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Sesqui- and diterpenoids from *Ptilidium ciliare* and *Barbilophozia* species (liverworts)

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Abstract

A new acorane-type sesquiterpenoid has been isolated from Finnish *Barbilophozia barbata*, together with one known dolabellane- and two known fusicoccane-type diterpenoids. *B. hatcheri* and *Ptilidium ciliare* afforded previously known barbatane-, daucane- and pinguisane-type sesquiterpenoids, as well as dolabellane- and fusicoccane-type diterpenoids. Their structures were identified by spectroscopic and X-ray crystallographic analyses.

Keywords: Liverworts; *Barbilophozia barbata*; *Barbilophozia hatcheri*; *Ptilidium ciliare*; Jungermanniales; Acorane-type; Pinguisane-type; Dolabellane-type; Fusicoccane-type; Sesqui- and diterpenoids

1. Introduction

Liverworts contain a broad rang of mono-, sesqui- and diterpenoids, and/or phenolic compounds (Asakawa, 1982, 1995). As a part of our systematic biochemical study of liverworts, we investigated the chemical constituents of three Finnish liverworts *Ptilidium ciliare*, *Barbilophozia hatcheri* and *Barbilophozia barbata*. In this paper, we report the isolation and structural determination of a new acorane-type sesquiterpene, named barbiacoradienone (**1**), and the distribution of the sesqui- (**2**–**7**) and diterpenoids (**8**–**10**).

2. Results and discussion

GC–MS analysis of the crude ether extract of *P. ciliare* indicated the presence of bicyclogermacrene (**2**) and β-caryophyllene (**3**) which had previously been isolated from *Conocephalum conicum* (Suire, Asakawa, Toyota, & Takemoto, 1982) and *Scapania undulata* (Anderson et al., 1977), respectively. This ether extract was chromatographed on Sephadex LH-20 and silica gel to give two previously known pinguisane-type sesquiterpenoids, deoxopinguisone (**4**) (Krutov, Samek, Benesova, & Herout, 1973) and pinguisanin (**5**) (Asakawa, Connolly, Fakunle, Rycroft, & Toyota, 1987). β-Barbatene (**6**) (Connolly, Harding, & Thornton, 1972) was detected by GC–MS analysis of the crude extracts of *B. hatcheri* and *B. barbata*. A known daucane-type sesquiterpenoid, hercinolactone (**7**) (Huneck, Cameron, Connolly, McLaren, & Rycroft, 1982) was isolated from *B. hatcheri*. A new acorane-type sesquiterpenoid barbiacoradienone (**1**) was isolated from *B. barbata*, along with three previously known diterpenoids, 10-deacetoxybarbilycopodin (**8**) (Huneck et al., 1986), barbifusicoccin A (**9**) (Tori, Nagai, & Asakawa, 1993) and barbifusicoccin B (**10**) (Tori et al., 1993).

The IR spectrum of compound **1** showed the presence of an acetoxyl (1740 cm^{-1}) and carbonyl group (1710 cm^{-1}). The molecular formula $C_{19}H_{26}O_5$ ($[M]^+$ m/z 334.1799) was determined by HR-EIMS. The ^1H NMR spectrum ((Table 1)) of **1** indicated the presence of a secondary methyl, three olefinic methyls, two acetoxy methyls and an olefinic proton. The ^{13}C NMR spectrum Table 1 displayed nineteen carbons and its

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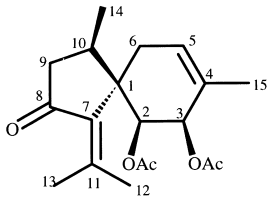

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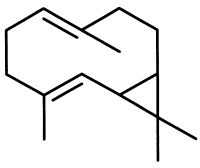

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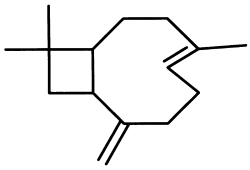

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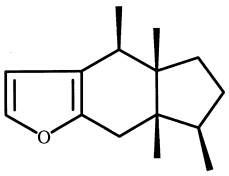

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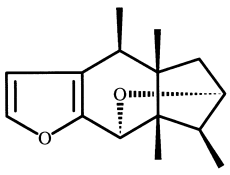

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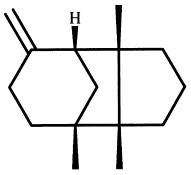

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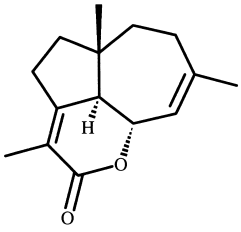

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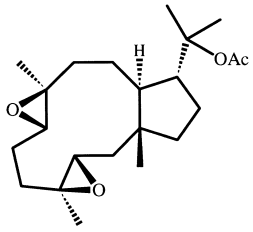

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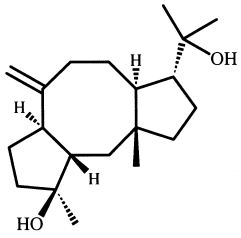

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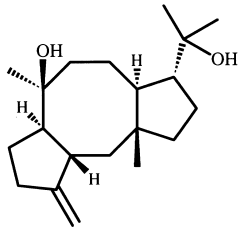

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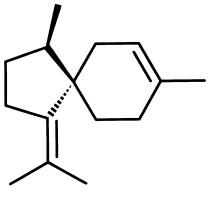

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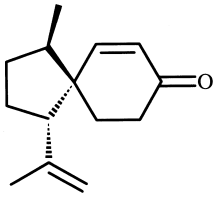

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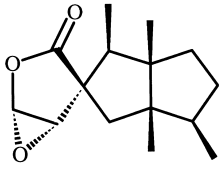

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DEPT spectrum showed the presence of a tri- and tetrasubstituted olefinic carbon and a ketone carbonyl carbon, together with six methyls, two methylenes, three methines and a quaternary aliphatic carbon. ^{13}C–^{1}H and ^{1}H–^{1}H COSY spectra revealed the presence of three partial structures [A]–[C] (Fig. 1). The connection of these partial structures could be obtained from the HMBC spectrum as shown in Fig. 1, suggesting that **1** is an acorane-type sesquiterpenoid. The NOESY spectrum showed NOEs between H-6 and H-5, H-2, H-12. However, the stereochemistry at C-10 could not be determined. The relative stereochemistry of **1** has been obtained from X-ray crystallographic analysis. Thus the structure of **1** was established as 2*R*,3*S*- or 2*S*,3*R*-diacetoxy-4,7(11)-acoradien-8-one.

Acorane-type sesquiterpenoids rarely occur in liverworts. Alaskene (**11**) has been found in *Barbilophozia attenuat* (Andersen, Ohta, Moore, & Tseng, 1978), *B.*

Table 1
The ^{13}C (100 MHz) and ^{1}H NMR (600 MHz) spectral data of **1**

	^{13}C	^{1}H
1	49.8	
2	72.5	5.71 1H, d, $J=4.4$ Hz
3	69.2	5.58 1H, br s
4	130.7	
5	124.9	5.62 1H, m
6	34.9	2.28 1H, ddd, $J=18.4$, 4.7, 2.5 Hz, α; 2.49 1H, dtd, $J=18.4$, 4.9, 1.4 Hz, β
7	134.3[a]	
8	206.2	
9	45.8	2.54 1H, dd, $J=17.3$, 7.4 Hz, α; 1.98 1H, dd, $J=17.3$, 4.4 Hz, β
10	35.2	2.37 1H, m
11	150.3[a]	
12	23.1	1.89 3H, s
13	24.4	2.25 3H, s
14	18.7	1.10 3H, d, $J=6.9$ Hz
15	19.5	1.69 3H, s
acetyl	21.0	1.99 3H, s
	170.1	2.09 3H, s
	21.1	
	169.7	

[a] May be interchanged.

barbata (Andersen, Costin, Kramer, Ohta, & Huneck, 1973) and *B. lycopodioides* (Andersen et al., 1978) and *Gymnocolea inflata* elaborates the nor-acorane-type sesquiterpene, inflatenone (**12**) (Tori et al., 1993). The presence of acoradienone (**1**) in *B. barbata* investigated in this study indicates that the acorane skeleton can serve as a significant chemical marker of the genus *Barbilophozia*.

Dolabellane- and fusicoccane-type diterpenoids are the most important chemical markers of the genus *Barbilophozia*. Previously, 10-deacetoxybarbilycopodin (**8**), and barbifusicoccins A (**9**) and B (**10**) were isolated from German B. barbata (Huneck & Overton, 1971; Huneck et al., 1986) and *B. floerkei* (Huneck & Overton, 1971; Tori et al., 1993), respectively.

Liverworts are rich sources of pinguisane-type sesquiterpenoids, which have not been found in other organisms. Pinguisanes are common constituents of *Porella* species (liverworts) (Asakawa, 1982, 1995). Deoxopinguisone (**4**) has been isolated from Czech *Ptilidium ciliare* (Krutov et al., 1973). French *P. pulcherrimum* also produces pinguisanes with pinguisanin (**5**) and pinguisanolide (**13**) as the major components (Asakawa, 1995; Asakawa, Matsuda, & Suire, 1981). Thus, the pinguisanes can be considered as chemical markers of the genus *Ptilidium*.

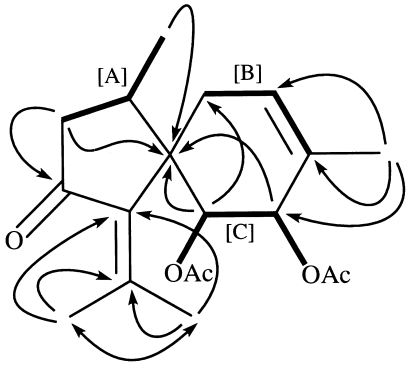

Fig. 1. ^{1}H–^{1}H (▬) and long-range ^{1}H–^{13}C (↘) of **1**.

3. Experimental

Melting points were uncorrected. ^{1}H NMR and ^{13}C NMR: TMS and $CHCl_3$ (δ 77.03 ppm) as int. standards. TLC spots were visualized under UV (254 nm) light and by spraying with Godin reagent (Godin, 1954) followed by heating.

3.1. Plant material

B. hatcheri (Evans) Loeske, *B. barbata* (Schreb.) Loeske and *P. ciliare* (L.) Hampe were collected by Y. A. in Turku, Finland, in June, 1996, and identified by Dr. S. Piippo. Voucher specimens were deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

3.2. Extraction and isolation

Dried ground *P. ciliare* (15 g) extracted with Et_2O for 1 month. The crude extract was filtered and evaporated *in vacuo* to give a residue (77 mg) which was divided into three fractions by column chromatog-

raphy on Sephadex LH-20 (CH_2Cl_2–MeOH 1:1). The presence of bicyclogermacrene (**2**) (Suire et al., 1982) and β-caryophyllene (**3**) (Anderson et al., 1977) was confirmed by GC–MS analysis of the crude extract. Chromatography on silica gel (*n*-hexane–EtOAc gradient) gave deoxopinguisone (**4**) (5 mg) (Krutov et al., 1973) and pinguisanin (**5**) (8 mg) (Asakawa et al., 1987).

GC–MS analysis of the crude extract showed the presence of β-barbatene (**6**) (Connolly et al., 1972). The crude extract (450 mg) of *B. hatcheri* (13 g) was treated in the same manner as described above to give three fractions. Hercinolactone (**7**) (15 mg) (Huneck et al., 1982) was isolated from fr. 2 using column chromatography on silica gel (*n*-hexane: EtOAc gradient).

The crude extract (620 mg) of *B. barbata* (5.8 g) was treated in the same manner as described above to yield fractions I–III. Fr II was rechromatographed on SiO_2 using *n*-hexane–EtOAc gradient to give 10 fractions. β-Barbatene (**6**) (Connolly et al., 1972) was detected by GC–MS analysis of frs. 1 and 2. Fr. 3 was rechromatographed on silica gel (CH_2Cl_2–EtOAc 4:1) and finally purified by prep. RP-18 TLC (CH_3CN) to give barbiacoratrienone (**1**) (12 mg). 10-Deacetoxybarbilycopodin (**11**) (12 mg) (Huneck et al., 1986), barbifusicoccin A (**12**) (1.5 mg) (Tori et al., 1993) and barbifusicoccin B (**13**) (1.5 mg) (Tori et al., 1993) were isolated from frs. 3 and 4 by MPLC (Si 60, *n*-hexane–EtOAc 4:1).

3.3. Barbiacoradienone (*1*)

mp. 144–147°; $[\alpha]_D$ −96.7° (*c* 3.18, $CHCl_3$); HR-EIMS: found 334.1799 $C_{19}H_{26}O_5$ requires 334.1781; CD: $\Delta\varepsilon_{249}$ −2.28, $\Delta\varepsilon_{209}$ −1.81 (*c* 0.34×10^{-3}, MeOH); UV λ_{max} nm (log ε): 250 (3.73) (*c* 0.34×10^{-3}, MeOH); FTIR ν_{max} cm^{-1}: 1740, 1710, 1240; ^1H and ^{13}C NMR: Table 1; EIMS *m*/*z* (rel. int.): 334 $[M]^+$ (3), 292(7), 274(24), 259(4), 232(72), 217(14), 208(27), 199(8), 177(5), 166(100), 135(14), 119(8), 105(5), 83(8), 69(5), 55(5), 43(27).

3.4. X-ray crystallographic analysis of *1*

Compound **1** was recrystallized from *n*-hexane. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer with Cu Kα radiation. The structure of **7** was solved by direct method using *CRYSTAN SIR92* and refined by full-matrix least-squares using *CRYSTAN*; $M_f = C_{19}H_{26}O_5$, $M_r = 334.00$, monoclinic, $P2_1$, $a = 12.158$ (3), $b = 8.951$ (2), $c = 9.039$ (2) Å, $\beta = 108.05$ (2)°, $V = 935.3$ (4) $Å^3$, $Z = 2$, $D_x = 1.185$ Mg m^{-3}, λ (CuKα) = 1.54178 Å, $\mu = 6.589$ mm^{-1}, Cell parameters from 20 reflections, $\theta = 1$–30°, absorption correction: DIFABS, $R_{int} = 0.024$, $\theta_{max} = 63.8°$, 1771 measured reflections, 1595 observed reflections, refinement on *F*, $R = 0.064$, $wR = 0.082$, $S = 1.373$, 1595 reflections, 216 parameters, Only coordinates of H atoms refined, $(\Delta/\sigma)_{max} = 0.3666$, $\Delta\rho_{max} = 0.25e$ $Å^{-3}$, $\Delta\rho_{min} = -0.29$ $Å^{-3}$, $w = \exp[3\sin^2\theta/\lambda]/[\sigma(F_0) + 0.003F_0^2]$.

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