

## Norditerpenoid Alkaloids and Other Components from the Processed Tubers of *Aconitum carmichaeli*

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A new norditerpenoid and a known alkaloid were isolated from the alkaloidal fraction of the processed tubers of *Aconitum carmichaeli*. The structure of the new norditerpenoid alkaloid was elucidated as lipoforesaconitine (**1**) on the basis of spectroscopic analysis. The known norditerpenoid alkaloid was characterized as lipoyunanaconitine (**2**). In addition, a new flavonoid, 6"-O-acetylquiritin (**7**), along with a known ceramide, (2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2'-hydroxylignoceroylamino]-8(*E*)-octadecene-1,3,4-triol (**3**), as well as a known steroid saponin, gracillin (**8**), and three known flavonoids, liquiritigenin (**4**), isoliquiritigenin (**5**), and liquiritin (**6**), were also isolated and characterized. All known compounds were isolated from this plant for the first time. The structures of the isolates were established by spectroscopic and chemical methods.

**Key words:** Prepared aconite, *Aconitum carmichaeli*, Ranunculaceae, Norditerpenoid alkaloid, Flavonoid, Steroid saponin

### INTRODUCTION

The aconite root is an indispensable and common drug in Chinese traditional medicine. The processed lateral root of *Aconitum carmichaeli* Debeaux (Ranunculaceae), now officially listed as "Prepared Aconite" in the *Korean Pharmacopoeia*, seventh edition, is used as an analgesic and anesthetic agent in the treatment of neuralgic and rheumatic conditions. The aconite plants are known to contain a number of norditerpenoid and diterpenoid alkaloids (Pelletier *et al.*, 1984; Pelletier and Joshi, 1991). During our work on diterpenoid alkaloids from *Aconitum* plants (Shim *et al.*, 2003a, 2003b; Shim *et al.*, 2005), we have reported the isolation of four new norditerpenoid alkaloids and five known norditerpenoid alkaloids from the processed tubers of *A. carmichaeli* (Shim *et al.*, 2003a). Being interested in this type of compound, this plant extract was further investigated to isolate another new norditerpenoid alkaloid; namely, lipoforesaconitine (**1**), together with a known compound, lipoyunanaconitine (**2**),

which was isolated for the first time from the title plant. In addition, a new flavonoid, 6"-O-acetylquiritin (**7**), was also isolated. Other unusual compounds, such as a ceramide, (2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2'-hydroxylignoceroylamino]-8(*E*)-octadecene-1,3,4-triol (**3**), and the flavonoids, liquiritigenin (**4**), isoliquiritigenin (**5**), and liquiritin (**6**), as well as a steroid saponin, gracillin (**8**), were also isolated and identified for the first time from this plant. The structures were determined by detailed NMR analyses, including <sup>1</sup>H-<sup>1</sup>H COSY, HMQC, and HMBC techniques. In this paper, we report the isolation and structural elucidation of these compounds.

### MATERIALS AND METHODS

#### General procedures

The optical rotations were determined on a JASCO P-1020 polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix, in the positive ion mode, on a VG-VSEQ spectrometer. The NMR spectra were measured on a Varian Gemini 2000 instrument (300 MHz) or Bruker AM-500 (500 MHz), and the chemical shifts were referenced

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to TMS. GC-MS analysis was performed, as previously described, using a Hewlett Packard 5989B mass spectrometer equipped with a 5890 Series II\* gas chromatograph (Kang *et al.*, 2001). TLC was performed on silica gel 60F<sub>254</sub> (Merck).

### Plant material

The processed tubers of *A. carmichaeli*, imported from Sichuan province, China, were purchased in April 2001 from the Sunheung Oriental Drug Store, Seoul, Korea, and authenticated by emeritus Professor H. J. Chi of the Natural Products Research Institute, Seoul National University, as well as by Professor J. H. Park of the College of Pharmacy, Pusan National University. A voucher specimen (no. KSS000403) was deposited at the Natural Products Research Institute, Seoul National University.

### Extraction and isolation

Powdered processed tubers of *A. carmichaeli* (7.1 kg) were extracted five times with MeOH at room temperature. The MeOH extracts were combined and evaporated to dryness under reduced pressure. This extract was partitioned with 3% aqueous NH<sub>4</sub>OH and CHCl<sub>3</sub>. The CHCl<sub>3</sub> extract (30 g) was separated into 10 fractions (frs. I-X) by chromatography on a silica gel column, with a gradient of MeOH in CHCl<sub>3</sub>. Fractions I and III were further separated by chromatography on a silica gel column, with EtOAc-MeOH (10:0.3) as eluent, to afford 10 subfractions (frs. 1-10), respectively. Subfraction I-10 was further purified by silica gel column chromatography, with cyclohexane-EtOAc-Et<sub>2</sub>NH (30:1:0.1) as eluent, to give lipoyunaconitine (**2**, 8 mg). Fractions III-3 (0.3 g) and III-4 underwent further chromatography on silica gel columns, employing the same eluent systems, to give a ceramide (**3**, 10 mg), and lipoforesaconitine (**1**, 15 mg), respectively. The aqueous layer was partitioned with EtOAc. The EtOAc extract (5 g) was purified by chromatography on a silica gel column, with a gradient of MeOH in CHCl<sub>3</sub> (100:0 → 0:100), to afford 10 subfractions (frs. 1-10). Subfractions 5-7 were further purified by a reversed phase chromatography on a RP-18 column, with MeOH-H<sub>2</sub>O (4:6) as eluent, to give liquiritigenin (**4**, 10 mg) and isoliquiritigenin (**5**, 2 mg), liquiritin (**6**, 28 mg), and 6''-O-acetylliquiritin (**7**, 8 mg), respectively. Subfraction 9 underwent further chromatography on a C-18 reversed phase column, with MeOH-H<sub>2</sub>O (35:65) as eluent, to give gracillin (**8**, 3 mg). The known compounds were identified by comparison of their physical and spectral data with published values.

### Lipoforesaconitine (1)

Colorless oil; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) δ: 0.86–0.90 (m, acyl CH<sub>2</sub>CH<sub>3</sub>), 1.07 (3H, t, *J* = 7.2 Hz, *N*-CH<sub>2</sub>CH<sub>3</sub>),

1.25 [br s, (CH<sub>2</sub>)<sub>n</sub>], 2.19 (1H, dd, *J* = 6.9, 15.6 Hz, H-15a), 3.05 (1H, dd, *J* = 7.2, 15.6 Hz, H-15b), 2.76 (1H, t, *J* = 5.7 Hz, H-9), 3.03 (1H, br s, H-7), 3.15 (3H, s, 1-OCH<sub>3</sub>), 3.25 (3H, s, 6-OCH<sub>3</sub>), 3.28 (3H, s, 18-OCH<sub>3</sub>), 3.38 (3H, s, 16-OCH<sub>3</sub>), 3.83 (3H, s, 4'-OCH<sub>3</sub>), 3.12, 3.62 (1H each, d, *J* = 8.4 Hz, H-18), 4.04 (1H, br d, *J* = 6.6 Hz, H-6β), 5.02 (1H, t, *J* = 4.8 Hz, H-14β), 5.27–5.42 (m, acyl CH=CH), 6.89 (2H, d, *J* = 9.0 Hz, H-3', 5'), 8.00 (2H, d, *J* = 9.0 Hz, H-2', 6'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz) δ: 85.0 (C-1), 26.4 (C-2), 34.8 (C-3), 39.3 (C-4), 49.0 (C-5), 82.9 (C-6), 45.0 (C-7), 85.6 (C-8), 49.0 (C-9), 44.0 (C-10), 50.4 (C-11), 28.9 (C-12), 39.1 (C-13), 75.4 (C-14), 38.1 (C-15), 83.5 (C-16), 61.5 (C-17), 80.3 (C-18), 53.8 (C-19), 48.9 (*N*-CH<sub>2</sub>CH<sub>3</sub>), 13.4 (*N*-CH<sub>2</sub>CH<sub>3</sub>), 56.6 (1-OCH<sub>3</sub>), 57.9 (6-OCH<sub>3</sub>), 56.1 (16-OCH<sub>3</sub>), 59.1 (18-OCH<sub>3</sub>), 14.0, 14.1 (acyl CH<sub>3</sub>), 22.5, 24.3, 25.6, 27.2, 28.9, 29.1, 29.3, 29.7, 31.5, 31.9, 34.8 (acyl CH<sub>2</sub>), 128.0, 128.6, 130.0, 130.2 (CH=CH), 172.4 (8-OC=O), 123.0 (C-1'), 131.7 (C-2', 6'), 113.7 (C-3', 5'), 163.3 (C-4'), 166.0 (C-7'), 55.4 (4'-OCH<sub>3</sub>); FAB-MS *m/z*: 880 (acyl = arachidoyl), 852 (acyl = stearoyl), 848 (acyl = linoleoyl), 824 (acyl = palmitoyl) [M + H]<sup>+</sup>, 792 [824 – CH<sub>3</sub>OH]<sup>+</sup>, 568 [M – lipoyl]<sup>+</sup>, 536 [568 – CH<sub>3</sub>OH]<sup>+</sup>, 135 [CH<sub>3</sub>OC<sub>8</sub>H<sub>4</sub>C≡O]<sup>+</sup>.

### Lipoyunaconitine (2)

Colorless oil; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) δ: 0.85–0.90 (m, acyl CH<sub>2</sub>CH<sub>3</sub>), 1.07 (3H, br t, *J* = 7.0 Hz, *N*-CH<sub>2</sub>CH<sub>3</sub>), 1.25 [br s, (CH<sub>2</sub>)<sub>n</sub>], 2.76 (1H, t, *J* = 6.3 Hz, H-9), 3.14 (3H, s, 1-OCH<sub>3</sub>), 3.24 (3H, s, 6-OCH<sub>3</sub>), 3.29 (3H, s, 18-OCH<sub>3</sub>), 3.54 (3H, s, 16-OCH<sub>3</sub>), 3.84 (3H, s, 4'-OCH<sub>3</sub>), 3.48, 3.61 (1H each, d, *J* = 9.0 Hz, H-18), 4.01 (1H, br d, *J* = 6.1 Hz, H-6β), 4.85 (1H, d, *J* = 4.5 Hz, H-14β), 5.27–5.40 (m, acyl CH=CH), 6.90 (2H, d, *J* = 9.0 Hz, H-3', 5'), 7.99 (2H, d, *J* = 9.0 Hz, H-2', 6'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 75.5 MHz) δ: 82.3 (C-1), 33.4 (C-2), 71.7 (C-3), 43.1 (C-4), 47.0 (C-5), 83.2 (C-6), 48.7 (C-7), 85.4 (C-8), 44.7 (C-9), 40.7 (C-10), 50.4 (C-11), 35.0 (C-12), 74.6 (C-13), 78.4 (C-14), 39.9 (C-15), 83.5 (C-16), 61.5 (C-17), 77.1 (C-18), 47.7 (C-19), 48.7 (*N*-CH<sub>2</sub>CH<sub>3</sub>), 13.3 (*N*-CH<sub>2</sub>CH<sub>3</sub>), 55.7 (1-OCH<sub>3</sub>), 58.0 (6-OCH<sub>3</sub>), 58.8 (16-OCH<sub>3</sub>), 59.1 (18-OCH<sub>3</sub>), 13.7, 14.0 (acyl CH<sub>3</sub>), 22.6, 24.2, 25.6, 27.2, 28.9, 29.1, 29.3, 29.7, 31.5, 31.9, 34.7 (acyl CH<sub>2</sub>), 127.8, 128.0, 129.9, 130.2 (CH=CH), 172.7 (8-OC=O), 123.0 (C-1'), 131.7 (C-2', 6'), 113.8 (C-3', 5'), 163.5 (C-4'), 165.9 (C-7'), 55.4 (4'-OCH<sub>3</sub>); FAB-MS *m/z*: 884 (acyl = stearoyl), 882 (acyl = oleoyl), 880 (acyl = linoleoyl), 856 (acyl = palmitoyl) [M + H]<sup>+</sup>, 600 [M – lipoyl]<sup>+</sup>, 135 [CH<sub>3</sub>OC<sub>8</sub>H<sub>4</sub>C≡O]<sup>+</sup>.

### (2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2'-Hydroxylignoceroylamino]-8(*E*)-octadecene-1,3,4-triol (3)

Amorphous powder (MeOH); m.p. 134–136°C; [α]<sub>D</sub><sup>23</sup> = +7.5° (*c* = 0.1, pyridine); IR *v*<sub>max</sub> (KBr) 3345 (OH), 2918, 2851, 1647, 1560 (amide), 1471, 1049, 720 cm<sup>-1</sup>; <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>, 300 MHz) δ: 0.81 (6H, br t, *J* = 7.0 Hz,

2 × Me), 1.20 – 1.26 [(CH<sub>2</sub>)<sub>n</sub>], 4.24 (1H, m, H-4), 4.31 (1H, dd, *J* = 4.5, 6.3 Hz, H-3), 4.38 (1H, dd, *J* = 5.1, 11.1 Hz, H-1a), 4.47 (1H, dd, *J* = 4.5, 10.8 Hz, H-1b), 4.58 (1H, dd, *J* = 3.5, 7.5 Hz, H-2'), 5.08 (1H, dd, *J* = 4.5, 9.0 Hz, H-2), 5.42 (1H, dd, *J* = 6.0, 15.6 Hz, H-8), 5.52 (1H, dd, *J* = 5.7, 15.6 Hz, H-9), 8.55 (1H, d, *J* = 8.7 Hz, NH); <sup>13</sup>C-NMR (pyridine-*d*<sub>5</sub>, 75.5 MHz) δ: 61.9 (C-1), 52.9 (C-2), 76.7 (C-3), 72.8 (C-4), 33.7 (C-5), 25.9 (C-6), 33.2 (C-7), 130.6 (C-8), 130.8 (C-9), 32.9 (C-10), 175.3 (C-1'), 72.4 (C-2'), 35.6 (C-3'), 26.7 (C-4'), 29.5, 29.8, 29.9 (CH<sub>2</sub>), 22.9 (C-17, 23'), 14.2 (C-18, 24'); (+)-FAB-MS *m/z*: 704 [M + Na]<sup>+</sup>, 682 [M + H]<sup>+</sup>; (+)-HR-FAB-MS *m/z*: 682.6350. Calcd for C<sub>42</sub>H<sub>83</sub>O<sub>5</sub>N + H: 682.6304.

### 6''-O-Acetylliquiritin (7)

Amorphous yellow powder (MeOH); m.p. 215–217°C; [α]<sub>D</sub><sup>23</sup> = -52.0° (*c* = 0.3, CH<sub>3</sub>OH); IR ν<sub>max</sub> (KBr) 3425 (OH), 1742, 1253 (acetate), 1709, 1655 (α,β-unsaturated C=O), 1616, 1583, 1466 (aromatic C=C), 1283, 1082, 1043 (glycosidic C-O) cm<sup>-1</sup>; UV λ<sub>max</sub> (MeOH) (log ε) 275 (4.19), 312 (3.94) nm; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 300 MHz) δ: 2.12 (3H, s, OCOCH<sub>3</sub>), 2.74 (1H, dd, *J* = 3.3, 16.8 Hz, H-3eq), 3.04 (1H, ddd, *J* = 1.2, 12.9, 16.8 Hz, H-3ax), 4.23 (1H, dd, *J* = 6.6, 12.0 Hz, H-6''), 4.40 (1H, dd, *J* = 2.4, 12.0 Hz, H-6''), 4.92 (1H, d, *J* = 7.5 Hz, H-1''), 5.46 (1H, dd, *J* = 3.0, 12.9 Hz, H-2), 6.36 (1H, d, *J* = 2.1 Hz, H-8), 6.50 (1H, dd, *J* = 2.1, 8.7 Hz, H-6), 7.11 (2H, d, *J* = 8.7 Hz, H-3', 5'), 7.44 (2H, d, *J* = 8.7 Hz, H-2', 6'), 7.72 (1H, d, *J* = 8.7 Hz, H-5); <sup>13</sup>C-NMR: see Table I; (+)-FAB-MS *m/z*: 461 [M + H]<sup>+</sup>.

### Gracillin (8)

<sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>, 500 MHz) δ: 0.68 (3H, d, *J* = 5.5 Hz, 27-CH<sub>3</sub>), 0.82 (3H, s, 18-CH<sub>3</sub>), 1.05 (3H, s, 19-CH<sub>3</sub>), 1.13 (3H, d, *J* = 7.5 Hz, 21-CH<sub>3</sub>), 1.76 (3H, d, *J* = 6.0 Hz, Rha CH<sub>3</sub>), 3.57 (1H, dd, *J* = 3.0, 11.5 Hz, H-26a), 3.82 (1H, ddd, *J* = 2.0, 5.5, 9.5 Hz, H-3), 3.96 (1H, m, H-26b), 4.95 (1H, d, *J* = 7.5 Hz, Glc H-1), 5.11 (1H, d, *J* = 8.0 Hz, Glc H-1), 5.32 (1H, d, *J* = 5.0 Hz, H-6), 6.39 (1H, br s, Rha H-1); <sup>13</sup>C-NMR (pyridine-*d*<sub>5</sub>, 125.5 MHz) δ: 37.5 (C-1), 30.1 (C-2), 77.9 (C-3), 38.7 (C-4), 140.8 (C-5), 121.9 (C-6), 32.3 (C-7), 31.7 (C-8), 50.3 (C-9), 37.2 (C-10), 21.1 (C-11), 39.9 (C-12), 40.5 (C-13), 56.7 (C-14), 32.2 (C-15), 81.1 (C-16), 62.9 (C-17), 16.3 (C-18), 19.4 (C-19), 42.0 (C-20), 15.0 (C-21), 109.2 (C-22), 31.8 (C-23), 29.3 (C-24), 30.6 (C-25), 66.9 (C-26), 17.3 (C-27), 100.0, 77.0, 89.6, 69.6, 77.7, 62.5 (inner Glc C-1 — C-6), 102.2, 72.5, 72.8, 74.2, 69.6, 18.7 (terminal Rha C-1 — C-6), 104.6, 75.0, 78.6, 71.5, 78.7, 62.5 (terminal Glc C-1 — C-6).

### Alkaline hydrolysis of 1 and 2 followed by fatty acid analysis by GC-MS

Compounds **1** and **2** (ca. 2 mg each) were each dissolved in 10 mL of 5% KOH in MeOH, and allowed to

stand for 20 h. After removal of the MeOH by evaporating *in vacuo*, 15 mL of water was added to each of the mixtures from **1** and **2**, which were then acidified with 3% HCl and extracted 3 times with *n*-hexane. The hexane layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to yield fatty acid mixtures. These were identified as containing methyl palmitate [*t*<sub>R</sub> 12.66 min], methyl stearate [*t*<sub>R</sub> 14.63 min], methyl linoleate [*t*<sub>R</sub> 18.53 min], and methyl arachidoate [*t*<sub>R</sub> 16.48 min] from **1**, and methyl palmitate, methyl oleate, methyl stearate, and methyl linoleate from **2**, using GC-MS after methylation with CH<sub>2</sub>N<sub>2</sub>.

### Acid hydrolysis of 3

Compound **3** (5 mg) was refluxed with 0.9N HCl in 82% aqueous MeOH (20 mL) for 24 h. The resulting solution was extracted with *n*-hexane, and the combined organic phase dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the hexane yielded a fatty acid methyl ester. The H<sub>2</sub>O layer was neutralized with NH<sub>4</sub>OH, filtered, and then concentrated to yield a long-chain base, which was identical to (2*S*,3*S*,4*R*,8*E*)-2-amino-8(*E*)-octadecene-1,3,4-triol by direct comparison with an authentic sample (Kang *et al.*, 1999). The fatty acid methyl ester was identified as (2*R*)-2-hydroxy-lignoceric acid methyl ester (*t*<sub>R</sub> 25.86 min) by direct comparison with an authentic sample from poke-weed cerebroside (Kang *et al.*, 2001).

### Acetylation of the long-chain base

The above long-chain base was acetylated with equal volumes of pyridine and acetic anhydride (0.5 mL each) at room temperature overnight. The reaction products were purified by chromatography on a silica gel column, with a mixture of hexane-EtOAc (10:1) as eluent, to afford a long-chain base tetraacetate. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz) δ: 0.81 (6H, br t, *J* = 7.0 Hz, 2 × CH<sub>3</sub>), 1.25 [(CH<sub>2</sub>)<sub>n</sub>], 2.03, 2.05, 2.05, 2.08 (3H each, s, 4 × OAc), 4.00 (1H, dd, *J* = 3.0, 11.5 Hz, H-1b), 4.29 (1H, dd, *J* = 4.8, 11.6 Hz, H-1a), 4.47 (1H, m, H-2), 4.93 (1H, dt, *J* = 3.3, 9.3 Hz, H-4), 5.10 (1H, dd, *J* = 3.0, 8.1 Hz, H-3), 5.33 (1H, dd, *J* = 5.7, 15.3 Hz, H-8/9), 5.42 (1H, dd, *J* = 5.4, 15.3 Hz, H-8/9), 5.98 (1H, d, *J* = 9.6 Hz, NH); (+)-FAB-MS *m/z*: 506 [M + Na]<sup>+</sup>, 446 [M + Na - HOAc]<sup>+</sup>.

## RESULTS AND DISCUSSION

Compound **1** was isolated as a colorless oil. The <sup>1</sup>H-NMR spectrum of compound **1** resembled the reported spectrum of foesaconitine, but showed two additional multiplets between δ 5.27 – 5.42 and 1.25 – 1.80 and a distorted triplet at δ 0.86 – 0.90 due to the lipo ester side-chains (Hanuman and Katz, 1994). Analysis of the <sup>13</sup>C-NMR spectra indicated that its structure to be quite similar

to that of foresaconitine (Shim *et al.*, 2003a; Kang *et al.*, 2004), with the exception of a fatty acid acyl group ( $\delta$  14.0, 14.1, 22.5, 24.3, 25.6, 27.2, 28.9, 29.1, 29.3, 29.7, 31.5, 34.8, 128.0, 128.6, 130.0, 130.2, and 172.4) instead of an acetoxyl ( $\delta$  21.7, 169.6) group at C-8. The FAB-MS showed four protonated molecular ions  $[M + H]^+$  of the arachidoyl, stearoyl, linoleoyl, and palmitoyl ester alkaloids, and a fragment ion at  $m/z$  568 due to the loss of an acylium ion from the molecular ion peaks. Substitution of the C-14 hydroxyl group with an anisoyl group was also deduced by the appearance of a peak at  $m/z$  135  $[(CH_3OC_6H_4C=O)^+$ , 100%] in the FAB-MS. The anisoyl moiety was assessed to be positioned at the C-14 of the chasmanine skeleton (Pelletier *et al.*, 1976; Pelletier and Djarmati, 1976) on the basis of the HMBC correlation between H-14 and the anisoyl C=O. Alkaline hydrolysis of **1** yielded a mixture of long-chain fatty acids, which displayed molecular ion peaks for the methyl esters of palmitic  $[M^+$ ,  $m/z$  270], linoleic  $[M^+$ ,  $m/z$  294], stearic  $[M^+$ ,  $m/z$  298], and arachidic  $[M^+$ ,  $m/z$  326] acids in the GC-MS. The proportion of each acid in the acid mixture was in the ratio 18:62:15:5, respectively. On the basis of these data, the structure of **1** was assigned as lipoforesaconitine, a previously undescribed alkaloid.

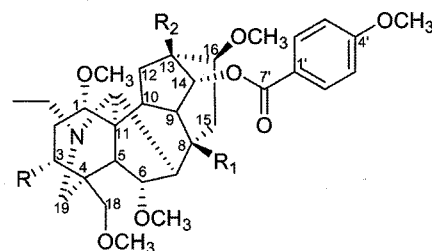
The  $^1H$ - and  $^{13}C$ -NMR spectra (Table I) of compound **7**

**Table I.** Carbon NMR chemical shifts for **4**, **6**, and **7** in  $CD_3OD^*$

Carbon no.	<b>4</b>	<b>6</b>	<b>7</b>	$\Delta\delta_C$ (7 - 6)
C-2	81.05	80.70	80.68	
C-3	44.96	44.98	44.96	
C-4	193.55	193.18	193.18	
C-5	129.86	129.87	129.88	
C-6	111.81	111.84	111.85	
C-7	166.96	166.87	166.87	
C-8	103.83	103.85	103.85	
C-9	165.60	165.41	165.41	
C-10	114.93	115.00	115.01	
C-1'	131.37	134.46	134.62	
C-2',6'	129.02	128.77	128.75	
C-3',5'	116.31	117.81	117.85	
C-4'	158.99	159.25	159.06	
C-1''		102.22	102.07	
C-2''		74.91	74.84	
C-3''		77.99	77.81	
C-4''		71.38	71.58	
C-5''		78.20	75.34	-2.86
C-6''		62.51	64.70	+2.19
OAc			172.70	
			20.75	

\*Chemical shifts are referenced to  $CD_3OD$  ( $\delta$  49.0 ppm)

were also similar to those of **6**, with the exception of the presence of an acetoxyl group [ $\delta_H$  2.12 (3H, s,  $OCOCH_3$ );  $\delta_C$  172.70 (C=O), 20.75 ( $CH_3$ )]. It also showed typical deshielded methylene signals at  $\delta_H$  4.23 (1H, dd,  $J = 6.6$ , 12.0 Hz, H-6'') and 4.40 (1H, dd,  $J = 2.4$ , 12.0 Hz, H-6''), and  $\delta_C$  64.70 in the NMR spectra compared to those of

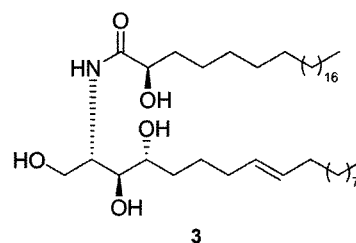


**1** R = R<sub>2</sub> = H, R<sub>1</sub> = OLip.<sup>a</sup>

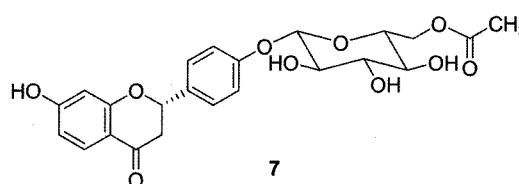
**2** R = R<sub>2</sub> = OH, R<sub>1</sub> = OLip.<sup>b</sup>

Lip.<sup>a</sup> : linoleoyl, palmitoyl, stearoyl, arachidoyl

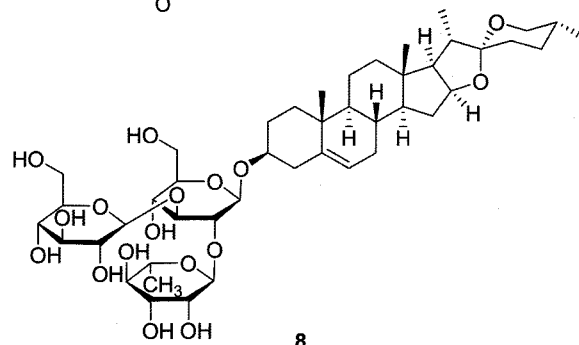
Lip.<sup>b</sup> : linoleoyl, palmitoyl, stearoyl, oleoyl



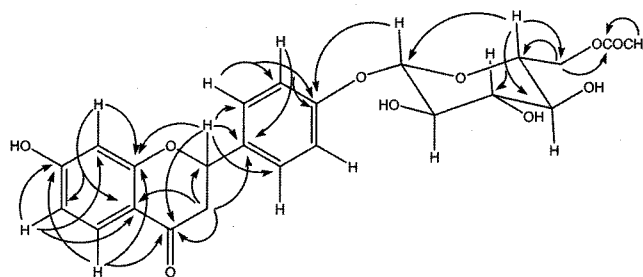
**3**



**7**



**8**



**Fig. 1.** Key correlation of **7** in HMBC spectrum

compound **6**. An HMBC correlation (glucose H-6 and acetoxy C=O) confirmed the acetoxy group was attached at the glucose C-6 position of **7**, as indicated in Fig. 1. Therefore the structure of **7** was deduced to be 6"-O-acetyllicquiritin, which was the first isolation of this compound from natural sources.

The known compounds, lipoyunaconitine (**2**) (Hanuman and Katz, 1994) (2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2'-hydroxylignoceroylamino]-8(*E*)-octadecene-1,3,4-triol (**3**) (Kraus and Spiteller, 1991; Su *et al.*, 2002), liquiritigenin (**4**) (Nakanishi *et al.*, 1985; Kang and Son, 2000), isoliquiritigenin (**5**) (Kang and Son, 2000), liquiritin (**6**) (Nakanishi *et al.*, 1985) and gracillin (**8**) (Kim *et al.*, 1989; Liu *et al.*, 2001) were also isolated and identified by comparison of their physical and spectral data with those of reported values. All these isolates were isolated from this species for the first time. The isolation of flavonoids, as well as steroid saponins, from the underground parts of *Aconitum* spp. have not been reported to date, and the present elucidation seems to be the first example of this occurrence.

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## REFERENCES

- Hanuman, J. B. and Katz, A., New lipo norditerpenoid alkaloids from root tubers of *Aconitum ferox*. *J. Nat. Prod.*, 57, 105-115 (1994).
- Kang, S. S., Kim, J. S., Xu, Y. N., and Kim, Y. H., Isolation of a New Cerebroside from the Root Bark of *Aralia elata*. *J. Nat. Prod.*, 62, 1059-1060 (1999).
- Kang, S. S. and Son, K. H., Structure Elucidation of Natural Products by Spectroscopy. Seoul National University Press, Seoul, (2000).
- Kang, S. S., Kim, J. S., Son, K. H., Kim, H. P., and Chang, H. W., Cyclooxygenase-2 Inhibitory Cerebrosides from *Phytolacca Radix*. *Chem. Pharm. Bull.*, 49, 321-323 (2001).
- Kang, S. S., Kim, Y. S., Bae, K.-H., Seo, E. K., Son, K. H., Shin, K. H., and Choi, J. S., Spectroscopic Data of Natural Products. Hanrimwon, Seoul, pp. 1094-1097, (2004).
- Kim, S. W., Chung, K. C., Son, K. H., and Kang, S. S., Steroidal Saponins from the Rhizomes of *Smilax china*. *Kor. J. Pharmacogn.*, 20, 76-82 (1989).
- Kraus, R. and Spiteller, G., Ceramides from *Urtica dioica* Roots. *Liebigs Ann. Chem.*, 125-128 (1991).
- Liu, H. W., Kobayashi, H., Qu, G. X., and Yao, X. S., A New Spirostanol Saponin from *Dioscorea futshauensis*. *Chin. Chem. Lett.*, 12, 613-616 (2001).
- Nakanishi, T., Inada, A., Kambayashi, K., and Yoneda, K., Flavonoid Glycosides of the Roots of *Glycyrrhiza uralensis*. *Phytochemistry*, 24, 339-341 (1985).
- Pelletier, S. W. and Djarmati, Z., Carbon-13 Nuclear Magnetic Resonance: Aconitine-Type Diterpenoid Alkaloids from *Aconitum* and *Delphinium* Species. *J. Am. Chem. Soc.*, 98, 2626-2636 (1976).
- Pelletier, S. W., Djarmati, Z., Lajšić, S., and De Camp W., Alkaloids of *Delphinium staphisagria*. The Structure and Stereochemistry of Delphisine, Neoline, Chasmanine, and Homochasmanine. *J. Am. Chem. Soc.*, 98, 2617-2625 (1976).
- Pelletier, S. W., Mody, N. V., Joshi, B. S., and Schramm, L. C., <sup>13</sup>C and Proton NMR Shift Assignments and Physical Constants of C<sub>19</sub>-Diterpenoid Alkaloids, in Pelletier, S. W. (Ed.). *Alkaloids: Chemical and Biological Perspectives*. John Wiley and Sons, New York, pp. 205-462, (1984).
- Pelletier, S. W. and Joshi, B. S., Carbon-13 and Proton NMR Shift Assignments and Physical Constants of Norditerpenoid Alkaloids, in Pelletier, S. W. (Ed.). *Alkaloids: Chemical and Biological Perspectives*. Springer-Verlag, New York, pp. 297-564, (1991).
- Shim, S. H., Kim, J. S., and Kang, S. S., Norditerpenoid Alkaloids from the Processed Tubers of *Aconitum carmichaeli*. *Chem. Pharm. Bull.*, 51, 999-1002 (2003a).
- Shim, S. H., Kim, J. S., Kang, S. S., Son, K. H., and Bae, K.-H., Alkaloidal Constituents from *Aconitum jaluense*. *Arch. Pharm. Res.*, 26, 709-715 (2003b).
- Shim, S. H., Kim, J. S., Kang, S. S., Son, K. H., and Bae, K.-H., A New Diterpenoid Alkaloid from *Aconitum jaluense*. *J. Asian Nat. Prod. Res.*, in press (2005).
- Su, B.-N., Misico, R., Park, E. J., Santarsiero, B. D., Mesecar, A. D., Fong, H. H. S., Pezzuto, J. M., and Kinghorn, A. D., Isolation and characterization of bioactive principles of the leaves and stems of *Physalis philadelphica*. *Tetrahedron*, 58, 3453-3466 (2002).