Three New and Antitumor Anthraquinone Glycosides from *Lasianthus acuminatissimus* MERR

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Received July 5, 2005; accepted October 28, 2005

Three new anthraquinone glycosides, lasianthoside A (1), B (2), and C (3), were isolated from the root of *Lasianthus acuminatissimus* MERR. The structural elucidation of these anthraquinones was mainly established on the basis of 1D and 2D NMR and HR-MS spectroscopic analysis. Ten known compounds, dammacanthol (4), dammacanthol 11-methyl ether (5), dammacanthol-3-O-β-D-primveroside (6), asperuloside (7), asperulosidic acid (8), deacetyl asperulosidic acid (9), a nonglycosidic iridoid (10), 2,6-dimethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside (11), tachioside (methoxyhydroquinone-4-O-β-D-glucopyranoside) (12), and isolactoside (methoxyhydroquinone-1-O-β-D-glucopyranoside) (13) were also identified for the first time from this plant in the course of the phytochemical and spectroscopic investigation. In addition to this report, a preliminary evaluation of 13 compounds in treating rheumatoid arthritis and antitumor effects of six anthraquinones are presented.

Key words *Lasianthus acuminatissimus;* lasianthoside A; lasianthoside B; lasianthoside C; iridoid; bioactivity

*Lasianthus acuminatissimus* MERR. is a plant of the Rubiaceae family, distributed in the southern provinces of China. It is used in traditional Chinese folk medicine for the treatment of rheumatoid arthritis. However, the investigations on the chemical constituents of plant have been few, studies were limited to species taxonomy, and no compound was reported.1,2 In a search for the active constituents in the treatment of rheumatoid arthritis, the acetone extract of the roots was chromatographed on a silica gel column, and the obtained eluates were subjected to chemical investigation leading to the isolation and the structural elucidation of three new anthraquinone glycosides, lasianthoside A (1), B (2), and C (3). Ten known compounds, dammacanthol (4),3,4 dammacanthol 11-methyl ether (5),5 dammacanthol-3-O-β-D-primveroside (6),3 asperuloside (7),6,7 asperulosidic acid (8),6,7 deacetyl asperulosidic acid (9),8 a nonglycosidic iridoid (10),7 2,6-dimethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside (11),9 tachioside (12),3 and isolactoside (13)10 are also reported for the first time from this plant. In addition to a preliminary evaluation in treating rheumatoid arthritis, we found that the iridoid glycosides are the bioactive compounds of the plant. The antitumor activity of six anthraquinones against human 2780 cells was also evaluated, and the three new anthraquinone glycosides showed significant bioactivity.

The HR-FAB mass spectrum of compound 1 showed a molecular ion at *m/z* 469.1087 (Calcd 469.1111) which corresponded to C21H22O11 Na [M+Na], while the UV maxima at 203.2, 267.0, and 358.0 nm and IR bands at 3510, 1670, and 1577 cm−1 suggested the presence of a hydroxyanthraquinone. A singlet signal at δH 3.76 (3H, s), an AB spin system at δH 4.58 and 4.67 (each H, d, J = 11.0 Hz), and the signals at δC 161.52 and 161.37 (two oxygenated aromatic carbons) and δC 52.66 (one oxygenated secondary aliphatic carbon) indicated an anthraquinone aglycone with three substituents, a methoxy, a phenolic hydroxyl, and a hydroxymethyl in the 1H-NMR and 13C-NMR spectra. Furthermore, the carbon signals of the glycosyl group suggested a glucose residue attached to one of the hydroxyls. Four aromatic protons in a symmetrical AA’BB’-type pattern indicated that one aromatic ring in the anthraquinone was unsubstituted, and one isolated aromatic proton at δH 7.74 exhibited cross-peaks with the carbons at 132.31 (C-2), 161.52 (C-3), 121.19 (C-9a), and 182.77 (C-10) in HMBC, indicating that the other ring was unsubstituted at C-4 and substituted at C-1, C-2, and C-3.

Most anthraquinones isolated from the Rubiaceae have no substituent in one aromatic ring and always have a carbon substituent at position 2 in the other ring.3,11 Therefore the hydroxymethyl group in 1 was placed at C-2 on comparative and biogenetic grounds, consistent with the HMBC data. The hydroxymethyl proton signals at δH 4.58 and 4.67 exhibited cross-peaks with the carbons at 161.37 (C-1), 132.31 (C-2), 161.52 (C-3), and 63.42 (MeO) further confirming that they are linked to C-2. The signals at ν 1670 and 1631 cm−1 and δC 181.13 and 182.77 indicated that the quinine carbonyls were not chelated with the phenol group. Thus the OH had to occupy a β-position of the anthraquinone, and thus the phenol hydroxyl had to be at C-3, and the methoxy group was placed at C-1 because the methoxy proton exhibited a cross-peak with the carbon at 161.37 (C-1) in HMBC. The δC of MeO (63.42, i.e., greater than 60) was in agreement with an ortho-disubstituted arrangement.

To locate the glycosylated hydroxyl group (OH-3 or OH-11), the 13C-NMR data of 1 were compared with those of the reference compound dammacanthol-11-O-β-D-glucoside.3 Several significant differences involving the δC values of 1 were noted: 1) C-11 was shifted to a higher field (δC 52.66, cf. δC 59.2 for reference) and C-2 to a lower field (δC 132.31, cf. δC 125.2 for reference). Such changes were in agreement with the α-carbonyl carbon of the aglycone moiety being lower field shifted (ca. 7), while the β-carbon is higher field shifted after glycosylation.10,11 2) The upfield shift of C-3 and downfield shift of C-9a in 1 relative to the corresponding signals in the reference compound can be attributed to the substituted OH-3 and its shielding effect on the para carbon C-9a. 3) C-1′ was shifted to higher field (δC 101.74, cf. δC 103.0 in the reference compound), and this change was due to the increased shielding of the anomeric carbon from the
secondary pyranoside in C-11 to the tertiary pyranoside in C-3. Comparing 1 with the known compound damnacanthol-3-O-β-α-primeveroside (compound 6), their aromatic carbonyl and glucose carbonyl data were rather similar, which strongly suggested glycosylation was located in C-3.

Furthermore, the δ of the anomic proton of 1 was shifted to δ 5.11 from δ 4.31 in the reference compound, which also strongly suggested that C-1 was linked to a phenolic oxygen rather than an alcoholic one. The above data confirmed that OH-3 was glucosylated and that OH-11 was free. Based on the J value (7.0 Hz) of the doublet of H-1, the anomic configuration of 1 was β, and the 13C-NMR signals of the sugar portion correspond well to a α-glucose. The proposed structure was further confirmed by HMBC. The proton at δ 5.11 (H-1) exhibited cross-peaks with the carbon at δ 161.52 (C-3), indicating the glycosylation was occupied to C-3. Thus 1 was deduced to be 3-hydroxy-1-methoxy-2-hydroxymethyl-9,10-anthraquinone-3-O-β-D-glucopyranoside, i.e., lasianthuoside A. All the 1H and 13C data of the aglycone were unambiguously assigned by means of HMBC.

The HR-FAB mass spectrum of compound 2 showed a molecular ion at m/z 461.1427 (Calcd 461.1447) which corresponded to C23H25O10 [M]+, while UV maxima absorption at 207.2, 265.8, and 358.0 nm and IR bands at 3435, 1674, and 1599 cm⁻¹ suggested the presence of a hydroxyanthraquinone. The 1H- and 13C-NMR and DEPT spectral data of 2 showed that it was similar to compound 1, except for the additional presence of a MeO (δH at 3.29, δC at 58.63). HMBC indicated that the protons at δ 4.53 and δ 4.63 (CH2O) exhibited cross-peaks with the carbons at δ 162.23 (C-3), 128.48 (C-2), and 58.63 (MeO), and the protons at δ 3.29 with the carbon at δ 62.66 (CH2O), indicated that this MeO is substituted at C-11. The structure of this new anthraquinone glycoside we designated lasianthuoside B was thus 3-hydroxy-1-methoxy-2-methoxymethylanthraquinone 3-O-β-D-glucopyranoside.

The HR-FAB mass spectrum of compound 3 showed a molecular ion at m/z 592.1855 (Calcd 592.1870), while UV maxima at 202.4, 268.4, and 358.0 nm and IR bands at 3450, 1668, and 1585 cm⁻¹ suggested the presence of a hydroxyanthraquinone. Data for compound 3, when compared with 2, revealed the presence of additional signals for the sugar D-xylose (the anomeric proton and carbon are at δ 5.16 and 104.81, respectively). Comparison of the sugar carbon resonances in 3 with those in 1 indicated the presence of an additional anomeric carbon signal at δ 104.16, which corresponded to a D-xylose residue.
orons that DEPT and HMBC data revealed that the signal at 68.75 assigned to C-6’ of glucose was shifted downfield by ca. 7.6 from that of compound 2 (δ 61.13). Such changes were in agreement with the α-carbonyl carbon of the glycosyl moiety being lower field shifted (ca. 7) after glycosylation. The terminal xylose moiety is attached to the glucosidic acid (7) after glycosylation and the proton at δ 4.50 (J = 5.0 Hz). HMBC indicated that protons at δ 4.50 and δ 4.60 (CH₃O) exhibited cross-peaks with the carbons at δ 161.54 (C-1), 162.22 (C-3), 128.52 (C-2), and 58.63 (MeO), the protons at δ 3.86 with the carbon at δ 161.54 (C-1) indicated that the MeO was substituted at C-1, the other MeO protons at δ 3.31 with the carbons at δ 63.49 (CH₃O) indicated that it was substituted at C-11. The proton at δ 5.11 (H-1) exhibited cross-peaks with the carbon at δ 162.22 (C-3), showing that glycosylation was located at C-3. Thus 3 was identified as 3-O-β-D-xylopyranoxy-1 methoxyl-2-methoxyethylsulfonhriquinone-3-O-β-D-xylopyranoside, i.e., lasianthusoside C.

Ten known compounds, dammacanthol (4), dammacanthol 11-methyl ether (5), dammacanthol-3-O-β-D-primeveroside (6), asperuloside (7), asperulosidic acid (8), deacetyl asperulosidic acid (9), a nonglycosidic iridoid (10), 2,6-dimethoxy-4-hydroxyphe-1-O-β-D-glucopyranoside (11), tachioside (12), and isotachioside (13) were also isolated and characterized by comparison of their spectral data (NMR, MS) with the literature values.

Compounds 1—6 were evaluated against human 2780 cells; three new anthraquinone glycosides (compounds 1, 2, 3) showed inhibitory effects on human 2780 cells with estimated IC₅₀ values of 0.84, 1, and less than 0.1 μg/ml, respectively, while the other two anthraquinone glycosides showed no significant inhibition. This indicates that the glycosyl moiety is a necessary substituent for the antitumor potency. On the other hand, inhibitory values for compound 3 were higher than for compound 2. The results may suggest that the more glycosyl groups attached, the higher the activity against human ovarian cancer. In this respect, this preliminary biological evaluation of anthraquinones from Lasianthus acuminatus Merr., appears promising since some compounds reveal activity against human 2780 cells.

A preliminary evaluation in treating rheumatoid arthritis with the present compounds showed that compounds 7 and 9 exhibit an inhibitory effect on the release of TNF-α from cultured mouse peritoneal macrophages with IC₅₀ values of

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### Table 2. HMBC Data for Compounds 1—3

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<th>HMBC data of 2 (δ₂C)</th>
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<th>HMBC data of 3 (δ₃C)</th>
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<td>3.31 s</td>
<td>11</td>
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### Table 3. 13C-NMR Spectral Data for Compounds 7—9 (CD3OD)

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<td>6’</td>
<td>61.79</td>
<td>60.98</td>
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### Table 4. IC₅₀ Values (μg/ml) for Inhibitive on Human 2780 Cells of Compounds 1—6

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<th>Compound</th>
<th>Inhibitory (%)</th>
<th>IC₅₀ (μg/ml)</th>
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<td>1</td>
<td>60.2</td>
<td>0.84</td>
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<tr>
<td>2</td>
<td>50.8</td>
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</tr>
<tr>
<td>3</td>
<td>73.5</td>
<td>&lt;0.1</td>
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<tr>
<td>4</td>
<td>2.7</td>
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<td>5</td>
<td>35.4</td>
<td>&gt;10</td>
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<tr>
<td>6</td>
<td>43.8</td>
<td>&gt;10</td>
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### Table 5. Effect on Release of TNF-α from Cultured Mouse Peritoneal Macrophages of Compounds 1—13

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<th>OD</th>
<th>Inhibitory (%)</th>
<th>IC₅₀ (μg/ml)</th>
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<td>1</td>
<td>0.63±0.018</td>
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<td>2</td>
<td>0.501±0.017</td>
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<td>3</td>
<td>0.470±0.040</td>
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<td>4</td>
<td>0.505±0.017</td>
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<td>5</td>
<td>0.522±0.007</td>
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<td>6</td>
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<td>12</td>
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<td>14</td>
<td>0.464±0.039</td>
<td>9.4</td>
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0.52 and 1 μg/ml, respectively, while the other compounds did not show any significant inhibition. Because the release of TNF-α is one of the main factors in rheumatoid arthritis, the results may suggest that iridoid glycosides are the bioactive compounds of the plant in the treatment of rheumatoid arthritis. Inhibitory values for compound 7 were higher than those of 9, yet 8 showed no significant activity. Because the polarity of 8 is between that of 7 and 9, this may suggest that esterification of all or none of OH is beneficial for the activity and half-esterification is not favorable. The amount in iridoid glycosides is sufficient in the plant, and further investigations are underway in our laboratory.

Experimental

Melting points were determined with an XT4-100X micromelting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-260 spectrophotometer. IR spectra were obtained in KBr on a Perkin Elmer 683 infrared spectrometer. 1H- and 13C-NMR spectra and 2D NMR experiments (HMQC, HMBC) were recorded on an Inova-500 spectrometer at 500 and 125 MHz, respectively, with tetramethylsilane as an internal standard. HR-FAB-MS and EI-MS were determined on an Autoscope-Ultima ETOF spectrometer. ESI-MS were obtained on an Agilent 1100 Series LC/MSD Trap mass spectrometer separately. Silica gel (Qing Dao Hai Yang Chemical Group Co., Qing Dao, China) was used for column chromatography. Si gel GF<sub>254</sub>(Qing Dao Hai Yang Chemical Group Co.) was used for TLC, and compounds were detected by UV at 254 nm. Porous polymer gel was prepared from Shandong Lukang Chemical Factory, and Sephadex LH-20 from Bei Jing Jin Ou Ya Chemical Company, respectively. The optical density was measured with a Model 312 micrometer plate reader purchased from Biotech Research Laboratories, Inc., Rockville, MD, U.S.A.

Plant Material

The roots of <i>L. acuminatissimus</i> Merr. were collected from Yushan, Jiangxi, China, during December 2003, which were identified by Professor Xue-wen Lai (Jiangxi University of traditional Chinese medicine). A voucher is deposited in the Chinese Pharmacy Department, Jiangxi University of traditional Chinese medicine, Nanchang, China.

Extraction and Isolation

Air-dried and powdered roots of the plants (10 kg) were refluxed with 95% EtOH (501 l) three times, and the alcoholic extracts were concentrated and deposited to yield 4 (350 mg) and recrystallized with MeOH. The concentration was successively extracted with pet ether, ethyl ether, ethyl acetate, ace tone, and n-butanol, and the ethyl ether extracts yielded 5 (30 mg). The acetone extracts were combined and evaporated to dryness (43 g). All of these extracts were subjected to silica gel (250 g) column chromatography, and the ethyl extracts were subjected to silica gel (250 g) column chromatography. The concentration was successively extracted with pet ether, ethyl ether, ethyl acetate, acetone, and n-butanol, and the ethyl ether extracts yielded 5 (30 mg). The acetone extracts were combined and evaporated to dryness (43 g). All of these extracts were subjected to silica gel (250 g) column chromatography, and the ethyl extracts were subjected to silica gel (250 g) column chromatography.

The item is supported by Chinese national natural science fund (No. 20062030). The authors would like to thank Professor Jian-bei Li and Wen-ye He for recording the mass and NMR spectra, respectively, and thank Mr. You-shou Yu for collecting the plants.

References