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New Eudesmane and Eremophilane Derivatives from *Laggera Alata*

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Abstract: From the aerial part of *Laggera alata*, a novel eremophilanoid (**1)** as well as two new eudesmanoids (**2-3**) were isolated. Their structures were elucidated by 2D-NMR technique and X-ray diffraction studies. The cytotoxic activities of these sesquiterpenes were also investigated.

Keywords: *Laggera alata*, sesquiterpene, eremophilanoid, eudesmanoid, X-ray diffraction, cytotoxicity.

Laggera pterodonta and Laggera *alata* are the only two species of *Laggera* genus found in China. Both of them are used as traditional herbal medicines in southwestern China. Previous investigations of *L. pterodonta* have led to the isolation of 20 new eudesmane derivatives including some cytotoxic ones[1,2]. These interesting findings have prompted us to a phytochemical examination of *L. alata.* Three new compounds, along with 14 known compounds were isolated from the aerial part of the title plant.

Compound **1** was isolated as colorless needles, $[\alpha]_D^{25}$ - 83.3 (*c* 0.28, MeOH). Its HREIMS exhibited a $[M]^+$ at *m/z* 248.141 (calcd. 248.1412) corresponding to a molecular formula $C_{15}H_{20}O_3$. Its IR spectrum (KBr) revealed the presence of an allylic acid moiety (1707 cm^{-1})[3]. ^{13}C-NMR indicated that it should contain a α,β-unsaturated ketone (δ 201.9, 146.3, 126.0). ^{1}H-NMR featured it as an eremophilanoid compound[7]: Me-14 (δ1.16, s, 3H), Me-15 (δ1.05, d, 3H, J=6.0 Hz). Apart from the exomethylene signals observed at δ 6.26 (br s, 1H) and 5.70 ppm (br s, 1H), another singlet appeared at δ 5.91 suggesting the presence of a trisubstituted olefin conjugated to a carbonyl group. Based on the above information, the presence of a 1(10)-en-2-one moiety in **1** was deduced. HMBC also revealed that the ketone carbonyl was on C-2 and the olefin carbons were on C-1 and C-10. The stereochemistry of H-7 was presumed to be axial from the coupling constant (δ2.64, dddd, 1H, J=12.0, 9.0, 4.5, 4.5 Hz). The

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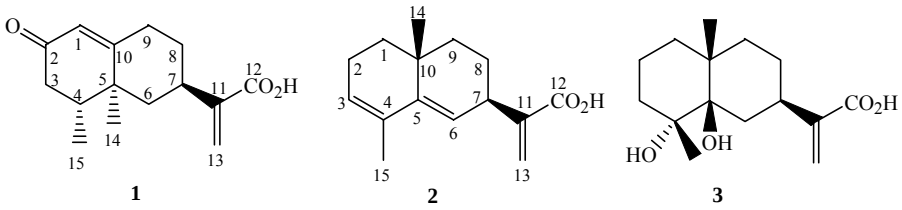
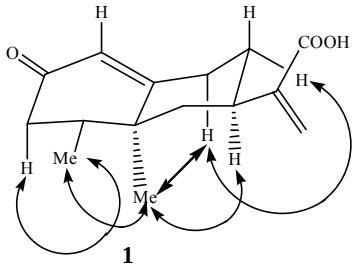
stereochemistry of Me-14 and Me-15 were deduced from the NOESY spectrum, from which clear correlations between H-14 and H-15; between H-14 and H-7α; as well as those between H-3α and H-15 could be observed (**Figure 2**). This was further supported by the CD spectrum that showed a positive Cotton effect at 245 nm and a negative one at 332 nm[4]. The structure of **1** was finally ascertained by X-ray diffraction analysis (**Figure 3**).

Figure 1 The structures of compounds **1-3**



Compound **2** was obtained as a colorless gum, $[\alpha]_D^{25}$ + 7.5 (*c* 0.4, $CHCl_3$). Its molecular formula was determined as $C_{15}H_{20}O_2$ by the fact that the HREIMS exhibited a $[M]^+$ at *m/z* 232.1459 (calcd. 232.1463). The ^1H and ^{13}C-NMR spectra of **2** showed close similarity to those of 11-cinnamoylloxyl-3,5-dien-eudesmane (**2a**)[5]. However, the tertiary methyl signals of Me-12 and Me-13 in **2a** were absent in the ^1H and ^{13}C-NMR spectra of **2**. Instead, signals of a methylene group was observed at δ 5.72 and 6.34 ppm, suggesting that the isopropyl group in **2a** was replaced by an allylic acid moiety in **2**. This was supported by the IR absorption band of **2** at 1693 cm^{-1}, and was further verified by the correlation peaks appearing at the 2D HMQC and HMBC experiments. Since no correlation between H-14 and H-7 was observed in the 2D NOESY spectrum of **2,** the configuration of H-7 should be of the α-orientation. Therefore, compound **2** was identified as 3,5,11(13)-trien-eudesma-12-oic acid.

Figure 2 Selective 2D-NOESY correlations of compounds **1**

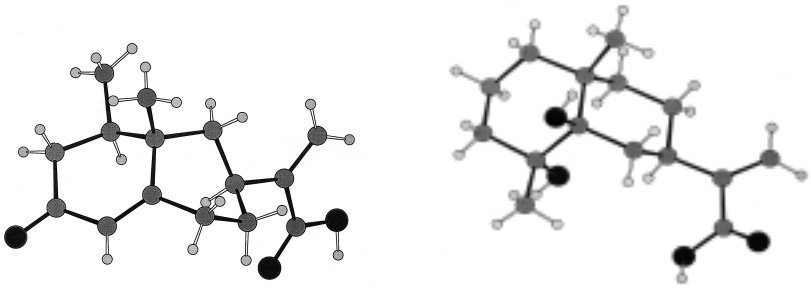


Compound **3** was isolated as colorless needles, $[\alpha]_D^{25}$ + 5.39 (*c* 0.15, MeOH). The ^1H and ^{13}C-NMR spectra of **3** bore close resemblance to those of ilicic acid[6,7]. However, the methine carbon signal of C-5 (δ 55.8) in ilicic acid did not appear in the ^{13}C-NMR spectrum of **3.** Instead, an oxygenated quaternary carbon resonance exhibited at δ 76.8. In addition, the C-4 and C-6 of **3** were downfield shifted when comparing with those of

ilicic acid[6,7]. These indicated that **3** was a 5-OH derivative of ilicic acid, consistent with the presence of six methylene signals in the DEPT spectrum of **3**. There was no correlation between H-14 and H-15 in the NOESY spectrum of **3,** suggesting that the A/B ring in the molecular structure of **3** was *cis*-fused. The stereochemistry of H-7 was presumed to be axial from the coupling constants (δ3.49, dddd, J=12.5, 12.5, 4.5, 4.5 Hz). Therefore, the structure of **3** was identified as 5β-hydroxyilicic acid. This was finally confirmed by X-ray diffraction analysis (**Figure 3**).

Cytotoxicity tests were conducted on KB cells. All the three new compounds exhibited some cytotoxic effects with $IC_{50}>10^{-4}$ μM.

Figure 3 X-ray structures of compounds **1** and **3**



1 **3**

Acknowledgments

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7. ^{13}C-NMR spectral data of compounds **1-3**. (**1**): C-1 – C-15: 126.0, 201.9, 42.9, 37.3, 41.7, 30.0, 33.8, 30.3, 40.7, 146.3, 170.2, 177.6, 123.6, 19.3, 15.7; (**2**): C-1 – C-15: 37.1, 22.8, 124.9, 131.0, 143.2, 121.6, 38.5, 26.3, 38.2, 31.3, 145.5, 126.0, 172.6, 23.4, 20.1; (**3**): C-1 – C-15: 38.8, 18.2, 38.7, 76.5, 76.8, 36.7, 38.3, 27.6, 35.2, 38.8, 148.4, 171.2, 122.4, 25.6, 26.8. ^{1}H-NMR spectral data of compounds **1-3**. (**1**): 5.94 (br s, 1H, H-1); 2.34 m, H-3; 2.40 m, H-3'; 2.36 m, H-4; 2.41 m, H-6α; 1.98 m, H-6β; 2.63 (dddd 1H J=11.0, 11.0, 4.5, 4.5Hz, H-7α);

2.00 m, H-8; 2.76 (dddd 1H J=13.5, 11.0, 11.0, 4.5Hz, H-8'); 1.70 (ddd 1H J=13.5, 11.0, 4.5Hz, H-9α); 1.92 (ddd 1H J=13.5, 4.5, 4.5Hz, H-9β); 6.26 (br s, 1H H-13); 5.70 (br s, 1H H-13'); 1.16 s, H-14; 1.05 d (6.0), H-15. (**2**): 1.60 m, H-1α; 2.05 m, H-1β; 2.06 ddd (12.5, 4.5, 4.5), H-2α; 2.64 ddd (12.5, 11.0, 4.5), H-2β; 5.56 br s, H-3; 5.39 br s, H-6; 3.42 ddd (10.0, 7.5, 3.0), H-7α; 1.44 m, H-8α; 1.40 m, H-8β; 1.54 m, H-9α; 1.56 m, H-9β; 6.34 br s, H-13; 5.70 br s, H-13'; 1.00 s, H-14; 1.79 s, H-15. (**3**): 1.02 ddd (13.5, 4.8, 4.8), H-1α; 1.76 ddd (13.5, 11.0, 4.8), H-1β; 1.90 (dddd 1H J=13.5, 12.0, 7.0, 4.5Hz, H-2α); 1.68 (dddd 1H J=13.5, 7.0, 4.5, 4.5Hz, H-2β); 1.38 d(dd 1H J=13.5, 6.8, 4.5Hz); 2.68 (ddd 1H J=13.5, 12.0, 6.8Hz, H-3β); 2.06 (dd 1H J=13.5, 4.5Hz, H-6α); 1.48 (dd J=13.5, 12.0Hz, H-6β); 3.49 (dddd 1H J=12.0, 12.0, 4.5, 4.5Hz, H-7α); 1.18 m, H-8α; 1.72 m, H-8β; 1.70 (ddd 1H J=13.2, 9.8, 3.5Hz, H-9α); 1.28 (ddd 1H, J=13.2, 3.5, 3.0Hz, H-9β); 6.10 (br s, H-13); 5.56 (br s, H-13'); 0.99 s, H-14; 1.26 s, H-15.

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