Eudesmane Derivatives and Other Sesquiterpenes from Laggera alata

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Three new eudesmane sesquiterpenes, 5α-hydroxyilicic acid (1), 5α-hydroxy-4-epi-illicic acid methyl ester (2), and 3α-hydroxyilicic acid (3), together with 12 known sesquiterpenes were isolated from the aerial part of Laggera alata. Their structures were elucidated primarily by NMR and mass spectroscopic methods. The structures of 1 and 2 were confirmed by X-ray crystallography.

Laggera (Compositae, tribe Inulea, subtribe Inulinea) is a small genus of about 20 species. Laggera alata (D. Don) Sch.-Bip. Ex. Olivier and Laggera pterodonta are the only two species of Laggera found in China. Previously, L. alata was reported to contain several new eudesmane sesquiterpenes.1,2 L. alata distributed in Madagascar and Namibia has also been shown to contain some characteristic eudesmane derivatives.3,4 However, no chemical study has been published on L. alata grown in China. This prompted us to investigate the title plant that is used traditionally in southwestern China as an herbal medicine. Investigation of the EtOH extract of the title plant led to the isolation of a number of compounds including three new eudesmanoids, 5α-hydroxyilic acid (1), 5α-hydroxy-4-epi-illicic acid methyl ester (2), and 3α-hydroxyilicic acid (3); a known eremophilane derivative, tessaric acid (4);5,6 and 11 eudesmane derivatives, 3,5,11(13)-trien-12-oxo-16-carboxylic acid,15 eudesma-4(14),11(13)-dien-12,5-carboxylic acid.16 The details of the isolation and structural elucidation of 1–3 are discussed in this paper.

![Figure 1. Selected HMBC correlations for compounds 1, 3, 4, and 5.](image)

Results and Discussion

The HREIMS spectrum of 1 exhibited its [M]+ at m/z 268.1684, corresponding to the molecular formula C15H24O4.

![Diagram](image)

Figure 1. Selected HMBC correlations for compounds 1, 3, 4, and 5.

Its IR spectrum exhibited bands at 3571 and 3523 cm−1 (hydroxyl), as well as a methylene conjugated with a carboxylic group at 1667 cm−1. The EIMS of 1 exhibited fragments at m/z 250 [M−H2O]+ and m/z 232 [M−2×H2O]+. Two oxygenated quaternary carbon signals at δ 76.4 and 76.8 in the 13C NMR spectrum indicated the presence of two tertiary hydroxyl groups. The 13C NMR spectrum of 1 disclosed a carboxyl signal at δ 170.2 and an olefinic methylene carbon signal at δ 122.4, indicating the presence of an allylic acid moiety. The above-mentioned 1H and 13C NMR spectra of 1 showed a close similarity to those of illicic acid.16 However, the methine carbon signal at δ 55.8 of C-5 in illicic acid was absent in the 13C NMR spectrum of 1. Instead, a quaternary carbon signal appearing at δ 76.8 was observed. This suggested that 1 was a 5-hydroxyl derivative of illicic acid. This conclusion was confirmed by detailed HMBC and HMBC experiments (Figure 1). The relative configuration of 1 was derived from NOESY correlations of H-14/H-6′, H-14/H-8′, and H-8/H-13 (Figure 2). These were consistent with the observed coupling constants, viz., J 6-7 = 4.5 Hz, J 6′-7′ = 12.0 Hz, J 7-8 = 4.5 Hz, and J 7-8′ = 12.0 Hz. Further evidence for the structure of 1 was subsequently obtained from the X-ray crystallography results (Figure 3), which showed a cis-fused A/B ring system with a chair–chair conformation. The crystal
study further showed an intermolecular H-bond between the hydroxyl group of 1 and the water molecule cocrystallized.

The $^1$H and $^{13}$C NMR spectral data of compound 2 showed close resemblance to those of 1 (see Experimental Section). However, a methoxy group was present in 2, as disclosed by signals appearing at $\delta$ 3.77 (3H, s) and at $\delta$

52.3 in the $^1$H and $^{13}$C NMR spectra of 2, respectively. The presence of a methoxy group was also supported by an IR absorption band at 1697 cm$^{-1}$. The HREIMS of 2 exhibited a molecular ion peak at m/z 282.1836 (calcld 282.1831), corresponding to a molecular formula of C$_{15}$H$_{24}$O$_4$ (14 mass units higher than that of 1), suggesting that 2 was a methyl ester of 1. The X-ray single-crystal diffraction experiment showed that 2 was 5a-hydroxy-4-epi-ilicic acid methyl ester and the A/B rings of 2 were found to be trans-fused (Figure 3). Only a few plants, such as Inula viscosa,$^{17}$ have been found containing 4-epi-eudesmane derivatives.

The HREIMS of 3 indicated a molecular formula of C$_{15}$H$_{24}$O$_4$. The observation of mass fragments due to [M – H$_2$O]$^+$ and [M – 2 x H$_2$O]$^+$ appearing at m/z 250 and 232, respectively, indicated the presence of at least two hydroxyl groups. This was supported by the IR spectrum of 3, which showed absorption bands at 3458 and 3469 cm$^{-1}$. The latter also suggested the presence of a conjugated carboxyl functionality, supported by the $^{13}$C NMR resonances at $\delta$ 170.5 (C=O), 147.6 (C), and 122.8 (CH$_2$) in the $^{13}$C NMR spectrum of 3. In the $^1$H NMR spectrum of 3, two tertiary methyl signals appeared at $\delta$ 1.03 and 1.13, suggesting that 3 was also an eudesmane derivative, especially in view of the strong similarity of the $^1$H NMR data of 3 with those of 3a-acetylilic acid (3a).$^{13}$ However, the oxymethylene proton (H-3) appearing at $\delta$ 4.75 as well as the acetyl signal at $\delta$ 2.12 in 3a-acetylilic acid were absent in the $^1$H NMR spectrum of 3. Instead, the H-3 signal of 3 appeared at $\delta$ 4.02. All the above suggested that 3 was most likely 3-hydroxyilicic acid. The $\beta$-orientation of H-3 was deduced from the coupling constants ($J_{2,3} = J_{2,4} = 4.5$ Hz) and was confirmed by 2D experiments including HMBC and NOESY (Figure 1) and NOESY (Figure 2). Additional evidence was provided by acetylation of 3; the $^1$H NMR and [$\alpha$]$_D$ data of acetylated 3 were in good accordance with those of 3a-acetylilic acid.$^{18}$ Therefore, 3 was identified as 3a-hydroxyilic acid, which is an epimer of 3/3'-hydroxyillic acid isolated from the Jordanian medicinal plant Inula viscosa.$^{17}$

Compound 4 was assigned as tessaric acid. This known sesquiterpene was identified by its IR, HREIMS, 1D and 2D NMR, [$\alpha$]$_D$, CD, and X-ray single-crystal diffraction (Figure 3). This is the first eremophilane compound reported from the genus Laggera.

Compound 5 was reported as a synthetic artifact from bromination of ilicic acid.$^{7}$ Compound 6 was obtained by biotransformation of ilicic acid using a cell culture of Cunninghamella echinulata.$^{8}$ To our knowledge, both 5 and 6 have not been isolated previously from a natural source.

It is notable that most eudesmane derivatives isolated from L. alata collected in China have a characteristic allylic moiety, and all of them have the 7a-H-orientation. However, the reported eudesmanoids obtained from Madagascar or Namibia possess no carboxylic group, and some possess the 7'/H-orientation. This difference could be of interest to plant taxonomists. The different environmental conditions under which the plant grows in China, however, could also explain this subtle phytochemical difference.

Compounds 1, 2, 3, 5, and 6 exhibited weak cytotoxicity against KB cells with IC$_{50}$ values larger than 10$^{-4}$ mol/L. Ilicic acid also exhibited an inhibition ratio of 23.6% to the SK-MEL cell line and of 10.3% to the A-549 cell line, respectively, at the concentration of 20 $\mu$g/mL.

**Experimental Section**

**General Experimental Procedures.** Melting points were recorded on a Kofler hot-stage instrument and were uncorrected. Optical rotations were determined on a Perkin-Elmer
NMR spectra were obtained on a Bruker AM-400 FT-NMR spectrometer with TMS as an internal standard. Preparative TLC was performed using silica gel GF 254 and RP-18 plates (Merck). Crystals were mounted on an Enraf-Nonius Kappa-CCD diffractometer. A full sphere of data was collected by ϕ axis rotation with an increment of 2° over 360° and 120 s of 1 (40 s of 2 and 4) exposure per degree. “Denzingering” was accomplished by measuring each frame twice. Data were analyzed using Kappa-CCD software. Cell dimensions were determined with H-atom placement, and data reduction, was performed with DENZO and SCALEPACK, and data processing was carried out with Denzo. The structure was solved by direct methods (SHELXS-86) and was refined on F^2 for all reflections by least-squares methods using SHELXL-93.

**Plant Material.** The whole plant of Laggera alata (Compositae) was collected in November 1994 at Queibe county, Yunnan province, China, and identified by Prof. Zhong-wen Lin. A voucher specimen (941102) is on deposit at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** The air-dried aerial parts of the plant (5.8 kg, dry weight) were powdered and extracted with MeOH (5.8 kg, dry weight) were extracted with petroleum ether containing gradually increasing polarity (20:1 – 1:0) followed by silica gel column chromatography again eluted with CHCl3–H2O (95:5) to give 62 mg of 1 and 53 mg of 2. Fraction 14 was separated on a Sephadex LH-20 column eluted with CHCl3–MeOH (1:1) followed by preparative TLC developed with CHCl3–MeOH (10:1) to give 48 mg of 4. All the known compounds were identified by comparing their physical and spectroscopic properties (mp, MS, IR, NMR, and 1H and 13C NMR) with literature values, and some were compared directly with authentic samples.

5/6-Hydroxyxilic acid (1): colorless needles (Me2CO), mp 160–161.5 °C; [a]_D^20 +5.39° (c 0.8, MeOH), IR νmax 3571, 3523, 3447, 2962, 2914, 2858, 2498, 1985, 1667, 1623, 1443, 1405 cm⁻¹; ¹H NMR (CD3OD, 400 Hz) δ 1.02 (1H, ddd, J 13.5, 4.8, 8.7 Hz, 1-β), 1.76 (1H, ddd, J = 13.5, 11.0, 4.8 Hz, H-β/), 1.90 (1H, ddd, δ = 13.5, 12.0, 7.0, 4.5 Hz, H-2α), 1.68 (1H, ddd, J = 13.5, 7.0, 4.5, 4.5 Hz, H-2β), 2.48 (1H, ddd, J = 13.5, 12.0, 12.0, 4.5 Hz, H-6), 3.50 (1H, ddd, J = 12.0, 12.0, 4.5, 4.5 Hz, H-7a), 1.18 (1H, m, H-8α), 1.72 (1H, m, H-8β), 1.70 (1H, ddd, J = 13.2, 9.8, 3.5 Hz, H-9α), 1.28 (1H, ddd, J = 13.2, 3.5, 3.0 Hz, H-9β), 6.10 (1H, br s, H-13), 5.56 (1H, br s, H-13), 0.99 (3H, s, H-14), 1.26 (3H, s, H-15), 19C NMR (CD3OD, 100 MHz) δ 171.1 (s, C-12), 148.4 (s, C-11), 122.4 (t, C-13), 76.8 (s, C-5), 76.4 (s, C-4), 38.8 (t, C-1), 38.8 (s, C-10), 38.3 (d, C-7), 37.2 (t, C-3), 36.7 (t, C-6), 35.2 (t, C-9), 27.6 (t, C-8), 26.8 (q, C-15), 25.6 (q, C-14), 18.2 (t, C-2); EIMS m/z 268 [M]+ (11), 250 (44), 232 (66), 204 (25), 192 (60), 180 (59), 111 (100), 84 (89), 71 (73), 55 (75); HREIMS m/z 268.1684 (calcd for C15H24O4, 268.1675).

**X-ray Crystallographic Analysis of 1.** Crystal data: C15H24O4•H2O, mol wt = 286.36, monoclinic, space group P21/c, a = 7.222(3) Å, b = 7.801(3) Å, c = 13.797(6) Å, β = 97.125(5) °, Z = 2, λ = 0.7107 Å. The asymmetric unit consisted of one molecule of 1 and one molecule of water cocrystallized.

5/6-Hydroxyxilic acid methyl ester (2): colorless needles (MeOH), mp 120–121 °C; [α]_D^20 +13.4° (c 0.49, MeOH); IR νmax 3483, 2942, 2864, 1697, 1620, 1440, 1380; 1H NMR (CD3OD) δ 1.02 (1H, m, H-1x), 1.98 (1H, m, H-1y), 2.00 (1H, m, H-2x), 1.92 (1H, m, H-2y), 1.37 (1H, m, H-3x), 1.98 (1H, m, H-3y), 1.92 (1H, m, H-6x), 1.59 (1H, m, H-6y), 3.03 (1H, m, H-7a), 1.59 (1H, m, H-8x), 1.68 (1H, m, H-8β), 0.99 (1H, m, H-9x), 1.40 (1H, m, H-9y), 6.17 (1H, br s, H-13), 5.68 (1H, br s, H-13), 1.16 (1H, s, H-14), 1.29 (1H, s, H-15), 3.77 (3H, s, CO2CH3); 13C NMR (CD3OD, 100 MHz) δ 169.5 (s, C-12), 147.5 (s, C-11), 123.6 (t, C-13), 77.1 (s, C-5), 75.8 (s, C-4), 52.3 (q, OCH3), 38.7 (t, C-1), 37.9 (s, C-10), 36.9 (t, C-3), 36.0 (d, C-7), 35.6 (t, C-6), 31.9 (t, C-9), 28.0 (t, C-8), 25.8 (q, C-15), 22.8 (q, C-14), 18.2 (t, C-2); EIMS m/z 282 [M]+ (36), 264 (34), 246 (26), 232 (60), 205 (56), 111 (100), 84 (79), 55 (80); HREIMS m/z 282.1837 (calcd for C15H23O5, 282.1837).

**X-ray Crystallographic Analysis of 2.** Crystal data: C15H23O5•H2O, mol wt = 282.37, orthorhombic, space group P212121, a = 8.937(4) Å, b = 9.455(4) Å, c = 19.139(7) Å, V = 1617.2(12) Å³, Z = 4, λ = 0.7107 Å. The asymmetric unit consists of one molecule of 2 and one molecule of water cocrystallized.

3α-Hydroxyxilic acid (3): colorless needles, mp 177–178 °C; [α]_D^20 –48° (c 0.3, CHCl3); IR νmax 3540, 3458, 3456, 2925, 2350, 1710, 1615 cm⁻¹; 1H NMR (CD3OD, 400 MHz) δ 1.38 (1H, m, H-1x), 1.18 (1H, m, H-1y), 1.60 (2H, ddd, J = 12.5, 12.5, 5.5 Hz, H-2x), 1.25 (1H, m, H-5x), 0.65 (1H, m, H-5y), 2.16 (1H, dd, J = 12.5, 4.0 Hz, H-5z), 1.00 (1H, m, H-8x), 1.50 (1H, m, H-8y), 0.75 (1H, H-9x), 5.61 (1H, br s, H-13), 5.56 (1H, br s, H-13), 1.03 (3H, s, H-14), 1.39 (3H, s, H-15); 13C NMR (MeOH, 100 MHz) δ 170.5 (s,
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Supporting Information Available: The X-ray datasets of compounds 1, 2, and 4 are available free of charge via the Internet at http://pubs.acs.org. In addition, another 13 known compounds have also been isolated from the title plant. Although they are not reported in this paper, detailed information on the isolation procedures of all the components including these 13 known compounds is also available free of charge at http://pubs.acs.org for reference.

References and Notes

(19) See further details of X-ray studies in the Supporting Information or Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk)