

Isolation of Enantiomeric Type Sesquiterpenoids from the Liverwort *Riccardia jackii*

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A series of the enantiomeric sesquiterpenoids corresponding to the antipodes of those obtained from higher plants were isolated from the liverwort *Riccardia jackii*. This result is an additional example suggesting the special position taxonomical of the liverworts in plant kingdom.

Introduction

In the course of our study on sesquiterpenoids of the liverworts which are placed in a special group considered an early stage of the evolution of the terrestrial plants, we found that almost all of the liverworts metabolite the enantiomeric (abbr. *ent*) sesquiterpenoids corresponding to the antipodal form of those in higher plants [1–5]. In general, the sesquiterpenoids special to plants are produced from *cis*-farnesyl pyrophosphate and *trans*-isomer through appropriate stereospecific cyclizations and rearrangements on enzyme surface. We have, therefore, recognized the phenomena, that is, the biogenesis of liverwort sesquiterpenoids, as one of the most important biochemical characteristics of the liverworts.

In this paper, as an additional result supporting the significant feature, we describe the isolation of a series of enantiomeric sesquiterpenoids from a thalloid liverwort, *Riccardia jackii* Schiffn. belonging to the Aneuraceae [6, 7].

Results and Discussion

The liverwort, *R. jackii*, was digested with methanol to afford a brownish oily substance. A neutral fraction was separated by usual manner from the methanol extract in a yield of 1.1% to the dried material. The neutral part was subjected to column chromatography using silica gel to separate

the hydrocarbon and alcohol fractions. From the hydrocarbon part *ent*-(+)- α -pompene (**1**) (syn. *ent*-(+)-isogymnomitrene), *ent*-(-)- β -pompene (**2**) (syn. *ent*-(-)-gymnomitrene), *ent*-(-)-cuparene (**3**), *ent*-(-)-bicyclogermacrene (**4**), and *ent*-(-)-ledrene (**7**) were isolated by a combination of column chromatography and preparative TLC in pure state, respectively. The two selinene isomers, *ent*-(+)- α -selinene (**5**) and *ent*-(-)- β -selinene (**6**), were obtained only by a preparative TLC using silica gel impregnated silver nitrate. From the polar fraction three sesquiterpene alcohols, *ent*-(-)-selin-11-en-4 α -ol (**8**), *ent*-(-)-spathulenol (**9**), and *ent*-(-)-aristolol-10 β -ol (**10**), were isolated by rechromatography through a silica gel column together with a diterpene alcohol phytol [8] and a new 9,10-dihydrophenanthrene derivative. The structure of the last compound was elucidated by X-ray analysis to be 3,4-dimethoxy-5-hydroxy-9,10-dihydrophenanthrene and the result will be reported elsewhere.

ent-(+)- α -Pompene (**1**) and *ent*-(-)- β -pompene (**2**) were identified to be the enantiomeric forms by coincidence of both the spectral data and the optical rotations (see Experimental Section) with those of the specimens obtained from other liverworts [9–12]. They are widely distributed in many liverworts, but are never isolated from other kinds of plants. These sesquiterpenoids are, therefore, one of the diagnostic components for showing chemotaxonomical position of the liverworts in plant kingdom. The structures including the absolute configurations of *ent*-(-)-cuparene (**3**) [13, 14] and *ent*-(-)-bicyclogermacrene (**4**) [4, 15] were presented

as the enantiomeric forms by determination of ^1H NMR, IR, and mass spectra as well as the optical rotations. The two bicyclic carbon skeletons are important precursors for converting into the tricyclic carbon skeletons such as gymnomitrane, aromadendrane, alloaromadendrane, and maaliane groups being common in the liverworts [5]. The identification of three selinane sesquiterpenoids, *ent*-(+)- α -selinene (5), *ent*-(—)- β -selinene (6) [16–18], and *ent*-(—)-selin-11-en-4 α -ol (8) [19], was performed by coincidence of the spectral data with those of the authentic compounds. The structures were also certified by determination of ^{13}C -NMR spectra [20] (see Experimental Section). The optical rotations of these selinene compounds, furthermore, indicated the stereostructures as the enantiomeric forms being the antipodes of those isolated from higher plants. The spectral data of the seventh hydrocarbon (7) exhibited the same properties as those of ledrene derived from bicyclogermacrene and ledrol [14]. The absolute configuration is not certain because of no report of the rotation value of the synthetic ledrene, but it is deduced to be the enantiomeric type (7) in relationship with the absolute configuration of *ent*-(—)-bicyclogermacrene (4) which is a possible precursor of the sesquiterpene ledrene. *ent*-(—)-Spathulenol (9) [21] was identified by superimposition of ^1H NMR and IR spectra to the reported ones, and the optical rotation showing an opposite sign to the value of normal stereostructure suggested the enantiomeric structure. The spectra of the last sesquiterpenoid were similar to those of *ent*-(+)-maalian-10 β -ol isolated from another liverwort *Plagiochila ovalifolia* [22], and the structure was tentatively proposed as *ent*-(+)-aristlan-10 β -ol (10) by analysis of the spectra features and by consideration of the biosynthetic sequence of all of

these sesquiterpenoids in this liverwort. However, the structure and absolute configuration of both compounds (7 and 10) should be certified in a future.

As described above, we isolated ten kinds of the enantiomeric sesquiterpenoids corresponding to the optical antipodes of those of higher plants from the thalloid liverwort, *R. jackii*. This result suggests the special position of the liverworts in plant kingdom, and furthermore supported that the enantiomeric sesquiterpenoids in the liverworts may be cyclized by action of the liverwort enzymes having some active sites in a contrary to the position of normal enzymes, *i.e.* the sesquiterpenoid synthetases of liverworts hold a reverse conformation to the sesquiterpenoid synthetic enzymes of higher plants.

Experimental

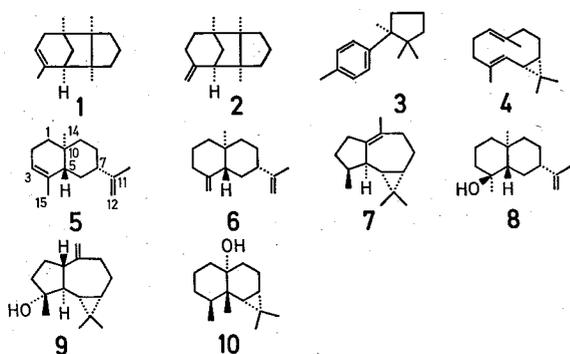
IR Spectra were recorded on a grating spectrometer for CCl_4 solutions and optical rotations were taken on an automatic polarimeter in CHCl_3 solutions. ^1H NMR Spectra were measured at 60 MHz and ^{13}C NMR at 22.63 MHz for CCl_4 solutions with TMS as the internal standard. Mass spectra were determined at 70 eV. For column chromatography Merk Kieselgel 60 was used and Merk Kieselgel PF₂₅₄ was used for TLC and preparative TLC. Analytical plates were visualized under UV light or were sprayed with 10% H_2SO_4 in EtOH and then heated at 100 °C for 10 min.

Material and its extraction

The liverwort, *Riccardia jackii*, was collected on granite rocks in a valley at the suburbs of Hiroshima city. The whole plant (1.7 kg), after washing with water and drying in the shade for several days, was digested with MeOH for a week at room temperature. The solvent was distilled off under reduced pressure to give a viscous brownish oil. A neutral part (18.2 g) was obtained by washing the extract with 5% NaOH solution for removing an acid part.

Isolation and identification of the constituents

The neutral part thus obtained was chromatographed through a silica gel-column to divide some fractions. The following compounds were isolated from the fractions by a further combination of column chromatography and preparative TLC using silica gel. For the selinene mixture the TLC plates impregnated with silver nitrate (10%) were used: *ent*-(+)- α -pompenone (1) (15 mg), *ent*-(—)- β -pompenone (2) (30 mg), *ent*-(—)-cuparene (3) (7 mg), *ent*-(—)-bicyclogermacrene (4) (35 mg), *ent*-(+)- α -selinene (5) (50 mg), *ent*-(—)- β -selinene (6) (160 mg), *ent*-(—)-



ledrene (7) (25 mg), *ent*-(—)-selin-11-en-4 α -ol (8) (70 mg), *ent*-(—)-spathulenol (9) (30 mg), *ent*-(+)-aristolol-10 β -ol (10) (60 mg), phytol (130 mg), and 3,4-dimethoxy-5-hydroxy-9,10-dihydrophenanthrene (150 mg). The physical and spectral properties of these compounds are listed below. *ent*-(+)-*a*-Pompene (1): C₁₅H₂₄ (M⁺ *m/z* 204, base peak *m/z* 96); [α]_D +45°; IR 1385, 1380, 835, 800 cm⁻¹; ¹H NMR 0.88, 0.93, 1.05 (each 3H, s), 1.69 (3H, br. s), 5.24 (1H, m). *ent*-(—)- β -Pompene (2): C₁₅H₂₄ (M⁺ *m/z* 204, base peak *m/z* 108); [α]_D -14°; IR 1645, 1387, 1375, 883 cm⁻¹; ¹H NMR 0.86, 0.91, 1.04 (each 3H, s), 4.53 (2H, br. s). *ent*-(—)-Cuparene (3): C₁₅H₂₂ (M⁺ *m/z* 202, base peak *m/z* 132); [α]_D -28°; IR 1520, 1385, 1375, 1365, 812 cm⁻¹; ¹H NMR 0.57, 1.08, 1.25, 2.29 (each 3H, s), 6.95, 7.12 (each 2H, d, *J* = 8.0). *ent*-(—)-Bicyclogermacrene (4): C₁₅H₂₄ (M⁺ *m/z* 204, base peak *m/z* 121); [α]_D -55°; IR 1650, 1385, 1370, 840, 820 cm⁻¹; ¹H NMR 0.4-0.9 (2H, complex), 1.03, 1.12 (each 3H, s), 1.46, 1.63 (each 3H, d, *J* = 1.5), 4.27, 4.73 (each 1H, m). *ent*-(+)-*a*-Selinene (5): C₁₅H₂₄ (M⁺ *m/z* 204, base peak *m/z* 41); [α]_D +15°; IR 1639, 1448, 1431, 891 cm⁻¹; ¹H NMR 0.80 (3H, s), 1.59 (3H, br. s), 1.72 (3H, d, *J* = 1.0), 4.65 (2H, br. s), 5.42 (1H, m); ¹³C NMR 15.7 (q: C-14), 20.9 (q: C-15), 21.0 (q: C-13), 23.0 (t: C-2), 26.9 (t: C-8), 29.0 (t: C-6), 32.4 (s: C-10), 38.1 (t: C-1), 40.4 (t: C-9), 46.9 (d: C-5), 46.9 (d: C-7), 108.3 (t: C-12), 121.0 (d: C-3), 135.2 (s: C-4), 150.8 (s: C-11). *ent*-(—)- β -Selinene (6): C₁₅H₂₄ (M⁺ *m/z* 204, base peak *m/z* 79); [α]_D -38°; IR 1649, 1443, 900 cm⁻¹; ¹H NMR 0.75 (3H, s), 1.95 (3H, d, *J* = 1.0), 4.45 (1H, br. s), 4.68 (1H, br. s), 4.68 (2H, br. s); ¹³C NMR 16.4 (q: C-14), 21.0 (q: C-13), 23.5 (t: C-2), 26.9 (t: C-8), 29.6 (t: C-6), 36.0 (s: C-10), 37.0 (t: C-3), 41.3 (t: C-1), 42.1 (t: C-9), 46.0 (d: C-7), 50.0 (d: C-5),

105.5 (t: C-15), 108.3 (t: C-12), 150.7 (s: C-4), 150.9 (s: C-11). *ent*-(—)-Ledrene (7): C₁₅H₂₄ (M⁺ *m/z* 204, base peak *m/z* 161); [α]_D -65°; IR 1440, 1128, 980, 958 cm⁻¹; ¹H NMR 0.5-0.75 (2H, complex), 0.94 (3H, d, *J* = 7.0), 1.00, 1.07 (each 3H, s), 1.57 (3H, br. s). *ent*-(+)-Selin-11-en-4 α -ol (8): C₁₅H₂₆O (M⁺ *m/z* 222, base peak *m/z* 67); [α]_D +26°; m.p. 93-94 °C; IR 3610, 3480, 1645, 890 cm⁻¹; ¹H NMR 0.90, 1.07 (each 3H, s), 1.75 (3H, d, *J* = 1.5), 4.70 (2H, br. s); ¹³C NMR 18.7 (q: C-14), 20.1 (t: C-2), 21.0 (q: C-13), 22.7 (q: C-15), 25.7 (t: C-8), 26.7 (t: C-6), 34.4 (s: C-10), 41.2 (t: C-1), 43.5 (t: C-3), 44.7 (t: C-9), 46.2 (d: C-7), 54.7 (d: C-5), 71.3 (s: C-4), 108.5 (t: C-12), 149.6 (s: C-11). *ent*-(—)-Spathulenol (9): C₁₅H₂₄O (M⁺ *m/z* 220, base peak *m/z* 43); [α]_D -22°; IR 3615, 3460, 1635, 890 cm⁻¹; ¹H NMR 0.3-0.9 (2H, complex), 1.07 (6H, s), 1.30 (3H, s), 4.70 (2H, br. s). *ent*-(+)-Aristolol-10 β -ol (10): C₁₅H₂₆O (M⁺ *m/z* 222, base peak *m/z* 161); [α]_D +6°; m.p. 87-88 °C; IR 3605, 3420, 1108, 987, 940, 893 cm⁻¹; ¹H NMR 0.3-0.7 (2H, complex), 0.92 (3H, d, *J* = 7.0), 0.99 (3H, s), 1.04 (6H, s). Phytol: C₂₀H₄₀O (M⁺ *m/z* 296); IR 3640, 3450, 1665, 1380, 1370 cm⁻¹; ¹H NMR 0.86 (12H, d, *J* = 7.0), 1.65 (3H, s), 4.04 (2H, d, *J* = 7.0), 5.33 (1H, t, *J* = 7.0). 3,4-Dimethoxy-5-hydroxy-9,10-dihydrophenanthrene: C₁₆H₁₆O₃ (M⁺ *m/z* 256); m.p. 125.5-126.5 °C; UV 218, 260, 267, 300 nm (ϵ 4.3 × 10⁴, 1.3 × 10⁴, 1.3 × 10⁴, 6.6 × 10³); IR 3430, 1605, 1581, 1485, 1263, 1128 cm⁻¹; ¹H NMR 2.65 (4H, s), 3.70, 3.85 (each 3H, s), 6.6-7.2 (5H, complex), 8.28 (1H, s).

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