The Tremulanes, a New Group of Sesquiterpenes from the Aspen Rotting Fungus *Phellinus tremulae*

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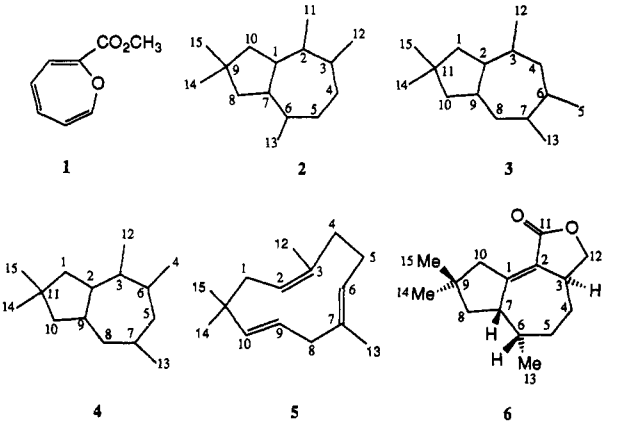
Received August 25, 1993•

A series of new sesquiterpenes, the tremulanes, possessing a previously unreported substituted perhydroazulene carbon skeleton, has been isolated from liquid cultures of the aspen (*Populus tremuloides*) rotting fungus *Phellinus tremulae*. The structures were determined by NMR techniques (^{1}H–^{1}H COSY, HMQC, and HMBC) and other physical methods including, in the case of tremulenolide A (6), X-ray crystallography. The chemical correlation of tremulenediol A (10) with tremulenolide A (6) is described as is the correlation of tremulenedial (8) with tremulenediol B (11). The absolute configuration of the compounds is assigned by application of the olefin octant rule to the allylic alcohol tremulenediol A (10). These new sesquiterpenes do not obey the biogenetic isoprene rule and it is suggested that they may not be derived from farnesyl pyrophosphate.

Introduction

Trembling or quaking aspen (*Populus tremuloides* Michx.) is the most widely distributed tree species in North America. It is a short-lived, pioneer species which grows rapidly on a wide range of soil and site conditions. In Canada, aspen represents 54% of the net merchantable hardwood timber and 11% of the entire Canadian timber resource.[1,2] *Phellinus tremulae* (Bond.) is a wood rotting pathogen which attacks the heartwood of aspen. In Canada, *P. tremulae* is the most serious fungal pathogen of aspen and greatly reduces the economic value of this resource.[3] As part of a program to develop methods for the control of fungal decay and staining in aspen, we have examined the metabolites produced when *P. tremulae* is grown in liquid culture. We have reported previously the isolation of 2-carbomethoxyoxepin (1) from these liquid cultures.[4] Previous workers have reported the isolation of methyl salicylate and methyl benzoate from cultures of the fungus.[5] The odor of wintergreen is characteristic of the rotted heartwood. In this article, we report on the isolation and structure determination of a series of new sesquiterpenes possessing a previously unreported perhydroazulene-type carbon skeleton. This skeleton, 2, which we call tremulane, is isomeric with the well-known lactarane skeleton (3)[6] and with the skeleton 4 produced by the fungus *Merulius tremellosus*.[7] Skeletons 3 and 4 may be derived by cyclization and rearrangement of humulene (5) which is obtained by cyclization of farnesyl pyrophosphate.[6,7] Biosynthetic studies indicate that the tremulane skeleton 2 may not be derived *via* the humulene pathway and that farnesyl pyrophosphate may not be the precursor of this group of sesquiterpenes.



Results and Discussion

P. tremulae was cultivated in shake culture on 2% malt extract broth for 16 days in the presence of DIAION HP 20,[8a] a nonionic highly porous resin capable of absorbing relatively nonpolar metabolites from aqueous media.[8b] The culture broth was filtered off and the resin and wet mycelium were extracted with dichloromethane. Flash chromatography of the crude extract using gradient elution with MeOH–CH_2Cl_2 gave first 2-carbomethoxyoxepin (1) and then the tremulanes 6–13. The separation and the yields of metabolites are summarized in Figure 1.

Tremulenolide A (6). Tremulenolide A (6) was obtained as colorless needles after crystallization from hexanes. The HREIMS and CIMS indicated a molecular formula $C_{15}H_{22}O_2$. The base peak at *m/z* 219 corresponds to the loss of a methyl radical. The presence of an α,β-unsaturated γ-lactone is apparent from UV absorption at 236 nm and an intense carbonyl absorption at 1753 cm^{-1} in the IR spectrum. This is supported by ^{13}C NMR signals (Table I) at δ 171.5 (C-11), δ 161.5 (C-1), and δ 120.7 (C-2). Since these carbon signals are singlets and there is evidence of coupling between the oxygenated methylene protons and an allylic proton in the ^{1}H NMR spectrum (Table II), it is clear that the double bond in the α,β-unsaturated γ-lactone is fully substituted and exocyclic to the lactone

• Abstract published in *Advance ACS Abstracts*, November 15, 1993.

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(2) Hunt, K.; Bosham, J. T.; Kemperman, J. A. *Can. J. For. Res.* **1978**, *8*, 181–187.

(3) Trifonov, L. S.; Chakravarty, P.; Hiratsuka, Y.; Ayer, W. A. *Eur. J. For. Pathol.* **1992**, *22*, 441–448.

(4) Ayer, W. A.; Cruz, E. R. *Tetrahedron Lett.* **1993**, *34*, 1589–1592.

(5) Collins, R. P.; Halim, A. F. *Can. J. Microbiol.* **1972**, *18*, 65–66.

(6) Ayer, W. A.; Browne, L. M. *Tetrahedron* **1981**, *37*, 2199–2248 and refs cited therein.

(7) Jonassohn, M.; Anke, H.; Sterner, O. *Abstracts*, 10th IUPAC Symposium on the Chemistry of Natural Products, Strasbourg, France, 1992, p 408.

(8) (a) Mitsubishi Chemical Industries Ltd., Tokyo, Japan. (b) Tokiwa, Y.; Miyoshi-saitoh, M.; Kobayashi, H.; Sunaga, R.; Konishi, M.; Oki, T.; Iwasaki, S. *J. Am. Chem. Soc.* **1992**, *114*, 4107–4110.

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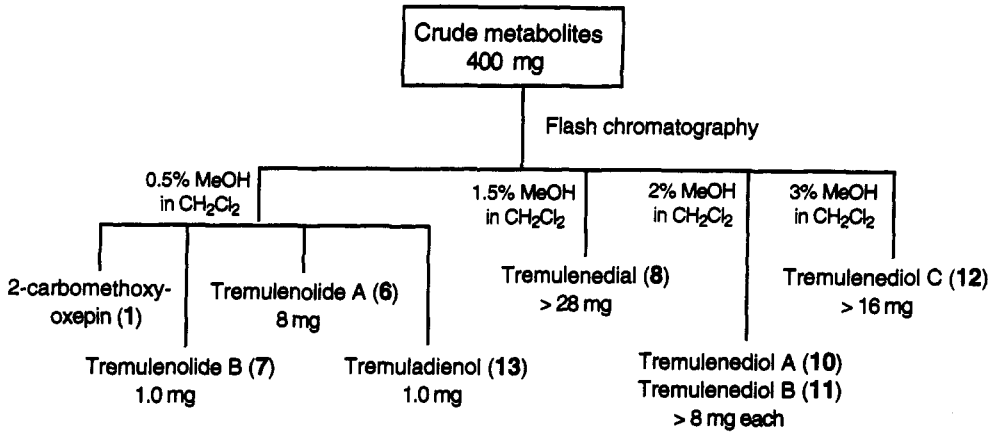
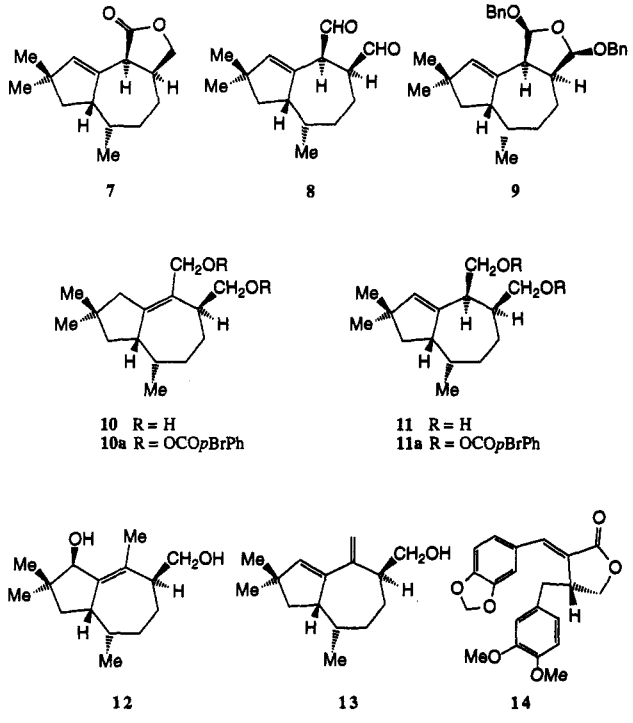


Figure 1. Sequence of elution of metabolites of *Phellinus tremulae* on silica gel flash chromatography.



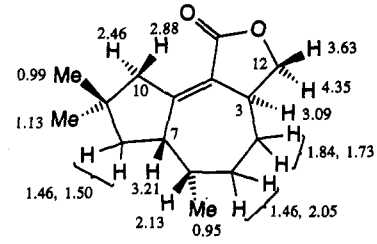


Figure 2. The ^1H NMR chemical shifts for tremulenolide A (**6**).

ring. The unsaturated lactone accounts for three of the five units of unsaturation, therefore tremulenolide A is tricyclic. ^1H NMR data (summarized in Figure 2) indicates the presence of three additional allylic protons, a methylene pair (δ 2.88 and 2.46, J_{gem} = 19 Hz) and a methine (δ 3.21) which is vicinally coupled to three hydrogens. The chemical shifts of the *geminal* methyl groups (δ 27.3, 29.0) are consistent with their location on a five-membered ring[9] and thus the remaining ring is seven-membered. Since the allylic proton at δ 3.21 is coupled (^1H–^1H COSY) to a methine at δ 2.13 which is further coupled to the secondary methyl hydrogens, constitution **6** is indicated for tremulenolide A. Analysis of the HMQC and HMBC spectra (summarized in Figure 3) supports the structural assignment. In particular, the long range ^1H–^{13}C correlation of H-7 with C-1, C-2, C-5, C-6, C-8, and C-13 (Figure 3A) and H-6 with C-1, C-4, C-5, and C-13 (Figure 3B), supports the positioning of the methyl at C-6 relative to the five-membered ring.

The relative stereochemistry of tremulenolide A (**6**) was initially established by NMR techniques and was confirmed by an X-ray crystallographic study. A computer-generated perspective drawing of the final X-ray model of tremulenolide A is shown in Figure 4.[10] The seven-membered ring exists in a distorted twist-boat conformation[11] with C-4 and C-6 as the out-of-plane atoms. The methyl group at C-6 is in a quasiaxial conformation. Ring A is in an envelope conformation with the *gem*-dimethyl groups at the flap of the envelope. Several interesting H–H couplings were important in establishing the relative stereochemistry. The coupling between H-3 and H-12β[12] is 10.5 Hz and that between H-3 and H-12α is 8.5 Hz, reflecting dihedral angles approaching 180° and 0°, respectively, and is consistent with the *trans* relationship of H-3 and H-12β. The assignment of H-12β at higher field (δ 3.63) than is H-12α (δ 4.35) is based on the fact that it is in the shielding region of the C-2,C-3 and C-11,O bonds and in an antiperiplanar relationship to one of the oxygen lone pairs, which causes an upfield shift due to an n–σ^* interaction.[13] The protons on C-10 (δ 2.88, 2.46) both show pronounced long-range couplings with H-3 (^5J = 2.5, 4.5 Hz, respectively). Since homoallylic coupling is at a

(9) The chemical shift difference between *gem*-dimethyl groups on five-membered rings is usually <3 ppm (ref 6, especially Daniewski, W.; Kocór, M.; Thorén, S. *Polish J. Chem.* **1978**, *52*, 561) while in six-membered rings it is usually >10 ppm (Herz, W.; Grisebach, H.; Kirby G. W. *Progress in the Chemistry of Organic Natural Products*; Springer-Verlag: New York, 1979; Vol. 36, pp 59–61).

(10) The X-ray crystallographic study was carried out by Dr. R. McDonald at the Structure Determination Laboratory, Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2. Inquiries regarding the crystallographic results may be directed to the above address quoting report reference code SDL:WAA9202. The authors have deposited atomic coordinates for this struture with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(11) Anet, F. A. L. In *Conformational Analysis of Medium-Sized Heterocycles*; Glass, R. S., Ed.; VCH: Weinheim, 1988; p 50.

(12) Hydrogens above the general plane of the molecule as written are referred to as β, those below the plane as α.

(13) Lambert, J. B.; Shurvell, H. F.; Verbit, L.; Cooks, R. G.; Stout, G. H. *Organic Structural Analysis*. Macmillan: New York, 1976; p 39.

Table I. ^{13}C NMR Chemical Shift Data ($CDCl_3$) for Tremulanes 6–13

atom	6	7	9	10	10a	11	11a	12	13
C-1	161.5	137.0	139.5	145.6	150.3	145.6	144.2	140.1	142.8
C-2	120.7	45.8	49.2	132.3	126.5	47.4	42.4	134.5	147.4
C-3	41.0	38.7	47.9	45.4	41.3	48.2	44.7	50.4	50.5
C-4	28.1	28.4	27.5	22.5	21.2	29.5	29.7	20.8	26.6
C-5	32.9	28.8	29.6	32.6	31.7	30.8	30.4	32.0	32.2
C-6	32.7	33.0	34.0	31.6	31.4	35.7	35.7	32.4	33.6
C-7	45.5	48.0	48.4	46.0	46.6	48.5	48.2	43.7	50.4
C-8	44.9	42.2	43.2	45.5	45.3	42.8	42.7	41.2	45.0
C-9	37.2	42.6	42.1	37.0	37.2	41.6	41.6	40.7	42.9
C-10	48.4	143.5	142.0	48.0	48.3	138.6	139.9	83.5	139.5
C-11	171.5	177.0	107.5	65.6	69.0	60.8	63.4	22.8	112.6
C-12	70.9	71.9	107.9	63.2	63.2	66.7	68.1	62.8	63.7
C-13	17.7	19.3	18.5	11.6	11.7	19.4	19.6	12.4	12.0
C-14	29.0	29.4	29.3	28.5	28.4	29.4	29.2	26.5	29.1
C-15	27.3	27.1	27.9	26.9	26.8	26.8	26.4	21.0	27.2

Table II. ^{1}H NMR Data ($CDCl_3$) for Tremulanes 6–13

	δ (multiplicity, J in Hz)						
proton	6	7	9	10	11	12	13
H-2		3.63, (d, 9.5)	3.52 (dd, 8, 5.2)		2.85 (ddd, 8.5, 5, 4)		
H-3	3.09 (m)	2.71 (m)	2.48 (m)	2.53 (m)	1.70 (m)	2.24 (m)	2.63 (m)
H-4	1.84 (m)	1.92 (m)	1.84 (m)	1.80 (bd, 11.5)	1.60 (m)	1.78 (dm, 11)	1.78 (m)
	1.73 (dddd, 13, 6.5, 2.5, 1.5)	1.50 (m)	1.52 (m)	1.59 (dd, 11.5, 3.5)	1.52 (m)	1.58 (m)	1.62 (m)
H-5	2.05 (m)	1.43 (m)	1.44 (m)	1.83 (m)	1.34 (m)	1.78 (dm, 11)	1.78 (m)
	1.46 (m)	1.43, (m)	1.44 (m)	1.61 (dd, 12, 3)	1.34 (m)	1.54 (m)	1.62 (m)
H-6	2.13 (pd, 7.5, 2)	1.81 (m)	1.83 (m)	1.76 (m)	1.88 (ddq, 10, 7.5, 7.5)	1.69 (m)	1.80 (m)
H-7	3.21 (tm, 10)	3.16 (m)	2.93 (m)	3.10 (tm, 9)	2.94 (dddd 11.5, 10, 6.5, 2.5)	2.97 (m)	3.13 (ddt, 10, 7.5, 2.5)
H-8	1.50 (d, 10)	1.72 (dd, 12.5, 7.5)	1.73 (dd, 13, 7.5)	1.52 (ddd, 12.5, 8, 2.5)	1.61 (dd, 11.5, 6.5)	1.46 (dd, 12, 8.5)	1.71 (dd, 12, 7.5)
	1.46 (m)	1.37 (dd, 12.5, 10)	1.37 (dd, 13, 9)	1.38 (dd, 12.5, 12)	1.32 (dd, 11.5, 11.5)	1.22 (dd, 12, 12)	1.50 (dd, 12, 10)
H-10α	2.46 (ddd, 19, 4.5, 3.5)			1.93 (bd, 15.5)			
H-10β	2.88 (dd, 19, 2.5)	5.50 (d, 2.2)	5.47 (d, 2.2)	2.29 (dd, 15.5, 2.5)	5.32 (d, 2.5)	4.03 (dd, 2, 2)	5.71 (d, 2.8)
H-11			5.20 (d, 5.2)	4.24 (d, 11)	3.90 (dd, 11, 8.5)	1.94 (dd, 2, 2)	5.22 (d, 2.5)
				3.83 (bd, 11)	3.64 (dd, 11, 5)		4.80 (d, 2.5)
H-12α	4.35 (dd, 8.5, 8.5)	4.36 (dd, 9.5, 7)		4.01 (dd, 10, 9)	3.62 (dd, 11, 4)	3.79 (dd, 10, 8)	3.76 (dd, 10.5, 9.5)
H-12β	3.63 (dd, 10.5, 8.5)	4.09 (dd, 9.5, 3.2)	4.98 (d, 1.5)	3.61 (dd, 9, 5)	3.58 (dd, 11, 6.5)	3.64 (dd, 10, 7)	3.61 (dd, 10.5, 6)
13-Me	0.95 (d, 7.5)	0.85 (d, 7)	0.83 (d, 7)	0.82 (d, 7)	0.84 (d, 7.5)	0.78 (d, 7)	0.79 (d, 7.2)
14-Me	1.13 (s)	1.10 (s)	1.09 (s)	1.07 (s)	1.04 (s)	1.02 (s)	1.09 (s)
15-Me	0.99 (s)	1.06 (s)	1.02 (s)	0.87 (s)	0.98 (s)	0.82 (s)	1.03 (s)

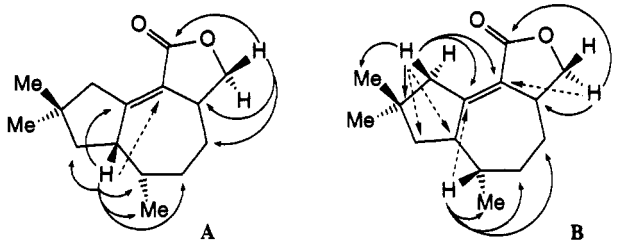


Figure 3. Significant HMBC correlations of tremulenolide A (6).

maximum for C–H bonds parallel to the π orbitals,[14] the proton at δ 2.46, exhibiting the larger coupling constant, should be that in the α position. The β H-10, which is in the deshielding cone of the lactone carbonyl, is at δ 2.88. Another important long-range coupling of H-10α is with H-7, which can be explained in terms of an axial–axial zig-zag coupling. This diaxial configuration permits maximum σ–π overlap involving the central π-bond at C-1, enhancing the normal spin transmission across four bonds.[15] The large magnitude of these coupling constants

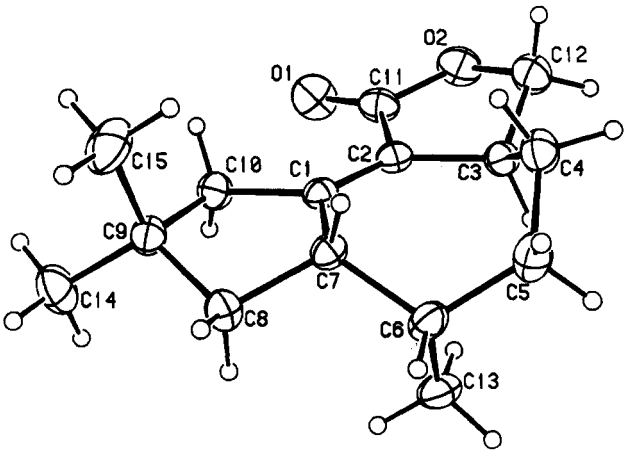


Figure 4. A computer generated drawing of the final X-ray model of tremulenolide A (6).

can be attributed to the rigid geometry of this molecule which is optimal for σ–π overlap.[14] The small *vicinal* coupling between H-7 and H-6 (J = 2 Hz, dihedral angle approaching 90°) indicates that H-6 is also β. In NOE

(14) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon: Oxford, 1969; p 338.

(15) Reference 14; pp 72–73.

experiments on tremulenolide A, the H-7 and H-6 protons exhibit 4.5% NOE, and H-3 shows a 10% NOE upon irradiation of the methyl at C-13. Additional NOEs have been observed between H-3 and H-12α (5.6%), and between H-10α and the methyl at δ 1.13 (8.7%). This latter NOE allows us to assign the chemical shifts of the *gem*-methyls at C-9.

Tremulenolide B (7). Tremulenolide B (7) was obtained as colorless needles after crystallization from hexanes. The HREIMS showed that it is isomeric with tremulenolide A (6). The IR spectrum reveals that the lactone carbonyl (1775 cm^{-1}) is not conjugated. The ^1H NMR spectrum shows the presence of a single olefinic proton (δ 5.50). This hydrogen exhibits long-range coupling (2.2 Hz) to H-7 (δ 3.16) showing that 7 is the β,γ-unsaturated lactone. Comparison of the ^1H and ^{13}C NMR data (Tables II and I, respectively) with those for tremulenolide A (6) confirms the relationship. The signal for the hydrogen at C-2 appears as a doublet (J = 9.5 Hz) at δ 3.63. This, however, does not allow the assignment of configuration at C-2, since both *cis* (dihedral angle ~0°) and *trans* (dihedral angle ~180°) couplings may be large.[16] The assignment of the *cis* stereochemistry is based on the assumption that tremulenolide B (7) has the same stereochemistry at C-2 as tremulenedial (8) where NOE experiments clearly reveal the *cis* relationship of H-2 and H-3 (see below).

Tremulenedial (8). The compound which eluted after tremulenolide B (7) was rather unstable as shown by its TLC behavior. The ^1H NMR spectrum of the crude material indicated the presence of a dialdehyde, and it was discovered that treatment of the crude compound with either methanol or benzyl alcohol in the presence of acid gave a diacetal which was amenable to purification. The structural studies were carried out on the dibenzyl derivative **9**, but is assumed that the natural product is the dialdehyde, tremulenedial (8).

The CIMS of the dibenzyl acetal 9 indicates a molecular weight of 432, consistent with the formula $C_{29}H_{36}O_3$. The highest peak in the HREIMS corresponds to $C_{22}H_{29}O_2$, reflecting the loss of a benzyloxy radical from the molecular ion. The cyclic acetal was apparent from the ^{13}C NMR spectrum which shows two dioxygenated carbons (δ 107.5, 107.9) each carrying a hydrogen. The ^1H NMR spectrum shows the alkenic H at δ 5.47 (long-range coupled to H-7, J = 2.2 Hz), and a readily analyzed spin system correlating H-11, H-2, H-3, and H-12. The signal for H-2, for example, appears as a double doublet (J = 8.0 and 5.2 Hz) and shows NOE to H-3 and H-11, establishing the *cis–cis* relationship. NOE enhancement is also observed between H-2 and the alkenic proton and between H-3 and H-12. The remaining connectivities were secured by selective decoupling, APT, HMQC, and HMBC experiments. Figure 5 shows the significant HMBC correlations observed for tremulenedial dibenzyl acetal (9).

Tremulenediols A (10) and B (11). Tremulenediols A and B were not separable by chromatography, both exhibiting the same R_f in several solvent systems. Crystallization from hexanes gave partial separation of tremulenediol A (10). However, the derived di-*p*-bromobenzoates (**10a** and **11a**) were separable by PTLC. The HREIMS of 10 indicated a formula $C_{15}H_{26}O_2$, whereas that of 11 shows the highest mass peak at *m/z* 220

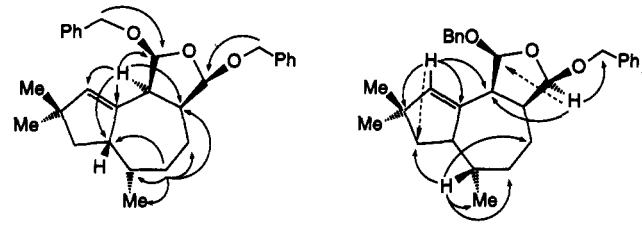


Figure 5. Significant HMBC correlations of tremulenedial dibenzyl acetal 9.

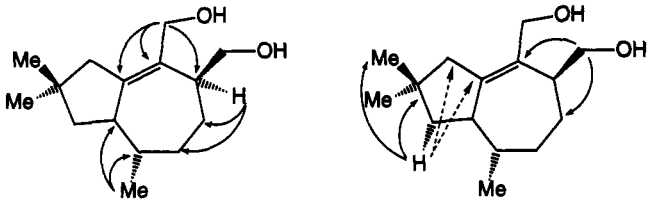


Figure 6. Significant HMBC correlations of tremulenediol A (10).

corresponding to the loss of water from the molecular ion. CIMS of the di-*p*-bromobenzoates confirmed the molecular formulas. The ^{13}C NMR spectra of 10 shows the presence of a fully substituted double bond, while that of 11 shows a trisubstituted double bond. The ^1H NMR of 11 shows the alkenic proton at δ 5.32, long-range coupled (2.5 Hz) to H-7. In tremulenediol A (10) the C-11 methylene protons appear as an AB quartet (δ 4.24, 3.83) which is not further coupled. HMBC correlation of the C-11 methylene hydrogens with C-1, C-2, and C-3 confirms the location of this primary alcohol function (Figure 6). The H's of the other primary alcoholic group show HMBC correlations with C-2, C-3, and C-4. In tremulenediol B (11), the nonequivalent H-11's are further split by coupling to the allylic H-2 (δ 2.85). The remaining connectivities were established by appropriate NMR techniques. The complete assignments of the NMR spectra are provided in Tables I and II. Confirmation of the structure and stereochemistry of tremulenediol A (10) was obtained by direct correlation with tremulenolide A (6). Deprotection of the di-*p*-bromobenzoate **10a** by hydrolysis under basic conditions[17] followed by oxidation with manganese dioxide[18] gave tremulenolide A (6).

The structure of tremulenediol B (11) was secured by its preparation from tremulenedial (8). The crude dialdehyde 8 was reduced with $NaBH_4$ to give diol 11, isolated as the di-*p*-bromobenzoate **11a**. Since it was possible that the crude dialdehyde 8 contained some of the diol 11 (they elute close together), the reduction was also carried out with $NaBD_4$. The ^1H and ^{13}C NMR spectra of the di-*p*-bromobenzoate obtained are consistent with the incorporation of one deuterium at both C-11 and C-12. The signals at δ 4.53 and δ 4.30 each integrate for a single hydrogen and the multiplicity of each is changed from a doublet to an apparent triplet due to additional *geminal* coupling with deuterium. The multiplicity of the signal for H-2 (δ 3.26) is changed from a triple doublet to a double doublet indicating *vicinal* coupling with a single hydrogen at C-11. In the ^{13}C NMR (APT) the resonances for C-11 and C-12 are shifted upfield (0.3 ppm) due to the isotope effect of deuterium, the phase is reversed, and the signals appear as triplets due to coupling with ^2H.

(16) Gaudemer, A. In *Stereochemistry: Fundamentals and Methods*; Kagan, H. B., Ed.; Georg Thieme: Stuttgart, 1977; Vol. 1, p 90.

(17) Mashimo, K.; Sato, Y. *Tetrahedron* **1970**, *26*, 803.

(18) Carlson, R. M.; White, L. L. *Synth. Commun.* **1983**, *13*, 237.

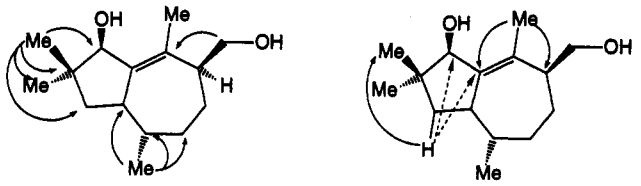


Figure 7. Significant HMBC correlations of tremulenediol C (12).

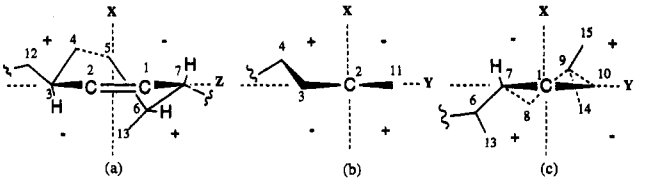


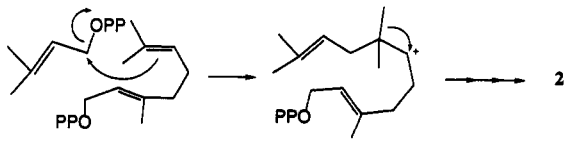
Figure 8. Olefin octant rule projections for tremulenediol A (10): (a) rear octants viewed along Y-axis; (b) front octants viewed along Z-axis from C-2 to C-1; (c) rear octants viewed along Z-axis from C-2 to C-1.

Tremulenediol C (12) and Tremuladienol (13). Tremulenediol C (12) was the last sesquiterpene to be eluted from the flash chromatogram. HREIMS showed it to be isomeric with diols A and B (10 and 11). The presence of an alkenic methyl group (δ 1.94) long-range coupled (2 Hz) to the methine proton (δ 4.03) of an allylic secondary alcohol suggested that this diol was the allylic rearrangement analog 12 of tremulenediol B (11). The proton at C-10 shows further long-range coupling (2 Hz) to the proton at C-7, similar to that shown by H-10α in tremulenolide A (6). Assuming that the five-membered ring in 12 is in the envelope conformation, this indicates the C-10 hydroxyl is β-oriented. The HMBC correlations summarized in Figure 7 support the structural assignment.

On standing in chloroform at room temperature for some days tremulenediol C (12) underwent dehydration to give the dienol 13, also isolated directly from the original chromatography. The transformation of 12 to 13 has been monitored by NMR where we observe the disappearance of the methyl signal at δ 1.94 with the concomitant appearance of the alkenic signals at δ 5.71, 5.22, and 4.80 characteristic of dienol 13. The ^1H and ^{13}C NMR spectra of 13 (Tables II and I, respectively) are consistent with the assigned structure. The UV spectrum shows conjugated diene absorption at 244 nm. The molecular formula has been verified by HREIMS.

Absolute Stereochemistry. The X-ray crystallographic study of 6 did not establish its absolute configuration and we have used CD techniques for this purpose. Tremulenolide A (6) exhibits a positive Cotton effect at 252 nm. However, the chirality-sign relationship of ene lactones is not straightforward to analyze, since it seems to be dependent on the ring size and the *cisoid/transoid* nature of the C=C—C=O system and a general quadrant rule cannot be applied.[19] Another approach is to reduce the ene lactone to the corresponding diol.[20] In this case, the chirality-sign relationship of allylic alcohols can be used and the sign of the Cotton effect can be predicted using the olefin octant rule.[21,22] Tremulenediol A (10) is the reduced equivalent of 6 and was used for this study. The CD spectrum of 10 shows a positive Cotton effect at 210 nm, which is associated with a right-handed helical arrangement of the C=C—C=O chromophore.[21] If an *S*-configuration is assigned to the chiral center at C-3 of 10 and the molecule is viewed along the *Y*-axis (Figure 8a) the heavily populated regions of the rear octants are in positive octants. The same is true when the molecule is viewed along the *Z*-axis from C-2 to C-1 (Figure 8b,c). If a 3*R*-configuration is assigned, the populated regions are in negative octants. This analysis suggests that the absolute stereochemistry of 10 is *S* at all three chiral centers (C-3, C-6, and C-7) by relating the relative configuration of the other asymmetric centers to C-3. Therefore, tremulenediol A has the absolute configuration shown in structure 10. The positive Cotton effect observed for tremulenolide A (6) is consistent with this absolute stereochemistry. Comparison of the CD spectrum of 6 (positive) with that of (−)-(3*R*)-hibalactone (14),[23] a model *cisoid* ene γ-lactone, shows that they have Cotton effects of opposite sign, indicating the opposite (and thus *S*) absolute stereochemistry at C-3 of 6. Extrapolation of the results to all the natural tremulanes indicates they possess the absolute stereochemistry indicated in the structural formulas 6-13.

Scheme I



Biogenesis. As mentioned in Introduction, the tremulane skeleton 2 appears to be a new sesquiterpene skeleton. It does not obey the classical isoprene rule and it cannot be derived in a straightforward manner by cyclization–rearrangement of farnesyl pyrophosphate (FPP), the usual precursor of sesquiterpenes in fungi.[6,24] Biosynthetic studies are currently underway in our laboratories, and preliminary results are consistent with a biogenetic pathway involving the head-to-tail addition of geranyl pyrophosphate (GPP) to dimethylallyl pyrophosphate (Scheme I) rather than the usual[25] tail-to-head addition of isopentenyl pyrophosphate to GPP which gives rise to FPP.[26]

Experimental Section

General Methods.[27] CD spectra were measured on a JASCO SS-20-2 spectropolarimeter and are reported in molar ellipticity [θ] units. HMQC and HMBC experiments were recorded on a Varian Unity 500 spectrometer. Long-range CH correlations were established using the HMBC experiment optimized for $^nJ_{CH}$ = 10 Hz. All NMR spectra were recorded in $CDCl_3$ solution. Universal Scientific Inc. silica gel 60 (40 μm) was used for flash chromatography. Preparative thin-layer chromatography (PTLC) was performed on E. Merck precoated 20 × 20 glass plates of silica gel 60 F-254. Hexanes refers to light petroleum (bp 60–68 °C). Molecular formulas of title compounds were established by HREIMS unless otherwise noted. The ^1H NMR spectra of all

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title compounds are provided as supplementary material. Small amounts of impurities are detected in the spectra of compounds 7 and 9–13.

Growth of *Phellinus tremulae* and Isolation of the Metabolites. Cultures of *P. tremulae* (strain NOF 1464) were obtained from Dr. Y. Hiratsuka, Forestry Canada, Northern Forestry Centre, Edmonton, and are deposited at the University of Alberta Microfungus Herbarium (UAMH 7005). Two 4-liter Erlenmeyer flasks, each containing 2 L of malt extract medium (40 g of malt extract broth DIFCO, 40 g of DIAION HP 20 resin,[8a] and 2 L of redistilled water) were inoculated with *ca.* 30 mL of a mycelial suspension of *P. tremulae* in water and were shaken at room temperature for 16 days. The culture broth was filtered and the combined resin and wet mycelium transferred into a sintered funnel and extracted with dichloromethane (3 × 200 mL). The organic extract was dried over $MgSO_4$ and the solvent evaporated under reduced pressure to afford 2.0 g of a crude organic extract (dark yellow oil), which was subjected to flash chromatography using a stepwise gradient of 0.5–5% (v/v) MeOH in CH_2Cl_2. Fractions of similar composition, as determined by TLC and ^1H NMR, were pooled (Figure 1). Further purification is described for the individual components.

Tremulenolide A (6): recrystallized from hexanes to give colorless crystals; mp 110–112 °C; $[\alpha]_D$ +110.7° (*c* 0.14, MeOH); R_f 0.4 (CH_2Cl_2); UV (MeOH) 236 nm (ϵ 8270); CD (0.0012 M, MeOH) $[\theta]_{252}$ +17820; IR 1753, 1673 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HMBC data, Figure 3; differential NOE, 14-Me to H-10α 8.7%, H-7 to H-6 4.5%, H-12α to H-3 5.6%, 13-Me to H-3 10%; HREIMS obsd 234.1616, calcd for $C_{15}H_{22}O_2$ 234.1613, base peak at *m/z* 219.1377 $[M - CH_3]^+$.

Tremulenolide B (7): recrystallized from hexanes to give colorless needles; R_f 0.4 (CH_2Cl_2); IR 1775 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HREIMS obsd 234.1619, calcd for $C_{15}H_{22}O_2$ 234.1618, base peak at *m/z* 219.1387 $[M - CH_3]^+$.

Tremulenedial dibenzyl acetal (9): the fractions eluted with 1.5% MeOH in CH_2Cl_2 exhibited an aldehydic signal at δ 9.72 twice as intense as the alkenic proton signal (δ 5.56). The unstable dialdehyde (8, 63 mg dissolved in 8 mL of $CHCl_3$) was isolated in the form of the diacetal by treatment with BnOH (78 mg dissolved in 2 mL of $CHCl_3$) and traces of TsOH, at room temperature. Flash chromatography of the crude reaction mixture (hexanes/CH_2Cl_2, 3:1) afforded **9** as a colorless oil: R_f 0.6 (hexanes/CH_2Cl_2, 1:1); IR 1454, 1358, 1094, 1075 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HMBC data, Figure 5; differential NOE, H-2 to H-3 11.4%, H-2 to H-10 14.3%, H-2 to H-11 3.4%, H-3 to H-12 4.1%; CIMS (NH_3) 450 $(MH + NH_3)^+$; HREIMS 325.2157 ($C_{22}H_{29}O_2$, $[M - OBn]^+$).

Tremulenediol A (10): recrystallized from hexanes to give colorless crystals; mp 95–97 °C; $[\alpha]_D$ +41.7° (*c* 0.24, MeOH); R_f 0.4 (benzene/acetone/AcOH, 75:25:1); UV (MeOH) 212 nm (ϵ 4350); CD (0.002 M, MeOH) $[\theta]_{210}$ +4620; IR 3310 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HMBC data, Figure 6; HREIMS obsd 238.1932, calcd for $C_{15}H_{26}O_2$ 238.1931.

Tremulenediol B (11): colorless oil, >85% yield of 11, but still showing NMR signals for **10**; R_f 0.4 (benzene/acetone/AcOH, 75:25:1); IR 3300 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HREIMS $[M - H_2O]^+$ obsd 208.1822, calcd for $C_{15}H_{24}O$ 208.1817.

***p*-Bromobenzoylation of 10 and 11**: the di-*p*-bromobenzoates of **10** (**10a**, 35 mg) and **11** (**11a**, 16 mg) were prepared by stirring a mixture of **10** and **11** (88 mg) dissolved in CH_2Cl_2 (2 mL) with a suspension of *p*-Br-BzCl (178 mg) in CH_2Cl_2 (2 mL) and pyridine (0.8 mL) for 1 h at room temperature. The residue was filtered off and the filtrate was evaporated to dryness. Most of the remaining *p*-Br-BzOH was removed by crystallization from hexanes. Separation of **10a** and **11a** was achieved by PTLC (hexanes/CH_2Cl_2, 1:1, developed several times).

Di-*p*-Bromobenzoyl derivative of 10 (10a): $[\alpha]_D$ +6° (*c* 0.84, MeOH); R_f 0.4 (CH_2Cl_2/hexanes, 1:1); UV (MeOH) 243 nm (ϵ 11400); IR 1718, 1590, 1268 cm^{-1}; ^1H NMR δ (integration, multiplicity, *J* in Hz) 7.89 (2H, d, 8), 7.86 (2H, d, 8), 7.56 (4H, d, 8), 4.82 (2H, d, 4), 4.56 (1H, d, 4), 4.54 (1H, bs), 3.16 (1H, bt, 8.5), 2.93 (1H, m), 2.35 (1H, dd, 15, 2), 2.04 (1H, bd, 15), 1.98 (1H, dt, 14, 2), 1.89 (1H, dt, 14, 2.5), 1.83 (1H, m), 1.75 (1H, dm, 14), 1.66 (1H, dm, 14), 1.57 (1H, bt, 10.5), 1.44 (1H, dd, 11), 1.09 (3H, s), 0.88 (3H, s), 0.86 (3H, d, 7); ^{13}C data, Table I; CIMS (NH_3) 624 (17.4%), 622 (35%), 620 (17.9%, $[C_{29}H_{32}O_4Br_2 + H + NH_3]^+$.

Di-*p*-Bromobenzoyl derivative of 11 (11a): R_f 0.4 (hexanes/CH_2Cl_2, 1:1); IR 1720, 1590, 1270 cm^{-1}; ^1H NMR δ (integration, multiplicity, *J* in Hz) 7.87 (2H, d, 8.5), 7.82 (2H, d, 8.5), 7.57 (4H, d, 8.5), 7.53 (2H, d, 8.5), 5.41 (1H, d, 2.5), 4.53 (2H, d, 7.5), 4.30 (2H, d, 7), 3.26 (1H, td, 8, 3.5), 3.08 (1H, m), 2.11 (1H, m), 2.04 (1H, bq, 7), 1.85 (1H, m), 1.66 (1H, dd, 12.5, 7), 1.49–1.34 (4H, dm, 14), 1.07 (3H, s), 0.98 (3H, s), 0.89 (3H, d, 7); ^{13}C data, Table I; CIMS (NH_3) 624 (23.4%), 622 (46.4%), 620 (26.1%, $[C_{29}H_{32}O_4Br_2 + H + NH_3]^+$.

Tremulenediol C (12): colorless oil; R_f 0.3 (benzene/acetone/AcOH, 75:25:1); IR 3340 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HMBC data, Figure 7; HREIMS obsd 238.1926, calcd for $C_{15}H_{26}O_2$ 238.1933.

Tremuladienol (13): pale yellow oil; this compound is also obtained when tremulenediol C (**12**) is kept in $CDCl_3$ solution for several days; R_f 0.3 (CH_2Cl_2); UV (MeOH) 244 nm (ϵ ~5000); IR 3410 cm^{-1}; ^{13}C and ^1H NMR data, Tables I and II, respectively; HREIMS obsd 220.1830, calcd for $C_{15}H_{24}O$ 220.1833.

Preparation of Tremulenolide A (6) from Tremulenediol A (10). A solution of **10a** (10 mg) in CH_2Cl_2 (0.5 mL) was added to a solution of 1% NaOH in MeOH (2 mL) and stirred for 1 h at rt.[17] The MeOH was evaporated under reduced pressure, and the residue was suspended in water and extracted with CH_2Cl_2. MnO_2 (35 mg) was added to the dry solution and the mixture was refluxed for 3 days.[18] The resulting suspension was filtered through anhydrous $MgSO_4$ and the solvent evaporated. The ^1H NMR spectrum of the crude product indicated a mixture of **6** and methyl *p*-bromobenzoate. PTLC (hexanes/CH_2Cl_2/MeOH, 50:50:1) of this mixture provided tremulenolide A (**6**) which showed the same R_f and ^1H NMR spectrum as the natural product.

Preparation of Tremulenediol B (11) from Tremulenedial (8). Dialdehyde **8** was dissolved in CH_2Cl_2, and $NaBH_4$ or $NaBD_4$ was added. Treatment of the resultant diol with *p*-bromobenzoyl chloride as above and purification by PTLC (hexanes/CH_2Cl_2, 1:1) provided **11a**, identical (R_f and ^1H NMR) with that previously described. The data for the deuterated diol are similar to those of **11a**, except for the signals at δ 4.53 (1H, t, 7.5), δ 4.30 (1H, t, 7.5), and δ 3.26 (1H, dd, 7.5, 3) in the ^1H NMR and the resonances for C-11 and C-12 in the ^{13}C NMR (APT). These are shifted upfield by 0.3 ppm, appear as triplets, and have the phase reversed because of the presence of deuterium.

Acknowledgment. We wish to thank the Natural Sciences and Engineering Research Council of Canada and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil) for financial support and Y. Hiratsuka and L. Hutchison, Forestry Canada, Northern Forestry Centre, Edmonton for cultures of *Phellinus tremulae* and for helpful advice. We also thank Drs. T. T. Nakashima and M. G. Pausler for assistance with NMR measurements and Mr. J. Olekszyk for the mass spectra.

Supplementary Material Available: 400-MHz ^1H NMR spectra of **6**, **7**, **9**, **10**, **10a**, **11**, **11a**, **12**, and **13** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.