



C-glycosylflavone from *Aletris spicata*

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ABSTRACT

The genus *Aletris* L., originally belonging to Liliaceae, was attributed to the family Nartheciaceae by Dahlgren et al. in 1985. Three flavones (1–3) and two steroidal saponins (4–5) were isolated from *Aletris spicata*, which C-glycosylflavone were the first reported from Liliaceae (s.l.). This result supports the APG system classified the genus into Nartheciaceae of Dioscoreales.

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1. Subject and source

Aletris spicata (Thunb.) Franch., a perennial herb distributed mainly in the south of China and adjacent regions of Japan and Philippines (Liang and Turland, 2000), was collected from Gongshan County in Yunnan Province of China, in July 2011, and identified by Dr. Rong Li, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (Li Rong & Dao Zhiling 517) of the collection was deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN) (Fig. 1).

2. Previous work

The genus *Aletris* L. comprises 25 species, mostly distributed in eastern Asia (20 species) and eastern North America (5 species) (Wu, 2006; Mabberley, 2008). Among them, 15 species are distributed in China, nine of which are endemic, and the Hengduan Mountains in southwest China is its diversity hotspot, where 13 species grow (Liang and Turland, 2000; Zhao et al., 2006; Li and Zhang, 2011).

This genus was established in 1753 with *Aletris farinose* L. from eastern North America as the type. However, the status of *Aletris* has been controversial. Historically, the group *Aletris* has been classified into the family Liliaceae of Liliales by Engler and Prantl (1930); Dahlgren et al. (1985) placed it into tribe Narthecieae (Melanthiaceae). The updated system of flowering plant classification put the genus *Aletris* in the family Nartheciaceae of Dioscoreales (APG III, 2009). Previous chemical investigation has demonstrated the presence of steroidal saponins in Liliales and Dioscoreales plants. So far, there is no chemical investigation on the plants from the genus *Aletris*.

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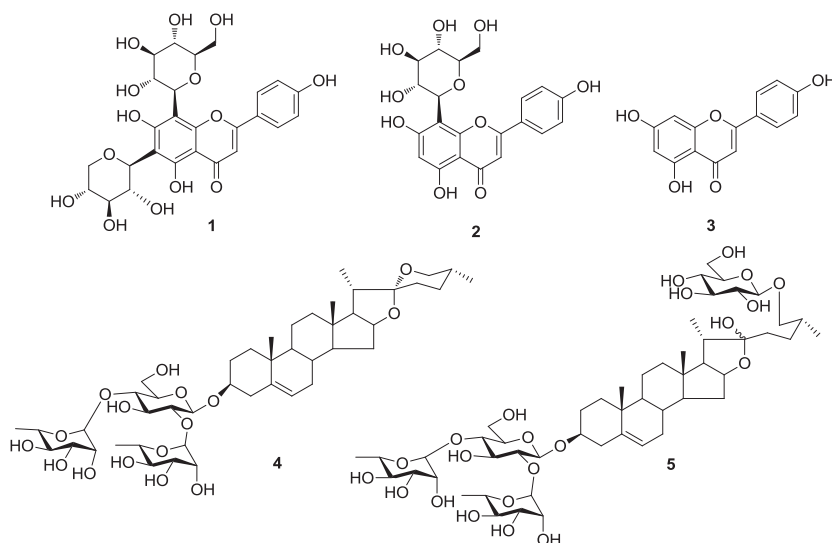


Fig. 1. Structural of compounds 1–5.

3. Present study

Phytochemical investigation of the whole plant of *A. spicata* led to the isolation of five compounds (1–5), including three flavones (1–3) and two steroidal saponins (4–5). Their structures were elucidated on the basis of MS, 1D and 2D NMR spectroscopic analysis and chemical evidence.

The air-dried and powdered whole plant of *A. spicata* (120 g) was ultrasonic extracted with 70% MeOH (3 × 800 mL, 30 min, each) at room temperature. The extracting solution was evaporated under reduced pressure to yield an extract, which was suspended in H₂O and then partitioned with petroleum ether and *n*-BuOH respectively.

The *n*-BuOH fraction (5.7 g) was chromatographed over MCI gel column eluting with MeOH/H₂O (0:1–3:7–6:4–1:0) to give four fractions (I–IV). Fraction II (830 mg, MeOH/H₂O 3:7) was subjected to repeated CC over Sephadex LH-20 (MeOH/H₂O 1:9–0:1), ODS open column (MeOH/H₂O 3:7–4:6) and semi-preparative HPLC (MeOH/H₂O 4:6 v/v) to yield compounds 1 (4.6 mg) and 2 (2 mg). Repeated CC over ODS open column (MeOH/H₂O 4:6–6:4), followed by Sephadex LH-20 (MeOH/H₂O 1:1) afforded compound 3 (2 mg) from Fraction III (417 mg). Fraction IV (940 mg, MeOH/H₂O 9:1) was subjected to ODS open column (MeOH/H₂O, 1:9–9:1) to give two subfractions. Each subfraction was finally purified by chromatography over silica gel (200–300 mesh, CHCl₃/MeOH/H₂O 8:2:0.2) to yield compounds 4 (7 mg) and 5 (3 mg), respectively.

Compound 1 was obtained as a yellow powder, with a molecular formula of C₂₆H₂₈O₁₄ as determined by high-resolution (HR)-electrospray ionization (ESI)-MS (m/z 563.1403, [M – H][–]). FeCl₃ reaction, HCl–Mg reaction and Molish reaction showed positive, which demonstrated that 1 was a flavonoid glycoside. The ¹H NMR spectrum revealed two characteristic proton signals at δ 13.69 and 6.51 belonging to 5-OH and H-3, respectively, and δ 7.86 (2H, d, J = 8.5 Hz) and 6.89 (2H, d, J = 8.5 Hz) arising from the typical A₂X₂ system of the B ring. Analysis of the ¹³C NMR spectrum suggested that 1 contained 26 carbons. Since the skeleton of flavonoids occupied 15 carbons, the remaining 11 resonances should be assignable to two sugar moieties, which was confirmed by signals of the anomeric proton at δ 4.72 (1H, d, J = 9.7 Hz) and 4.63 (1H, d, J = 9.7 Hz). In the HMQC–TOCSY spectrum, the correlations from the anomeric proton signal at δ 4.72 to five carbon signals at δ 79.1, 74.4, 71.7, 70.4 and 69.6, suggested the presence of β -xylopyranosyl. In the same case, another sugar was ascribable to β -glucopyranosyl, of which the ¹³C NMR data were δ 80.8, 78.8, 75.5, 71.0, 70.6, and 60.7. Moreover, the HMBC correlations of H-1'' of glucose with C-5/C-7, and H-1''' of xylose with C-7/C-9 indicated the two sugar unit was linked to C-6 and C-8, respectively. Thus, the structure of 1 was established as apigenin-6-C- β -D-xylopyranosyl-8-C- β -D-glucopyranoside (Fiorentino et al., 2008).

Additionally, on the basis of the NMR spectroscopic data and comparison with the literatures or authentic samples, the structures of other compounds were determined to be apigenin-8-C- β -D-glucopyranoside (2) (Rayyan et al., 2010), apigenin (3) (Csupor-Löffler et al., 2011), dioscin (4) and protodioscin (5) (Zheng et al., 2004), respectively. It is the first time to report the C-glycosylflavone from the genus *Aletris*.

Apigenin-6-C- β -D-xylopyranosyl-8-C- β -D-glucopyranoside (1). HR-ESI-MS: m/z 563.1403, [M – H][–] (calcd. C₂₆H₂₈O₁₄: 563.1401). ¹H NMR (500 MHz, DMSO-*d*₆): δ _H 13.70 (1H, s, 5-OH), 7.86 (2H, d, J = 8.5 Hz, H-2', 6'), 6.89 (2H, d, J = 8.5 Hz, H-3', 5'), 6.51 (1H, s, H-3), 4.63 (1H, d, J = 9.7 Hz, H-1''), 4.72 (1H, d, J = 9.7 Hz, H-1''') and 3.1–3.9 (sugar part); ¹³C NMR (125 MHz, DMSO-*d*₆): δ _C 180.4 (C-4), 164.0 (C-2), 161.0 (C-5), 160.7 (C-4'), 159.6 (C-7), 155.6 (C-9), 128.1 (C-2', 6'), 122.2 (C-1'), 115.8 (C-3', 5'), 108.8 (C-6), 104.8 (C-10), 101.9 (C-3), 98.1 (C-8), 80.8 (C-5''), 79.1 (C-3'''), 78.8 (C-3''), 75.5 (C-1''), 74.4 (C-1'''), 71.7 (C-2'''), 71.0 (C-2''), 70.6 (C-4''), 70.4 (C-4'''), 69.6 (C-5''') and 60.7 (C-6'').

4. Chemotaxonomic significance

To the best of our knowledge, it is the first report on the chemical constituents of the genus *Aletris*. The isolated compounds from *A. spicata* are assigned as two C-glycosylflavones (**1–2**), one flavonoid (**3**) and two steroidal saponins (**4–5**).

The family Liliaceae (Liliales) and Nartheciaceae (Dioscoreales) are characterized by steroidal saponins as the major chemical constituents, of which the dioscin (**4**) and protodioscin (**5**) have been reported in both families (Yang et al., 2007). However, it is the first time to isolation of the flavonoid C-glycosides (**1–2**) from the genus *Aletris*, which may support the APG system classified the genus into Nartheciaceae of Dioscoreales from the point view of chemotaxonomy.

In conclusion, the results indicate that *A. spicata* has some similar steroidal saponins (**4–5**) with other species of Dioscoreales plants. C-glycosylflavones (**1–2**) should serve as the chemosystematic markers of the genus *Aletris*. Further chemical investigations around the family Nartheciaceae are currently underway, which will provide important information on chemotaxonomy of the family.

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References

- Angiosperm Phylogeny Group (APG), 2009. Bot. J. Linn. Soc. 161, 105.
- Csupor-Löffler, B., Hajdú, Z., Zupkó, I., Molnár, J., Forgo, P., Vasas, A., Kele, Z., Hohmann, J., 2011. Planta Med. 77, 1183.
- Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F., 1985. The Families of the Monocotyledons, Structure, Evolution and Taxonomy. Springer-Verlag, Berlin, New York.
- Engler, A., Prantl, K., 1930. Die natürlichen Pflanzenfamilien, second ed., vol. 15. Wilhelm Engelmann, Leipzig.
- Fiorentino, A., D'Abrosca, B., Pacifico, S., Izzo, A., Letizia, M., Esposito, A., Monaco, P., 2008. Biochem. Syst. Ecol. 36, 691.
- Li, R., Zhang, S.D., 2011. Nord. J. Bot. 29, 440.
- Liang, S., Turland, N.J., 2000. *Aletris* Linnaeus. In: Wu, Z.Y., Raven, P.H. (Eds.), Flora of China, vol. 24. Science Press, Beijing.
- Mabberley, D.J., 2008. Mabberley's Plant-book: A Portable Dictionary of Plants, Their Classification and Uses. Cambridge Univ. Press, Cambridge.
- Rayyan, S., Fossen, T., Andersen, Ø.M., 2010. J. Agr. Food Chem. 58, 7211.
- Wu, Z.Y., 2006. The Areal-types of Seed Plants and Their Origin and Differentiation. Yunnan Science and Technology Press, Kunming.
- Yang, C.R., Zhang, Y., Wang, D., Zhang, Y.J., 2007. Acta Bot. Yunnan. 29, 591.
- Zhao, Y.M., Zhang, S.R., Bi, H.Y., 2006. Acta Bot. Boreal.-Occident. Sin. 26, 2131.
- Zheng, Q.A., Zhang, Y.J., Li, H.Z., Yang, C.R., 2004. Steroids 69, 111.