Momilactones A (1) and B (2), which have been identified as phytoalexins in rice, were isolated from extracts of the moss *Hypnum plumaeforme*. This is the first isolation and identification of momilactones as allelochemicals from a bryophyte. *H. plumaeforme* produces considerable amounts of momilactones (isolated yield: 8.4 mg/Kg plant for 1; 4.2 mg/Kg for 2). EtOAc extracts from *H. plumaeforme* and 2 showed growth inhibitory activity against angiosperms, moss, and liverwort plants. On the other hand, the growth of *H. plumaeforme* was insensitive to its extract and 2. Our finding suggests that momilactones play an important role as allelochemicals in this moss.

Key words: *Hypnum plumaeforme*; moss; momilactone; allelochemicals

Bryophytes, which represent the oldest lineages among extant land plants, are the second largest phylum of land plants after angiosperms and, they inhabit every continent. Among bryophytes, which are divided into three classes (mosses, liverworts, and hornworts), mosses are the most diverse class, comprising 10,000 or more species.1,2) Mosses differ from liverworts and hornworts in a number of characteristics, including microscopic features such as a gametophyte composed of stems with undivided and often costate leaves, and a sporophyte terminated by a capsule. Over the last few decades, investigation of the chemical constituents of bryophytes has focused mainly on liverworts, which contain several oil bodies characteristic of the species in each cell of the gametophyte. It has been found that these oil bodies usually contain sesquiterpenoids and diterpenoids, as well as esters of fatty acids and aromatic acids, as the major lipophilic constituents.2–4) On the other hand, it is thought that mosses contain no oil bodies, and there are only a few reports of the chemical constituents of mosses.5–7)

*Hypnum plumaeforme* (Hypnaceae) is a species of moss that is widely distributed, from Hokkaido to the southwest islands of Japan, and forms large colonies on dry ground and rocks.8) *H. plumaeforme* has oil droplets in the leaf cell, similaly to liverworts (Fig. 1), and few other plants grow close to *H. plumaeforme*. Furthermore, ethyl acetate extracts of *H. plumaeforme* showed potent inhibition of *Arabidopsis* growth, but did not affect the growth of *H. plumaeforme* itself. These observations suggest that *H. plumaeforme* contains allelochemicals, and that prompted us to investigate the active agents involved. We describe the identification of momilactones A (1) and B (2) from *H. plumaeforme* (Fig. 2),9) and discuss the allelopathic effects of 2 on different plant species, including angiosperms, liverworts and mosses.

*Hypnum plumaeforme* was collected in Yoshinaga-cho, Okayama Prefecture, Japan. Dried aerial parts of *H. plumaeforme* (2,400 g) were extracted twice with acetone (2 × 15 liters) for 2 weeks at room temperature. The acetone extract showed potent growth inhibitory activity against *Arabidopsis* plants (Fig. 3A), and the

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active principles were purified by activity-guided fractionation. The extract (23.4 g) was suspended in water (150 ml) and then extracted with EtOAc (3 × 100 ml). The EtOAc extract was washed with water and then dried in vacuo to yield 18 g of active residue, which was subjected to column chromatography on silica gel (70–230 mesh, Merck silica gel 60, 9 × 80 cm) with a gradient of 5–100% EtOAc in hexane (95.5:9:1:82:3:1:1:EtOAc, each 3 liters), from which 11 fractions were collected. Fractions 8 and 9, which showed inhibitory activity, were combined and chromatographed on a silica gel column (230–400 mesh, Merck silica gel 60, 3 × 60 cm) developed with benzene/EtOAc (4:1, v/v, 1.6 liters), followed by 10% (w/v) AgNO₃-silica gel preparative TLC to furnish compounds 1 and 2 (20 mg and 10 mg respectively).

Compounds 1 [colorless needles; mp 238–239 °C; EI-MS m/z 314 [M]+; [α]D = −279° (c = 0.8, CHCl₃); IR (KBr) v max: 1762, 1700, 1660, 1640, 990, 905 cm⁻¹; ¹H NMR (CDCl₃): δH 2.33 (d, J = 5.2, 1H, H-5), 4.85 (d, J = 5.2, 1H, H-6), 5.71 (d, J = 5.2, 1H, H-7), 2.03 and 2.22 (d, J = 12.5, 2H, H-14), 5.85 (dd, J = 17.7 and 10.7, 1H, H-15), 4.93 (dd, J = 10.7 and 0.9, 1H, H-16), 4.95 (dd, J = 17.7 and 0.9, 1H, H-16), 0.90 (s, 3H, H17), 1.53 (s, 3H, H-18), 1.00 (s, 3H, H-20)] and 2 [colorless needles; mp 243–245 °C; EI-MS m/z 330 [M]+; [α]D = −169° (c = 0.1, CHCl₃); IR (KBr) v max: 3450, 1738, 1660, 1635, 1130, 980, 905 cm⁻¹; ¹H NMR (CDCl₃): δH 2.21 (dd, J = 6.9 and 2.0, 1H, H-5), 4.93 (dd, J = 6.9 and 4.9, 1H, H-6), 5.69 (d, J = 4.9, 1H, H-7), 5.83 (dd, J = 17.4 and 10.7, 1H, H-15), 4.93 (dd, J = 10.7 and 1.2, 1H, H-16), 4.96 (dd, J = 17.4 and 1.2, 1H, H-16), 0.87 (S, 3H, H-17), 1.41 (s, 3H, H-18), 3.55 (dd, J = 9.0 and 2.1, 1H, H-20), 4.07 (bd, J = 9.0, 1H, H-20)] were identified by spectral analysis, including 2D-NMR, as momilactone A and momilactone B respectively (Fig. 2). This was further confirmed by direct comparison with authentic samples using a silica gel and an ODS reversed phase TLC. Optical rotation studies confirmed that the absolute stereochemistry of 1 and 2 was identical to that of momilactones A and B.¹⁰

Momilactones A and B have been isolated from the seed husk of rice (Oryza sativa) and found to be inhibitors of plant germination and growth.¹⁰,¹¹ To the best of our knowledge, this is the first isolation of momilactones A and B from a plant other than rice. Momilactones are accumulated in rice during vegetative growth¹²,¹³ and are released into the soil, causing growth inhibition of neighboring plants.¹²,¹³ It has been reported that accumulation of momilactones in rice plants increases in response to UV irradiation and pathogenesis such as fungal invasion, suggesting that momilactones in rice also play a role in plant defense.¹⁴

H. plumaeforme prefers to grow in relatively sunlit places, and exclusive colony formation of this moss has frequently been observed, implying that H. plumaeforme exerts allelopathic effects against the growth of other plants. In addition, H. plumaeforme produces large amounts of momilactone B (4,100 µg/kg yield isolated from dry weight), which has been reported at levels of 80 and 21 µg/kg (quantitative analysis of fresh plant) in the shoots and roots of rice respectively.¹³ Several studies have reported that momilactone B plays an important role in rice allelopathy, suggesting that momilactones in H. plumaeforme might also act as allelochemicals.¹²,¹⁴

To confirm our hypothesis, we examined the growth inhibitory effects of EtOAc extracts of H. plumaeforme and momilactone B (2) on various plant species. Arabidopsis thaliana (ecotype Columbia), tobacco (Nicotiana tabacum cv Petit Habana), the liverwort Jungermannia subulata cultured cells, and mosses Physcomitrella patens and H. plumaeforme were used in growth assays. The plants (n = 8–12) were grown under continuous light in 6-well microtiter plates containing 5 ml of medium with or without EtOAc extract of H. plumaeforme or 2 for the times indicated. In this assay, plants were grown on the following media: germination medium for Arabidopsis,¹⁵ MS medium for tobacco,¹⁵ MSG-2 medium for J. subulata cells,¹⁶ and

Fig. 1. Optical Micrograph of Hypnum plumaeforme. Arrows indicate oil droplets. The scale bar represents 20 µm.

Fig. 2. Structure of Momilactone A (1) and Momilactone B (2).
BCDATG medium for *P. patens* and *H. plumaeforme*.17) After cultivation, photographs of representative individuals were taken using an optical microscope.

The EtOAc extract of *H. plumaeforme* showed growth inhibitory activity against the angiosperms tobacco and *Arabidopsis*, the liverwort *J. subulata*, and the moss *P. patens* at 100–500 μg/ml (Fig. 3A). Surprisingly, the growth of *H. plumaeforme* was insensitive to the extract at the same concentration (Fig. 3A). This suggests that momilactones in the extract act as allelochemicals, suppressing the growth of other plant species. The allelopathic properties of 2 rather than 1 have been studied in depth owing to the more potent activity of 2.12–14) Hence we examined the inhibitory effects of 2 on plant growth. As expected, 2 did not affect the growth of *H. plumaeforme* at 20 μM, but exhibited potent inhibitory activity against the germination and growth of the other test plants at 2–20 μM (Fig. 3B). These results indicate that momilactones play an important role in allelopathy in *H. plumaeforme*. Allelochemicals from a bryophyte have not been described. Therefore, our finding is the first example of allelochemicals in moss, and also suggests that this moss is a potential natural source of herbicides for use in weed control.18)

Momilactones A and B are pimarane-type diter-

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**Fig. 3.** Alleropathic Effects of *H. plumaeforme* Extracts and Momilactone B.

A, Plant growth inhibitory activity of the extract from *H. plumaeforme*. Plants (n = 8–12) were grown in the absence (left panel, mock) or presence of the extract (right panel, 500 μg/ml) for the following times: *Arabidopsis* for 14 d; the moss *P. patens* for 0 and 13 d; the liverwort *J. subulata* for 0 and 9 d; tobacco for 0 and 10 d; and *H. plumaeforme* for 0 and 12 d. B, Plant growth inhibitory activity of momilactone B (2). Plants (n = 8–12) grown in the absence (left panel, mock) or presence of 2 (middle panel, 2 μM and right panel, 20 μM) for the following times: *Arabidopsis* for 8 d; *P. patens* for 13 d; *J. subulata* for 9 d; tobacco for 10 d; and *H. plumaeforme* for 0 and 13 d. The scale bar represents 5 mm.

**Fig. 4.** Biosynthetic Pathway of Diterpenes.
penes biosynthesized from geranylgeranyl diphosphate (GGDP) in rice (Fig. 4). The first cyclization from GGDP produces syn-copalyl diphosphate (syn-CDP) by the action of syn-CDP synthase (syn-CPS), and the second cyclization converts syn-CDP into syn-pimara-7,15-diene by the action of syn-pimara-7,15-diene synthase (OsDTS2). 

Remarkably, this function is different from that of higher plants, which synthesize ent-kaulane successively using two cyclases, ent-copalyl diphosphate synthase and ent-kaurene synthase, from GGDP. Methylketones have been found only in rice, moss, *H. plumaeformis* is a lower land plant and phylogenetically quite distinct from rice. How does the moss biosynthesize methylketones and regulate the expression of their biosynthetic genes? The answers would not only provide new insight into the evolution of diterpenoids, but also shed light on the evolution of plant defense system by allelochemicals. Hence we are very much interested in the diterpene cyclase that catalyzes the first committed step in the biosynthesis of methylketones in *H. plumaeformis*.

**References**