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TRITERPENES FROM DOUGLAS FIR SAPWOOD

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Key Word Index—*Pseudotsuga menziesii*; Pinaceae; Douglas fir; triterpenes; extractives; tall oil.

Abstract—The saponified ether-soluble extractives of Douglas fir sapwood contained (24*R*)-4α,14α,24-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol (24*R*-cycloeucalanol), a new natural product; 4α,14α-dimethyl-9β,19-cyclo-24-methylene-5α-cholestan-3β-ol (cycloeucalenol); and (24*R*)-4α,24-dimethyl-5α-cholest-7-en-3β-ol (24*R*-methyllophenol); this is the first time they have been reported from Douglas fir.

INTRODUCTION

Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] is a major conifer species used by many kraft mills in western United States and Canada for producing pulp. Tall oil, a commercially important chemical by-product of kraft pulping, originates from extractable components of wood. While investigating Douglas fir extractives and their relation to tall oil obtainable from wood [1], an unidentified triterpene alcohol (*ca* 3–4% of the Et_2O extract) was isolated from the non-saponifiable fraction. Further investigation has shown this material is a mixture of three triterpenes: 24*R*-cycloeucalanol [(24*R*)-4α,14α,24-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol] (**1a**), the major constituent, and cycloeucalenol [4α,14α-dimethyl-9β,19-cyclo-24-methylene-5α-cholestan-3β-ol] (**2a**) and 24*R*-methyllophenol [(24*R*)-4α,24-dimethyl-5α-cholest-7-en-3β-ol] (**3a**), both minor constituents. This is the first reported occurrence of cycloeucalanol as a natural product, and of cycloeucalenol and 24-methyllophenol from Douglas fir.

RESULTS AND DISCUSSION

24*R*-Cycloeucalanol (**1a**), cycloeucalenol (**2a**) and 24*R*-methyllophenol (**3a**) were isolated and characterized as their respective acetates (**1b**, **2b** and **3b**). 24*R,S*-Cycloeucalanyl acetate has been previously synthesized by hydrogenation of cycloeucalenyl acetate [2]. This is the first reported occurrence of 24*R*-cycloeucalanyl acetate as a pure diastereoisomer and of 24*R*-cycloeucalanol as a natural product. Cycloeucalenol has been isolated from numerous natural sources, and is a postulated intermediate in the biosynthesis of phytosterols from squalene [3]. 24ξ-Methyllophenol has been isolated from *Larix decidua* leaves [4], from *Sorghum vulgare* grain [5, 6], *Digitalis pupurea* seeds [7], avocado oil [8] and grapefruit peel [9]; however, in each isolation the proof of structure was based solely on GC/MS data. This is the first report of cycloeucalenol and 24*R*-methyllophenol as natural products from Douglas fir.

24R-*Cycloeucalanyl acetate* (**1b**)

The molecular formula of this compound is $C_{32}H_{54}O_2$ as determined by high-resolution MS. The MS fragmentation pattern (Fig. 1) is consistent with the proposed structure and with MS data reported for compounds of this type [10]. The ions at *m/z* 343 [M^+ − side chain (C_9H_{19})], *m/z* 302 and *m/z* 175 [*m/z* 302 − side chain (C_9H_{19})] support the presence of the C(24)-Me. The ^{1}H NMR (Table 1) is also consistent with the proposed structure (**1b**). From the position of the 9β,19-cyclopropane AB Hs, the methyl at C(4) must have the 4α-configuration [11]. The C(24)-Me is confirmed by the magnetic nonequivalence of the isopropyl methyls [C(26) and C(27)] in the ^{1}H NMR because of the asymmetric center at C(24) [12]. The chemical shifts for the C(26), C(27) and C(28) protons are the same as those reported for similar compounds having the 24*R*-configuration [13].

Optical rotation measurements also establish the stereochemistry of cycloeucalanyl acetate as 24*R*. The molecular rotation ($[M]_D$) of a 24*R*-Me compound is *ca* 29° more dextrorotatory than is the corresponding 24-H compound; the 24*S*-Me compound is about 29° more levorotatory [14]. The $[M]_D$ of cycloeucalanyl acetate (**1b**) is +333°; the $[M]_D$ of 31-norcycloartanyl acetate (**4a**), the corresponding 24-H compound, is +301° [15]. Thus, the $[M]_D$ of cycloeucalanyl acetate is about 32° more dextrorotatory than is the 31-norcycloartanyl acetate, as expected for the 24*R*-Me configuration.

The identity of this compound was further confirmed by comparison with 24*R,S*-cycloeucalanyl acetate (**4b**) obtained by hydrogenation of cycloeucalenyl acetate (**2b**). The two materials were identical by TLC and GLC. In addition, the ^{1}H NMR spectra were identical; however, the relative intensities of various peaks in the methyl region of the spectra (*ca* δ 0.75–1.00) were not the same. This difference is expected because 24*R,S*-cycloeucalanyl acetate is a mixture of C(24)-epimers in which the chemical shifts of the C(26), C(27), C(28) and possibly the C(21) protons, are slightly different because of the asymmetric center at C(24).

Cycloeucalenyl acetate (**2b**)

The compound isolated from Douglas fir was identical to authentic cycloeucalenyl acetate by mp, $[\alpha]_D$, NMR, IR, TLC, GLC and MS.

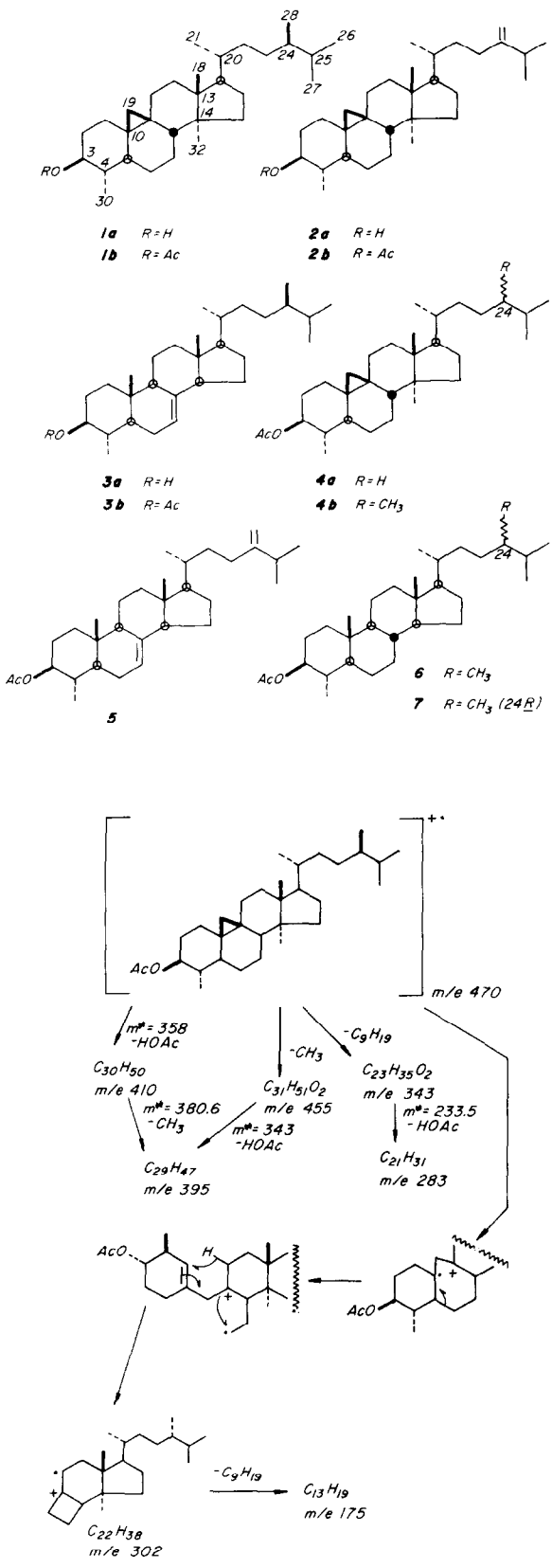

Fig. 1. Mass spectral EI fragmentation of 24*R*-cycloeucalanyl acetate.

24R-*Methyllophenyl acetate* (**3b**)

The high-resolution MS indicated a molecular formula of $C_{31}H_{52}O_2$. The MS (Fig. 2) contained peaks with the approximate relative intensity as those reported for 24ξ-methyllophenyl acetate [5]. The peaks at *m/z* 329, 269, and 161 indicate a C(24)-Me in the side chain. The low intensity of the *m/z* 288 peak, formed by retro-Diels–Alder from M^+, is expected for the 5α-Δ^7 configuration [16].

The ^{1}H NMR data are also consistent with the proposed structure (Table 1). The chemical shifts of the =CH at δ 5.21 and of the C(18) protons at δ 0.53 are fully consistent with a Δ^7 double bond. The chemical shifts for the C(26), C(27), and C(28) protons are the same as those reported for similar compounds having the 24*R* configuration [13].

Biogenetically, the stereochemistry at C(24) should be the same as that for 24*R*-cycloeucalanyl acetate. Because this compound was obtained only in about 90% purity, the optical rotation must be interpreted with caution to establish the stereochemistry at C(24). The $[M]_D$ is about 52° more dextrorotatory than that of lophenyl acetate (**5**) [17]; this difference is comparable to the 41° difference between cholest-7-en-3β-yl acetate ($[M]_D$ + 18°) and ergost-7-en-3β-yl acetate (24*S*-Me; $[M]_D$ −23°) [18], but is larger than the 29° expected. However, the direction of the $\Delta[M]_D$ is consistent with the 24*R* configuration.

The identity of this compound was further confirmed by comparing its hydrogenation product [24*R*-methyllophanyl acetate (**7**)] with 24*R*,*S*-methyllophanyl acetate (**6**) obtained by hydrogenation of 24-methylenelophenyl acetate (**5**). The 24*R*- and 24*R*,*S*-methyllophanyl acetates were identical by TLC and GLC. In addition, the ^{1}H NMR spectra were identical; however, the relative intensities of various peaks in the methyl region between *ca* δ 0.75–1.00 were not the same. This difference is expected because 24*R*,*S*-methyllophanyl acetate is a mixture of the C(24)-epimers.

EXPERIMENTAL

Mps were measured in evacuated capillaries and are corrected. ^{1}H NMR spectra were determined in $CHCl_3$. GLCs (Table 2) were obtained with a 10-m glass capillary coated with SP 2100 operated at 250°. TLCs were obtained on Si gel impregnated with 20% $AgNO_3$ using petrol (PE)–Et_2O (90:10).

Initial isolation of triterpenes. Chips of Douglas fir sapwood (*ca* 1.5 kg) were ground in a Wiley Mill, and the resulting milled wood continuously extracted with Et_2O in a Soxhlet apparatus for 1 week. The Et_2O soln was concd, extracted with dil. NaOH to remove the acidic components, evapd to dryness to yield the neutrals, and saponified by refluxing with KOH in EtOH for 4 hr. The EtOH was evapd and the residue taken up in Et_2O and extracted with dilute NaOH to remove any acidic components. The Et_2O was evapd to yield 1.3 g of non-saponifiable neutrals.

The non-saponifiables were chromatographed over Si gel with toluene and toluene–Et_2O mixtures. Toluene–Et_2O (95:5 and 90:10) eluted a mixture of 24-methylenecycloartanol and cycloartenol identified by TLC, GLC and ^{1}H NMR (60 MHz). Further elution gave a mixture of cycloeucalanol, cycloeucalenol and 24-methyllophenol, followed by a mixture of campesterol, stigmastanol and sitosterol. The center portion of the fractions containing cycloeucalanol, cycloeucalenol and 24-methyllophenol (127 mg) was acetylated with Ac_2O–pyridine. The mixed acetates were chromatographed over Si gel–20% $AgNO_3$. PE and PE–Et_2O (99:1 and 99:2) eluted 79 mg of a mixture of cycloeucalanyl acetate and 24-methyllophenyl acetate. PE–Et_2O (95:5) eluted 35 mg of impure cycloeucalenyl acetate.

Table 1. ^{1}H NMR spectral data of Douglas fir triterpenes*

Proton	24*R*-Cycloeucalanyl acetate (**1b**)	Cycloeucalenyl acetate (**2b**)	24*R*-Methyllophenyl acetate(**3b**)	24*R*-Methyllophanyl acetate (**7**)
C(3)—H	4.50, 1 *H*, *m*	4.50, 1 H, *m*	4.43, 1 H, *m*	4.46, 1 H, *m*
C(3)—O—C(=O)—CH$_3$	2.04, 3 H, *s*	2.04, 3 H, *s*	2.04, 3 H, *s*	2.04, 3 H, *s*
C(4α)—CH$_3$	0.88, 3 H, *d*, J = 8 Hz	0.89, 3 H, *d*, J = 7 Hz	0.92, 3 H, *d*, J = 7 Hz	0.92, 3 H, *d*, J = 7 Hz
C(7)—H			5.21, 1 H, *br. d*, J = 5 Hz	
C(14)—CH$_3$	0.90, 3 H, *s*	0.90, 3 H, *s*	—	—
C(18)—H$_3$	0.96, 3 H, *s*	0.96, 3 H, *s*	0.53, 3 H, *s*	0.71, 3 H, *s*
C(19)—H	0.27, 2 H, AB*dd* (δ_A = 0.15, δ_B = 0.40) J = 4 Hz	0.27, 2 H, AB*dd* (δ_A = 0.14, δ_B = 0.39) J = 4 Hz	0.83, 3 H, *s*	0.82, 3 H, *s*
C(21)—H$_3$	0.85, 3 H, *d*, J = 7 Hz	0.83, 3 H, *d*, J = 7 Hz	0.85, 3 H, *d*, J = 7 Hz	0.84, 3 H, *d*, J = 7 Hz
C(26)—H$_3$	0.80, 3 H, *d*, J = 7 Hz	1.015, 3 H, *d*, J = 7 Hz	0.80, 3 H, *d*, J = 7 Hz	0.80, 3 H, *d*, J = 7 Hz
C(27)—H$_3$	0.84, 3 H, *d*, J = 7 Hz	1.020, 3 H, *d*, J = 7 Hz	0.84, 3 H, *d*, J = 7 Hz	0.84, 3 H, *d*, J = 7 Hz
C(28)—H$_3$	0.78, 3 H, *d*, J = 7 Hz	4.66, 1 H, *s* 4.71, 1 H, *s*	0.78, 3 H, *d*, J = 7 Hz	0.77, 3 H, *d*, J = 7 Hz

*At 270 MHz; results given in δ (ppm).

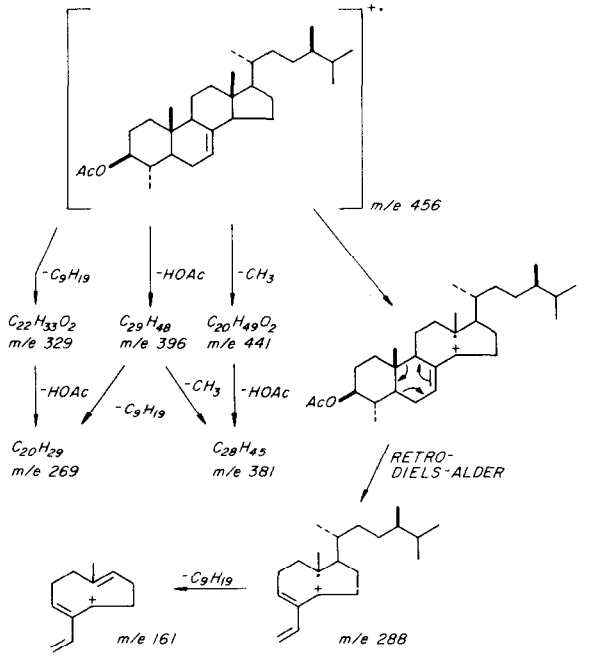

Fig. 2. Mass spectral EI fragmentation of 24*R*-methyllophenyl acetate.

24R-*Cycloeucalanyl acetate* (**1b**). The mixture of cycloeucalanyl acetate and 24-methyllophenyl acetate was rechromatographed over 40 g Si gel–20% $AgNO_3$. PE–Et_2O (98:2) eluted 51 mg of cycloeucalanyl acetate that was crystallized from MeOH: mp 107.5–108°, $[\alpha]_D^{25}$ +70.9° (*c* 0.9). Reported for 24*R*,*S*-cycloeucalanyl acetate [2]: mp 112–113°, $[\alpha]_D$ +62°. This material was homogeneous by TLC and GLC. ν_{max}^{KBr} cm^{-1}: 1735 and 1252 (acetate). NMR—see Table 1. MS (probe, 175°, 60 eV) *m/z* (rel. int.): 470 (10; M^+; $C_{32}H_{54}O_2$), 455 (10; $C_{31}H_{52}O_2$), 411 (31), 410 (100; $C_{30}H_{50}$), 396 (22; $C_{29}H_{48}$), 395 (76; $C_{29}H_{47}$), 380.9 (M*, 410 → 395), 358 (M*; 470 → 410), 355 (14; $C_{26}H_{43}$), 343 (M*; 455 → 395), 302 (18; $C_{22}H_{38}$), 283 (28; $C_{21}H_{31}$), 233.5 (M*; 343 → 283), 220 (13; $C_{16}H_{28}$), 189 (18; $C_{14}H_{21}$), 188 (15; $C_{14}H_{20}$), 175 (29; $C_{13}H_{19}$), 173 (16; $C_{13}H_{17}$), 163 (22; $C_{12}H_{19}$), 161 (24; $C_{12}H_{17}$), 149 (15; $C_{11}H_{17}$), 148 (11; $C_{11}H_{16}$), 147 (24; $C_{11}H_{15}$), 145 (14; $C_{11}H_{13}$), 137 (11; $C_{10}H_{17}$), 136 (16; $C_{10}H_{16}$), 135 (28; $C_{10}H_{15}$), 134 (16; $C_{10}H_{14}$), 133 (25; $C_{10}H_{13}$), 131 (12; $C_{10}H_{11}$), 123 (19; C_9H_5), 121 (35; C_9H_{13}), 120 (21; C_9H_{12}), 119 (29; C_9H_{11}), 109 (39; C_8H_{13}), 108 (21; C_8H_{12}), 107 (41; C_8H_{11}), 105 (26). M^+ *m/z* 470.4099. Required for $C_{32}H_{54}O_2$, M^+ *m/z* 470.4122.

24R-*Methyllophenyl acetate* (**3b**). Continued elution of the previous column with PE–Et_2O (98:2) gave 7 mg of a compound that was 80% pure by GLC. This material was cryst. from methanol to give 3 mg of 24*R*-methyllophenyl acetate (+90% purity): mp 135–135.5°, $[\alpha]_D^{25}$ +38.6° (*c* 0.3). NMR—see Table 1. MS (probe, 220°, 70 eV) *m/z* (rel. int.): 456 (100; M^+, $C_{31}H_{52}O_2$), 441 (20; M^+ – Me, $C_{30}H_{49}O_2$); 396 (15; M^+ – HOAc, $C_{29}H_{48}$), 381 (19; M^+ – Me – HOAc, $C_{28}H_{45}$), 329 (8; M^+ – C_9H_{19}), 288 (4; $C_{21}H_{36}$), 287 (9; $C_{18}H_{39}O_2$), 270 (14; $C_{20}H_{30}$), 269 (67, M^+ – HOAc – C_9H_{19}), 243 (23; $C_{18}H_{27}$), 227 (41; $C_{17}H_{23}$), 161 (20; $C_{12}H_{17}$). M^+ *m/z* 456.3943. Required for $C_{31}H_{52}O_2$ M^+ *m/z* 456.3966. Reported MS [5] *m/z* (rel. int.): 456 (100), 381 (25), 329 (7), 287 (9), 269 (90), 227 (45).

Cycloeucalenyl acetate (**2b**). The impure sample of cycloeucalenyl acetate was chromatographed over Si gel-20% $AgNO_3$. PE–Et_2O (90:10) eluted 20 mg of cycloeucalenyl acetate that was cryst. from methanol: mp 108–109°, $[\alpha]_D^{25}$ +62.2° (*c* 0.9). Reported: mp 110°, $[\alpha]_D$ +63° [2]; mp 105–109°, $[\alpha]_D$ +61.6° [19]. NMR—see Table 1. This compound was identical to authentic cycloeucalenyl acetate by NMR, IR, TLC and GLC. MS (probe, 200°, 70 eV) *m/z* (rel. int.): 468 (8; M^+), 453 (9; M^+ – Me), 425 (4), 409 (36), 408 (100; M^+ – HOAc), 394 (27), 393 (85), 365 (7), 353 (12), 325 (8), 324 (5), 300 (24), 283 (22), 281 (15). Reported MS *m/z* (rel. int.) [19]: 468 (12), 453 (9), 425 (5), 408 (100), 393 (52), 365 (5), 353 (9), 325 (7), 324 (5), 300 (12), 283 (14), 281 (10).

Table 2. GLC of Douglas fir triterpenes

Compound	*RRT**
24*R*-Methyllophanyl acetate (**7**)	0.92
Sitosteryl acetate	1.00
24*R*-Methyllophenyl acetate (**3b**)	1.03
Cycloeucalenyl acetate (**2b**)	1.05
24*R*-Cycloeucalanyl acetate (**1b**)	1.07

**RRT*, relative retention time compared to sitosteryl acetate.

24R,S-*Cycloeucalanyl acetate* (**4b**) *from cycloeucalenyl acetate* (**2b**). Cycloeucalenyl acetate (14 mg) in dimethoxyethane (20 ml) was hydrogenated over 10% Pd–C (20 mg) for 2 hr at room temp. The reaction mixture was filtered through a small bed of Si gel with Et_2O washings. The solvent was evapd *in vacuo* to yield 24*R,S*-cycloeucalanyl acetate (13 mg). This material was chromatographed over Si gel. PE–Et_2O (95:5) eluted chromatographically pure material identical with 24*R*-cycloeucalanyl acetate by TLC and GLC. This compound was cryst. from MeOH: mp 103–104°, $[\alpha]_D^{24}$ +66° (*c* 0.5). Reported [2]: mp 112–113°, $[\alpha]_D$ +62°.

24R-*Methyllophanyl acetate* (**7**) *from* 24R-*methyllophenyl acetate* (**3b**). 24*R*-Methyllophenyl acetate (2 mg) in dimethoxyethane (20 ml) was hydrogenated over 10% Pd–C (15 mg) for 3 hr at room temp. The reaction mixture was filtered through a small bed of Si gel with Et_2O washings. The Et_2O was evapd to give 24*R*-methyllophanyl acetate (**7**) (2 mg) that was chromatographed over Si gel. PE–Et_2O (98:2) eluted 2 mg of material: $[\alpha]_D^{23}$ +46° (*c* 0.1). NMR—see Table 1.

24R,S-*Methyllophanyl acetate* (**6**) *from* 24-*methylenelophenyl acetate* (**5**). 24-Methylenelophenyl acetate [20] (14 mg) in dimethoxyethane (20 ml) was hydrogenated over 10% Pd–C (10 mg) at room temp. for 1.5 hr. The reaction mixture was filtered through a small bed of Si gel with Et_2O washings.

The Et_2O was evapd to give 24*R,S*-methyllophanyl acetate (14 mg). This material was chromatographed over Si gel. PE–Et_2O (98:2) eluted 8 mg of a compound that was cryst. from MeOH: mp 106–107°, $[\alpha]_D^{24}$ +36° (*c* 0.4). This compound was identical to 24*R*-methyllophanyl acetate by TLC and GLC.

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