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TERPENOIDS AND BIBENZYLS FROM SOME NEW ZEALAND LIVERWORTS*

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Key Word Index—*Marchantia berteroana*; *M. foliacea*; *Plagiochila stephensoniana*; *Porella elegantula*; Marchantiales; Jungermanniales; Hepaticae; terpenoids; 2-hydroxycuparene; perrottetianal; bibenzyls; 4-hydroxy-3′-methoxybibenzyl; chemosystematics.

Abstract—Four liverworts, *Marchantia berteroana*, *M. foliacea*, *Plagiochila stephensoniana* and *Porella elegantula* collected in New Zealand were chemically investigated. *M. berteroana* contains cuparene and (−)-2-hydroxycuparene as the major components. γ-Cadinene is the major component of *M. foliacea*. 4-Hydroxy-3′-methoxybibenzyl is a chemical marker for *P. stephensoniana* which belongs to the non-pungent type of *Plagiochila* species. *P. elegantula* synthesizes perrottetianal and belongs to the non-pungent type of *Porella* species.

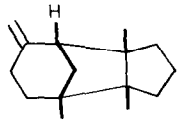
INTRODUCTION

Most liverworts contain terpenoids and/or lipophilic aromatic compounds as the major components. In previous papers [1–15] we reported that terpenoids and aromatic compounds are important endogeneous characteristics of species and can be used as chemosystematic indicators. In this communication, we wish to report the distribution of terpenoids and bibenzyl derivatives in the previously unreported four liverworts, *Marchantia berteroana*, *M. foliacea*, *Plagiochila stephensoniana* and *Porella elegantula* collected in New Zealand.

*Part 13 in the series "Chemosystematics of Bryophytes". For Part 12, see ref. [12].

RESULTS AND DISCUSSION

Air-dried and ground materials of each species were extracted with diethyl ether. The crude extracts were examined by TLC and GLC, and then analysed by GC/MS. The major components were isolated by prep. TLC and their structures confirmed by spectral evidence or synthesis. The mono- and sesquiterpene hydrocarbons and the other minor components were identified by GC-coinjection of standard compounds and direct comparison of mass spectra with those of the authentic samples. Table 1 summarizes the chemical components detected in each species. The major components of *M. berteroana* belonging to the Marchantiales are cuparene (**3**) and (−)-2-hydroxycuparene (= δ-cuparenol) (**4**) which have been isolated from *M. polymorpha* L. as the major sesquiterpenoids [1, 7, 16]. Lunularin (**9**) which has also



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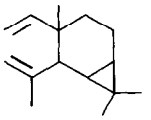

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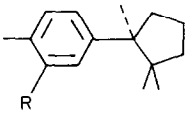

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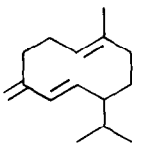
4 R = OH



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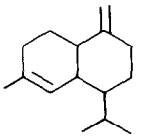

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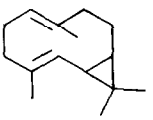

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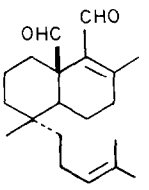

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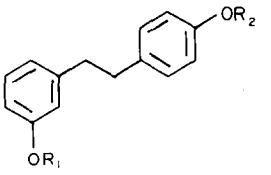

9 $R_1 = R_2 = H$
10 $R_1 = R_2 = Me$
11 $R_1 = Me, R_2 = H$
12 $R_1 = H, R_2 = Me$
13 $R_1 = Me, R_2 = Ac$

Table 1. Terpenoids and aromatic compounds detected in *Marchantia berteroana* and *M. foliacea*

M. berteroana	Content*	*M. foliacea*	Content*
β-Barbatene (**1**)	+	α-Cubebene	+
Bicycloelemene (**2**)	+	α-Copaene	+
β-Caryophyllene	+	γ-Muurolene	+
Cuparene (**3**)	+ + + + + + +	Germacrene-D (**5**)	+
M^+ ? (69†)	+ +	M^+ 204 (161)	+
(−)-2-Hydroxy-	+ + + + + + +	γ-Cadinene (**6**)	+ + + + +
cuparene (**4**)		M^+ 204 (165)	+ + + + +
M^+ 272 (191)	+	Calamenene	+
M^+ 272 (191)	+	M^+ 222 (43)	+
M^+ 272 (191)	+	Phytol	+ + + + +
M^+ 272 (95)	+ +	M^+ 308 (265)	+ +
Lunularin (**9**)	+ +	M^+ 310 (55)	+ + +
M^+ 290 (209)	+ + +	M^+ 318 (110)	+ + + + + + +
		M^+ 320 (43)	+
		M^+ 362 (205)	+
		M^+ ? (205)	+
		M^+ 396 (363)	+
Campesterol	+ +	Campesterol	+ + + + +
Stigmasterol	+ +	Stigmasterol	+ + + + +
Sitosterol	+ +	Sitosterol	+

*The symbols +, ++, + + + etc. are relative concentrations estimated by GC.
†Base peak of mass spectrum.

been isolated from *M. polymorpha* [16, 17] was detected in *M. berteroana* as a minor component. *M. foliacea* produces γ-cadinene (**6**), phytol and two unidentified compounds (M^+ 204 and M^+ 318) as main components. Except for the presence of phytosterols, there is no chemical affinity between *M. berteroana* and *M. foliacea*, and the former species is chemically similar to *M. polymorpha*. Recently, we reported that *M. berteroana* was closely related to *M. polymorpha*, but not to *M. foliacea*, by comparative flavonoid chemistry [18]. The present results further support the chemosystematics of the above three *Marchantia* species.

Plagiochila and *Porella* species belonging to the Jungermanniales synthesize various sesquiterpenoids. *Plagiochila* species can be divided into two types: those containing pungent or non-pungent 2,3-secoaromadendrane-type sesquiterpenoids (Type A) and those containing no secoaromadendrane-type sesquiterpenoids (Type B) [6]. The GC of the crude extract of *P. stephensoniana* was quite simple. The major component is a new bibenzyl (**11**), $C_{15}H_{16}O_2$ (M^+ 228.1151; calc. 228.1150). The IR and UV spectra showed the presence of a hydroxyl group (3400 cm^{-1}) and an aromatic ring (1600, 1510 cm^{-1}; λ_{max} 274.5, 280.5 nm). The ^1H NMR spectrum (see Experimental) contained the signals of a methoxyl group, two benzylic methylenes, four protons on a *p*-substituted benzene ring and four protons on a *m*-substituted benzene ring. Methylation of **11** gave a dimethyl ether (**10**), $C_{16}H_{18}O_2$ (M^+ 242) whose mass spectrum and chromatographic behavior were identical to the natural bibenzyl detected in *P. stephensoniana* as a minor component. The above chemical and spectral evidence coupled with the molecular formula and the intense fragment ions at *m/z* 121 (62%) and *m/z* 107 (85%) showed that the new bibenzyl might be 4-hydroxy-3′-methoxybibenzyl (**11**) or 3-hydroxy-4′-methoxybibenzyl (**12**). The structures **12** and **10** for the natural bibenzyls were confirmed by the synthesis of **10**–**12**. Wittig condensation of *m*-methoxybenzyltriphenylphosphonium bromide (**14**) with *p*-benzyloxybenzaldehyde (**15**) prepared from *p*-hydroxybenzaldehyde gave stilbene mixtures (**16**) which were hydrogenated in the presence of Pd–C to afford a dihydrostilbene whose spectral data and chromatographic behavior were identical to those of the natural bibenzyl (**11**). The alternative bibenzyl (**12**) was also synthesized by Wittig reaction of *p*-methoxybenzyltriphenylphosphonium bromide (**17**) with *m*-benzyloxybenzaldehyde (**18**), followed by hydrogenation. The spectral data and chromatographic behavior of **12** were not identical to those of the major bibenzyl isolated from *P. stephensoniana*. Methylation of **11** and **12** gave the same dimethyl ether (**10**), identical to the natural bibenzyl (**10**) in all respects. The bibenzyl (**10**) was also synthesized by Wittig reaction of *p*-methoxybenzyltriphenylphosphonium bromide (**17**) with *m*-methoxybenzaldehyde (**20**), followed by hydrogenation, as shown in Fig. 1. Secoaromadendrane-type sesquiterpenoids have not been detected in *P. stephensoniana* even by GC/MS analysis, indicating that *P. stephensoniana* belongs to the species of Type B. *P. stephensoniana* is chemically similar to Japanese *P. arbuscula* (Bird.) Lehm. et Lindenb. since the latter species elaborates **11** as the major component [Asakawa, Y., Toyota, M. and Takemoto, T., unpublished results]. Although the bibenzyl (**11**) is a chemical marker for some *Plagiochila* species, it has also been found in some *Frullania* species [2, 9].

Porella species are divided into two types: those

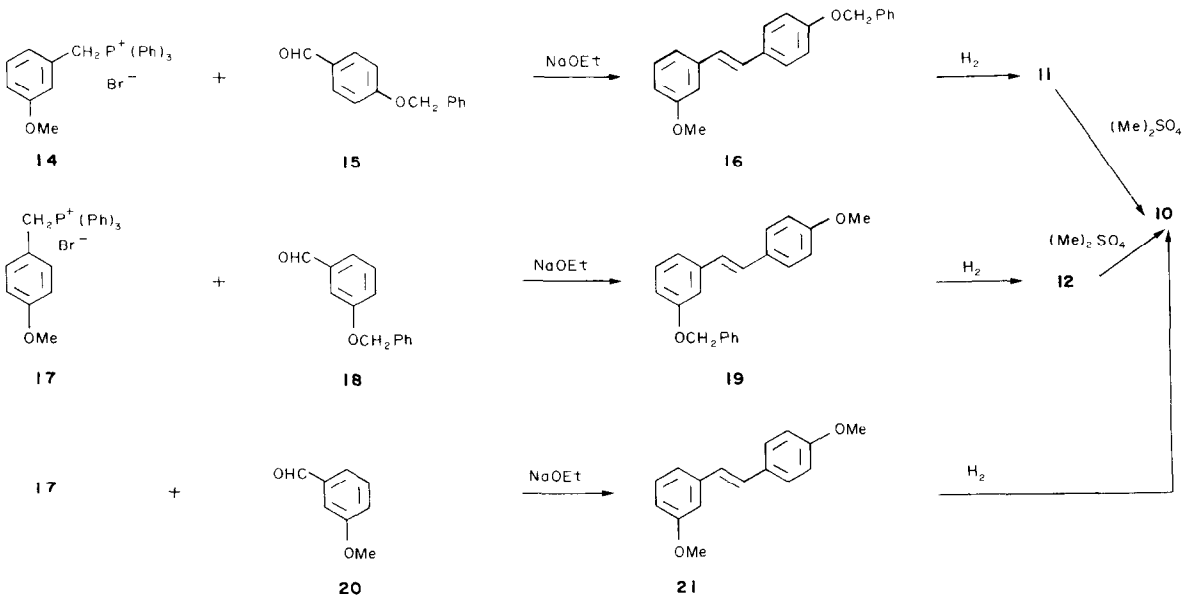

Fig. 1. Synthesis of bibenzyls **10–12**

Table 2. Terpenoids and aromatic compounds detected in *Plagiochila stephensoniana* and *Porella elegantula*

P. stephensoniana	Content*	*P. elegantula*	Content*
α-Pinene	+		
β-Pinene	+		
Allomadendrene	+		
Bicyclogermacrene (**7**)	+	Bicyclogermacrene (**7**)	++
3,4′-Dimethoxybibenzyl (**10**)	+	M^+ 218 (107)	+
4-Hydroxy-3′-methoxybibenzyl (**11**)	+++++	M^+ 218 (41)	+
Bibenzyl (M^+ 228, 107)	+++	M^+ ? (69)	+
Bibenzyl (M^+ ? 107)	+	Phytol	+
M^+ 288 (107)	+	Perrottetianal A (**8**)	++++++++
		M^+ 312 (69)	+
		M^+ ? (73)	+
		Paraffin (M^+ ? 57)	+
		Paraffin (M^+ ? 57)	+
		Campesterol	+
		Stigmasterol	+
		Sitosterol	+

*See Table 1.

containing the intense pungent sesquiterpene dialdehyde, polygodial and its related drimane-type sesquiterpenoids (Type A) and those containing sacculatane-type diterpenoids (Type B) [5]. The crude extract of *P. elegantula* showed a simple GC with one large peak and 12 small ones. The major component is perrottetianal (**8**), a unique diterpene dialdehyde [19]. Thus, *P. elegantula* belongs to the species of Type B. Among the *Porella* species examined so far, *P. elegantula* is chemically quite similar to Japanese *P. perrottetiana* (Mont.) Trev. [19].

EXPERIMENTAL

The solvents used for spectral determinations were TMS–$CDCl_3$ [^{1}H NMR (60 and 90 MHz)]; EtOH (UV); $CHCl_3$ (IR and $[\alpha]_D$) unless otherwise stated. TLC and prep. TLC: precoated Si gel (0.25 mm) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1 or 1:1) as solvents. Spots were visualized in UV (254 and 360 nm) by spraying with 30% H_2SO_4. GC: 1 or 5% SE-30, 3 m × 2 mm glass column, temp. programme 50–270° at 5° min, injection temp. 270°, N_2 30 ml/min. GC/MS: 70 eV, 1% SE-30, 3 m × 2 mm glass column, temp.

programme 50–270° at 5°/min, injection temp. 260°, He 30 ml/min. EI/MS (direct inlet), 70 eV.

Plant material. *Marchantia berteroana* Lehm. et Lindenb., *M. foliacea* Mitt., *Plagiochila stephensoniana* Mitten. and *Porella elegantula* (Mont.) Hodgs. identified by E. O. C. are deposited in the Herbarium of Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. *M. berteroana*, *M. foliacea*, *P. stephensoniana* and *P. elegantula* collected on Mt. Egmont, New Zealand in Feb. and May 1981 were air-dried and ground. The ground material was extracted with Et_2O for 1 week. The green extracts were filtered through a short column packed with Si gel (230–400 mesh). Each filtrate after the solvent was evaporated was analysed by TLC, GC and computerized GC/MS. The components obtained by GC/MS (Table 1) were identified by co-injection of authentic samples and by the direct comparison of MS with those of authentic samples. The crude extract (65 mg) from *M. berteroana* was purified by prep. TLC to give (−)-2-hydroxycuparene (**4**) (8 mg) whose spectral and physical data were in good agreement with those of an authentic sample [20]. The crude extract (56 mg) from *P. stephensoniana* was also purified by prep. TLC to afford pure 4 - hydroxyl - 3' - methoxybibenzyl (**11**) (9 mg). UV λ_{max} nm (ϵ): 224.5 (7000), 274.5 (1500), 280.5 (1500), 287 sh (700); IR $\nu_{max}^{liq.}$ cm^{-1}: 3400 (OH), 1600, 1515, 1490 (aromatic ring); 1260, 1165, 1155, 1100, 1040, 820, 790, 775, 690; ^1H NMR: δ 2.78 (4H, *s*), 3.71 (3H, *s*), 5.26 (1H, *brs*, disappeared on addition of D_2O), 6.70 (2H, *d*, $J = 8$ Hz), 6.86 (2H, *d*, $J = 8$ Hz), 6.55–6.77 (3H, *m*), 7.06 (1H, *dd*, $J = 7$, 2 Hz); MS *m/z* (rel. int.): 228 $[M]^+$ $C_{15}H_{16}O_2$ (100), 121 $[MeO-C_7H_6]^+$ (62), 107 $[HO-C_7H_6]^+$ (85). Acetate (**13**) of **11**: UV λ_{max} nm (ϵ): 268.5 sh (1600), 273 (2000), 280 (1700), 286 sh (600); IR ν_{max} cm^{-1}: 1760 (OAc), 1600, 1590, 1495, 1370, 1260, 1220, 1200, 1050, 1020, 910, 850, 780, 695; ^1H NMR: δ 2.25 (3H, *s*), 2.90 (4H, *s*), 3.63 (3H, *s*), 6.66–7.21 (8H, *m*). The spectral and chromatographic behavior of **11** and **13** were identical to those of the synthetic samples (see below). The crude extract (76 mg) from *P. elegantula* was purified by prep. TLC to afford perrottetianal (**8**) (10 mg) whose spectral and chromatographic properties were in good agreement with those of an authentic sample [19].

Synthesis of 4-*hydroxy*-3'-*methoxybibenzyl* (**11**). To $LiAlH_4$ (460 mg) in THF (20 ml) was added *m*-methoxybenzaldehyde (2.1 g) and the mixture refluxed for 5 hr. Work-up as usual gave *m*-methoxybenzyl alcohol (1.80 g) which was treated with 47% HBr (2.5 ml) in C_6H_6 (10 ml) to give *m*-methoxybenzyl bromide (2.4 g). To triphenylphosphine (3.1 g) in DMF (2.5 ml) was added *m*-methoxybenzyl bromide (2.0 g) and the mixture refluxed at 160° for 4 hr. Work-up as usual gave *m*-methoxybenzyltriphenylphosphoniumbromide (**14**) (1.8 g), mp 246–248°; UV λ_{max} nm (ϵ): 263 (2700), 269.5 (3700), 277 (3700), 286 (2000); IR ν_{max} cm^{-1}: 1585, 1105, 1030, 995, 920, 830; ^1H NMR: δ 3.48 (3H, *s*), 6.66–7.71 (19H, *m*). A mixture of the phosphonium salt (**14**) and *p*-benzyloxybenzaldehyde (**15**) (820 mg) derived from *p*-hydroxybenzaldehyde was refluxed with NaOEt (530 mg) in EtOH (20 ml) to give the *cis*- and *trans*-stilbene derivatives (800 mg) which were purified by a combination of Si gel CC and prep. TLC to give the *cis*- (150 mg) and the *trans*-isomer (430 mg). *Cis*-isomer: mp 56–57°; UV λ_{max} nm (ϵ): 287 (10300); IR ν_{max} cm^{-1}: 1600, 1485, 1450, 1435, 1380, 1250, 1170, 1130, 1110, 1080, 1040, 870, 840, 780, 750, 695; ^1H NMR: δ 3.63 (3H, *s*), 5.00 (2H, *s*), 6.48 (2H, *s*), 6.70–7.32 (8H, *m*), 7.33 (5H, *s*). *Trans*-isomer: mp 116–117°; UV λ_{max} nm (ϵ): 298 (7100), 307 (7500), 323.5 (7400), 340 sh (4400); IR ν_{max} cm^{-1}: 1590, 1580, 1485, 1450, 1430, 1380, 1250, 1150, 1110, 1080, 1010, 955, 840, 810, 680; ^1H NMR: δ 3.79 (3H, *s*), 5.03 (2H, *s*), 6.87 (2H, *d*, $J = 8.5$ Hz), 6.91 (2H, *s*), 6.71–7.43 (*m*), 7.30 (5H, *s*). The *trans*-isomer (**16**) (330 mg) in EtOH (5 ml) was hydrogenated in the presence of 10% Pd–C (337 mg). Work-up as usual gave a pure dihydrostilbene (**11**) (300 mg). The spectral and chromatographic properties of **11** and its acetate (**13**) were identical to those of the natural bibenzyl (**11**) and its acetate (**13**).

Synthesis of 3-*hydroxy*-4'-*methoxybibenzyl* (**12**). *p*-Methoxybenzyl alcohol (3.37 g) was treated with 47% HBr (4.4 ml) in C_6H_6 (10 ml) with stirring for 3 hr at room temp. Work-up as usual gave *p*-methoxybenzyl bromide (4.75 g). To triphenylphosphine (6.6 g) in DMF (40 ml) was added *p*-methoxybenzyl bromide and the mixture refluxed at 160° for 4 hr. Evaporation of the solvent gave a white mass which was recrystallized from C_6H_6–$CHCl_3$ (1:2) to afford *p*-methoxybenzyltriphenylphosphonium bromide (**17**) (6.48 g), mp 226–228°; UV λ_{max} nm (ϵ): 225.5 (1600), 269.5 (3000), 276.5 (2600); IR ν_{max} cm^{-1}: 1600, 1575, 1475, 1450, 1425, 1290, 1205, 1170, 1100, 1020, 940; ^1H NMR: δ 3.66 (3H), 5.23 (2H, *br d*, $J = 14$ Hz), 6.60 (2H, *d*, $J = 9$ Hz), 6.98 (2H, *d*, $J = 9$ Hz), 7.60–7.70 (15H, *m*). *m*-Hydroxybenzaldehyde (1.51 g) in Me_2CO (25 ml) was refluxed with benzyl bromide (25 ml) in the presence of K_2CO_3 (6.5 g) for 5 hr. Work-up as usual gave *m*-benzyloxybenzaldehyde (850 mg), mp 147–148°. A mixture of the phosphonium salt (**17**) (860 mg) and *m*-benzyloxybenzaldehyde (360 mg) was treated in the same manner as that described for the synthesis of **11**, to afford the *cis*- and *trans*-stilbene mixtures (**19**) (831 mg), mp 88–92°; UV λ_{max} nm (ϵ): 297.5 (4400), 304 (4500), 320.5 (4420), 336.5 sh (2700); IR ν_{max} cm^{-1}: 1595, 1440, 1380, 1250, 1175, 1160, 1030, 965, 840, 820. This was hydrogenated in the presence of 10% Pd–C without purification to afford dihydrostilbene. The crude product was chromatographed on Si gel using a C_6H_6–EtOAc gradient to afford 3 - hydroxy - 4' - methoxybibenzyl (**12**) (230 mg), mp 76–78°; UV λ_{max} nm (ϵ): 276.5 (1700), 283.5 (1400); IR ν_{max} cm^{-1}: 3450 (OH), 1600, 1510, 1450, 1350, 1300, 1280, 1250, 1180, 1170, 1155, 1100, 1050, 950, 870, 860, 815, 790, 770, 695; ^1H NMR: δ 2.78 (4H, *s*), 3.73 (3H, *s*), 5.30 (1H, *br s*, disappeared on addition of D_2O), 6.76 (2H, *d*, $J = 8$ Hz), 7.01 (2H, *d*, $J = 8$ Hz), 6.56–7.16 (4H, *m*). The MS was very similar to that of **11**.

Synthesis of 3,4'-*dimethoxybibenzyl* (**10**). The bibenzyl (**11**) (120 mg) was methylated with $(Me)_2SO_4$ to give the dimethyl ether (**10**) (75 mg). UV λ_{max} nm (ϵ): 274 (1200), 279 (1300); IR ν_{max} cm^{-1}: 1600, 1585, 1505, 1490, 1450, 1435, 1300, 1250, 1175, 1150, 1110, 1085, 1030, 870, 840, 820, 780, 720, 695; ^1H NMR: δ 2.86 (4H, *s*), 3.73 (6H, *s*), 6.76 (2H, *d*, $J = 7$ Hz), 7.08 (2H, *d*, $J = 7$ Hz), 6.66–7.17 (4H, *m*); MS *m/z* (rel. int.) 242 $[M]^+$ $C_{16}H_{18}O_2$ (15), 121 (100). Compound **10** was also obtained from **12** by the same method as that described above and by the following Wittig reaction. The phosphonium salt (**17**) (1.43 g) was treated with *m*-methoxybenzaldehyde (**21**) (840 mg), followed by hydrogenation to afford **10** (1.33 g).

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