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Iridoid and phenylethanol glycosides from *Scrophularia umbrosa* with inhibitory activity on nitric oxide production

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ABSTRACT

Three new iridoid glycosides, namely umbrosides A–C (**1–3**); ten known iridoid glycosides (**4–8** and **16–20**); and seven known phenylethanoid glycosides (**9–15**) were isolated from *Scrophularia umbrosa* Dumortier, and their structures were established through spectroscopic methods including 1D and 2D NMR experiments and ESIMS analysis. The inhibitory activities of the isolated compounds on LPS-stimulated nitric oxide (NO) production in RAW 264.7 cells were investigated. Compounds **1–3**, **7**, **9–10**, **13–16**, and **19–20** inhibited NO production, with IC_{50} values ranging from 9.0 to 40.3 μM. Their structure–activity relationships are preliminarily discussed in this paper.

1. Introduction

Scrophularia is a large genus in the Scrophulariaceae family, and approximately 200 species of this genus are distributed in Asia, Europe, and North America, and few species grow tropical deserts, such as *S. deserti* Delile. Thirty species are found in China, and many of them growin high mountains, forests, and riverine areas (Chinese Plant Science Editorial Board of the Chinese Academy of Science, 1979a,b). Some species are widely used as anti-inflammatory and anticancer remedies. *Shen Nong's Herbal Classic* describes the heat-clearing and detoxifying effects of the traditional Chinese herb *S. ningpoensis* Hemsl., a representative plant of this genus (Zhang and Li, 2011). In Europe, *S. aquatica* L. is used as heart and circulatory stimulants, as is *S. lucida* L. in Iran. Iridoid and phenylethanol glycosides are vital chemical constituents of *Scrophularia*, and a pharmacological evaluation demonstrated that these compounds exhibit more than 10 bioactivities, including anti-inflammatory, antioxidative, antitumour, and heart protective activity. Among these, anti-inflammatory activity has been the most popular research topic in recent years (Abbas, 2016; Pasdaran and Hamedi, 2017; Pieroni et al., 2004; Wei et al., 2017; Zhu et al., 2015).

S. umbrosa is distributed in the Xinjiang Autonomous Region of China as well as in Russia and Europe (Chinese Plant Science Editorial Board of the Chinese Academy of Science, 1979a,b). According to previous studies, the content of iridoids is as high as 1.1% in *S. umbrosa* Dumortier; among them, catapol, aucubin, harpagide, and ajugol have been isolated (Damtoft et al., 1993; Swann and Melville, 1972). However, thus far, no study has investigated the constituents or pharmacology of this plant. In this study, three new iridoid glycosides (**1–3**) and seventeen known compounds (**4–20**) were isolated from *S. umbrosa* (Fig. 1). Among them, compounds **4–6**, **14–17**, **19**, and **20** were identified for the first time in the genus *Scrophularia*. The inhibitory activities of these compounds on lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in the RAW 264.7 cell line were investigated.

2. Results and discussion

Compound **1** was isolated as a white amorphous solid. Its molecular formula was determined to be $C_{35}H_{44}O_{17}$ through LRESIMS at *m/z* 735.2506 $[M-H]^-$ (calcd for 735.2506). The ^1H NMR data of **1** (Table 1) showed signals at δ_H 4.98 (d, J = 6.9 Hz), 6.36 (d, J = 6.2 Hz), 5.92 (brs), and 2.98 (brt, J = 7.4 Hz), which were easily distinguishable as the signals of cyclopentene-type iridoid aglycone. Two methine proton signals at δ_H 4.70 (d, J = 7.9 Hz) and 4.97 (brs) indicated that **1** was an iridoid diglycoside. The signals of four protons coupled in an AA'BB' system (δ_H 7.56, 2H, d, J = 8.8 Hz; 6.97, 2H, d, J = 8.8 Hz), together with two *trans* olefinic protons (δ_H 7.60, 1H, d, J = 15.9 Hz; 6.29, 1H, d, J = 15.9 Hz) and a methoxyl group (δ_H 3.85, 3H, s), indicated the presence of a *trans-p*-methoxy-cinnamoyl moiety. Two methyl proton signals at δ_H 2.16 (s) and 2.04 (s) indicated the presence of two acetyl moieties. The NMR spectral data of **1** were quite similar to those of unduloside III (Bedir and Khan, 2004), except for the two acetyl moieties, which were identified through HSQC and HMBC spectrum analysis. Moreover, HMBC experiments revealed the linkage

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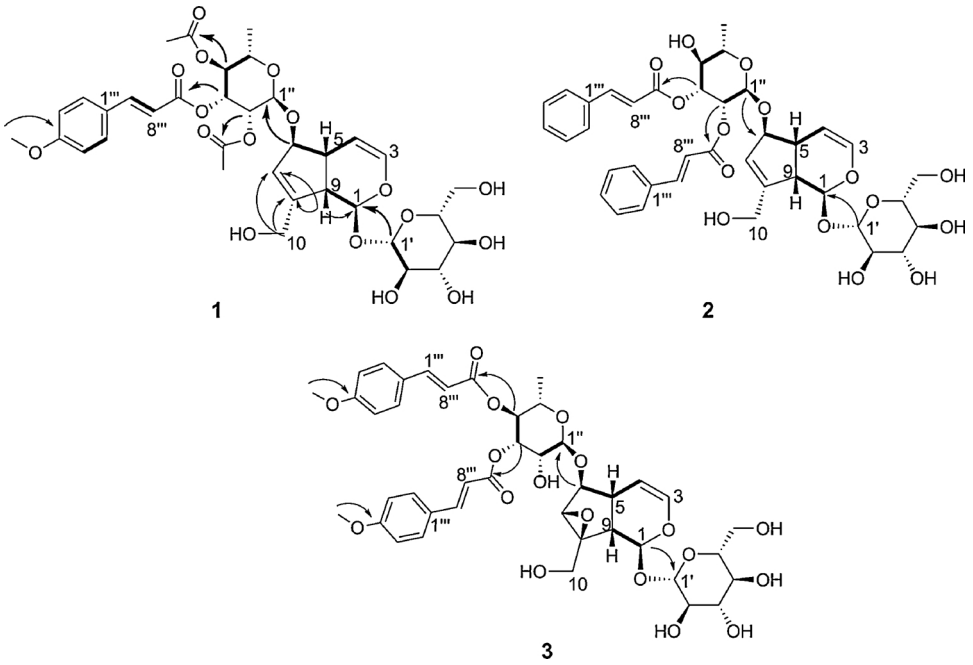

Fig. 1. Key ^{1}H-^{1}H COSY (—) and HMBC (→) correlations of **1**, **2** and **3**.

position of *β*-glucopyranosyl on C-1, α-rhamnosyl on C-6, two acetyls on C-2″ and C-4″, *trans-p*-methoxycinnamoyl on C-3″, and one methoxyl on C-4‴. Accordingly, the structure of **1** was elucidated as 6-*O*-(2″,4″-diacetyl-3″-*trans-p*-methoxycinnamoyl) rhamnosyl aucubin, and it was designated as umbroside A.

Compound **2** was obtained as a white amorphous solid; through HRESIMS ($[M+Na]^+$ *m/z* 775.2568, calcd for 775.2572), its molecular formula was established as $C_{39}H_{44}O_{15}$. Comparison of the NMR data of **2** with those of **1** revealed that compound **2** had the same 6-*O*-rhamnosyl aucubin skeleton as **1**. The most notable differences in NMR spectra were two *trans*-cinnamoyl moieties (Table 1). Comprehensive analysis of the 2D NMR spectra of **2**, specifically ^{1}H ^{1}H COSY and HMBC data, was conducted. The hydrogens at δ_H 5.39 (H-2″) and 5.27 (H-3″) displayed correlations to the carbons at δ_C 167.7 (C-9‴) and 168.0 (C-9⁗). Accordingly, the structure of **2** was elucidated as 6-*O*-(2″,3″-*trans*-cinnamoyl) rhamnosyl aucubin, and it was designated as umbroside B.

Compound **3** was obtained as a white amorphous solid, and its molecular formula was determined to be $C_{41}H_{48}O_{18}$ through HRESIMS at *m/z* 851.2723 $[M+Na]^+$ (calcd for 851.2733). The ^{1}H NMR data of **3** (Table 1) showed signals at δ_H 5.13 (m), 6.42 (dd, *J* = 6.1/1.7 Hz), 4.10 (d, *J* = 8.2 Hz), 3.71 (brs), and 2.61 (dd, *J* = 9.7/7.6 Hz), which were easily distinguishable as the signals of 7,8-epoxy cyclopentanoid iridoid aglycone. Two methine proton signals at δ_H 4.80 (d, *J* = 7.9 Hz) and 5.07 (d, *J* = 1.7 Hz) indicated that **3** was an iridoid diglycoside. The signals of eight aromatic protons (δ_H 6.92, 4H, d, *J* = 8.8 Hz; 7.50, 2H, d, *J* = 8.8 Hz; 7.52, 2H, d, *J* = 8.8 Hz), together with four *trans* olefinic protons (δ_H 7.65, 2H, d, *J* = 15.9 Hz; 6.35, 2H, d, *J* = 15.9 Hz) and two methoxyl groups (δ_H 3.82, 6H, s), indicated the presence of two *trans-p*-methoxycinnamoyl moieties. The NMR spectral data of **3** were similar to those reported for 6-*O*-*α*-L-(3″-*O*-*trans-p*-methoxycinnamoyl) rhamnopyranosylcatalpol (Damtoft et al., 1993), except for the signal of an additional *trans-p*-methoxycinnamoyl moiety.

The ^{1}H ^{1}H COSY spectrum displayed two coupled spin systems of H-1″/H-2″ and H-4″/H-5″. In the HMBC spectra, the correlation between δ_H 5.13 (H-1) and δ_C 99.9 (Glc-1′) and between δ_H 4.10 (H-6) and δ_C 100.6 (Rha-1″) indicated the presence of 6-*O*-*α*-L-rhamnopyranosylcatalpol. Moreover, the clear correlation of 5.37 or 5.38 (Rha-3″ or Rha-4″) and δ_C 168.4 (C-9‴ or C-9⁗) indicated that two *trans-p*-methoxycinnamoyl groups were linked to C-3″ and C-4″ of rhamnopyranosyl, respectively. Accordingly, the structure of **3** was elucidated as 6-*O*-(3″,4″-*trans-p*-methoxycinnamoyl) rhamnosyl catalpol, and it was designated as umbroside C.

Furthermore, 17 known compounds were isolated and identified from *S. umbros*. By comparing their NMR and MS data with reported data, their structures were identified as 6-*O*-(*α*-L-rhamnopyranosyl) catalpol (**4**) (Bedir and Khan, 2004), 6-*O*-*α*-L-(3″-*O*-*trans-p*-methoxycinnamoyl)rhamnopyranosylcatalpol (**5**) (Otsuka et al., 1991), 6-*O*-*α*-L-(2″-*O*-*trans-p*-methoxycinnamoyl)rhamnopyra-nosylcatalpol (**6**) (Otsuka et al., 1991), scropolioside A (**7**) (Calis et al., 1998), scrophuloside A_8 (**8**) (Miyase and Mimatsu, 1999), isoverbascoside (**9**) (Xin et al., 2008), verbascoside (**10**) (Xiao, 2003), martynoside (**11**) (Calis et al., 1984), angoroside C (**12**) (Zhang et al., 1994), lipedoside A-I (**13**) (He et al., 1994), leucosceptoside A (**14**) (Hiroshi et al., 1989), campneoside II (**15**) (Hiroshi et al., 1989), verbaspinoside (**16**) (Kalpoutzakis et al., 1999), 6-*O*-*α*-L-(3″-*O*-cis-*p*-methoxycinnamoyl)rhamn-opyranosylcatalpol (**17**) (Miyase et al., 2008), verb-ascoside A (**18**) (Miyase et al., 2008), *cis*-6-*O*-[4-*O*-(methoxycinnamoyl)-*α*-L-rh-amnopyranosyl] catalpol (**19**) (Cogne et al., 2003), and 6-*O*-*α*-L-(2″-*O*-*cis-p*-methoxycinnamoyl)rhamnopyranosylcatalpol (**20**) (Otsuka et al., 1991). Among them, compounds **4–8** and **16–20** were iridoid glycosides, and compounds **9–15** were phenylethanoid glycosides. Compounds **4**, **5**, **6**, **14–17**, **19**, and **20** were identified for the first time in the genus *Scrophularia*.

Inflammation is a defensive reaction of the body that accompanies most diseases, including atherosclerosis, allergies, and rheumatic diseases. NO is a main inflammation target, and excessive NO production destroys the functioning of normal tissues during acute and chronic inflammation. At a concentration of 100.0 μM, all these compounds exhibited no cytotoxicity against the RAW 264.7 cell line, as revealed by the MTT assay. Inhibitory activities on NO production are summarised in Table 2. Compounds **1–3**, **7**, **9–10**, **13–16**, and **19–20** inhibited NO production, with IC_{50} values ranging from 9.0 to 40.3 μM; compounds **3** and **14** had more potent inhibitory activities than aminoguanidine. Based on these results, preliminary structure–activity relationships for these compounds were determined. Comparing compounds **1–3**, **7**, **16**, and **19–20** with compounds **4–6** and **17–18**, the inhibitory activities of iridoid glycosides on NO production may be related to linkage sites, cinnamoyl numbers, and cinnamoyl