Occurrence of a Bis-bibenzyl Derivative in the Japanese Fern *Hymenophyllum barbatum*: First Isolation and Identification of Perrottetin H from the Pteridophytes

Yasushi OISO, Masao TOYOTA and Yoshinori ASAKAWA*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770–8514, Japan.

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The methanol extract of the Japanese fern *Hymenophyllum barbatum* was chromatographed on Sephadex LH-20 and HPLC to give perrottetin H, an acyclic bis-bibenzyl derivative which has been isolated from the liverwort *Jubula japonica*. This is the first isolation and identification of bis-bibenzyl from the pteridophytes. The occurrence of bis-bibenzyl in both pteridophytes and liverwort is very important in determining the evolutionary ladder of both terrestrial spore-forming green plants.

Key words *Hymenophyllum barbatum*; fern; perrottetin H; bis-bibenzyl; evolutionary process

The bryophytes, which are taxonomically placed between the algae and the pteridophytes, are divided into three classes, the Musci (moss), Hepaticae (liverwort), and Anthocerotae (hornwort) and there are more than 20,000 species worldwide. It is suggested that the bryophytes occurred in Gondwanaland and were distributed in northern hemisphere after this land mass broke up.^{1,2)} It is thought that the bryophytes have accumulated bitter and pungent substances which inhibit the growth of microorganisms and provide a defense against herbivores. We have focused on the bioactive constituents^{3–7)} and the chemosystematics of liverworts as well as the evolutionary relationship between terrestrial spore-forming green plants and algae using their characteristic chemical indicators.^{3,4,8–10)} Generally the chemical constituents of the Musci, Hepaticae, and Anthocerotae differ from each other, and the Hepaticae produce constituents very similar to those found in the Phaeophyceae (brown algae).^{4,9)} More recently, we isolated *ent*-sesqui- and dolabellane diterpenoids from the Japanese moss, *Plagiomnium acutum*.¹¹⁾ This evidence shows that some mosses are evolutionarily related to the Hepaticae.

There are two main hypotheses regarding the evolution of the bryophytes (Fig. 1).¹⁾ The first is the progressive theory, which states that the bryophytes have their origins in the filamentous fresh green algae or a unicellular green alga and

that the pteridophytes (ferns) are in turn derived from the bryophytes. The second hypothesis, the reductive theory, regards the green algae as being the ancestors of primitive vascular plants, such as the extinct class Rhyniophytina, from which evolved both bryophytes and pteridophytes. However, no conclusive scientific evidence for these theories has been obtained from paleontology because of the absence of bryophyte fossils. In order to understand the evolutionary stages between the bryophytes and the pteridophytes, we are currently studying the chemical constituents of the pteridophytes. Here we report the isolation and identification of an acyclic bis-bibenzyl derivative from the Japanese pteridophyte *Hymenophyllum barbatum* and the chemical correlation between this fern and some liverworts.

Air-dried and ground *H. barbatum* (2.40 kg) was extracted with methanol and the ethyl acetate-soluble fraction (70.0 g) was chromatographed on Sephadex LH-20 and HPLC (column: ODS; eluent: MeCN:H₂O; 2:8) to afford an acyclic bis-bibenzyl derivative (**1**), C₂₈H₂₆O₆ (HRMS *m/z*: 458.1717 [M]⁺; calcd for 458.1729). Methylation of **1** in anhydrous acetone with methyl iodide gave a pentamethyl ether (**2**), C₃₃H₃₆O₆ (HRMS *m/z*: 528.2495 [M]⁺; calcd for 528.2512). The spectral data of **1** and **2** were identical to those of perrottetin H and its permethylated product, respectively.¹²⁾ Perrottetin H (**1**) has been isolated from the Japanese liverwort *Jubula japonica* and characterized by spectroscopic evidence.¹²⁾ This is the first isolation and identification of a bis-bibenzyl derivative from the pteridophytes. The occurrence of the bis-bibenzyl in ferns is significant. Previously, various cyclic and acyclic bis-bibenzyls have been isolated only from the Hepaticae and are recognized as one of the most important chemical markers of the Hepaticae.⁴⁾ A number of monomeric bibenzyls, such as lunularic acid (**3**), its decarboxyl derivative, lunularin (**4**), and prenyl bibenzyl (**6**), have also been found in the Hepaticae and they are also valuable for the chemosystematics of the Hepaticae.⁴⁾ It is noteworthy that bibenzyl (**5**) and prenyl bibenzyl (**7**) similar to those of

Fig. 1. Two Hypotheses for the Evolutionary Process of Bryophytes and Pteridophytes

* To whom correspondence should be addressed.

3, 4, and 6 have been isolated from the ferns, *Notholaena dealbata*, *N. limitanea*,¹³⁾ and *Sceptridium ternatum*,¹⁴⁾ respectively. These bibenzyls and bis-bibenzyls might be considered to be living chemical fossils that could link the evolutionary stage between the bryophytes and the pteridophytes. It is still difficult to conclude which theory mentioned above better explains the evolutionary processes of the bryophytes, although on the basis of the present results, it is clear that there are significant chemical affinities between some ferns and liverworts in the Jungermanniales, Marchantiales, and Metzgeriales which produce bibenzyl and bis-bibenzyl derivatives.

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References

- 1) Schofield W. B., *Introduction to Bryology*, Macmillan, New York, 1985.
- 2) Inoue H., *Kagaku Asahi*, 116—121 (1988).
- 3) Asakawa Y., *Progress in the Chemistry of Organic Natural Products*, Vol. 42, ed. by Herz W., Grisebach H., Kirby G. W., Springer, Vienna, 1982, pp. 1—285.
- 4) Asakawa Y., *Progress in the Chemistry of Organic Natural Products*, Vol. 65, ed. by Herz W., Kirby G. W., Moore R. E., Steglich W., Tamm Ch., Springer, Vienna, 1995, pp. 1—562.
- 5) Asakawa Y., *Bryophytes: Their Chemistry and Chemical Taxonomy*, ed. by Zinsmeister H. D., Mues R., Clarendon Press, Oxford, 1990, pp. 369—410.
- 6) Asakawa Y., *Bioactive Natural Products: Detection, Isolation, and Structural Determination*, ed. by Colegate S. M., Molyneux R. J., CRC Press, Boca Raton, 1993, pp. 259—287.
- 7) Asakawa Y., *J. Hattori Bot. Lab.*, **88**, 91—104 (1998).
- 8) Asakawa Y., Toyota M., Takeda R., Suire C., Takemoto T., *Phytochemistry*, **20**, 725—728 (1981).
- 9) Asakawa Y., *J. Bryol.*, **14**, 59—70 (1986).
- 10) Asakawa Y., *J. Hattori Bot. Lab.*, **76**, 293—311 (1994).
- 11) Toyota M., Kimura K., Asakawa Y., *Chem. Pharm. Bull.*, **46**, 1488—1489 (1998).
- 12) Toyota M., Asakawa Y., *Phytochemistry*, **34**, 1135—1137 (1993).
- 13) Wollenweber E., Favre-Bonvin J., *Phytochemistry*, **18**, 1243—1244 (1979).
- 14) Tanaka N., Wada H., Murakami T., Sahashi N., Ohmoto T., *Chem. Pharm. Bull.*, **34**, 3727—3732 (1986).