Geranyl Phenyl Ethers from the New Zealand Liverwort *Trichocolea hatcheri*

Seung-Hwa Baek,[†] Nigel B. Perry,*[,‡] Rex T. Weavers,[‡] and Raymond S. Tangney[§]

Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Limited, Department of Chemistry, and Department of Botany, University of Otago, Box 56, Dunedin, New Zealand, and Department of Chemistry, Wonkwang University, Iksan 570-749, Korea

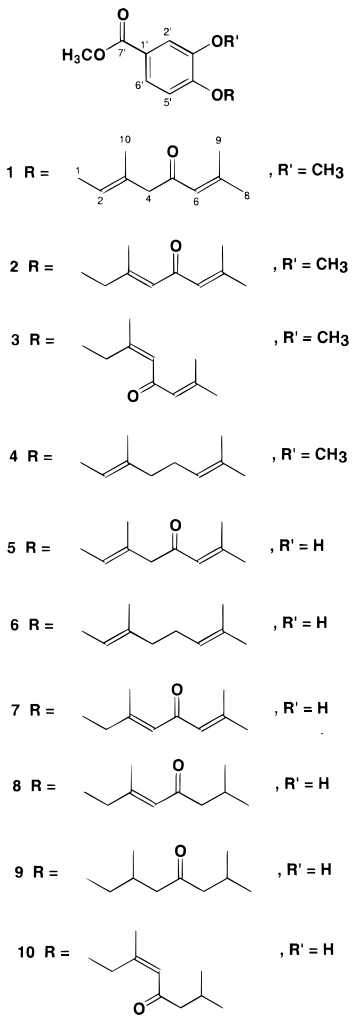
Received August 8, 1997[⊗]

Methyl 4-(geranyloxy)-3-hydroxybenzoate (**6**), previously unreported, has been identified from the New Zealand liverwort *Trichocolea hatcheri*. Four new related 3-hydroxybenzoates **7**−**10** were also found, with a carbonyl at C-5 of the geranyl group and various double bond arrangements. Two known geranyl phenyl ethers **1** and **3**, found in other *Trichocolea* species, were also identified.

Liverworts have yielded a rich array of secondary metabolites, which are mainly terpenoids or aromatic compounds.[1] A few of these metabolites contain combinations of these two groups, such as the geranyl phenyl ether **1** that we recently reported as the main cytotoxic component in the New Zealand liverwort *Trichocolea mollissima* (Hook. f. and Tayl.) Gott. (family Trichocoleaceae).[2] Double-bond isomers **2** and **3** were found at lower levels. Japanese collections of *Trichocolea tomentella* (Ehrh.) Dum. also yielded **1** and **3**, as well as compound **4**, with an unoxidized geranyl chain, and compound **5**, with a free hydroxyl group.[2] These discoveries prompted us to investigate another of New Zealand's *Trichocolea* species, *T. hatcheri* Hodgs. This species, which grows throughout New Zealand, is distinguished from *T. mollissima* by its smaller size, dark green color, and prostrate habit.[3] Microscopically, *T. hatcheri* is characterized by tapered leaf cilia, which lack swollen septae, and weak or absent cuticular ornamentation.[4] However, the taxonomy of *Trichocolea* in New Zealand is not settled. Hybrids between *T. rigida* and *T. mollissima* have been reported by Ratkowsky as having a morphology similar to that of *T. hatcheri*.[5] The nature of this morphological variation and associated chemotaxonomy is the subject of ongoing research (see below). We now report five new geranyl phenyl ethers **6**−**10** from *T. hatcheri*, all with free hydroxyl groups.

An extract of *T. hatcheri* showed cytotoxic effects against monkey kidney (BSC) cells. Reversed-phase flash chromatography concentrated the cytotoxic activity in fractions eluted with MeCN−H_2O 3:1 and 9:1. Si gel column chromatography spread this activity across several fractions containing geranyl phenyl ethers **1**, **3**, and **6**−**10**. These compounds were obtained pure in quantities too small for biological assays, but syntheses and biological activities of some will be reported separately.

The least polar compound (**6**), purified by preparative TLC, had UV and IR spectra appropriate for a 3,4-dioxygenated benzoic acid derivative. The MS supported a molecular formula of $C_{18}H_{24}O_4$. ^{1}H- and ^{13}C-NMR spectra of **6** (Tables 1 and 2) showed the presence of only one methoxyl group, with a ^{13}C-NMR shift (51.9 ppm) very close to the methyl ester shift (52.0 ppm) in **1**. The ^{1}H- and ^{13}C-NMR signals of the benzoate portion of **6** were very similar to those in the 3′-hydroxy compound **5**.[2] The phenolic OH of **6** was observed in the ^{1}H-NMR spectrum as a broad exchangeable signal at 5.7 ppm. The remaining ^{1}H- and ^{13}C-NMR signals



* To whom correspondence should be addressed. Phone: +64-3-4798354. FAX: +64-3-4798543. E-mail: perryn@crop.cri.nz.
[†] Department of Chemistry, Wonkwang University.
[‡] Department of Chemistry, University of Otago.
[§] Department of Botany, University of Otago.
[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1997.

S0163-3864(97)00379-0 CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/23/1998

Table 1. ^{1}H-NMR Data for Compounds from *Trichocolea hatcheri*[a]

signal	6	7	8	9	10
1	4.67 (d, 7)	4.27 (t, 7)	4.27 (t, 7)	4.1 (m)	4.26 (t, 6)
2	5.47 (br t, 7)	2.66 (br t, 7)	2.65 (br t, 6)	1.85 (m), 1.7 (m)	3.04 (br t, 6)
3				2.3 (m)	
4	2.1 (m)	6.12 (br s)	6.12 (br s)	2.4 (m)	6.23 (br s)
5	2.1 (m)				
6	5.07 (m)	6.07 (br s)	2.31 (d, 7)	2.29 (d, 7)	2.33 (d, 7)
7			2.16 (m)	2.16 (m)	2.16 (m)
8	1.68 (br s)	1.91 (d, 1)	0.93 (d, 7)	0.92 (d, 7)	0.94 (d, 7)
9	1.61 (br s)	2.19 (d, 1)	0.93 (d, 7)	0.92 (d, 7)	0.94 (d, 7)
10	1.75 (br s)	2.24 (d, 1)	2.21 (br s)	1.01 (d, 6)	1.97 (d, 1)
2′	7.6 (m)	7.6 (m)	7.6 (m)	7.6 (m)	7.6 (m)
5′	6.87 (d, 9)	6.88 (d, 9)	6.88 (d, 9)	6.84 (d, 9)	6.90 (d, 9)
6′	7.6 (m)	7.6 (m)	7.6 (m)	7.6 (m)	7.6 (m)
7′-OMe	3.88 (s)	3.89 (s)	3.89 (s)	3.86 (s)	3.87 (s)
3′-OH	5.7 (br s)	5.6 (br s)	5.6 (br s)	6.3 (br s)	6.1 (br s)

[a] In $CDCl_3$, δ in ppm (J in Hz), 300 MHz spectrometer.

Table 2. ^{13}C-NMR Data for Compounds from *Trichocolea hatcheri*[a]

signal	6[b]	7[b]	8[c]	9[b]	10[c]
1	65.9	66.5	66.4	66.9	67.4
2	118.4	40.4	40.3	35.7	33.8
3	142.7	N.O.[d]	152.2	26.3	152.7
4	39.5	127.8	125.8	50.7	126.9
5	26.2	N.O.[d]	201.0	210.6	201.5
6	123.5	125.9	53.6	52.4	53.5
7	132.0	N.O.[d]	25.2	24.6	25.1
8	25.7	27.9	22.7	22.7	22.7
9	17.7	20.8	22.7	22.7	22.7
10	16.7	19.2	19.2	20.2	26.0
1′	123.2	N.O.[d]	123.9	N.O.[d]	123.5
2′	115.5	115.9	116.0	115.8	115.7
3′	145.5	N.O.[d]	145.5	N.O.[d]	145.7
4′	149.7	N.O.[d]	149.3	N.O.[d]	149.9
5′	111.0	110.9	110.9	110.6	110.9
6′	122.6	122.8	122.8	122.6	122.6
7′	166.8	N.O.[d]	166.8	166.6	166.9
7′-OMe	51.9	52.1	52.1	52.0	52.0

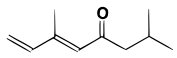
[a] In $CDCl_3$, δ in ppm. [b] 75 MHz spectrometer. [c] 50 MHz spectrometer. [d] Not observed.

of **6** were very similar to those in the unoxidized geranyl group of **4**.[2] An NOE interaction between the aromatic H-5′ and the H-1 protons of the geranyl group showed compound **6** to be the previously unreported methyl 4-geranyloxy-3-hydroxybenzoate.

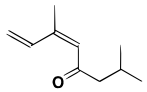
Six more polar compounds were purified by reversed-phase HPLC. Two of these were identified as compounds **1** and **3** by comparison with our data on samples from *T. mollissima*.[2] The other compounds, **7**−**10**, were all 3′-OH methyl benzoates, by comparison of their ^{1}H- and ^{13}C-NMR data with those of **6** (Tables 1 and 2).

The most polar compound (**7**) had the molecular formula $C_{18}H_{22}O_5$, isomeric with the previously reported compound **5**.[2] However, the ^{1}H-NMR spectrum of **7** differed from that of **5** in having both olefinic proton signals as broad singlets (6.12 and 6.07 ppm, Table 1). These shifts showed the presence of the cross-conjugated dienone unit previously found in **2** and **3**. The geometry of the side chain in **7** was shown to be 3*E* by the similarity of the ^{13}C NMR shifts of C-8, C-9, and C-10 to those of **2**.[2]

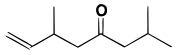
Two of the other new compounds from *T. hatcheri*, **8** and **10**, were isomers with the molecular formula $C_{18}H_{24}O_5$. The ^{1}H-NMR spectra of **8** and **10** (Table 1) showed signals for only one olefinic proton and one allylic methyl, plus a six-proton doublet of a $(CH_3)_2CH$ group. Therefore, the 6,7 double bond found in the other *Trichocolea* geranyl ethers was reduced in these compounds. The shift of the olefinic proton signal (6.12 ppm in **8** and 6.23 ppm in **10**) showed the presence of a 3,4 double bond, conjugated to a carbonyl group at C-5. An NOE interaction between the olefinic proton and the allylic methyl group suggested the 3*Z* geometry for compound **10**, so compound **8** should have the 3*E* geometry. These assignments were supported by close matches of the ^{13}C-NMR shifts of C-1, C-2, and C-10 signals of **8** with those of **2**, and of **10** with those of **3** (compare Table 2 and data in Perry et al.[2]). We could find no references to any (3,7-dimethyl-5-oxo-3-octenyl)-oxy compounds in the literature. The nearest related compounds seem to be (*E*)- and (*Z*)-tagetones (**11**) and (**12**), which co-occur with dihydrotagetone (**13**) and (*E*)- and (*Z*)-ocimenones in the essential oil of the higher plant *Tagetes minuta*.[6] The ^{13}C-NMR shifts of C-5 to C-10 of **10** closely matched those reported for the corresponding carbons in (*Z*)-tagetone (**12**).[6]



11



12



13

The final new compound obtained pure from *T. hatcheri*, compound **9**, had the molecular formula $C_{18}H_{26}O_5$. The ^{1}H-NMR spectrum (Table 1) showed no olefinic proton or allylic methyl signals, so **9** was proposed to be the 3,7-dimethyl-5-oxooctyl ether. This structure contains a chiral center at C-3. The natural product **9** showed an optical rotation, but its absolute stereochemistry was not determined. The ^{1}H-NMR spectrum of **9** (Table 1), which was relatively complex because of the chiral center, was assigned from a COSY spectrum. The ^{13}C-NMR shifts of C-4 to C-10 of **9** (Table 2) closely matched those reported for the corresponding carbons in dihydrotagetone (**13**).[6,7]

The discovery of compounds **1**, **3**, and **6**−**10** in *T. hatcheri* supports the proposal that isoprenyl phenyl ethers are characteristic of the genus *Trichocolea*.[2] The

predominance of 3′-OH benzoates in *T. hatcheri*, compared with 3′-OMe benzoates in *T. mollissima*, could indicate a chemical division between these species; however, valid chemotaxonomic conclusions require analysis of multiple samples combined with careful morphological examinations. We are currently taking this approach to the intraspecific and interspecific chemotaxonomy of *Trichocolea*.

Experimental Section

General Experimental Procedures. All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 45 °C. Octadecyl-functionalized Si gel (Aldrich) was used for reversed-phase flash chromatography, and Davisil, 35−70 μm, 150 Å, was used for Si gel flash chromatography. TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F_{254}, visualized with a UV lamp, then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH), and heating. MS, UV, and IR spectra were recorded on Kratos MS-80, Shimadzu UV 240, and Perkin−Elmer 1600 FT-IR instruments, respectively. NMR spectra, of $CDCl_3$ solutions at 25 °C, were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian VXR-300 spectrometer, and at 200 MHz for ^{1}H and 50 MHz for ^{13}C on a Varian Gemini spectrometer ($CDCl_3$). Chemical shifts are given in parts per million on the δ scale referenced to the solvent peak C*H*Cl_3 at 7.27 and *C*DCl_3 at 77.08. Optical rotation was measured on a Perkin−Elmer 241 polarimeter. BSC cytotoxicity assays were performed as described previously.[8]

Plant Material. *T. hatcheri* was collected from a steep earth bank in the Morrisons Creek area, Dunedin, New Zealand, in February 1996 [University of Otago Herbarium (OTA) specimen no. 048094].

Isolation of 1, 3, and 6−10. Dried *T. hatcheri* (13.2 g) was extracted with EtOH (600 mL) and $CHCl_3$ (200 mL) by homogenizing and filtering to give a dark green gum (443 mg, 50% BSC cytotoxicity at 150 μg/disk, abbreviated as 50% cyt at 150 μg). Reversed-phase flash chromatography over C18 (443 mg precoated on 1.0 g C18, loaded on a 10-g C18 column) was developed in 20-mL steps from H_2O through CH_3CN to $CHCl_3$. Fractions eluted with H_2O−CH_3CN 1:3 and 1:9 (86 mg, brown oil, 75% cyt at 60 μg) were chromatographed over Si gel (precoated on 172 mg Si gel, loaded on a 1.0-g column). This column was developed in steps from EtOAc−cyclohexane 3:97 to EtOAc−cyclohexane 20:80.

Si gel column fractions eluted with EtOAc−cyclohexane 3:97 and 5:95 showed the same UV active spot on TLC (lilac with vanillin−H_2SO_4). These were combined and the solvent removed to produce a residue (14 mg, yellow oil). Final purification was by Si gel TLC with EtOAc−hexane 20:80. A UV-active band at R_f 0.30 was eluted with Et_2O to give **6** (4.5 mg).

Si gel column fractions eluted with EtOAc−cyclohexane 5:95, 7:93, and 10:90 showed the same UV active spots on TLC (total mass 31.1 mg, yellow gums). These fractions were subjected to preparative reversed-phase HPLC (Merck Lichrospher 100 C18, 256 × 10 mm, with 25 × 4 mm guard column). The mobile phase was H_2O−CH_3CN 40:60 (5 mL/min) with UV detection at 280 nm. Samples, as 31.1 mg/mL solutions in CH_3CN, were injected in amounts of up to 100 μg per injection. Combined fractions from the 8.04-min peak yielded **7** (2.5 mg); the 9.25-min peak yielded **8** (2.0 mg), the 11.50-min peak yielded **1** (4.6 mg), the 12.19-min peak yielded **9** (0.6 mg), the 12.71-min peak yielded **10** (5.7 mg), and the 18.03-min peak yielded **3** (0.2 mg).

Methyl 4-[[(2*E*)-3,7-dimethyl-2,6-octadienyl]oxy]-3-hydroxybenzoate (6): colorless oil; UV (MeOH) λ_{max} (log ϵ) 261 (4.55), 297 (4.28) nm; IR (dry film) ν_{max} 3412, 2920, 1716, 1615, 1599, 1509, 1436, 1287, 1212, 1127, 990, 765 cm^{-1}; ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; CIMS (C_4H_{10}) *m/z* 305.1751 $[MH]^+$ (17, calcd for $C_{18}H_{25}O_4$, 305.1753), 209 (10), 169 (57), 138 (13), 137 (100).

Methyl 4-[[(3*E*)-3,7-dimethyl-5-oxo-3,6-octadienyl]oxy]-3-hydroxybenzoate (7): colorless oil; UV (MeOH) λ_{max} (log ϵ) 262 (3.35) nm; IR (dry film)ν_{max} 3412, 2932, 1714, 1622, 1513, 1436, 1382, 1355, 1284, 1213, 1126, 1033, 989, 886, 761 cm^{-1}; ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (30 eV) *m/z* 318.1475 $[M]^+$ (2.5, calcd for $C_{18}H_{22}O_5$, 318.1467), 168.0416 (25, $[M]^+ - C_{10}H_{14}O$), 152 (13), 151 (100), 150 (35), 137 (49), 135 (39), 109 (17), 95 (27), 83 (76).

Methyl 4-[[(3*E*)-3,7-dimethyl-5-oxo-3-octenyl]oxy]-3-hydroxybenzoate (8): colorless oil; UV(MeOH) λ_{max} (log ϵ) 221 (4.31), 255 (4.09), 296 (3.71) nm; IR (dry film) ν_{max} 3408, 2956, 1716, 1690, 1615, 1590, 1511, 1461, 1436, 1286, 1214, 1128, 1096, 1043, 993, 892, 766 cm^{-1}; ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (30 eV) *m/z* 320.1612 $[M]^+$ (2, calcd for $C_{18}H_{24}O_5$, 320.1624), 289.1421 (5, $[M]^+ - OCH_3$), 263.0869 (4, $[M]^+ - C_4H_9$), 168.0428 (23, $[M]^+ - C_{10}H_{16}O$), 154 (12), 153 (100), 152 (23), 137 (39), 109 (20), 95 (90), 85 (28).

Methyl 4-[[(2*E*)-3,7-dimethyl-5-oxo-2,6-octadienyl]oxy]-3-methoxybenzoate (1): colorless oil; UV, IR, ^{1}H NMR, and EIMS match those reported previously.[2]

Methyl 4-[(3,7-dimethyl-5-oxo-3-octyl)oxy]-3-hydroxybenzoate (9): colorless oil; $[\alpha]^{21.4}_D = -11.4°$ (*c* 0.25 in MeOH); UV (hexane) λ_{max} (log ϵ) 252 (3.39), 285 (3.11) nm; IR (dry film) ν_{max} 3349, 2924, 2856, 1712, 1514, 1460, 1376, 1288, 1215, 1127, 766 cm^{-1}; ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) *m/z* 322.1782 $[M]^+$ (3, calcd for $C_{18}H_{26}O_5$, 322.1780), 168.0401 (7, $[M]^+ - C_{10}H_{18}O$), 167.0350 (8, $[M]^+ - C_{10}H_{19}O$), 156 (10), 155 (100), 137 (12), 99 (8), 85 (27).

Methyl 4-[[(3*Z*)-3,7-dimethyl-5-oxo-3-octenyl]oxy]-3-hydroxybenzoate (10): colorless oil; UV (MeOH) λ_{max} (log ϵ) 223 (4.93), 257 (4.83), 296 (4.49) nm; IR (dry film) ν_{max} 3408, 2955, 1712, 1616, 1512, 1439, 1289, 1133, 1020, 992, 764 cm^{-1}; ^{1}H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (30 eV) *m/z* 320.1624 $[M]^+$ (1, calcd for $C_{18}H_{24}O_5$, 320.1624), 168.0396 (17, $[M]^+ - C_{10}H_{16}O$), 155 (19), 153 (55), 152 (26), 151 (18), 149 (13), 137 (36), 109 (28), 95 (100), 85 (12).

Methyl 4-[[(3*Z*)-3,7-dimethyl-5-oxo-3,6-octadienyl]oxy]-3-methoxybenzoate (3): colorless oil; ^{1}H and EIMS match those reported previously.[2]

Acknowledgment. We thank the Dunedin City Council for permission to collect; E. Burgess for assistance with HPLC; G. Ellis for biological assays; B. Clark for MS; and M. Thomas for NMR spectra. This research was supported in part by the New Zealand Foundation for Research, Science and Technology; and in part by Wonkwang University.

References and Notes

(1) Asakawa, Y. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds., Springer: Wien, 1995; Vol. 65.
(2) Perry, N. B.; Foster, L. M.; Lorimer, S. D.; May, B. C. H.; Weavers, R. T.; Toyota, M.; Nakaishi, E.; Asakawa, Y. *J. Nat. Prod.* **1996**, *59*, 729−733.
(3) Allison, K. W.; Child, J. *The Liverworts of New Zealand*; University of Otago Press: Dunedin, 1975.
(4) Schuster, R. M. *Nova Hedwigia* **1968**, *15*, 437−529.
(5) Ratkowsky, D. A. *Bryologist* **1980**, *83*, 78−82.
(6) Thappa, R. K.; Agarwal, S. G.; Kalia, N. K.; Kapoor, R. *J. Essent. Oil Res.* **1993**, *5*, 375−379.
(7) Ruberto, G.; Spadaro, A.; Piatelli, M.; Piozzi, F.; Passannanti, S. *Flav. Fragr. J.* **1991**, *6*, 225−227.
(8) Lorimer, S. D.; Perry, N. B.; Tangney, R. S. *J. Nat. Prod.* **1993**, *56*, 1444−1450.

NP970379B