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TERPENOIDS AND AROMATIC COMPOUNDS FROM SIX LIVERWORTS

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Key Word Index—*Porella platyphylla*; *Barbilophozia floerkei*; *B. lycopodioides*; *B. barbata*; *Riccardia multifida*; *Jungermannia comata*; liverwort; perrottetianal B; 3,4-dimethoxy-5-hydroxy-9,10-dihydrophenanthrene; sacculatane type; fusicoccane-type; dollabellane-type; sesquiterpenoid; diterpenoid; bis-bibenzyl; phenanthrene derivative.

Abstract—The structure of perrottetianal B, a sacculatane-type diterpene dialdehyde, which has previously been isolated from the liverwort *Porella perrottetiana* was revised to 15-hydroxyperrottetianal by the analysis of NMR spectra. The distribution of the known sesqui- and diterpenoids, as well as bis-bibenzyl derivatives, in Swiss liverworts *Barbilophozia lycopodioides*, *B. floerkei* and French *B. barbata* and Japanese *Jungermannia comata* is discussed. Perrottetin E, a cyclic bis-bibenzyl isolated from *Jungermannia comata* showed inhibitory activity for thrombin (IC_{50} 18 μM).

INTRODUCTION

Liverworts contain oil bodies which comprise mainly the mono-, sesqui- and diterpenoids and/or aromatic compounds [1–3]. As part of a chemosystematic study and search for biologically active substances of liverworts, we are studying their chemical constituents [1, 2]. Here we report the distribution of terpenoids and aromatic compounds of four European liverworts, *Porella platyphylla*, *Barbilophozia floerkei* and *B. lycopodioides* and *B. barbata*, and two Japanese liverworts, *Riccardia multifida* and *Jungermannia comata* and revision of the structure of perrottetianal B (**2**), previously isolated from Japanese liverwort *P. perrottetiana* [4].

RESULTS AND DISCUSSION

The ether extract of *P. platyphylla* was chromatographed on silica gel and Sephadex LH-20 to give perrottetianal B (**2**) [4], pinguisanin (**3**) [5], phytol and stigmasterol.

The GC-CI mass spectrum of **2** showed the quasimolecular ion peak $[M + H]^+$ at *m/z* 319. Its IR, ^{1}H and ^{13}C NMR spectra (Table 1) indicated the presence of a secondary hydroxyl (3550 cm^{-1}; δH 3.24 *dd*, $J = 8.3$ Hz, 4.2 Hz; δC 75.7 *t*) and two aldehyde groups (1730, 1675 cm^{-1}; δH 9.73, 10.22 each *s*; δC 189.6, 203.5 each *t*). Furthermore, the ^{1}H and ^{13}C NMR spectra showed four tertiary methyls, six methylenes, a methin, two quaternary carbons and a trisubstituted olefinic (δH 5.05 *t*, $J = 8.1$ Hz; δC 122.4 *d*, 135.3 *s*) and a tetrasubstituted olefinic (δC 139.0, 156.9 each *s*) carbons. These spectral data were completely identical with those of perrottetianal B (**1**), previously isolated from *P. perrottetiana* [4]. However, the complete assignments of the ^{1}H and ^{13}C NMR spectra have not yet been reported. Thus, the detailed analysis of ^{1}H–^{1}H COSY, ^{1}H Detected Single Quantum Coherence (HSQC) and HMBC spectra were carried out. The HMBC spectrum (Table 2) of **2** indicated that the methine proton (H-15) bearing the hydroxyl group was correlated with a methyl (C-14), a methylene (C-16), a methine (C-5) and a quaternary carbon (C-4) and further with a methine (C-17) on the trisubstituted olefinic carbon, respectively. The NOESY spectrum (Fig. 1) of **2** clearly showed the presence of NOE between H-15 and a trisubstituted olefinic proton at H-17. On the basis of the above spectral evidence, the secondary hydroxyl group was attached to C-15, but not to C-3. Thus, the former structure **1** of perrottetianal B should be revised to **2**. The absolute configuration of H-15 remained to be clarified.

Barbilophozia species has been analysed chemically and dollabellane- and fusicoccane-type diterpenoids have been found [6–8]. Further fractionation of the ether extract of *B. floerkei* collected in Switzerland resulted in the isolation of β-barfatene (**4**) [9], *ent*-spathulenol (**5**) [1], (1*S*, 7*R*)-germacra-4(15),5, 10(14)-trien-10-ol (**6a**) ($[\alpha]_D - 108.4°$) [10], 18-hydroxydollabell-7*E*-en-3-one (**8**) [6] and stigmasterol. The sign of optical rotation of compound **6a** was in agreement with that of the same compound isolated from brown alga [10]; compound **6b** ($[\alpha]_D + 146°$) has already been isolated from the

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Table 1. ^{13}C (50 MHz) and ^1H NMR (600 MHz) spectrum data of perrottetianal B (**2**) (in C_6D_6)

	^{13}C	^1H
1	31.8	0.92 *ddd*, J = 13.4, 13.4, 4.2 Hz, α 3.07 *br d*, β
2	18.9	1.50 *m*, α 1.72 *m*, β
3	30.5	1.43, *ddd*, J = 13.2, 13.2, 4.9 Hz, α 1.08 *br d*, β
4	39.8	
5	47.6	2.02–2.08 *m*
6	16.6	1.62 *m*, α 1.85 *m*, β
7	35.8	1.89–1.94 *m* 2.02–2.08 *m*
8	156.9	
9	139.0	
10	50.5	
11	189.8	9.73 *s*
12	19.0	1.51, 3H, *s*
13	203.5	10.22 *s*
14	18.8	0.58, 3H, *s*
15	75.7	3.24 *dd*, J = 8.3, 4.2 Hz
16	30.2	1.89–1.94, 2H, *m*
17	122.4	5.05 *t*, J = 8.1 Hz
18	135.3	
19	26.0	1.63, 3H, *s*
20	17.8	1.49, 3H, *s*

Table 2. The long-range C–H correlations of **2** by the HMBC spectrum

H	Correlated carbons
1	3, 5, 10
3	1, 5, 14
5	4, 10, 13
7	8, 9, 12
11	9, 10
12	7, 8, 9
13	1, 9, 10
14	3, 4, 5, 15
15	3, 4, 5, 14, 17
16	15, 17, 18
17	15, 16, 19, 20
19	17, 18, 20
20	17, 18, 20

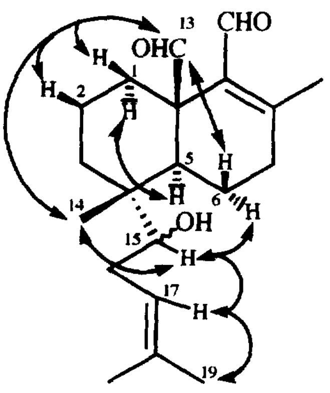


Fig. 1. NOEs of **2** observed by the NOESY spectrum.

liverwort *Jackiella javanica* [11]. The isolation of **6a** and its antipode **6b** is very interesting from the viewpoint of the biosynthesis of terpenoids in the liverworts. The ether extracts of Swiss *B. lycopodioides* and French *B. barbata* were also chromatographed on column chromatography using silica gel or Sephadex LH-20, respectively and 18-hydroxydollabell-7*E*-en-3-one (**8**) and barbifusicoccine A (**10**) [8] were isolated from the former species and a daucane-type sesquiterpenoid, hercinolactone (**7**) [12] and diterpenoids, 18-hydroxydollabell-7*E*-en-3-one (**8**) [10] and 10-deacetoxybarbilycopodin (**9**) [6] from latter species. The dollabellane- and fusicoccane-type diterpenoids have been isolated as main compounds from European *Barbilophozia* species [6–8] and the present three species contained the same type diterpenoids. Thus, the dollabellane- and fusicoccane-type diterpenoids might be the significant chemical markers of the Barbilophoziaceae.

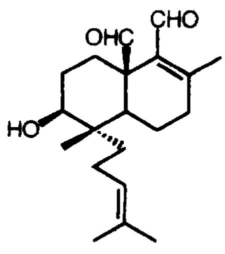
From the ether extract of Japanese *Riccardia multifida*, 3,4-dimethoxy-5-hydroxy-9,10-dihydrophenanthrene (**11**) was obtained together with two bis-bibenzyl compounds, riccardins A (**12**) and B (**13**) [13]. Compound **11** has been isolated from *Riccardiajackii* [14] but the ^1H and ^{13}CNMR data of **11** were not described in the reference. Therefore, the assignments of protons and carbons in **11** were carried out by the ^{13}C–^1HCOSY and HMBC experiments shown in Table 3. As the same compounds **12** and **13** have been isolated from the other Japanese *R. multifida* collected in a different location [15, 16]. *R. multifida* is chemically similar to *R. jackii* because both species produce the same phenanthorene **11**, although the bis-bibenzyl derivatives **12** and **13** have not been found in the species.

Perrottetin E (**14**) has been found in *Jungermannia comata* collected in Kagoshima, Japan, as a main product [8]. Further investigation of the same species from Tokushima, Japan, resulted in the isolation of perrottetin E (**14**) [17] as the major compound. This result suggests that *J. comata* is chemically quite different from the other *Jungermannia* species, which are composed of sesqui- and diterpenoids as major compounds [1, 3]. Perrottetin E (**14**) exhibited inhibitory activity for thrombin (IC_{50} = 18 μM), which is associated with blood coagulation [18].

EXPERIMENTAL

All mps uncorr. The solvents used for spectra were TMS-$CDCl_3$ (^1H and ^{13}C NMR), $CHCl_3$ ($[\alpha]_D$), MeOH (UV). TLC was carried out as previously reported [19].

Plant materials. Porella platyphylla (L.) Dum., *Barbilophozia floerkei* (Web. et Mohr) Loeske and *B. lycopodioides* (Wallr.) Loeske were collected in Champex, in Switzerland, in July, 1993 and identified by



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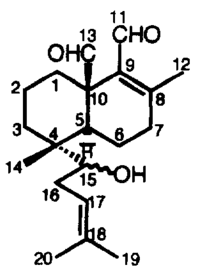

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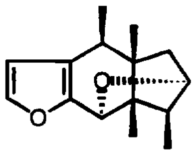

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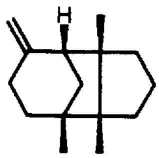

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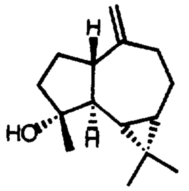

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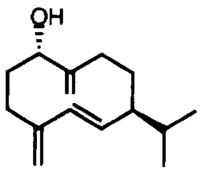

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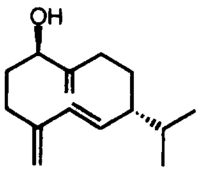

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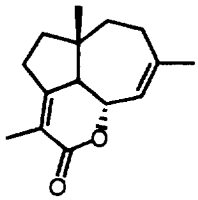

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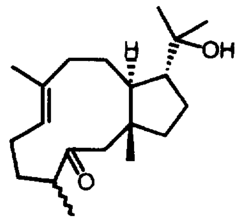

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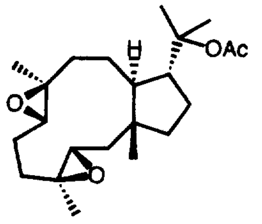

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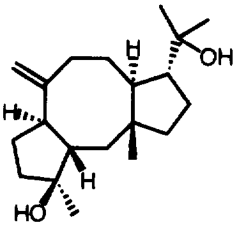

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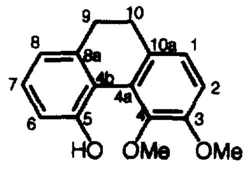
Dr L. Meinunger. *B. barbata* (Schreb.) Loeske were collected in France and identified by Dr R. Muse. *Riccardia multifida* (L.) S. Gray and *Jungermannia comata* Nees were collected in Tokushima, Japan, in August, 1994 and identified by Dr M. Mizutani. The voucher specimens were deposited at the Institute for Plant Biochemistry, Germany and the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation of Porella platyphylla. The ether extract (2.55 g) of *P. platyphylla* was divided into five fractions by CC on silica gel using a *n*-hexane–AcOEt gradient. Fr. 3 was rechromatographed on Sephadex LH-20 (CH_2Cl_2 : MeOH; 1 : 1) and silica gel (*n*-hexane–EtOAc gradient) to give pinguisanin (**3**) (303 mg) [5] whose spectral data were identical with those of authentic sample. Stigmasterol (43 mg) and phytol (6 mg) were isolated from fr. 4. Fr. 5 was rechromatographed on Sephadex LH-20 (CH_2Cl_2 : MeOH, 1 : 1 and MeOH), silica gel (*n*-hexane–AcOEt gradient) and finally purified by prep. HPLC (NUCLEOSIL 50-5; *n*-hexane: EtOAc, 1 : 1) to give perrottetianal B (**2**) (25 mg) [4]; oil; $[\alpha]_D + 274^\circ$ (c 2.20) (lit. [4] + 250°); GC-CIMS, 319 $[M + H]^+$; UV λ_{max} nm (log ε), 245 (4.09), 206 (3.93); ^1H and ^{13}C NMR (Table 1).

Barbilophozia floerkei. The ether extract (3.28 g) of *B. floerkei* was treated in the same manner as described above to give 13 fractions. Fr. 1 was rechromatographed on silica gel, followed by purification on prep. TLC (*n*-hexane-benzene; 95 : 5) impregnated with 5% $AgNO_3$ to give β-barbatene (**4**) (26 mg) [9, 20]. From fr. 8



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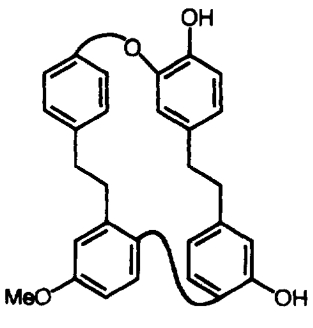

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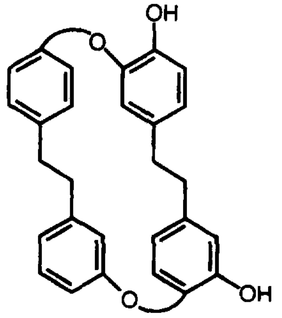

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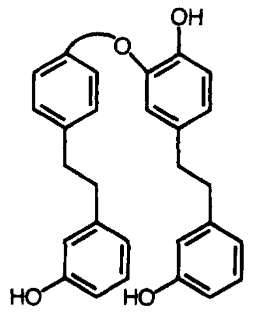

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Table 3. ^{13}C (50 MHz) and ^{1}H NMR (400 MHz) data of **12**

C	^{13}C	H	^{1}H
1	123.8	1	7.02 *d*, *J* = 8.3 Hz
2	110.4	2	6.82 *d*, *J* = 8.3 Hz
3	151.3	6	6.97 *dd*, *J* = 7.8, 1.0 Hz
4	143.7	7	7.18 *t*,-*J* = 7.8 Hz
4a	126.7	8	6.84 *dd*, *J* = 7.8, 1.0 Hz
4b	119.9	9	2.70 2H, *m*
5	154.1	10	2.63 2H, *m*
6	118.0	3-OMe	3.87 3H, *s*
7	128.9	4-OMe	3.69 3H, *s*
8	119.6		
8a	141.2		
9	31.3		
10	29.9		
10a	133.3		
3-OMe	55.8		
4-OMe	61.7		

ent-spathulenol (**5**) was isolated (23 mg) [20] by CC on Sephadex LH-20 ($CHCl_3$: MeOH, 1 : 1), silica gel (*n*-hexane : Et_2O, 85 : 15) and prep. HPLC (NUCLEOSIL 50-5; *n*-hexane : Et_2O, 4 : 1). Fr. 9 was rechromatographed on Sephadex LH-20, silica gel (*n*-hexane–AcOEt gradient) and then purified prep. HPLC (NUCLEOSIL 50-5; *n*-hexane-Et_2O; 1 : 1) to give 18-hydroxydollabell-7*E*-en-3-one (**7**) (10 mg) [6] and (1*S*,7*R*)-germacra-4(15),5,10(14)-trien-10-ol (**6**) (18 mg) [10], respectively. The spectral data of compounds **4**–**7** were identical to those of authentic samples and references [6, 10].

Barbilophozia lycopodioides. The ether extract (2.35 g) of *B. lycopodioides* was chromatographed on Sephadex LH-20 ($CHCl_3$: MeOH; 1 : 1) to give four fractions. Frs 2 and 3 were rechromatographed on silica gel, Sephadex LH-20 and prep. HPLC (NUCLEOSIL 50-5; CH_2Cl_2 : Et_2O, 4 : 1) to give 18-hydroxydollabell-7*E*-en-3-one (**7**) (35 mg) [6] and barbifusicoccine A (**8**) (12 mg) [8, 20]. These spectral data were identical to those of references [6, 8, 20].

Barbilophozia barbata. The ether extract (1.9 g) of *B. barbata* was chromatographed on silica gel using a *n*-hexane–AcOEt gradient to divide six fractions. Fr. 4 was rechromatographed on Sephadex LH-20 and silica gel (*n*-hexane : AcOEt, 4 : 1 and benzene : AcOEt, 95 : 5) to give 18-hydroxydollabell-7*E*-3-one (**8**) (11 mg) [6] and a sesquiterpene lactone-containing fraction which was recrystallized from *n*-hexane to produce hercinolactone (**7**) (155 mg) [12, 20]. The CC on silica gel (benzene : EtOAc, 95 : 5) and MPLC (benzene : EtOAc, 9 : 1) of fr. 5 provided 10-deacetoxybarbilycopodin (**9**) (40 mg) [6]. The spectral data of these compounds were identical to those of authentic samples [6, 12, 20].

Riccardia multifida. The ether extract (2.4 g) of *R. multifida* was chromatographed on silica gel using *n*-hexane–AcOEt gradient to give seven fractions. Fr. 3 was rechromatographed on Sephadex LH-20 (CH_3Cl_3–MeOH;

1 : 1) and then silica gel (CH_2Cl_2–Et_2O gradient) to give 3,4-dimethoxy-5-hydroxy-9,10-dihydrophenanthrene (**12**) (149 mg); Crystal; mp 74–76°; HREIMS, found. $[M]^+$ 256.1100; $C_{16}H_{16}O_3$ requires 256.1099; UV λ_{max} nm (log ε): 300 (3.84), 260 (4.11), 216 (4.57); ^1H and ^{13}C NMR, Table 3; EIMS *m/z* (rel. int.): 256 $[M]^+$ (100), 241 (13), 225 (7), 209 (40), 181 (19), 153 (9), 128 (6), 99 (2), 76 (4). Riccardin A (**9**) (107 mg) and B (**10**) (102 mg) [13] were isolated from rechromatography on Sephadex LH-20 (CH_3Cl_3 : MeOH, 1 : 1) and silica gel (*n*-hexane–Et_2O gradient) of fr. 5. These spectral data were identical to those of authentic spectral data [20].

Jungermannia comata. The ether extract (700 mg) of *Jungermannia comata* was repeatedly chromatographed on Sephadex LH-20 to afford perrottetin E (**11**) (405 mg) [11], the spectral data of which were identical to those of authentic sample.

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