence of GA_{36} as an endogenous GA in vascular plants. GA_{36} is considered to be a direct precursor of GA_4 which is one of the most common GAs. The accumulation of GA_{36} in a pteridophyte may suggest that biological conversion of GA_{36} to GA_4 is either very slow as compared with that of higher plants or blocked.

EXPERIMENTAL

Plant material. Sporangia-bearing fronds of *Psilotum nudum* were harvested at the greenhouse of Kyoto University, Kyoto city, Japan, in November 1982.

Extraction and fractionation. Fresh plant material (250 g fr. wt) was homogenized and extracted with 500 ml of Me_2CO (× 2). The mixture was filtered and the Me_2CO removed *in vacuo*. The resultant aq. residue was fractionated by the same method as that of Yamane *et al.* [5] to give an acidic EtOAc fraction (317.30 mg).

Partition column chromatography. The acidic EtOAc fraction adsorbed on celite (2 g) was placed on a column packed with silica gel (16.0 g) impregnated with 0.5 M aq. HCOOH (10 ml) and eluted with EtOAc–*n*-hexane (0–100% EtOAc in 10% steps/50 ml).

HPLC. Nucleosil $5N(Me)_2$ column (6 mm i.d. × 100 mm) eluted with MeOH containing 0.05% AcOH was used. The flow rate was 1.5 ml/min, and the column temp. was 50°.

Bioassay. The dwarf rice test was carried out at a concn of 10 g fr. wt eq./ml according to the water culture method used by Yokota *et al.* [6] at 30° under continuous illumination (fluorescent light). Seeds of *Oryza sativa* L., dwarf cv. Tan-ginbozu and Waito-C were used for the assay.

GC/MS analysis. A Hitachi M80-A equipped with a glass column (3 mm i.d. × 1 m) packed with 2% OV-1 on Chromosorb W was used. Ionizing voltage, 20 eV; oven temp, 210°; He, 50 ml/min. A sample for GC/MS was silylated with pyridine–BSA–TMCS (1:2:1) at room temp. R_t of GA_{36}–TMS–TMS in GC: 4.3 min. GA_{36}–TMS–TMS from *P. nudum* showed the following mass spectrum in GC/MS: 578 $[M]^+$ (12%), 563 (51), 550 (19), 488 (79), 460 (58), 432 (45), 398 (55), 370 (84), 342 (100), 314 (38), 280 (46), 224 (89). The mass spectrum of authentic GA_{36}–TMS–TMS in GC/MS: 578 $[M]^+$ (20), 563 (51), 550 (18), 488 (75), 460 (64), 432 (54), 398 (66), 370 (82), 342 (100), 314 (51), 280 (58), 224 (99).

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REFERENCES

1. Yamane, H., Watanabe, M., Satoh, Y., Takahashi, N. and Iwatsuki, K. (1980) *Plant Cell Physiol.* **24**, 1027.
2. Yamane, H., Satoh, Y., Takahashi, N., Takeno, K., and Furuya, M. (1980) *Agric. Biol. Chem.* **44**, 1967.
3. Yamaguchi, I., Fujisawa, S. and Takahashi, N. (1982) *Phytochemistry* **21**, 2049.
4. Murakami, Y. (1968) *Bot. Mag. Tokyo* **81**, 33.
5. Yamane, H., Takahashi, N., Takeno, K. and Furuya, M. (1979) *Planta* **147**, 251.
6. Yokota, T., Murofushi, N., Takahashi, N. and Katsumi, M. (1971) *Phytochemistry* **10**, 2943.