DOI 10.5012/bkcs.2011.32.4.1368

Lupane-type Triterpenoids from the Leaves of *Heteropanax fragrans*

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Received December 7, 2010, Accepted February 8, 2011

Key Words : *Heteropanax fragrans*, Lupane-type triterpenoid, Cytotoxic activity, KB cell

Lupane-type triterpenoids, a very large and interesting family of compounds, are widely distributed in food, herbs, and other plants throughout the world. This class of compounds possess a wide range of biological properties including cytotoxic,[1] antitumor,[2] antiviral,[3] anti-inflammatory effects[4] and the ability to induce apoptosis in the most prevalent cancer cell lines, such as HepG2, Hep3B and MCF7 cells.[2,5] The betulinic acid, one of the known lupane-type triterpenoids, showed the selective cytotoxic activity toward melanoma cell lines and is being examined in Phase I trials as a cancer chemoprevention agent.[6] For their various pharmacological and medicinal properties, these pentacyclic triterpenes of the lupane-type have recently been investigated by the scientific community.

Heteropanax fragrans (Roxb.) Seem (Araliaceae), an ornamental plant and important source of herb, is distributed in the southern China. The rhizomes of *H. fragrans* have been used for acesodyne, hemostasia and detumescence by local people.[7] Previous studies reported that four lupane-type triterpenoids and glycosides were isolated from the rhizomes of *H. fragrans*.[7] However, chemical constituents of the leaves of *H. fragrans* have not been studied. In this paper, we report the isolation and structure elucidation of one new lupane-type triterpenoid, 3β-hydroxylup-20(29)-ene-23,27,28-trioic acid (**1**), as well as four known lupane-type derivatives, melaleucic acid (**2**),[7] 3-oxolup-20(29)-ene-27,28-dioic acid (**3**),[8] cylicodiscic acid (**4**),[9] and 3β,23-dihydroxylup-20(29)-ene-27,28-dioic acid (**5**)[7] from the leaves of the title plant (Fig. 1). All the compounds were tested for the cytotoxic activity against KB cell.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{30}H_{44}O_7$ based on HRESIMS ([M-H]$^-$, *m/z* 515.2996), ^{13}C NMR and DEPT data, indicating nine degrees of unsaturation. The IR spectrum of **1** indicated the existence of hydroxyl (3399 cm^{-1}) and carboxyl groups (1696 cm^{-1}). Analysis of the ^{1}H NMR data (Table 1) and the correlations observed in the HSQC experiment showed that the molecule contained four methyl singlets [δ_H 0.97 (3H, s), 1.18 (3H, s), 1.62 (3H, s), 1.96 (3H,

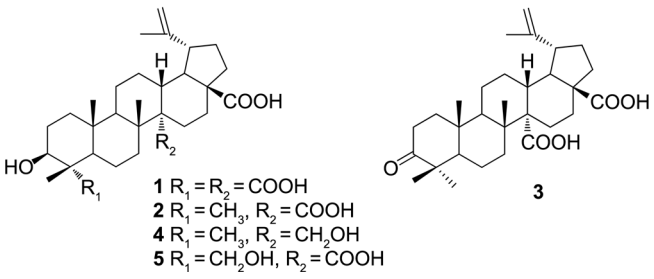

Figure 1. Structures of compounds **1-5** from the leaves of *Heteropanax fragrans.*

Table 1. ^{13}C NMR and ^{1}H NMR data of compound **1** (pyridine-d_5, δ in ppm, *J* in Hz)[a]

Positions	δ_H (mult *J* in Hz)	δ_C (mult)
1	1.82 (1H, ddd, *J* = 13.2, 7.2, 3.0 Hz), 1.18 (1H, m)	39.0 (t)
2	1.98 (1H, m), 1.74 (1H, m)	28.4 (t)
3	4.57 (1H, d, *J* = 11.1, 5.1 Hz)	75.8 (d)
4	–	55.0 (s)
5	2.07 (1H, m)	52.5 (d)
6	1.98 (1H, m), 1.74 (1H, m)	22.4 (t)
7	2.03 (1H, m), 1.55 (1H, m)	37.8 (t)
8	–	41.6 (s)
9	2.01 (1H, m)	52.5 (d)
10	–	38.0 (s)
11	1.74 (1H, m), 1.68 (1H, m)	21.9 (t)
12	2.50 (1H, brd, *J* = 13.2 Hz), 1.81 (1H, m)	29.1 (t)
13	2.99 (1H, ddd, *J* = 13.2, 10.8, 4.3 Hz)	41.0 (d)
14	–	60.6 (s)
15	2.74 (1H, ddd, *J* = 12.9, 8.1, 3.3 Hz), 2.69 (1H, m)	27.4 (t)
16	1.74 (1H, m), 1.68 (1H, m)	40.1 (t)
17	–	57.0 (s)
18	2.17 (1H, dd, *J* = 12.0, 10.8 Hz)	52.7 (d)
19	3.72 (1H, ddd, *J* = 12.0, 8.4, 3.7 Hz)	48.4 (d)
20	–	151.9 (s)
21	2.03 (1H, m), 1.55 (1H, m)	31.4 (t)
22	2.90 (1H, brd, *J* = 13.0 Hz), 1.98 (1H, m)	36.2 (t)
23	–	181.3 (s)
24	1.62 (3H, s)	12.6 (q)
25	0.97 (3H, s)	17.8 (q)
26	1.18 (3H, s)	17.8 (q)
27	–	179.0 (s)
28	–	180.0 (s)
29	5.08 (1H, s), 4.83 (1H, s)	111.2 (t)
30	1.96 (3H, s)	19.6 (q)

[a]The assignments were based on DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC experiments.

s)] and two olefinic protons at δ_H 5.08 (1H, s), 4.83 (1H, s). The ^{13}C NMR and DEPT spectra displayed 30 signals including four methyl groups (δ_C 12.6, 17.8, 17.8 and 19.6), one oxygen-bearing methylene group at δ_C 75.8, two olefinic carbons arising from a terminal double bond (δ_C 111.2 and 151.9), as well as three carboxyl groups (δ_H 179.0, 180.0, 181.3). The above NMR data was very similar to that of the known lupane-type triterpenoid, melaleucic acid (**2**).[7] The main differences between **1** and **2** included Mw and the chemical shifts of the carbons associated with the methyl attached to C-4. The MW of **1** was 30 Da greater than that of melaleucic acid (**2**). In the ^{13}C NMR spectrum of **1** an additional signal due to carboxyl group (δ_C 181.3) was observed. In addition, the carbon at C-4 signal resonated further downfield (δ_C 55.0), compared to **2** (δ_C 39.7). However, the methyl connected with C-4 signal resonated further upfield (δ_C 12.6), compared to **2** (δ_C 16.8). These data indicated the presence of an additional carboxyl group attached to C-4 of compound **1**. The location of the additional carboxyl group was further confirmed by the HMBC correlation of the CH_3-24 with the carboxyl carbon (C-23) at δ_C 181.3 and the ROESY correlation of CH_3-24 with CH_3-25. This ROESY correlation also indicated that the methyl group at C-24 was β oriented. Moreover, the large coupling constant of H-3 (δ_H 4.57, dd, J = 5.1, 11.1 Hz) suggested a 3β-hydroxyl substituent. The proton signal at δ_H 3.72 (ddd, J = 12.0, 8.4, 3.7 Hz) further revealed a typical H-19β lupane structure. The larger coupling constant of H-18 [δ_H 2.17 (dd, J = 10.8, 12.0 Hz)] and H-13 [δ_H 2.99 (ddd, J = 10.8, 8.4, 3.7 Hz)] showed the equatorial and axial orientations of protons at C-18 and C-13, respectively. In addition, in the ROESY spectrum, the correlation (Fig. 2) between H-13 and CH_3-26 and the absence of the correlation between H-13 and H-18 further confirmed the orientations of protons at C-18 and C-13 as above mentioned. To the best of our knowledge, the naturally occurring lupane-type triterpene has rings C/D and D/E in trans fused system.[2] These aforementioned data suggested that the C-27 and C-28 carboxyl groups were α and β oriented, respectively. Therefore, the structure of **1** was determined to be 3β-hydroxylup-20(29)-ene-23,27,28-trioic acid, which was a new one due to the existence of an additional carboxyl moiety linked with C-4 in molecule. This compound was the only member of this family of lupane-type triterpenoids.

All the compounds were tested the cytotoxicity against KB cell line. Compounds **1-5** exhibited 11.6, 21.9, 11.4, 20.5 and 25.0% inhibitory effects at a minimum inhibition concentration of 10^{-5} mol/L in cytotoxic assay, respectively.

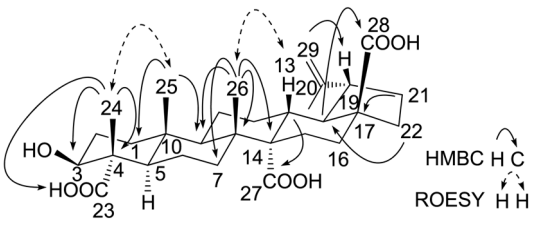

Figure 2. Key HMBC and ROESY correlations of compound **1**.

However, the LC_{50} values of all the compounds were more than 40 μM. Taxol was used for a control experiment with LC_{50} value < 0.008 μM.

Experimental

General Procedures. Optical rotations were measured at 25 °C on a JASCO P1010 polarimeter. IR spectra were measured on a Nicolet FTIR 205 spectrophotometer. NMR spectra were recorded on a Bruker spectrometer (400 MHz for ^1H, 125 MHz for ^{13}C, and 600 MHz for 2D NMR) at 25 °C, using TMS as an internal standard. Chemical shifts (relative to TMS) are in ppm, and coupling constants (in parentheses) in Hz. The 2D ROESY and NOESY spectra were recorded at mixing times of 500 and 600 ms, respectively. ESIMS spectra were obtained on a Navigator Mass Thermoquest. HRESIMS were obtained on a MALDI-TOF spectrometer (Voyager-De STR; Perseptive Biosystems). Precoated silica gel plates (Merck) were used for TLC. Detection was done by spraying plates with 5% anisaldehyde-sulfuric acid, followed by heating.

Plant Material. Leaves of *Heteropanax fragrans* were collected in November 2000 in the high altitude scrubland of Xishuangbanna, Yunnan province, China. It was identified by Dr. YH Zhang. The voucher specimen was deposited in Herbarium of Yunnan Normal University.

Extraction and Isolation. The powdered, air-dried leaves of *H. fragrans* (20 g) was extracted three times with EtOAc at room temperature to afford an EtOAc extract (2.0 g), which was subjected to flash column chromatography on silica gel (30-70 μm) eluting with CH_2Cl_2-MeOH (100/0-90/10) to give ten fractions (1-10). Fraction 3 (459.6 mg) was subjected to flash column chromatography on silica gel (30-70 μm) CH_2Cl_2-MeOH (100/0-90/10) to yield **3** (49.6 mg). Fractions 4 (47.8 mg) and 7 (837.4 mg) were separately chromatographed over silica gel eluting with CH_2Cl_2-MeOH (100/0-90/10) afforded **3** (9.6 mg) and **4** (2.1 mg) from fraction 4 and **2** (657.7 mg) from fraction 7. Column chromatography of fractions 10 (99.8 mg) on silica gel eluting with heptane-EtOAc (1:0-1:1) and CH_2Cl_2-MeOH (100/0-90/10) furnished **5** (8.4 mg) and **1** (3.4 mg).

3β-Hydroxylup-20(29)-ene-23,27,28-trioic Acid (1): White amorphous powder. $[\alpha]_D^{25}$ –21.0 (*c* 0.06, pyridine). IR (KBr) ν_{max}: 3399, 2947, 2352, 1696, 1459, 1231, 1168 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1; ESIMS (negative ion) *m/z*: 515 $[M-H]^-$; HR-ESIMS *m/z* 515.2996 (Calcd for $C_{30}H_{43}O_7$, 515.3009).

Cytotoxic Assay. All compounds were tested for their cytotoxic effects against the KB cell line (mouth epidermoid carcinoma) from the ATCC. The cytotoxicity assays were performed according to the published procedure.[10]

Acknowledgments. We are grateful to the Dr. YH Zhang for the identification of the plants. This research Project was partially supported by Natural Scientific Foundation of Yunnan Province, China (2010CD046) and the Educational

Commission of Yunnan Province, China (2010Z088).

Rerferences

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