Isolation of Seven Verrucosane Diterpenoids from the Liverwort *Scapania bolanderi*

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From the liverwort *Scapania bolanderi* eight kinds of diterpenoids have been isolated together with two kinds of sesquiterpenoids, and seven of them have been identified as the verruconsane and neoverrucosane diterpenoids with 3,6,6,5-tetracyclic carbon framework.

Introduction

Liverworts (Hepaticae) form a special group considered to be an early stage in the evolution of terrestrial plants, and produce terpenoids and/or aromatic compounds characteristic to the species as their major lipophilic constituents. During the last two decades many types of sesquiterpenoids have been obtained from the liverworts and most of the liverwort sesquiterpenoids are the enantiomeric forms corresponding to antipodes of those from higher plants [1–5]. However, few investigations have been conducted on diterpenoids of the liverworts: about twenty kinds of diterpenoids with acyclic [3, 6], labdane [7–9], pimarane [10], kaurane [11–15], clerodane [16], and dolabellane [17, 18] frameworks have been isolated from several liverworts. All of these carbon skeletons are known in other kinds of organisms. In general, the diterpenoids, distributed widely in plants, are classified by difference of the initial step in biogenetical cyclization of geranylgeranyl pyrophosphate: the first group is formed by a proton-initiated reaction at the double bond of the distal unit, in a manner similar to those for triterpenoids and steroids. Alternative path of diterpenoid biosynthesis has a similarity to that observed in sesquiterpenoids.

Recently, several diterpenoids consisting of the new carbon skeletons, sacculatane [19–21], verrucosane [22–25], and neoverrucosane [26], have been isolated from the liverworts. In continuing our study on the diterpenoid constituents of liverworts, using as the diagnostic components to chemotaxonomy of the liverworts, we here isolated eight kinds of diterpenoids including two new compounds from the leafy liverwort *Scapania bolanderi* Aust. belonging to the Scapaniaceae of the Jungermanniales. The structures of these diterpenoids were assigned on the basis of the following spectral evidence as (−)-2β-hydroxyverrucosane (**1**), (−)-5β-hydroxyneoverrucosane (**2**), (−)-2β,9α-dihydroxyverrucosane (**3**), (−)-2β-hydroxy-9-oxoverrucosane (**4**), (−)-9α-acetoxy-2β-hydroxyverrucosane (**5**), (−)-2β-acetoxy-11α-hydroxyverrucosane (**6**), and (−)-2β,9α-dihydroxyverrucos-13-ene (**7**), consisting of the tetracyclic verrucosane or neoverrucosane skeleton, except for (−)-methyl-13-hydroxycleroda-3,14-diene-18-carboxylate (**8**) [27].

Results and Discussion

The liverwort, *Scapania bolanderi,* was digested with methanol and a neutral portion was separated by usual manner from the extract. Retention times of the gas chromatogram suggested that it was mainly consisted of several kinds of diterpenoids as well as a few kinds of sesquiterpenoids (see Fig. 1). This was

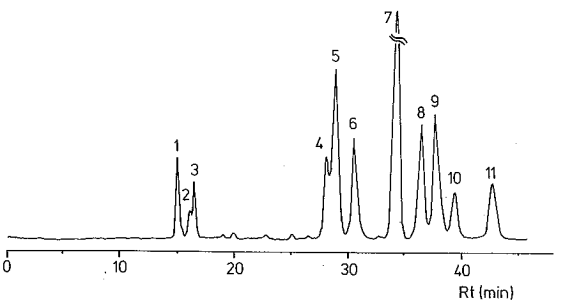

Fig. 1. Gas chromatogram of the neutral part from MeOH extract of *Scapania bolanderi,* Column: SE-30 (2% on Chromosorb AW), Programming at 3 °C/min from 130 °C to 270 °C.

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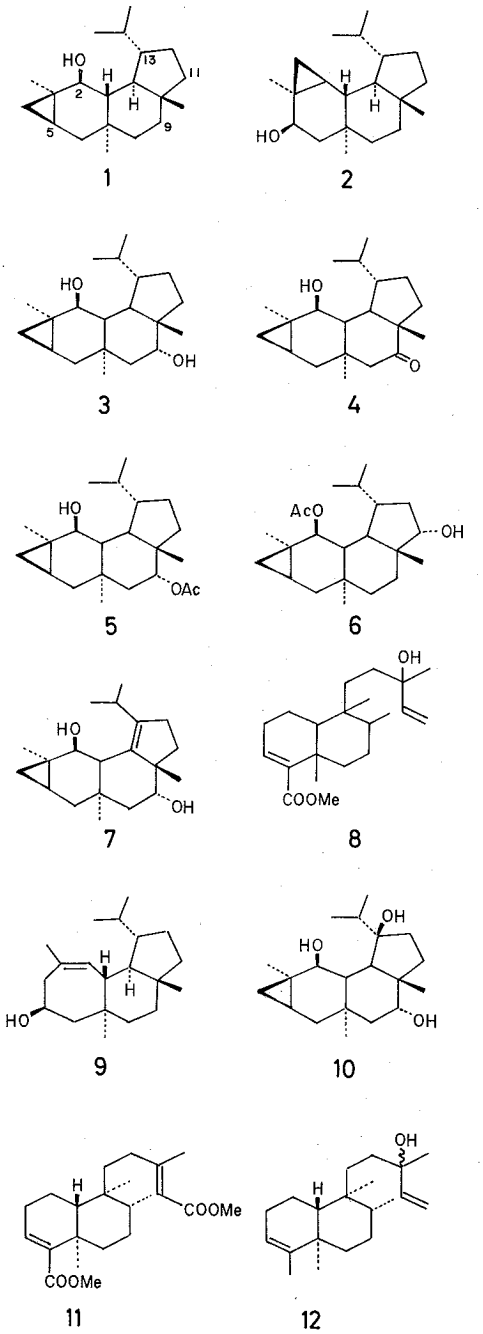
subjected to column chromatography and preparative TLC using silica gel to isolate each compound in pure state. Two sesquiterpenoids (peak 1 and 3) obtained from a less polar fraction were identified by spectroscopic evidence as β-gymnomitrene and cuparene [3], the most common sesquiterpenoids in liverworts. From a more polar fraction the two diterpene alcohols (peak 5 and 6) were first isolated. The spectroscopic properties of these two compounds revealed that they were tetracyclic diterpenoids having a cyclopropane ring as well as a secondary hydroxy group, an isopropyl group, and three tertiary methyls. These cyclopropyl alcohols were, respectively, converted by ring expansion reaction with acid into the known homoallylic alcohol, (+)-5β-hydroxyhomoverrucosane (**9**) [24, 26]. The structures and absolute configurations were confirmed by coincidence of both of the physical constants and spectroscopic data with those of (−)-2β-hydroxyverrucosane (**1**) and (−)-5β-hydroxyneoverrucosane (**2**), respectively [24, 26]. The peak 7 was constituted by two compounds which were isolated as the diol (**3**) and the keto-alcohol (**4**). On oxidation of the former (**3**) with Jones reagent the latter (**4**) was produced in a higher yield. Acetylation of the diol (**3**) gave the acetoxyalcohol (**5**) corresponding to peak 8 of the gas chromatogram. Physical constants and spectral data of these three compounds were, respectively, coincident with those of (−)-2β,9α-dihydroxyverrucosane (**3**), (−)-2β-hydroxy-9-oxoverrucosane (**4**), and (−)-9α-acetoxy-2β-hydroxyverrucosane (**5**) [24]. Another acetoxy-alcohol (peak 9) was identified by the same methods to be (−)-2β-acetoxy-11α-hydroxyverrucosane (**6**) which was one of the three known verrucosanoids with acetoxy-alcohol moiety [23–25].

We furthermore isolated a new diterpene diol (**7**) (peak 11) whose spectroscopic features resembled to those of the major diterpenoid 2β,9α-dihydroxyverrucosane (**3**). Its NMR spectrum showed the characteristic signals assigned to the two carbinyl protons of 2α (δ 3.82, d, J = 10.0 Hz) and 9β (δ 3.62, t, J = 2.5 Hz) on verrucosane skeleton. Since the molecular weight was two less than that of the diterpene diol (**3**) but no signals of vinyl protons appeared on the NMR spectrum, this compound was deduced to have a tetrasubstituted double bond on C_{13}–C_{14} or C_1–C_{14} of 2β,9α-dihydroxyverrucosane (**3**). The NMR spectrum gave the 2α-proton signal (δ 3.82, d, J = 10.0 Hz) coupling with 1β-hydrogen and the mass spectrum was very similar to that of the known



triol, 2β,9α,13β-trihydroxyverrucosane (**10**) [25]. Accordingly, the structure was proposed to be 2β,9α-dihydroxyverrucos-13-ene (**7**). The spectral features of the last compound (**8**) (peak 10) was different from those of verrucosanoids. The spectra suggested a clerodane structure containing an α,β-

unsaturated methyl ester, a vinyl group, a tertiary hydroxy, three tertiary methyls, and a secondary methyl. The structure of this minor diterpenoid was deduced to be methyl 13-hydroxycleroda-3,14-diene-18-carboxylate (**8**) by comparison of the spectral properties with those of dimethyl kolavate (**11**) and kolavelool (**12**) which had been isolated from the higher plant *Hardwicka pinnata* [28, 29].

The 3,6,6,5-tetracyclic verrucosane and neoverrucosane frameworks mentioned above might be biosynthesized by cyclization reaction of the 11,5-bicyclic dolabellane carbon skeleton, the diterpenoids with which had been obtained from not only liverworts [17, 18] but also marine invertebrates [30]. It is chemotaxonomically interesting that the major constituents of this liverwort are consisted of the diterpenoids, though many species of the genus *Scapania,* such as *S. aequiloba, S. ampliata, S. aspera, S. nemorea, S. parvitexta, S. stephanii, S. subalpina,* and *S. undulata,* contain mainly sesquiterpenoids [31, 32]. In addition, occurrence of the verrucosane diterpenoids is very rare in nature; they have been first isolated from the liverwort *Mylia verrucosa* and now, as the second case, have been isolated from the liverwort which is classified in a separate family from the first liverwort [33].

Experimental

Melting points are uncorrected. Optical rotations were taken on an automatic polarimeter in $CHCl_3$ solutions at room temperature. IR Spectra were recorded on a grating spectrometer for $CHCl_3$ solutions and NMR spectra were determined at 60 MHz for $CDCl_3$ solutions with TMS as the internal standard. Mass spectra were obtained at 70 eV. GLC was carried out on an apparatus with a flame-ionization detector in connection with a separation column (0.3×200 cm) packed with SE-30 (2%) on Chromosorb AW (60–80 mesh). For column chromatography Merk Kieselgel 60 was used and Merk Kieselgel PF_{254} 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, *Scapania bolanderi,* was collected in a forest at Ashiyasu-mura, Yamanashi-ken. The whole plant (550 g), after washing with water and drying in the shade for serveral days, was digested with MeOH for a week at room temperature. The solvent was distilled off under reduced pressure to afford a viscous brownish oil. A neutral part (3.1 g) was obtained by washing the extract with 5% NaOH solution for removing an acid part.

Isolation of the constituents

A gas chromatogram of the neutral part showed three peaks of sesquiterpenoids and eight peaks of diterpenoids. This neutral part was first chromatographed through a silica gel column to separate several fractions. By a further combination of column chromatography and preparative TLC using silica gel the following eight diterpenoids as well as two sesquiterpenoids were isolated from the fractions. The physical and spectral properties of these compounds isolated in pure state are listed below. (−)-2β-Hydroxyverrucosane (**1**) (peak 5): $C_{20}H_{34}O$;m.p. 76.5–78 °C; $[\alpha]_D$ −58°; IR 3610, 3050, 1380, 1370, 1035, 1005, 950 cm^{-1}; NMR δ 0.82 and 0.91 (each 3H, d, J = 7.0), 0.75, 0.89, and 1.22 (each 3H, s), 3.65 (1H, d, J = 9.5 Hz). (−)-5β-Hydroxyneoverrucosane (**2**) (peak 6): $C_{20}H_{34}O$; m.p. 174–175 °C; $[\alpha]_D$ −10°; IR 3270, 3060, 1380, 1373, 1045, 1022, 997 cm^{-1}; NMR δ 0.81 and 0.88 (each 3H, d, J = 5.5), 0.73, 0.82, and 1.17 (each 3H, s), 4.01 (1H, d.d, J = 7.0 and 4.5). (−)-2β,9α-Dihydroxyverrucosane (**3**) (peak 7): $C_{20}H_{34}O_2$; m.p. 153–154 °C; $[\alpha]_D$ −72°; IR 3525, 3400, 3060, 1385, 1375, 1170, 1030 cm^{-1}; NMR δ 0.83 and 0.90 (each 3H, d, J = 7.0), 0.77, 1.03, 1.20 (each 3H, s), 3.4–3.8 (2H, complex). (−)-2β-Hydroxy-9-oxoverrucosane (**4**) (peak 7); $C_{20}H_{32}O_2$; m.p. 111–112 °C; $[\alpha]_D$ −103°; IR 3540, 3075, 1695, 1382, 1372, 1410, 1038 cm^{-1}; NMR δ 0.81 and 0.91 (each 3H, d, J = 7.0), 0.88, 1.08, and 1.24 (each 3H, s), 1.88 and 2.48 (each 1H, d, J = 15.0), 3.67 (1H, d, J = 8.5). (−)-9α-Acetoxy-2β-hydroxyverrucosane (**5**) (peak 8); $C_{22}H_{36}O_3$; $[\alpha]_D$ −83°; IR 3600, 3525, 3060, 1735, 1390, 1380, 1250 cm^{-1}; NMR δ 0.86 and 0.91 (each 3H, d, J = 7.0), 0.87, 0.97, 1.22, and 2.00 (each 3H, s), 3.55 (1H, d, J = 10.0), 4.75 (1H, t, J = 3.0). (−)-2β-Acetoxy-11α-hydroxyverrucosane (**6**) (peak 9): $C_{22}H_{36}O_3$; m.p. 203–204 °C; $[\alpha]_D$ −103°; IR 3495, 3060, 1707, 1388, 1375, 1260, 1028 cm^{-1}; NMR δ 0.82 and 0.87 (each 3H, d, J = 6.0), 0.73, 0.95, 1.32, and 2.07 (each 3H, s), 3.61 (1H, d, J = 5.0), 4.95 (1H, d, J = 9.0). (−)-2β,9α-Dihydroxyverrucos-13-ene (**7**) (peak 11): $C_{20}H_{32}O_2$; m.p. 169–170 °C; $[\alpha]_D$ −26°; IR 3645, 3575, 3030, 1385, 1368, 1155, 1025 cm^{-1}; NMR δ 0.93 and 0.97 (each 3H, d, J = 7.0), 1.03, 1.03, 1.23 (each 3H, s), 3.62 (1H, t, J = 2.5), 3.82 (1H, d, J = 10.0); MS m/z 304 (M^+, 8%), 286(9), 279(10), 268(8), 261(71), 243 (59), 225(9), 201(12), 175(23), 149(37), 119(27), 109(36),

95(31), 81(39), 71(38), 55(41), 43(100). (−)-Methyl-13-hydroxycleroda-3,14-diene-18-carboxylate (**8**) (peak 10): $C_{21}H_{34}O_3$; $[\alpha]_D$ −4°; UV λ (EtOH) 214 nm (ε 6900); IR 3610, 3500, 1720, 1640, 1255, 995, 920 cm^{-1}; NMR δ 0.73 (3H, d, J = 6.0), 0.79, 1.20, 1.23, and 3.64 (each 3H, s), 4.97 (1H, d.d, J = 10.0 and 2.0), 5.11 (1H, d.d, J = 17.0 and 2.0), 5.83 (1H, d.d, J = 17.0 and 10.0), 6.40 (1H, t, J = 3.0); MS *m/z* 334 (M^+, 4%), 316(19), 303(8), 301(13), 286(14), 273(9), 264(10), 248(8), 245(12), 235(40), 219(12), 203(42), 175(28), 153(19), 139(52), 119(49), 107(44), 95(40), 81(43), 69(34), 55(46), 43(100). β-Gymnomitrene (peak 1): $C_{15}H_{24}$(M^+ *m/z* 204, base *m/z* 96); IR 1645, 1385, 1375, 885 cm^{-1}; NMR δ 0.86, 0.91, and 1.04 (each 3H, s), 4.53 (2H, br.s). Cuparene (peak 3): $C_{15}H_{22}$(M^+ *m/z* 202, base *m/z* 132); IR 1520, 1385, 1375, 1365, 812 cm^{-1}; NMR δ 0.57, 1.08, 1.25, and 2.29 (each 3H, s), 6.95 and 7.12 (each 2H, d, J = 8.0).

Acid treatment of (−)-2β-hydroxyverrucosane (**1**) *and (−)-5β-hydroxyneoverrucosane* (**2**)

The cyclopropyl alcohol (**1**) (20 mg) was dissolved in acetone (5 ml) and heated with 0.5 N H_2SO_4 (1 ml) under reflux for 4 h. The reaction mixture was treated in a usual way to produce the known (+)-5β-hydroxyhomoverrucosane (**9**), $C_{20}H_{34}O$; m.p. 152−153 °C; $[\alpha]_D$ +20°, which was identified by coincidence of the IR and NMR spectra with the reported values. By the same procedure another cyclopropyl alcohol (**2**) was converted into (+)-5β-hydroxyhomoverrucosane (**9**).

Jones oxidation of (−)-2α,9β-dihydroxyverrucosane (**3**)

To the diol (**3**) (15 mg) in acetone (2 ml) Jones reagent (0.5 ml) was added and the mixture was stored at 0 °C for 5 min. The reacted mixture was then poured into ice-water and the aqueous solution was extracted with ether. The mixture was worked up in the usual way to give a crude hydroxy-ketone which was recrystallized from a mixed solvent of hexane and ethyl acetate. The product, $C_{20}H_{32}O_2$; m.p. 111−112 °C; $[\alpha]_D$ −120°, was identical with the naturally occurring (−)-2β-hydroxy-9-oxoverrucosane (**4**) (IR and NMR spectra).

Acetylation of (−)-2β,9α-dihydroxyverrucosane (**3**)

Acetic anhydride (0.2 ml) was added to the diol (**3**) (15 mg) in pyridine (0.5 ml) and the mixture was set aside overnight at room temperature. The reacted mixture was then worked up in the usual way to afford a crude monoacetate which was purified by means of preparative TLC. The spectral data (IR and NMR spectra) of the product, $C_{22}H_{36}O_3$; $[\alpha]_D$ −75°, were coincided with those of (−)-9α-acetoxy-2β-hydroxyverrucosane (**5**) isolated from the liverwort.

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