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VOLATILE COMPONENTS AND PINGUISANE-TYPE SESQUITERPENOIDS FROM THE LIVERWORT *PORELLA CORDAEANA**

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Key Word Index—*Porella cordaeana*; *Jungermanniales*; Hepaticae; dimethyl sulphide; monoterpenoids; porellapinguisanolide; porellapinguisenone; spiropinguisanin; norpinguisone methyl ester; pinguisanin; striatenone; squalene; perrottetianal; pinguisane-type sesquiterpenoids; chemosystematics; flavour; mossy note.

Abstract—Three new pinguisane-type sesquiterpenoids, porellapinguisanolide, porellapinguisenone and spiropinguisanin have been isolated from the American liverwort *Porella cordaeana*, together with the previously known pinguisanin, norpinguisone methyl ester, striatenone and squalene and their structures established by extensive ^{1}H and ^{13}C NMR spectroscopy. *Porella cordaeana* belongs to pinguisane-type species of Porellaceae.

INTRODUCTION

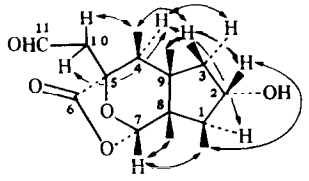
The *Porella* species of liverworts are rich sources of drimane, pinguisane, guaiane, aromadendrane and striatane-type sesquiterpenoids [1]. One of the American *Porella* species, *P. cordaeana* emits a potent, characteristic mossy odour when it is crushed. In the present paper, we wish to report the isolation and structures of three new pinguisane-type sesquiterpenoids named porellapinguisanolide (**1**), porellapinguisenone (**3**) and spiropinguisanin (**5**) and the volatile components from *P. cordaeana* and to discuss its chemosystematics.

*Part 31 in the series 'Chemosystematics of Bryophytes'. For part 30, see ref. [11].

RESULTS AND DISCUSSION

A combination of the silica gel and Sephadex LH-20 column chromatography of the methylene chloride extract of *Porella cordaeana* resulted in the isolation of three pinguisane-type sesquiterpenoids **1**, **3** and **5**, along with the known pinguisane-type sesquiterpenoids, pinguisanin (**7**) [2], norpinguisone methyl ester (**8**) [3], a striatane-type sesquiterpene, striatenone (**9**) [4], a sacculatane-type diterpene, perrottetianal A (**10**) [5], and squalene.

The molecular formula of **1** was determined as $C_{15}H_{22}O_5$ by its high resolution mass spectrum. The IR, ^{1}H (Table 1) and ^{13}C NMR spectra indicated the presence of a secondary hydroxyl group [3450 cm^{-1}; δ3.96 (*ddd*, J = 9.5, 4.8, 1.9); δ77.9, *d*], an aldehyde group [1730 cm^{-1}; δ9.68 (*d*, J = 3.7); δ197.5] and a lactone



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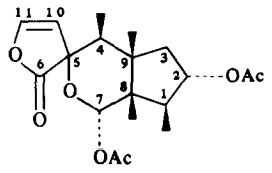

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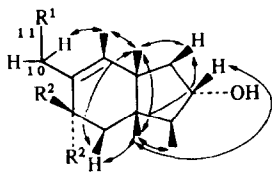
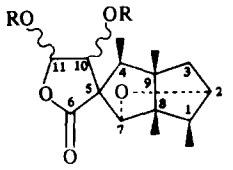


3 R^1 = CHO, R^2 = O
4 R^1 = CH_2OH, R^2 = H



5 R = H
6 R = Ac

NOEs ( ) observed by NOE difference spectra

Table 1 ^{1}H NMR spectral data of compounds* **1–4** and **6** (400 MHz, $CDCl_3$, TMS)

H	1	2	3	4	6
1	2.67 *dddd* (13.9, 7.0, 7.0, 7.0)	2.92 *ddd* (7.0, 7.0, 7.0)	1.97 *dddd* (13.9, 6.7, 6.7, 6.7)	1.85 *m*	2.08 *m*
2	3.96 *ddd* (13.9, 9.5, 4.8)	4.81 *ddd* (14.3, 9.5, 4.4)	3.83 *m*	3.81 *ddd* (11.7, 7.3, 4.4)	3.98 *m*
3α	1.66 *dd* (14.7, 4.8)	1.57 *dd* (15.0, 4.4)	2.18 *dd* (15.0, 4.4)	1.89 *m*	1.69 *d* (15.1)
β	1.88 *dd* (14.7, 9.5)	2.02 *dd* (15.0, 9.5)	2.05 *dd* (15.0, 8.1)	1.83 *m*[b]	2.05 *d* (15.1)
4	2.44 *q* (7.3)	2.23 *dddd* (14.2, 7.3, 7.3, 7.3)			2.45 *q* (7.7)
6				4.07 *m*	
7	5.48 *s*	5.48 *s*	2.37 *d* (1.5)	1.83 *m*[b]	3.96 *s*
				1.36 *dd* (13.2, 10.3)	
10	2.92 *d* (16.5)	5.70 *d* (12.8)	3.35 *d* (17.6)	2.41 *ddd* (14.7, 9.8, 5.4)	5.82 *s*
			3.70 *d* (17.6)	2.52 *ddd* (14.7, 5.0, 5.0)	
11	9.68 *dd* (3.7, 0.7)	7.54 *d* (12.8)	9.64 *s* .	3.73 *m*	6.29 *s*
C-1-Me	1.00 *d* (7.0)	0.96 *d* (7.3)	0.99 *d* (6.7)	0.98 *d* (8.3)	0.96 *d* (7.4)
C-4-Me	0.94 *d* (7.3)	0.97 *d* (7.0)	1.92 *s*	1.73 *d* (1.5)	1.15 *d* (7.7)
C-8-Me	0.90 *s*	0.93 *s*[a]	0.85 *s*	0.72 *s*	1.28 *s*
C-9-Me	0.95 *s*	0.95 *s*[a]	1.15 *s*	0.99 *s*	1.03 *s*
OAc		2.07			2.12 *s*
		2.17			2.17 *s*

*Figures in parentheses are coupling constants in Hz.

[a]May be interchanged.

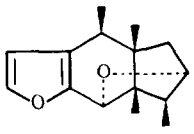
[b]Overlapped signal within the same column.

carbonyl group (1790 cm^{-1}; δ172.8), two tertiary methyls (δ0.95, 0.90) and two secondary methyl groups (δ0.95, 1.00) and a quarternary carbon bearing an oxygen atom (δ79.8) and an acetal group [δ5.48 (*s*); δ107.1]. The ^{13}C NMR spectrum further contained the signals of four methyls, two methylenes and two methine groups. On the basis of the above spectral data and the molecular formula, one of five oxygen atoms was confirmed to be the ether oxygen. Irradiation at δ2.67 (H-1) caused the doublet at δ1.00 (C-1 methyl) to collapse to a singlet and the disappearance of the coupling ($J = 13.9$ Hz) of δ3.96 (H-2). Irradiation of the double doublet signal at δ3.96 (H-2) caused collapse of the double double doublets at 1.66 (Ha-3) and 1.88 (Hb-3) to a doublet, respectively. From the above data, compound **1** had the partial structure –CH(Me)–CH(OH)–CH_2–. When the quartet signal at δ2.44 (H-4) was irradiated, the doublet signal at δ1.94 (C-4 methyl) was changed to a singlet, indicating that **1** had the partial structure –CH(Me)–. Irradiation at δ9.68 (H-11) caused the broad doublet at δ2.92 (Ha-10) to collapse to a sharp doublet and a double doublet at δ2.80 (Hb-10) became a doublet, respectively, suggesting **1** to have another partial structure –CH_2–CHO. The connectivity of the three partial structures and the remaining functional groups depicted in **1** were established by the difference NOE experiment. The NOEs were observed between (i) C-4 methyl and H-10, (ii) C-4 methyl and H-3, (iii) C-9 methyl and H-3, (iv) H-7 and C-8 methyl, (v) H-7 and C-1 methyl (vi) H-7 and H-1, H-4 and H-3, (vii) H-4 and H-1, (viii) H-4 and H-10, (ix) H-2 and H-3, (x) H-2 and C-1 methyl. Acetylation of **1** in acetic anhydride–pyridine gave a rearrangement lactone diacetate (**2**), $C_{19}H_{26}O_7$ (*m/z* 366.1663) [1790, 1755, 1730 cm^{-1}; δ2.07, 2.17 (each *s*, 3H)] in which the aldehyde group present in **1** disappeared. The diacetate contained an ethylenic double bond [δ5.70 (*d*, $J = 12.8$) and 7.54 (*d*, $J = 12.5$); δ109.8 and 138.6 (each *d*)], an acetal carbon (δ106.5) and a lactone carbonyl (δ167.6), and a quaternary carbon (δ79.8) and a methine bearing oxygen atom, whose assignments were confirmed by off-resonance measurements. The assignments of C-6 and C-7 were carried out by selective coupling and long-range selective decoupling of the ^{13}C NMR spectrum. From the above spectral data, the spirolactone which might be formed through the opening of the lactone ring of **1**, followed by relactonization between aldehyde and carboxylic acid in the presence of pyridine, was proposed for the diacetate. On the basis of the above chemical and spectral evidence, the stereostructure of porellapinguisanolide was established as **1**.

Porellapinguisenone (**3**), had the molecular formula $C_{15}H_{22}O_3$ (*m/z* 251.1594, CIHRMS) indicating five degrees of unsaturation. The IR, ^1H NMR (Table 1) and ^{13}C NMR spectra indicated the presence of a secondary hydroxyl group (3450 cm^{-1}; δ3.83; δ78.2 *d*), an aldehyde group (1720 cm^{-1}; δ9.64; δ199.1), an α,β-unsaturated carbonyl group (1655 cm^{-1}; δ196.1), a vinylic methyl (δ1.92) and a tetrasubstituted double bond (δ126.9, 164.8) and two tertiary methyl groups (δ0.85, 1.15) and one secondary methyl group (δ0.99) and an AB-type proton (δ3.35, 3.70). The ^{13}C NMR spectrum further contained signals of three methylenes, two quaternary carbons and a methine carbon. The above spectral data showed that **3** was a bicyclic sesquiterpene. The ^1H NMR spectrum of **3** was similar to that of **1** and the previously reported porellapinguisenol [4], indicating that **3** was a pinguisane-type sesquiterpenoid. Irradiation of the multiplet at δ3.83 (H-2) caused the double doublet signals at δ2.05 and 2.18 (H-3) to collapse to a doublet ($J = 15$ Hz) and the quadruple doublet signal at δ1.97 (H-1) to collapse to a quartet. Irradiation at δ1.97 (H-1) caused the doublet signal at δ0.99 (C-1 methyl) to collapse to a singlet and the multiplet signal at δ3.83 (H-2) was simplified. The above irradiation results indicated the presence of the same partial structure as **1**. The location of the other functional groups were determined by the difference NOE experiment. The NOEs were observed between (i) vinyl methyl and C-9 methyl, (ii) vinyl methyl and H-10, (iii) C-1 methyl and C-9 methyl, (iv) H-3 and C-9 methyl (v) H-7 and C-9 methyl, (vi) C-8 methyl and C-9 methyl, (vii) C-1 methyl and C-8 methyl, (viii) H-2 and C-8 methyl, H-7 and C-8 methyl. Reduction of **3** with lithium aluminium hydride gave an allyl alcohol (**4**) (δ3.81, 3.68–3.78, 4.07; δ61.2, 66.9 and 78.1), confirming the presence of a ketone at C-6 and the aldehyde group at C-11. The above evidence was also supported by the decoupling experiment of **4**. Thus, the structure of porellapinguisenone was depicted as **3**.

The molecular formula, $C_{15}H_{22}O_5$, for **5** was determined from its high resolution mass spectrum. The presence of a hydroxyl and γ-lactone ring was confirmed by the IR and ^{13}C NMR spectra (3450, 1750 cm^{-1}; δ176.2). The ^{13}C NMR spectrum indicated the signals due to four carbons bearing oxygen atoms one of which was assigned to be an acetal carbon (δ102.5). Compound **5** was acetylated by acetic anhydride and pyridine to give a diacetate (**6**) indicating that **5** possessed two hydroxyl groups. The ^1H NMR spectrum (Table 1) contained the signals of two secondary acetoxyl groups (δ2.12, 2.17, each 3H; 5.82 and 6.29, each 1H), two tertiary methyls and two secondary methyls. On the above spectral and chemical evidence, as well as the molecular formula, one of the five oxygen atoms was suggested to be the ether. This assumption was confirmed by the presence in the ^1H NMR spectrum of **6** of signals at δ3.96 and 3.98. The signal pattern of the ^1H NMR spectrum of **6** was quite similar to that of pinguisanin except for the absence of a furane-ring indicating that **5** had the same partial structure C-1 to C-7 of pinguisanin. This was further supported by the double resonance experiments. Irradiation of the multiplet at δ3.98 (H-2) caused the multiplets at δ1.69 and 2.05 (H-3) to collapse to an AB doublet, and the multiplet at δ2.05 (H-1) to give a quartet. Irradiation of the multiplet at δ2.05 (H-1), caused the doublet signal at δ0.96 (C1-Me) and multiplet at δ3.98 (H-2) to collapse to a singlet and double doublet, respectively. the remaining partial structure thus possessed the γ-lactone and two acetoxyl groups, one of which was placed at the hemiacetal carbon (δ6.29; δ102.5). The two partial structures were connected to give the structure for spiropinguisanin. The absolute configuration of the lactone moiety remained to be clarified. A similar compound, ptycantanolide (**11**), has been isolated from the liverwort *Ptychantus striatus*, belonging to Lejeuneaceae [1].

Porella species are divided into two major chemotypes, pungent and non-pungent. The former type produces a drimane-type sesquiterpene dial, polygodial [1] and its related compounds together with pinguisane- and norpinguisanes; the latter type elaborates pinguisane-type sesquiterpenoids [1, 7–9]. The present species belongs to the latter chemotype. *P. cordaeana* is chemically close to *P. navicularis*, but the former contained more highly oxidized pinguisane-type sesquiterpenoids than those isolated from *P. navicularis* [4]. *Porella cordaeana* emits a



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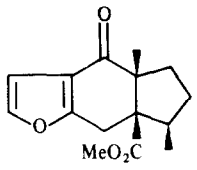

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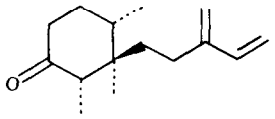

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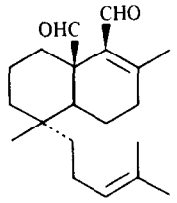

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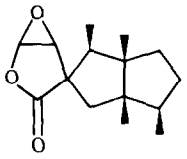

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strong mossy odour. Dimethyl sulphide, the monoterpene hydrocarbons α-thujene, α- and β-pinene, camphene, selinene, β-sabinene, myrcene, γ-terpinene as well as 2-methylbutanol, were detected in the head space gas of fresh *P. cordaeana* by GC-MS. The presence of dimethyl sulphide was also confirmed by the FPD detector used for GC. It is suggested that the potent mossy odour of *P. cordaeana* is due to the mixture of these components. Dimethyl sulphide is one of the important flavours of seaweed. This is the first report of the detection of dimethyl sulphide in a bryophyte.

EXPERIMENTAL

The solvents used for spectral measurements were TMS–$CDCl_3$ [^{1}H NMR (400 MHz); ^{13}C NMR (100 MHz)]; $CHCl_3$ (IR; $[\alpha]_D$) and MeOH (CD). TLC, GC and GC-MS were carried out as previously described [10].

Plant material. Porella cordaeana (Hüb.) Moore was collected in Oregon State, U.S.A. in 1985 and identified by Prof. W. C. Schofield. The voucher specimen was deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

Head space gas analysis. Head space gas obtained from fresh *P. cordaeana* (1 g) at 90° for 30 min was analysed by capillary GC [column: DB-1 (MEGABORE), 0.53 mm × 30 m; column temp. 50° (5 min hold)–240° temp. programme at 3°/min; carrier gas: He, 6.0 psi; detector and detector temp. FID or FPD at 280°; inject temp. 250°]. Capillary GC-MS: 70 eV; GC condition, the same condition as described above.

Extraction and isolation. Porella cordaeana was dried for 3 days and mechanically ground and extracted with CH_2Cl_2 for one month. The CH_2Cl_2 extract was filtered and removal of the solvent gave a green oil (9 g). The crude extract was divided into 12 fractions by CC on silica gel using an *n*-hexane – EtOAc gradient. A combination of chromatography on Sephadex LH-20 ($CHCl_3$–MeOH 1:1) and silica gel (*n*-hexane–EtOAc and $CHCl_3$) of fr. 6 (2 g) gave **10** (15 mg), **8** (10 mg), **9** (12 mg), **1** (28 mg); **1**: $[\alpha]_D$ +55.5° (*c* 1.08); IR ν_{max} cm^{-1}: 3530, 1790, 1730; CIHRMS: Found: $[M+H]^+$ 283.1534; $C_{15}H_{23}O_5$ requires 283.1545; ^{1}H NMR: Table 1; ^{13}C NMR; δ9.8, 12.0, 14.5, 19.1 (each *q*), 45.2, 49.0 (each *t*), 42.1, 45.9 (each *d*), 77.8 (CH–O), 107.1 (O–CH–O), 197.5 (–CHO), 45.8, 47.8 (each *s*), 79.8 (–C–O), 172.9 (C=O); EIMS (rel. int.): *m/z* 282 $[M]^+$ (1), 264 (6), 236 (7), 208 (9), 190 (27), 175 (8), 165 (40), 136 (45), 121 (53), 109 (69), 95 (21), 83 (15), 67 (17), 55 (41), 42 (100); Fr. 10 (875 mg) was rechromatographed on Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to give an unidentified perrottetianal-type diterpene dialdehyde (18 mg). Fr. 11 (950 mg) was rechromatographed on Sephadex LH-20 to give pinguisanin (**7**) (15 mg). A combination chromatography of Sephadex LH-20 and silica gel ($CHCl_3$) of fr. 12 (2.5 g) gave **5** (19 mg). Furthermore, the fraction including sesquiterpenes was purified by prep. TLC (C_6H_6–EtOAc 1:1) to give porellapinguisenone (**3**) (3 mg): **3**; IR ν_{max} cm^{-1}: 3450, 1720, 1655; CIHRMS: Found: $[M+H]^+$ 251.1594; $C_{15}H_{23}O_3$ requires 251.1647; ^{1}H NMR; Table 1; ^{13}C NMR; δ12.7, 18.7, 18.9, 20.4 (each *q*), 41.0, 43.0, 45.2 (each *t*), 49.2, (*d*), 78.2 (CH-O), 199.1 (–CHO), 46.7, 51.0 (each *s*), 126.9, 164.8 (each C=C), 196.4 (C=O); EIMS (rel. int.): *m/z* 232 $[M-H_2O]^+$ (3), 222 (100), 204 (37), 189 (75), 161 (32), 149 (76), 135 (40), 122 (54), 109 (34), 97 (41), 83 (38), 79 (28), 67 (27); **5**; amorphous; IR ν_{max} cm^{-1}: 3450, 1755, 1750; HRMS: Found: $[M]^+$ 282.1478; $C_{15}H_{22}O_5$ requires 282.1467; ^{13}C NMR (pyridine-d_5); δ10.3, 12.1, 17.0, 21.3, 45.8, 47.7, 48.9, 51.5, 59.3, 63.1, 77.4, 78.6, 92.1, 102.5, 176.2; EIMS (rel. int.): *m/z* 282 $[M]^+$ (13), 264 $[M-H_2O]^+$ (17), 236 (8), 208 (35), 190 (8), 164 (18), 149 (24), 137 (34), 121 (21), 109 (100), 97 (71), 91 (32), 77 (25), 67 (43), 54 (62).

Acetylation of porellapinguisanolide (**1**). Compound **1** (21 mg) was dissolved in Ac_2O (1 ml) and pyridine (1 ml) and the mixture was kept at room temp. overnight. After work-up as usual it gave a diacetate (**2**) (8 mg); IR ν_{max} cm^{-1}; 1790, 1755, 1730, 1220, 1010: HRMS: Found: $[M]^+$ 366.1663; $C_{19}H_{26}O_7$ requires 366.1679; ^{1}H NMR: Table 1; ^{13}C NMR: δ10.2, 12.0, 14.3, 18.9, 20.6, 21.2, 42.8, 45.1, 46.0, 46.3, 47.2, 79.8, 106.8, 109.0, 138.6, 167.6, 171.2, 171.9: EIMS (rel.int.); *m/z* 366 $[M]^+$ (1), 322 (5), 279 (10), 225 (12), 170 (90), 128 (100), 110 (18), 94 (12), 82 (7), 72 (34).

Reduction of porellapinguisenone (**3**). To a suspension of $LiAlH_4$ (20 mg) in Et_2O was added **3** (77 mg) in Et_2O and the mixture stirred at 0° for 1 hr. The resulting mixture, after work-up as usual, was chromatographed on Sephadex LH-20 and rechromatographed on a Lobar column ($CHCl_3$–MeOH 9:1) to give a triol (**4**) (10 mg); ^{1}H NMR; Table 1; ^{13}C NMR; δ12.4, 16.3, 19.5, 22.0, 31.7, 37.5, 44.3, 45.3, 46.5, 49.1, 61.2, 66.9, 78.1, 129.2, 139.1.

Acetylation of spiropinguisanin (**5**). Compound **5** (16 mg) was dissolved in Ac_2O (0.1 ml) and pyridine (0.1 ml) and the mixture was kept at room temp., after work-up as usual it gave a diacetate (**6**) (15 mg); amorphous; ^{1}H NMR: Table 1.

Acknowledgements—We thank Prof. W. C. Schofield (Department of Botany, University of British Columbia) for the identification of the plant material.

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