New Eudesmane and Eremophilane Derivatives from *Laggera Alata*

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Abstract: From the aerial part of *Laggera alata*, a novel eremophilanoloid (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, eremophilanoloid, 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 ones1,2. 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, [α]25D - 83.3 (c 0.28, MeOH). Its HREIMS exhibited a [M]+ at m/z 248.141 (calcd. 248.1412) corresponding to a molecular formula C15H20O3. Its IR spectrum (KBr) revealed the presence of an allylic acid moiety (1707 cm–1)3. 13C-NMR indicated that it should contain a α,β-unsaturated ketone (δ 201.9, 146.3, 126.0). 1H-NMR featured it as an eremophilanoloid compound7: 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. 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, CHCl₃). Its molecular formula was determined as C₁₅H₂₀O₂ by the fact that the HREIMS exhibited a [M]+ at m/z 232.1459 (calcd. 232.1463). The ¹H and ¹³C-NMR spectra of 2 showed close similarity to those of 11-cinnamoylloxyl-3,5-dien-eudesmane (2a). However, the tertiary methyl signals of Me-12 and Me-13 in 2a were absent in the ¹H and ¹³C-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 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 ¹³C-NMR spectra of 3 bore close resemblance to those of ilicic acid. However, the methine carbon signal of C-5 (δ 55.8) in ilicic acid did not appear in the ¹³C-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
illicic acid\(^6\). These indicated that 3 was a 5-OH derivative of illicic 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 (83.49, dddd, J=12.5, 12.5, 4.5, 4.5 Hz). Therefore, the structure of 3 was identified as \(\beta\)-hydroxyillicic 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\(^{-4}\) \(\mu\)M.

Figure 3  X-ray structures of compounds 1 and 3

Acknowledgments

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References
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.
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-6\(^\alpha\); 1.98 m, H-6\(^\beta\); 2.63 (dddd 1H J=11.0, 11.0, 4.5, 4.5Hz, H-7\(^a\));
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 (6.0), 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 (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 (ddd 1H J=13.5, 12.0, 7.0, 4.5Hz, H-2α); 1.68 (ddd 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 (ddd 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 (dd 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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