Occurrence of *ent*-Sesquiterpene in the Japanese Moss-*Plagiomnium acutum*: First Isolation and Identification of the *ent*-Sesqui- and Dolabellane-type Diterpenoids from the Musci

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The ether extract of the Japanese moss *Plagiomnium acutum* was chromatographed on silica gel and Sephadex LH-20 to give *ent*-β-cedrene and 3,7-dolabelladiene-18-ol which has been isolated from brown algae. To the authors' knowledge, this is the first isolation and identification of sesquiterpenoids from the Musci and the first record of *ent*-β-cedrene from the plant kingdom.

Key words *Plagiomnium acutum*; moss; *ent*-β-cedrene; sesquiterpenoid; 3,7-dabelladien-18-ol; dolabellane-type diterpene

The bryophytes are taxonomically placed between the algae and the pteridophytes and more than 20000 species are known in the world. They are divided into three classes, Musci (mosses, 14000 species), Hepaticae (liverworts, 6000 species), and Anthocerotae (hornworts, 300 species).

Many bryophytes have been used as medicinal plants in China, Europe, and North America to cure cuts, burns, external wounds, convulsions, bacteriosis, scalds, urocystitis, tympanitis, neurasthenia, fractures, pulmonary tuberculosis, *etc.*[1] Some bryophytes have an intensely pungent and bitter taste, induce allergenic contact dermatitis, and inhibit the growth of microorganisms. We have been interested in the biological activity of bryophytes and continued to study their chemical constituents.[2,3] Among the bryophytes, the chemical constituents of the Hepaticae have been investigated in more detail, because liverworts possess cellular oil bodies which comprise mono-, sesqui-, and diterpenoids and lipophilic aromatic compounds, while the other two classes contain no oil bodies. More than 750 terpenoids, excluding the triterpenoids and carotenoids, and 220 aromatic compounds, excluding the flavonoids, have been isolated from or detected in the Hepaticae.[2,3] On the contrary, only three monoterpene hydrocarbons have been detected in the mosses or *Splachnum* species and only one kaurane-type diterpene isolated from *Saelania* moss.[3] No sesquiterpenoids have been detected in or isolated from the Musci.[3]

Some species belonging to the Mniaceae mosses show hemostatic activity and induce allergenic contact dermatitis.[1] In order to isolate such biologically active substances from mosses, we started to study the chemical constituents of the Japanese *Plagiomnium acutum*. Surprisingly, this moss biosynthesizes *ent*-sesquiterpene hydrocarbons and a dolabellane-type diterpene alcohol. Here we report the isolation and identification of these terpenoids.

A air-dried *P. acutum* (10.5 g) was extracted with ether to obtain the crude extract (160 mg). A small amount of the crude extract was analyzed by GC-MS to detect three sesquiterpene hydrocarbons, α- and β-cedrenes and α-acoradiene. The remaining extract was further chromatographed on silica gel and Sephadex LH-20 to afford *ent*-β-cedrene (**1a**)[4] (13.3 mg) and (+)-dolabella-3,7-dien-18-ol (**2**)[5] (10.0 mg). The structures of **1a** and **2** were established by 600-MHz ^{1}H- and 150-MHz ^{13}C-NMR spectra including 2D-spectra (^{1}H–^{1}H, HMQC, HMBC, NOESY), IR, EI-MS (high resolution) and comparison of the spectral data with those of an authentic sample (for β-cedrene) and reference data (for β-cedrene[4] and dolabella-3,7-dien-18-ol[5]). The absolute configuration of β-cedrene was established as follows. Commercially available (+)-β-cedrene (**1b**)-rich oil was chromatographed on silica gel impregnated with silver nitrate to give (+)-β-cedrene (**1b**) (lit.[4] +9.7). The same compound isolated from the moss showed $[\alpha]_D$ −10.9. Upon co-injection of **1a** and **1b** into a capillary column (DB-17; 30 m×0.25 mm i.d., film thickness 0.25 μm) one peak appeared on the total ion chromatogram. However, when both compounds were co-injected into a chiral capillary column (β-DEX 120; 30 m×0.25 mm i.d., film thickness 0.25 μm), two well-separated peaks appeared on the total ion chromatogram (Fig. 1). Thus it is clear that β-cedrene isolated from the moss is the enantiomer of that obtained from commercial oil. β-Cedrene has also been detected in the liverwort *Bazzania stolonifera*,[6] although, its absolute configuration

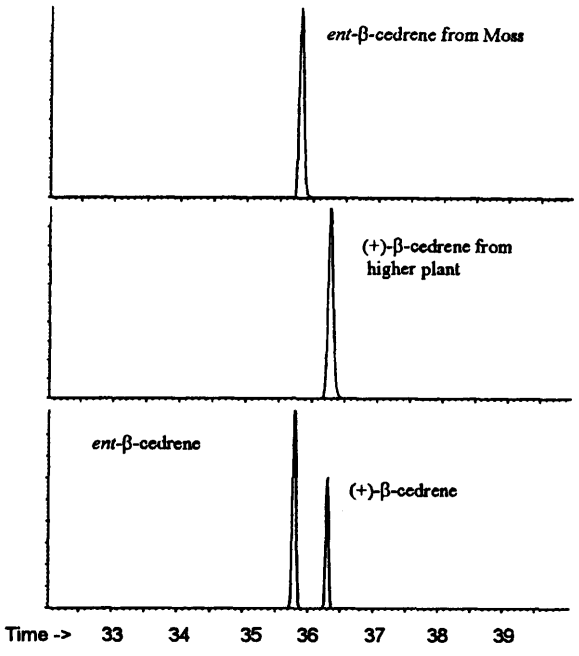

Fig. 1. Total Ion Chromatograms of (+)- and *ent*-β-Cedrenes, and the Co-injected Both Enantiomers

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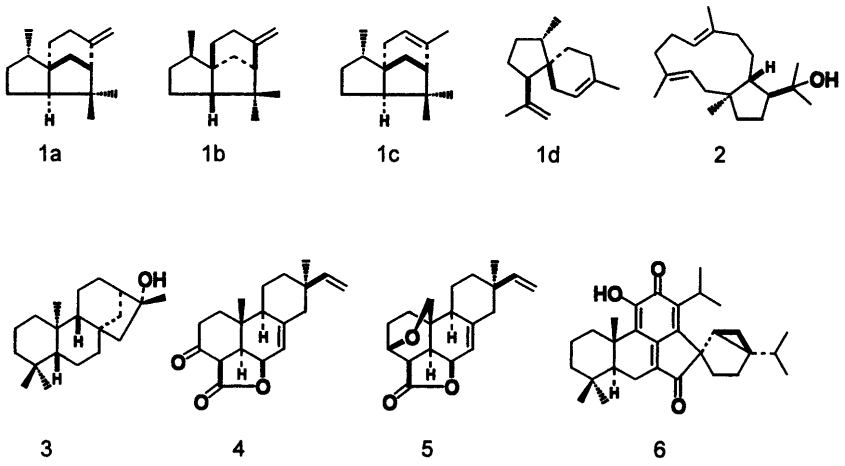

Chart 1

remained to be clarified. α-Cedrene (**1c**) and α-acoradiene (**1d**) detected by GC-MS might be the same enantiomers as those found in higher plants, since *ent*-β-cedrene (**1a**) coexisted within the present species. This is the first known isolation and identification of *ent*-sesquiterpenoid from the Musci. As far as we are aware, it is also the first record of *ent*-β-cedrene from the plant kingdom. The dolabellane-type diterpenoid **2** from the moss showed positive optical rotation $[\alpha]_D$+23.7 which was almost identical to that ($[\alpha]_D$+27.5) of the same compound isolated from the brown algae *Dictyota dichotoma*.[5] The occurrence of dolabellane-type diterpenoids is very rare in organisms. From the digestive gland of the opisthobranch mollusc *Dolabella californica*, 14 dolabellane-type diterpenoids have been isolated and their relative stereochemistries established by Ireland and Faulkner.[7] The brown algae *Dictyota* species are the plant source of dolabellanes.[5] The dolabellanes are distributed in the Jungermannales liverworts, *Barbilophozia*, *Odontoschisma*, and *Pleurozia* species.[3] This is the first isolation of dolabellane diterpenoids from the moss. Only one diterpene, *ent*-16β-hydroxykaurane (**3**), has been isolated from the exudate of the moss *Saelania glaucescens*.[8] The presence of three other diterpenes, momilactones A (**4**) and B (**5**) and chamaecydin (**6**), have orally been reported from mosses,[9] althuogh, their details have not been reported in published papers.

The present four terpenoids are previously known compounds, except for *ent*-β-cedrene (**1a**). However, these results have a significant meaning from the morphological and taxonomic viewpoints of mosses. The Hepaticae contain oil bodies which are responsible for lipophilic mono-, sesqui-, and diterpenoids as well as lipophilic aromatic compounds and 80% of the isolated sesquiterpenes are the enantiomers of those found in higher plants. These terpenoids and their absolute configurations are very important markers for classification of the Hepaticae.[2,3] It is noteworthy that the present moss is chemically very similar to some liverworts because it produces *ent*-sesqui- and dolabellane-type diterpenoids, although the two plants are morphologically quite different. There is no doubt that our moss sample was pure because we collected the mat of this plant from a location where neither other liverworts nor mosses grow. There are 18 *Plagiomnium* species in Japan.[10] The present compounds might be significant chemical markers of *Plagiomnium* species. We will attempt to analyze the chemical constituents of the other *Plagiomnium* species to determine whether further sesqui- and diterpenoids are detected in or isolated from this genus.

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