Isoprenyl Phenyl Ethers from Liverworts of the Genus *Trichocolea*: Cytotoxic Activity, Structural Corrections, and Synthesis

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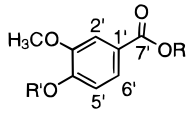
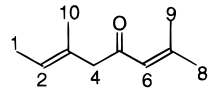
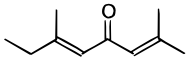
The main cytotoxic component in New Zealand collections of the liverwort *Trichocolea mollissima* was identified as methyl 4-[(5-oxogeranyl)oxy]-3-methoxybenzoate, a structure that has not been reported previously. Two double-bond isomers of this geranyl ether were present at lower levels. Reinvestigation of the benzoates from Japanese collections of *Trichocolea tomentella* led to the identification of four geranyl ethers (including two of the three compounds identified from *T. mollissima*), which had previously been assigned incorrect geranyl ester structures. One compound, previously reported as a 3,3-dimethylallyl ester, could not be re-isolated from *T. tomentella*, but was found in a New Zealand collection of *Trichocolea lanata*. It was shown to be a 3,3-dimethylallyl ether by synthesis from methyl vanillate. Several of these compounds were active in cytotoxic and antifungal assays.

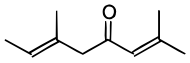
Liverworts have yielded many new compounds, mainly terpenoids or lipophilic aromatic compounds, a good proportion of which have been found to be bioactive.[1] Recently we began a screening program searching for new bioactive natural products from the rich liverwort flora of New Zealand, as lead compounds for new pharmaceuticals or agrochemicals.[2] Early in our liverwort research in Tokushima, we discovered from a Japanese collection of *Trichocolea tomentella* (Ehrh.) Dum. (family Trichocoleaceae) some compounds with the rare combination of both terpenoid and aromatic portions.[3,4] These were identified as isoprenyl esters **1**−**4** based on IR, UV, ^{1}H NMR, and MS data (but not ^{13}C NMR spectra), and on degradative reactions. Pathak and Khanna synthesized compound **1** (tomentellin) by reacting 5-oxogeranyl bromide with veratric acid (**5**), but they were not able to make a direct comparison of synthetic **1** with the natural product.[5] We later reported more isoprenyl benzoates from Japanese and European collections of *T. tomentella*, including geranyl ester (**6**).[6] Some of these compounds were also found in a collection of *Trichocolea pluma* Mont. from Malaysia, and 3,4-dimethoxybenzoates were proposed as significant chemical markers of the Trichocoleaceae.[7] Becker reported that compounds **1** and **2** were major products of cultured *T. tomentella*.[8]

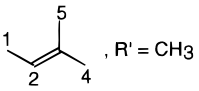
Renewed interest in *Trichocolea* was sparked by the cytotoxic activity of crude extracts from New Zealand collections of *T. mollissima*, a species whose chemistry has not been reported. This foliose liverwort grows throughout New Zealand in rainforest and beech forest, hanging from old tree trunks or growing erect on the forest floor.[9] Four other *Trichocolea* species have been recorded from New Zealand, of which *T. lanata* (Hook.) Nees and *T. hatcheri* Hodgs. are also common. We now report that the main cytotoxic component in New Zealand collections of *T. mollissima* is the monoterpene phenyl ether (**7**), and that closely related compounds **8** and **9** occur at lower levels. This result prompted the re-investigation of the aromatic compounds from Japanese collections of *T. tomentella*, for which the corrected structures **7**, **9**, **10**, and **11** are now reported. The compound previously reported as **4** could not be re-isolated from *T. tomentella*, but it was isolated from a New Zealand collection of *T. lanata*. Its correct structure has now been confirmed as **12** by synthesis from methyl vanillate (**13**).



1 R =  , R' = CH_3

2 R =  , R' = CH_3

3 R =  , R' = H

4 R =  , R' = CH_3

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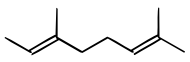
6 R =  , R' = CH_3

Results and Discussion

Crude extracts of various New Zealand collections of *T. mollissima* consistently showed cytotoxic effects against monkey kidney (BSC) cells and antifungal activity against the dermatophyte *Trichophyton mentagrophytes*. We used cytotoxicity against BSC cells to

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

signal	7[b]	8[b]	9[c]	10[c]	11[b]	12[c]
1	4.71(d, 6)	4.20(t, 7)	4.30(t, 7)	4.68(d, 7)	4.69(d, 7)	4.65(d, 7)
2	5.60(tm, 6, 1)	2.67(t, 7)	3.11(t, 7)	5.50(br t, 7)	5.56(t, 7)	5.52(br t, 7)
4	3.12(s)	6.13(m, 1)	6.16(br s)	2.10(m)	3.15(s)	1.79(s)
5				2.10(m)		1.75(s)
6	6.07(m, 1)	6.06(m, 1)	6.07(br s)	5.07(m)	6.08(br s)	
8	1.85(d, 1)	1.88(d, 1)	1.89(d, 1)	1.66(s)	1.86(br s)	
9	2.13(d, 1)	2.16(d, 1)	2.17(d, 1)	1.59(s)	2.14(br s)	
10	1.75(d, 1)	2.23(d, 1)	2.04(d, 1)	1.74(s)	1.76(br s)	
2′	7.53(d, 2)	7.54(d, 2)	7.53(d, 2)	7.54(d, 2)	7.56(m)	7.55(d, 2)
5′	6.86(d, 9)	6.80(d, 8)	7.06(d, 8)	6.88(d, 9)	6.85(d, 9)	6.89(d, 9)
6′	7.63(dd, 9, 2)	7.65(dd, 8, 2)	7.67(dd, 8, 2)	7.65(dd, 9, 2)	7.56(m)	7.66(dd, 9, 2)
7′-OMe	3.88(s)	3.885(s)	3.89(s)	3.89(s)	3.85(s)	3.90(s)
3′-OMe	3.91(s)	3.895(s)	3.90(s)	3.92(s)		3.92(s)

[a] In $CDCl_3$, δ in ppm (J in Hz). [b] 300 MHz spectrometer. [c] 400 MHz spectrometer.

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

signal	**7**[b,c]	**8**[b]	**9**[c,d]	**10**[c,d]	**11**[b,c]	**12**[c,d]
1	65.6	66.9	67.9	65.9	65.6	65.8
2	123.8	40.2	33.6	119.1	123.0	119.3
3	135.5	N.O.[e]	154.5	141.3	136.6	138.4
4	55.0	127.6[f]	127.6	39.5	54.7	25.8
5	197.9	191.4	190.7	26.2	197.7	18.3
6	122.7	126.1[f]	125.9	123.7	122.7	
7	156.6	N.O.	155.3	131.8	157.0	
8	27.7	27.8	27.8	25.6	27.7	
9	20.8	20.6	20.6	17.7	20.8	
10	17.1	19.5	27.0	16.7	17.2	
1′	122.6	123.0	122.4	122.4	123.3	122.5
2′	112.2	112.6	112.2	112.1	115.6[f]	112.1
3′	148.9	152.1[g]	148.8	148.9	145.4	148.9
4′	152.0	152.3[g]	152.4	152.3	149.5	152.3
5′	111.7	111.8	111.6	111.7	111.0	111.6
6′	123.4	123.4	123.6	123.4	122.6[f]	123.4
7′	166.8	166.8	167.0	167.0	166.7	167.0
7′-OMe	52.0	52.0	51.9	51.9	51.9	52.0
3′-OMe	56.0	56.1	56.0	56.0		56.0

[a] In $CDCl_3$, δ in ppm. [b] 75 MHz spectrometer. [c] Assignments confirmed by HMQC and HMBC experiments. [d] 100 MHz spectrometer. [e] Not observed. [f,g] Assignments interchangeable within columns.

direct the isolation of the main cytotoxic component. Reversed-phase flash chromatography on a crude extract of *T. mollissima* gave most of the cytotoxic activity in a fraction eluted with CH_3OH-H_2O 9:1. This fraction contained one main UV-active compound by TLC. This compound, which was responsible for the cytotoxic activity of this fraction, was purified by Si gel flash chromatography.

HRMS showed a molecular ion at 332.1624 daltons, corresponding to the formula $C_{19}H_{24}O_5$. The ^{1}H NMR spectrum (Table 1) and UV and IR spectra were similar to those reported for **1**.[4] The ^{13}C NMR spectrum (Table 2) of our compound showed signals appropriate for a trisubstituted aromatic ring, a 5-oxogeranyl group, an ester carbonyl, and two methoxyl groups, as expected for **1**. Because the ^{13}C NMR spectrum of **1** had not been reported, we rigorously assigned the ^{1}H- and ^{13}C NMR spectra with the aid of HMQC, HMBC, and NOE difference experiments.

The NOE difference experiments produced a surprising result. Irradiation of the two methoxyl signals, which were barely resolved, only gave enhancement of the H-2′ aromatic proton signal. Structure **1** would be expected to give enhancements of both the H-2′ and H-5′ signals. An NOE interaction between H-5′ and H-1 protons of the geranyl group was also inconsistent with structure **1**, but could be explained by structure **7**

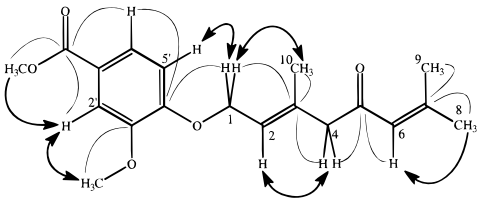


Figure 1. Important NMR correlations establishing the structure of **7**. ↔, selected NOE interactions; —, selected HMBC correlations.

(Figure 1). The critical features that distinguish these structures are the linking of the 5-oxogeranyl group to the aromatic ring via an ether linkage instead of an ester and the replacement of a methyl ether with a methyl ester. Unequivocal evidence of these features was obtained from the HMBC experiment, in which correlations were observed between the ester carbonyl (δ 166.8) and one methoxyl proton signal (δ 3.88, 3H) and between a quaternary oxygenated aromatic signal (δ 152.0) and the H_2-1 protons (δ 4.71, d, J = 6 Hz) of the geranyl group (Figure 1). A further NOE interaction between the geranyl H-10 and H-1 signals showed that the 2,3 double bond has *E* stereochemistry (Figure 1). We could find no references to this monoterpene phenyl ether (**7**) in the literature.

Another collection of *T. mollissima* was extracted to give more of compound **7** for biological assays. During this isolation, an additional TLC spot was noticed at higher R_f than **7** on Si gel, but with a similar color reaction to that of **7**. The compound responsible for this spot and another with a very similar R_f on Si gel to **7** were purified by preparative reversed-phase HPLC and shown to be isomeric with **7** by MS. Both of the new compounds had ^{1}H NMR (Table 1), UV, and IR spectra similar to those reported for a compound from Japanese *T. tomentella*, which was assigned monoterpene ester structure (**2**), with a cross-conjugated dienone in the monoterpene portion.[3,4] However, the ^{13}C NMR spectra (Table 2) showed aromatic and methoxyl signals very similar to those of **7**. Our suspicion that these compounds were also monoterpene phenyl ethers was confirmed by NOE interactions between H-5′ and H-1 for both new compounds. The compound with closest Si gel and C_{18} retention behavior to **7** showed NOE interactions that demonstrated *E* stereochemistry about the 3,4 double bond, and was assigned the previously unreported structure **8**. Comparison of the ^{13}C NMR spectra of **8** and **9** (Table 2) showed changes in chemical shift for C-2 and C-10 consistent with a change from *E* to *Z* stereochemistry about the 3,4 double bond.[10] As

Table 3. Biological Activity Data for Phenyl Ethers[a]

assay	**7**[b]	**7**[c]	**8**[b]	**9**[b]	**10**[c]	**11**[c]	**12**[d]	**13**[d]
BSC cytotoxicity[e]	100 75[f]	100 75[f]	10	10	0	100 75[f]	50	25
C. albicans[g]	0	0	0	0	0	0	1	0
T. mentagrophytes[g]	2	1	0	0	0	6	2	0

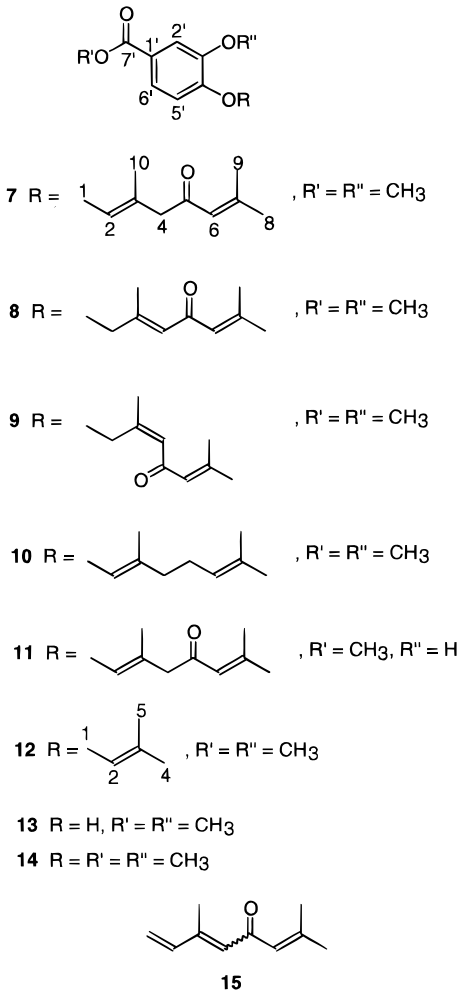
[a] Tested at 60 μg/disk, unless otherwise stated. [b] From *T. mollissima*. [c] From *T. tomentella*. [d] Synthetic. [e] Percentage of well showing cytotoxic effects. [f] At 15 μg/disk. [g] Width of growth inhibition zone (mm).

this stereochemistry was confirmed by NOE difference experiments, this compound has the previously unreported structure **9**. Purified samples of both **8** and **9** were found to undergo *E*−*Z* isomerization, to mixtures of **8** and **9**, on storage.

As the spectral data for compound **7** were very similar to those reported for tomentellin,[4] we re-isolated the compounds previously assigned structures **1** (tomentellin), **2** (isotomentellin), **3** (demethoxytomentellin), and **6** (deoxytomentellin) from Japanese collections of *T. tomentella*. Tomentellin proved identical to the cytotoxic compound **7** from *T. mollissima*. HMBC and NOE difference experiments showed that the structures of isotomentellin, demethoxytomentellin, and deoxytomentellin had also been incorrectly assigned as monoterpene esters. The correct structures are **9** for isotomentellin, **11** for demethoxytomentellin, and **10** for deoxytomentellin.

We did not find the compound assigned structure **4** (trichocolein) in our reinvestigation of Japanese *T. tomentella*. Therefore, we undertook a synthesis of compound **12**, previously unreported, which we expected to be the correct structure of trichocolein. Coupling of 4-bromo-2-methylbut-2-ene to methyl vanillate (**13**) gave **12** in good yield. Structure **12** was confirmed by spectral data (Tables 1 and 2), including HMBC and NOE difference experiments. Comparison of the original ^{1}H NMR spectrum of trichocolein with the ^{1}H NMR spectrum of the synthetic compound supported our conclusion that the correct structure of trichocolein is **12**. Compound **12** was confirmed as a natural product of *Trichocolea* when we isolated it as the main isoprenyl phenyl ether in a New Zealand collection of *T. lanata*, a species that had not been investigated previously. We are currently investigating the correlation of isoprenyl phenyl ether content with taxonomic divisions of the New Zealand *Trichocolea* species.

Compound **7** was the major cytotoxic component of *T. mollissima*, active against BSC cells at 15 μg/disk with no apparent antiviral effects against Herpes simplex or Polio viruses. The only other isoprenyl phenyl ether with similar cytotoxic potency was compound **11** (Table 3). Therefore, both the allylic ether and the conjugated enone substructures seem to be required for cytotoxic activity. It may be that the cytotoxic activity is due to hydrolysis of the ether linkage to release β-ocimenones (**15**), which we have found to be cytotoxic during our synthetic studies on **7** (unpublished results). Compounds **7** and **11** were less toxic to fast-growing P-388 leukemia cells, with IC_{50}'s > 25 μg/mL. The cytotoxic activity of **7** was tested in the U.S. National Cancer Institute's screen against 60 tumor cell lines,[11] but the results did not warrant further investigation. Natural products **7** and **11** were also mildly antifungal against the dermatophyte *Trichophyton mentagrophytes* (Table 3). Crude extracts of *T. lanata* were much less cytotoxic than extracts of *T. mollissima*, but showed some antifungal activity. This fact was explained by the presence of the simpler isoprenyl ether **12** in *T. lanata*, because **12** was less cytotoxic than **7** but had similar mild antifungal activity (Table 3).



Experimental Section

General Experimental Procedures. General experimental and instrumental methods have been described previously,[2] as have details of antimicrobial, BSC (antiviral), and P-388 assays.[12] Si gel TLC was carried out using Merck DC-Plastikfolien Kieselgel 60 F_{254}, with hexane−EtOAc (4:1) as the mobile phase, followed by visualization first with a UV lamp, and then by dipping in 1% vanillin + 1% H_2SO_4-EtOH solution and heating. NMR spectra, of $CDCl_3$ solutions at 25 °C, were recorded either at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian VXR-300 spectrometer, referenced to the solvent peaks C*H*Cl_3 at 7.25 ppm and *C*HCl_3 at 77.00 ppm; or at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a JEOL JNM GX400 spectrometer, referenced to TMS at 0.00 ppm.

Plant Material. *T. mollissima* was first collected from Lake Ellery, Haast, on the west coast of the South Island, New Zealand, in October 1992 [University of Otago Herbarium (OTA) specimen no. 046586]. Initial screening was carried out using an extract produced by shaking air-dried (30 °C), ground material (5.0 g) overnight in EtOH (50 mL). *T. mollissima* was recollected from the same location in June 1993 (OTA

046636). *T. lanata* was collected from the Catlins, on the coast of South Otago, New Zealand, in December 1994 (OTA 046799). *T. tomentella* was collected in December 1994, at Kamiyama-cho, Tokushima, Japan (dry wt 36.6 g, Tokushima specimen no. 94245); and in July 1994, at Ozaka-cho, Gifu, Japan (4 g, no. 94123).

Bioactivity-Directed Isolation of 7. Dried *T. mollissima* from the second collection (31.5 g) was extracted with EtOH (600 mL, then 3 × 150 mL) and $CHCl_3$ (1 × 100 mL) by homogenizing and filtering to give a dark green gum (1.4 g, 25% BSC cytotoxicity at 150 μg/disk, abbreviated as 25% cyt at 150 μg). Reversed-phase flash chromatography over C18 (1.4 g precoated on 5.6 g C18, loaded on a 14 g C18 column) was developed in 20 mL steps from H_2O through CH_3OH to $CHCl_3$. The most cytotoxic fraction was eluted with H_2O−CH_3OH 1:9 (75 mg, brown oil, 100% cyt at 150 μg). A subsample of this fraction was chromatographed over Si gel (58 mg precoated on 180 mg Si gel, loaded on a 2 g column) developed with hexane−EtOAc 9:1 (10 × 2 mL fractions) and then hexane−EtOAc 4:1 (15 × 2 mL fractions). Fractions 14 to 16, which showed a single UV active spot on TLC (lilac with vanillin/H_2SO_4), were combined and solvent removed to give **7** (12 mg).

Methyl 4-[(2*E*)-3,7-dimethyl-5-oxo-2,6-octadienyl)oxy]-3-methoxybenzoate (7): colorless oil; TLC R_f 0.25; UV (MeOH) λ max (log ϵ) 250 (4.39), 288 (3.99) nm; (MeOH/NaOH) 300 (4.59); IR (dry film) ν max 2950, 2833, 1715, 1687, 1620, 1600, 1511, 1435, 1293, 1271, 1219, 1174, 1134, 1107, 990, 764 cm^{-1} ; ^1H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) $[M]^+$ 332.1624 (3, $C_{19}H_{24}O_5$ req 332.1624), 301.1429 (3, $[M]^+ - OCH_3$), 264.0996 (29, $[M]^+ - C_5H_8$), 182.0558 (82, $[M]^+ - C_{10}H_{14}O$), 151.1123 (38, $C_{10}H_{15}O$), 151.0393 (49, $C_8H_7O_3$), 83 (100, C_5H_7O); CIMS (C_4H_{10}) $[MH]^+$ 333 (4), 301 (1), 265 (6), 264 (7), 183 (21), 182 (13), 151 (49), 83 (100); assay results in Table 3.

Isolation of 8 and 9. Dried *T. mollissima* from the first collection (40.0 g) was extracted with EtOH (2 × 300 mL) and $CHCl_3$ (2 × 200 mL) by homogenizing and filtering to give a combined dark green-brown solution. Most of the organic solvents were removed by evaporation, then the remaining suspension was partitioned between H_2O (150 mL) and $CHCl_3$ (150 mL). The $CHCl_3$-soluble material was subjected to reversed-phase flash chromatography over C18 (1.1 g precoated on 4.0 g of C18, loaded on a 20 g C18 column). This column was developed in steps of H_2O−CH_3CN 1:1 (4 × 20 mL), H_2O−CH_3CN 1:3 (2 × 20 mL), H_2O−CH_3CN 1:9 (2 × 20 mL), CH_3CN (2 × 20 mL), CH_3CN−$CHCl_3$ 3:1 (2 × 20 mL), and $CHCl_3$ (4 × 20 mL). Compound **7** was detected by TLC in fractions eluted with H_2O−CH_3CN, 1:3 so these were combined and the solvent removed (69 mg, yellow oil). TLC of fractions eluted with H_2O−CH_3CN 1:9 showed some **7** plus a higher R_f UV-active spot, lilac with vanillin/H_2SO_4. These fractions were combined and solvent removed (47 mg). The $CHCl_3$−soluble materials from these two samples were subjected to preparative reversed-phase HPLC (Merck Lichrospher 100 C_{18}, 250 × 10 mm, with 25 × 4 mm guard column). The mobile phase was H_2O:CH_3CN 1:3 (5 mL/min) with UV detection at 280 nm. Samples, as 100 mg/mL solutions in CH_3CN, were injected in amounts of up to 10 mg per injection. Peaks at 6.0, 6.5, and 8.3 min were collected separately. Combined fractions from the 6.0 min peak yielded **7** (18 mg), the 6.5 min peak yielded **8** (5 mg), and the 8.3 min peak yielded **9** (5 mg).

Methyl 4-[(3*E*)-3,7-dimethyl-5-oxo-3,6-octadienyl)oxy]-3-methoxybenzoate (8): brown oil; TLC R_f 0.3; UV (MeOH) λ max (log ϵ) 262 (3.78) nm; IR (dry film) ν max 2925, 2850, 1715, 1625, 1600, 1515, 1435, 1270, 1220, 1135, 1115, 1030, 875, 765 cm^{-1} ; ^1H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) $[M]^+$ 332.1619 (20, $C_{19}H_{24}O_5$ req 332.1624), 300.1339 (17, $[M]^+ - HOCH_3$), 182.0582 (48, $[M]^+ - C_{10}H_{14}O$), 151.1145 (97, $C_{10}H_{15}O$), 151.0424 (40, $C_8H_7O_3$), 83 (100) 55 (45); assay results in Table 3.

Methyl 4-[(3*Z*)-3,7-dimethyl-5-oxo-3,6-octadienyl)oxy]-3-methoxybenzoate (9): yellow oil; TLC R_f 0.4; UV (MeOH) λ max (log ϵ) 262 (3.89) nm; IR (dry film) ν max 3020, 2925, 2855, 1710, 1625, 1600, 1515, 1435, 1295, 1270, 1215, 1115, 1035, 880, 760 cm^{-1} ; ^1H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) $[M]^+$ 332.1629 (12, $C_{19}H_{24}O_5$ req 332.1624), 300.1329 (10, $[M]^+ - HOCH_3$), 182.0582 (53, $[M]^+ - C_{10}H_{14}O$), 151.1109 (100, $C_{10}H_{15}O$), 151.0419 (20, $C_8H_7O_3$), 83 (75) 55 (60); assay results in Table 3.

Re-isolation of 7, 9, and 10 from *T. tomentella* (no. 94245). The diethyl ether extract (1.2 g) was chromatographed on Si gel using an *n*-hexane−EtOAc gradient, giving eight fractions. Fraction 5 (446 mg) was chromatographed on Sephadex LH-20 using CH_2Cl_2−MeOH and further purified by Si gel HPLC using *n*-hexane−EtOAc 4:1 to give **7** (152 mg). Fraction 4 (70 mg) was chromatographed on Sephadex LH-20 using CH_2Cl_2−MeOH and further purified by Si gel HPLC using *n*-hexane−EtOAc 9:1 to give **9** (13 mg). Fraction 3 (76 mg) was chromatographed on Sephadex LH-20 using CH_2Cl_2−MeOH and further purified by Si gel HPLC using *n*-hexane−EtOAc 9:1 to give **10** (29 mg).

Methyl 4-[(2*E*)-3,7-dimethyl-2,6-octadienyl)oxy]-3-methoxybenzoate (10): yellow crystals, mp 41−42 °C; TLC R_f 0.5 (pale blue with vanillin/H_2SO_4); UV (EtOH) λ max (log ϵ) 223 (4.4), 262 (4.3), 293 (4.1) nm; IR (dry film) ν max 1720, 1600, 1510, 1440, 1300, 1140, 990, 760 cm^{-1} ; ^1H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) $[M]^+$ 318.1826 (1, $C_{19}H_{26}O_4$ req 318.1831), 287.1632 (1, $[M]^+ - OCH_3$), 182 (100), 167 (5), 151 (13), 136 (32), 121 (10), 93 (50), 81 (41), 69 (99); assay results in Table 3.

Re-isolation of 11 from *T. tomentella* (no. 94123). The diethyl ether extract (0.11 g) was directly chromatographed on Sephadex LH-20 to give a mixture containing **11** (63 mg). This mixture was re-chromatographed on Si gel using an *n*-hexane−EtOAc gradient and further purified by Si gel HPLC using *n*-hexane−EtOAc 7:3 to give **11** (25 mg).

Methyl 4-[(2*E*)-3,7-dimethyl-5-oxo-2,6-octadienyl)oxy]-3-hydroxybenzoate (11): colorless oil; TLC R_f 0.15 (lilac with vanillin/H_2SO_4); UV (EtOH) λ max (log ϵ) 212 (4.4), 238 (4.4), 250 (4.0), 293 (3.5) nm; IR (dry film) ν max 3550, 1710, 1690, 1620, 1590, 1510, 1460, 1440, 1290, 1130, 1000 cm^{-1} ; ^1H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) $[M]^+$ 318.1460 (0.3, $C_{18}H_{22}O_5$ req 318.1467), 287.1284 (1, $[M]^+ - OCH_3$), 250.0853 (2, $[M]^+ - C_5H_8$), 168.0424 (3, $[M]^+ - C_{10}H_{14}O$), 151.1158 (9, $C_{10}H_{15}O$), 137.0265 (8, $C_7H_5O_3$), 83 (100); assay results in Table 3.

Synthesis of 12. 4-Bromo-2-methylbut-2-ene (registry no. 870-63-3, 0.059 g, 0.4 mmol) in dry DMF (0.40

mL) was added dropwise, with stirring, to a flame-dried flask containing methyl 3-methoxy-4-hydroxybenzoate (**13**) (registry no. 3943-74-6, 0.060 g, 0.33 mmol) and NaH (0.013 g, 0.36 mmol) under N_2. Stirring was continued at 25 °C for 24 h, then the reaction mixture was partitioned between H_2O (10 mL) and CH_2Cl_2 (10 mL). The H_2O fraction was re-extracted with CH_2Cl_2 (10 mL), the organic fractions combined, dried with $MgSO_4$, and the solvent removed. The crude product (0.183 g) was precoated on Si gel (Merck 9385, 0.50 g), loaded onto a dry-packed Si gel column (5 g, 55 × 15 mm), which was developed with hexane−CH_2Cl_2 (1:4, 2 mL fractions). Fractions 1−5 were combined and the solvent removed to give **12** (0.060 g, 73%).

Methyl 4-(3-methyl-2-butenoxy)-3-methoxybenzoate (12): colorless oil; bp 120 °C/0.15 mm Hg; *anal.*, calcd for $C_{14}H_{18}O_4$, C 67.2%, H 7.2%, found C 67.4%, H 7.4%; TLC R_f 0.4 (pale blue with vanillin/H_2SO_4); UV (MeOH) λ max (log ϵ) 262 (4.03), 290 (3.73) nm; IR (dry film) ν max 2977, 2934, 1711, 1598, 1512, 1464, 1418, 1384, 1366, 1343, 1269, 1218, 1180, 1134, 1105, 992, 932, 877, 764 cm^{-1}; ^1H NMR in Table 1; ^{13}C NMR in Table 2; EIMS (70 eV) $[M]^+$ 250 (2), 182 (100), 151 (72), 69 (53); assay results in Table 3.

Isolation of 12 from *T. lanata*. Dried *T. lanata* (3.3 g) was extracted with EtOH (66 mL) by homogenizing and filtering, to give a dark green gum (76 mg). This extract was partitioned between H_2O (40 mL) and $CHCl_3$ (40 mL), the H_2O fraction re-extracted with $CHCl_3$ (40 mL), and the organic fractions combined and solvent removed to give a green solid (60 mg). This was subjected to reversed-phase flash chromatography over C18 (sample pre-coated on 0.17 g of C18, loaded on 2.00 g C18 column, 65 × 8 mm) developed in six steps from 1:1 H_2O−CH_3CN through CH_3CN to $CHCl_3$. ^1H NMR and Si gel TLC suggested that a fraction eluted with H_2O−CH_3CN 1:1 (2 mg) contained **12**, which was further purified by preparative reversed-phase HPLC using the conditions described above. The major peak (1 mg, retention time 6.2 min) was collected and proved to be **12** from ^1H NMR, TLC, and reversed-phase HPLC (coinjection) comparisons with synthetic **12**.

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