

SESQUITERPENOIDS FROM *PILGERODENDRON UVÍFERA*

M. LUISA OYARZÚN and JUAN A. GARBARINO

Departamento de Química, Facultad de Ciencia, Universidad Federico Santa María, Casilla 110-V, Valparaíso, Chile

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Abstract—A new sesquiterpenoid, 15-copaenol, and the known compounds (–)-*trans*-calamenene, (+)- δ -cadinene, (–)-caryophyllene-4,5-epoxide, (–)-humulene-1,2-epoxide, (–)-cubenol, (–)-epicubenol and (–)-torreyol has been isolated from the heartwood of *Pilgerodendron wifera*. The structures of the new and the known compounds were established by spectroscopic methods.

INTRODUCTION

Pilgerodendron wifera (D. Don) Florin [1] (Cupressaceae), commonly known as 'ciprés de las Guaitecas', is an endemic tree which grows abundantly in the Chiloé, Guaitecas and Chonos archipelagos, Chile. Its trunk wood is very resistant to rotting and is used for naval constructions, wharfs, etc [1]. No previous phytochemical work on this species has been published. We now report the isolation and structure elucidation of a new copaene sesquiterpenoid, 15-copaenol (**1a**), and the known (–)-*trans*-calamenene (**2**), (+)- δ -cadinene (**3**), (–)-caryophyllene-4,5-epoxide (**4**), (–)-humulene-1,2-epoxide (**5**), (–)-cubenol (**6**), (–)-*epi*-cubenol (**7**) and (–)-torreyol (**8**) present in *P. wifera*. Compounds **4** and **5** are also naturally co-occurring in *Zingiber zerumbet* [2]. Recently studies on biological activity of terpenoids from *Melampodium divaricatum* have been made [3] which showed that **4** is a potent ant-repellent (leafcutter ant *Atta Cephalotes*). This sesquiterpenoid is also present in *Hymenea coubaril* [4] and in *Dacrydium cupressinum* [5].

RESULTS AND DISCUSSIONS

The essential oil of the heartwood was obtained as described in the Experimental. It was chromatographed on a silica gel column, using increasing proportions of ethyl acetate in petrol as solvent, to afford some sesquiterpene-enriched fractions. Repeated chromatography on silica gel impregnated with silver nitrate led to the isolation of seven known sesquiterpenoids (**2–8**) and the new compound 15-copaenol (**1a**). The known compounds were identified by comparing their spectroscopic data with those reported in the literature. The previously unreported ^{13}C NMR spectra of these sesquiterpenes are listed in the Experimental. The new sesquiterpenoid **1a**, $[\alpha]_{\text{D}}^{25} -28.6^\circ$ (CHCl_3), was isolated as a colourless oil. The mass spectrum of **1a** suggested a molecular formula $\text{C}_{15}\text{H}_{24}\text{O}$ (molecular ion at m/z 220). A fragment at m/z 177 $[\text{M}-43]^+$ corresponded to the loss of an isopropyl group. Its IR spectrum contained absorption bands for hydroxyl (3400 cm^{-1}) and double bond groups (1655 cm^{-1}). The ^1H NMR spectrum of **1a** showed the presence of a broad singlet at δ 3.95 (2H) which corresponds to a primary alcohol, and a broad singlet at 5.48

(1H) was assigned to an olefinic proton of a trisubstituted double bond. In addition, the ^1H NMR spectrum showed a singlet at δ 0.80 (3H) which corresponds to an angular methyl group and two methyl doublets centered at 0.85 and 0.84 (3H each, $J=6.5\text{ Hz}$) were assigned to the isopropyl group. The molecular formula, $\text{C}_{15}\text{H}_{24}\text{O}$, suggested that **1a** had three rings with only one double bond. Among known sesquiterpenoid carbon skeletons only the copaene or ylangene types appeared to accommodate the structural elements indicated above [6]. The ^{13}C NMR spectrum corroborated this conclusion (Table 1). Examination of the ^{13}C NMR data confirmed the existence of a primary alcohol (δ 66.08, *t*, C-15) and a trisubstituted double bond (118.00, *d*, C-4 and 148.00, *s*, C-3), it also showed three methyls (C-12, C-13 and C-14), three methylenes (C-5, C-8 and C-9), five methines (C-1,

Table 1 ^{13}C NMR spectra of compounds **1a** and **1b** (90 MHz, CDCl_3 , TMS as int. standard)

C	1a	1b
1	44.54 <i>d</i>	44.42 <i>d</i>
2	50.41 <i>d</i>	50.62 <i>d</i>
3	148.00 <i>s</i>	142.51 <i>s</i>
4	118.00 <i>d</i>	121.26 <i>d</i>
5	36.40 <i>t</i>	36.04 <i>t</i>
6	44.54 <i>d</i>	44.58 <i>d</i>
7	39.37 <i>s</i>	39.42 <i>s</i>
8	29.89 <i>t</i>	30.06 <i>t</i>
9	21.73 <i>t</i>	21.68 <i>t</i>
10	37.34 <i>d</i>	37.18 <i>d</i>
11	32.20 <i>d</i>	32.15 <i>d</i>
12	19.60 <i>q</i> *	19.43 <i>q</i> *
13	19.70 <i>q</i> *	19.63 <i>q</i> *
14	20.00 <i>q</i> *	19.91 <i>q</i> *
15	66.08 <i>t</i>	66.99 <i>t</i>
MeCO		20.94 <i>q</i>
MeCO		170.92 <i>s</i>

*Assignments may be interchanged

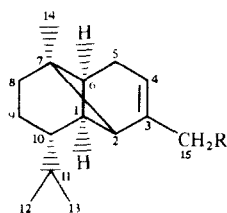
C-2, C-6, C-10 and C-11) and one quaternary carbon (C-7). Acetylation of **1a** gave a colourless oily acetate **1b**, the IR spectrum of which showed no hydroxyl absorption. The ^{13}C NMR spectrum of **1b** (Table 1) provided information allowing the localization of the primary alcohol. Comparison of the ^{13}C NMR spectrum of **1a** with that of **1b** indicated that the signal due to C-4 of **1b** was shifted to lower field (δ 121.26) and the one of C-3 to upper field (142.51). From the above data, it was deduced that the position of the primary alcohol was next to the double bond. On the other hand, the mass spectrum of **1b** was very similar to that of ylangenyl acetate (**9**) present in *Wunderlichia mirabilis* [7]. Comparison of the ^1H NMR spectrum of **1b** with that of **9**, indicated that the only difference between both signals was due to the methyls of the isopropyl group. The signals of **1b** were shifted up-field compared to those of **9**. Therefore, the compound **1a** is shown to be 15-copaenol.

EXPERIMENTAL

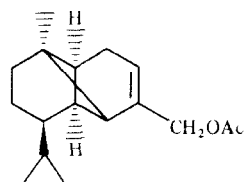
Mps uncorr., ^1H NMR and ^{13}C NMR spectra were recorded at 360 and 90 MHz (CDCl_3 soln and TMS as int. standard), IR film on NaCl or KBr pellets, MS direct inlet, 70 eV, silica gel 60 (70–230 mesh) was used for CC.

The wood of *P. uifera* was collected on the main island of Chiloe archipelago, in December 1985. A voucher specimen has been deposited at Universidad Federico Santa Maria. The heartwood (500 g) was dried finely powdered and subjected to steam dist. for 20 min at atm. pres. The distillates were extracted $\times 3$ with 100 ml of CHCl_3 , the extract was dried (Na_2SO_4) and coned. It was obtained as a colourless oil (5.5 g). This extract was chromatographed on a silica gel column (200 g) and eluted with mixtures of petrol and EtOAc of increasing polarity. The homogeneous fractions were repeatedly chromatographed on a 10% AgNO_3 silica gel column, yielding the following compounds in order of elution: (–)-*trans*-calamenene (**2**, 120 mg), (+)- δ -cadinene (**3**, 80 mg), (–)-caryophyllene-4,5-epoxide (**4**, 180 mg), (–)-humulene-1,2-epoxide (**5**, 70 mg), (–)-cubenol (**6**, 150 mg), (–)-epicubenol (**7**, 200 mg), (–)-torreyol (**8**, 280 mg) and 15-copaenol (**1a**, 170 mg).

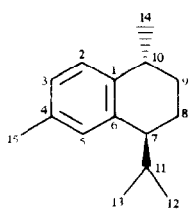
15-Copaenol (1a) Colourless oil, $[\alpha]_D^{25} -28.6$ (CHCl_3 , c 0.2), IR $\nu_{\text{max}}^{\text{KBr}}$ 3400, 1655, 1450, 1380, 1360, 1165, 1125, 1055, 1000, 800, ^1H NMR δ 0.80 (3H, s, H-14), 0.84 (3H, d, $J=6.8$ Hz, H-13), 0.86 (3H, d, $J=6.8$ Hz, H-12), 1.53 (1H, m, H-11), 1.70 (1H, br s, H-1), 2.26 (2H, br s, H-5), 3.98 (2H, br s, H-15), 5.48 (1H, br s, H-4), ^{13}C NMR see Table 1. MS m/z (rel. int.): 220 $[\text{M}]^+$ (18), 202 $[\text{M}-\text{H}_2\text{O}]^+$ (9), 177 $[\text{M}-\text{CH}(\text{Me})_2]^+$ (84), 159 $[\text{M}-\text{CH}(\text{Me})_2-\text{H}_2\text{O}]^+$ (45), 149 (20), 147 (31), 136 (37), 135 (95), 131 (23), 121 (40), 119 (27), 117 (33), 107 (30), 105 (75), 93 (75), 92



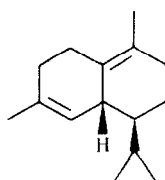
1a R = OH
1b R = OAc



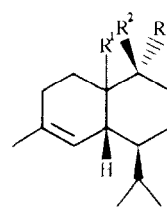
9



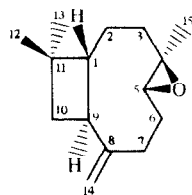
2



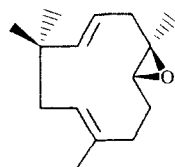
3



	R ¹	R ²	R ³
6	OH- α	H	Me
7	OH- β	H	Me
8	H- β	Me	OH



4



5

(33), 91 [C_7H_7]⁺ (100), 81 (35), 79 (49), 77 (31), 69 (22), 67 (28), 55 (41), 43 (30), 41 (52)

15-Copaenyl acetate (1b) Compound **1a** (80 mg) was treated with Ac_2O (3.0 ml) and pyridine (0.5 ml) at room temp for 24 hr. After addition of EtOH, the reaction mixture was evapd to dryness and the only residue was chromatographed on a 10% $AgNO_3$ -silica gel column, pure 15-copaenyl acetate (**1b**, 45 mg) was obtained as colourless oil, $[\alpha]_D^{25} -19.5^\circ$ ($CHCl_3$, c 0.20) IR ν_{max}^{KBr} cm^{-1} 1740, 1650, 1450, 1380, 1360, 1250, 1020 1H NMR δ 0.79 (3H, s, H-14), 0.83 (3H, d, $J=6.5$ Hz, H-13), 0.86 (3H, d, $J=6.5$ Hz, H-12), 1.55 (1H, m, H-11), 1.70 (1H, br s, H-2), 2.28 (2H, br s, H-5), 4.50 (1H, ddd, $J=1.5, 3.5, 12$ Hz, H-15), 4.46 (1H, ddd, $J=1.5, 3.5, 12$ Hz, H-15), 5.56 (1H, m, H-4), ^{13}C NMR see Table 1 Ms m/z (rel int) 262 [M]⁺ (3), 202 [$M-HOAc$]⁺ (30), 187 [$M-HOAc-Me$]⁺ (13), 159 [$M-HOAc-CH(Me)_2$]⁺ (100), 131 (32), 128 (21), 119 (27), 118 (30), 117 (40), 115 (26), 93 (27), 91 [C_7H_7]⁺ (75), 79 (33), 77 (40), 65 (21), 55 (34), 45 (35)

trans-Calamenene (2) Colourless oil, $[\alpha]_D^{25} -91^\circ$ ($CHCl_3$, c 0.15) (lit $[\alpha]_D^{25} -96^\circ$ [8]) 1H NMR δ 0.71 (3H, d, $J=6.7$ Hz, H-13), 1.00 (3H, d, $J=6.7$ Hz, H-12), 1.26 (3H, d, $J=7.0$ Hz, H-14), 2.30 (3H, s, H-15), 6.95 (1H, d, $J=7.5$ Hz, H-2), 7.02 (1H, br s, H-4) 7.13 (1H, d, $J=7.5$ Hz, H-1), ^{13}C NMR δ 140.14 (s, C-6 or C-1), 139.97 (s, C-6 or C-1), 134.52 (s, C-4), 128.81 (d, C-5), 126.85 (d, C-2), 126.24 (d, C-3), 43.87 (d, C-7), 32.57 (d, C-11), 31.97 (d, C-10), 30.12 (t, C-9), 22.38 (q, C-14), 21.55 (t, C-8), 21.35 (q, C-15), 21.35 (q, C-13), 17.42 (q, C-12) The 1H NMR spectrum of **2** was identical to that reported for *trans*-calamenene [8]

δ -Cadinene (3) Colourless oil, $[\alpha]_D^{25} = +75^\circ$ ($CHCl_3$, c 0.15) (lit $[\alpha]_D^{25} +93^\circ$ [9]) ^{13}C NMR δ 134.30 (s, C-4), 130.05 (s, C-10), 124.83 (d, C-5), 124.29 (s, C-1), 45.48 (d, C-7), 39.57 (d, C-6), 39.57 (d, C-6), 32.46 (t, C-9), 32.09 (t, C-2), 26.90 (t, C-3), 26.85 (d, C-11), 23.86 (q, C-13), 21.86 (q, C-12), 21.32 (t, C-8), 18.62 (q, C-14), 15.79 (q, C-15). The 1H NMR spectrum of **3** was identical to that reported for δ -cadinene [9]

Caryophyllene-4,5-epoxide (4) Mp 61–62° (lit 62° [5]) The IR [2], 1H NMR [10] and ^{13}C NMR [5, 11] spectra of **4** were identical to those reported for caryophyllene-4,5-epoxide.

Humulene-1,2-epoxide (5) Colourless oil $[\alpha]_D^{25} -29.0^\circ$ ($CHCl_3$, c 0.15) (lit $[\alpha]_D^{25} -31.2^\circ$ [12]) ^{13}C NMR δ 143.17 (d, C-1), 131.98 (s, C-8), 125.79 (d, C-2), 122.17 (d, C-9), 63.28 (s, C-4), 62.00 (d, C-5), 42.65 (t, C-10), 40.03 (t, C-3), 36.69 (t, C-6), 36.56 (s, C-11), 29.07 (q, C-12), 25.61 (q, C-13), 24.81 (t, C-7), 17.27 (q, C-15), 15.14 (q, C-14). The IR and 1H NMR spectra of **5** were identical to those reported for humulene-1,2-epoxide [12]

Cubenol (6) Colourless oil, $[\alpha]_D^{25} -28^\circ$ ($CHCl_3$, c 0.12) (lit $[\alpha]_D^{25} -33^\circ$ [13]) ^{13}C NMR δ 135.11 (s, C-4), 119.84 (d, C-5), 70.69 (s, C-1), 46.05 (d, C-10), 39.88 (d, C-6), 39.82 (d, C-7), 32.11 (t, C-9), 30.21 (t, C-2), 26.85 (t, C-8), 25.94 (d, C-11), 24.14 (t, C-3), 23.68 (q, C-13), 21.38 (q, C-12), 15.04 (q, C-15), 14.91 (q, C-14). The IR and 1H NMR spectra of **6** were identical to those reported for cubenol [14, 15]

Epicubenol (7) Colourless oil $[\alpha]_D^{25} -96^\circ$ ($CHCl_3$, c 0.12) (lit $[\alpha]_D^{25} -96^\circ$ [13]) ^{13}C NMR: δ 133.87 (s, C-4), 122.20 (d, C-5), 72.72 (s, C-1), 49.29 (d, C-10), 48.11 (d, C-6), 41.95 (d, C-7), 31.19 (t, C-9), 26.94 (d, C-11), 26.74 (t, C-2), 24.08 (t, C-8), 23.55 (q, C-13), 22.06 (t, C-3), 21.66 (q, C-12), 15.23 (q, C-14), 15.23 (q, C-15) The IR and 1H NMR spectra of **7** were identical to those reported for epicubenol [14, 15]. The ^{13}C NMR spectrum of **7** was identical to that reported for *ent*-epicubenol [16]

Torreyol (8) Mp 138–139°, $[\alpha]_D^{25} -98^\circ$ ($CHCl_3$, c 0.20) (lit $[\alpha]_D^{25} -100.4^\circ$ [17]) ^{13}C NMR δ 134.43 (s, C-4), 124.69 (d, C-5), 72.59 (s, C-10), 45.62 (d, C-1), 44.16 (d, C-7), 36.85 (d, C-6), 35.39 (t, C-9), 31.20 (t, C-2), 28.02 (q, C-14), 26.47 (d, C-11), 23.68 (q, C-13), 21.73 (q, C-12), 21.60 (t, C-3), 18.58 (t, C-8), 15.38 (q, C-15) The IR and 1H NMR spectra of **8** were identical to those reported for torreyol [17]

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