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Acylated iridoid glycosides and acylated rhamnopyranoses from *Gmelina arborea* flowers

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ABSTRACT

Nine acylated iridoid glycosides (**1**–**9**), five acylated rhamnopyranoses (**10**–**14**) and verbascoside (**15**) were isolated from *Gmelina arborea* flowers, including 5 new compounds (**1**, **2**, and **10**–**12**). The cytoprotective activity of 11 selected compounds (**1**–**8**, **10**, **11**, and **15**) against CCl_4-induced cytotoxicity on liver was determined. Compounds **1**, **2**, **4**, **7**, **8** and **15** displayed hepatoprotective activity. 6-*O*-α-L-(2″, 3″-di-*O*-*trans*-*p*-hydroxycinnamoyl)rhamnopyranosylcatalpol (**2**) exhibited the most potent cytoprotective effect with an EC_{50} value of 42.5 μM (SI = 19.3) compared with biphenyldimethylesterate (DDB, EC_{50} = 277.3 μM, SI = 9.8) and bicylo-ethanol (EC_{50} = 279.2 μM, SI = 12.2). Among the acylated iridoid glycosides, the compounds (**2** and **8**) containing phenolic hydroxy groups were more active than were those lacking them.

1. Introduction

The genus *Gmelina* (ca. 40 species), which belongs to the Verbenaceae family, is widely distributed in the tropical and subtropical regions of Australia, Asia and Africa (Greaves, 1981). In China, it is naturally distributed in the Xishuangbanna, Dehong, Pu'er and Lincang prefectures in Yunnan Province. The fruit is edible and the flower is used as coloring and flavoring ingredients for festival cakes in Water Splashing Festival by the Dai minority people in Xishuangbanna. The flower is also used for pigment extraction (Wang, 2004). In addition, *Gmelina arborea* is an important folk medicine for the Dai and Hani ethnic groups in Xishuangbanna. The folklore of India described the use of *G. arborea* bark and leaves to treat liver disorders, loosen phlegm, act as a diuretic or galactogogue and stimulate the appetite (Kawamura and Ohara, 2005). In the ethnobotanical regimes of the indigenous tribes in Tamil Nadu, India, the aerial part of the *G. arborea* is used to treat jaundice and other hepatic diseases (Merlin and Parthasarathy, 2011).

Previous phytochemical studies demonstrated the presence of flavonoids (Nair and Subramanian, 1975), lignans (Anjaneyulu et al., 1972, 1975, 1977; Satyanarayana et al., 1986; Kawamura et al., 2004), iridoid glycosides (Kawamura and Ohara, 2005; Hosny and Rosazza, 1998; Tiwari et al., 2008), and other chemical constituents (Olatunji, 1999; Barik et al., 1992; Satyanarayana et al., 1985; Rao et al., 1967; Falah et al., 2008) in the aerial parts of *G. arborea*; however, these chemical constituents from *G. arborea* have not been reported to possess cytoprotective activity. In this study, an investigation of the constituents of *G. arborea* flowers was performed, which led to the isolation of 15 glycosides, including 5 new ones. Selected compounds were then tested for activity against hepatic injury.

2. Results and discussion

2.1. Phytochemical investigation

The ethyl acetate and *n*-butanol fraction of *G. arborea* flowers extract was separated by a combination of column chromatography on silica gel, followed by further purification using reversed phase chromatography (RP-18) and Sephadex LH-20 column chromatography as well as high-pressure liquid chromatography

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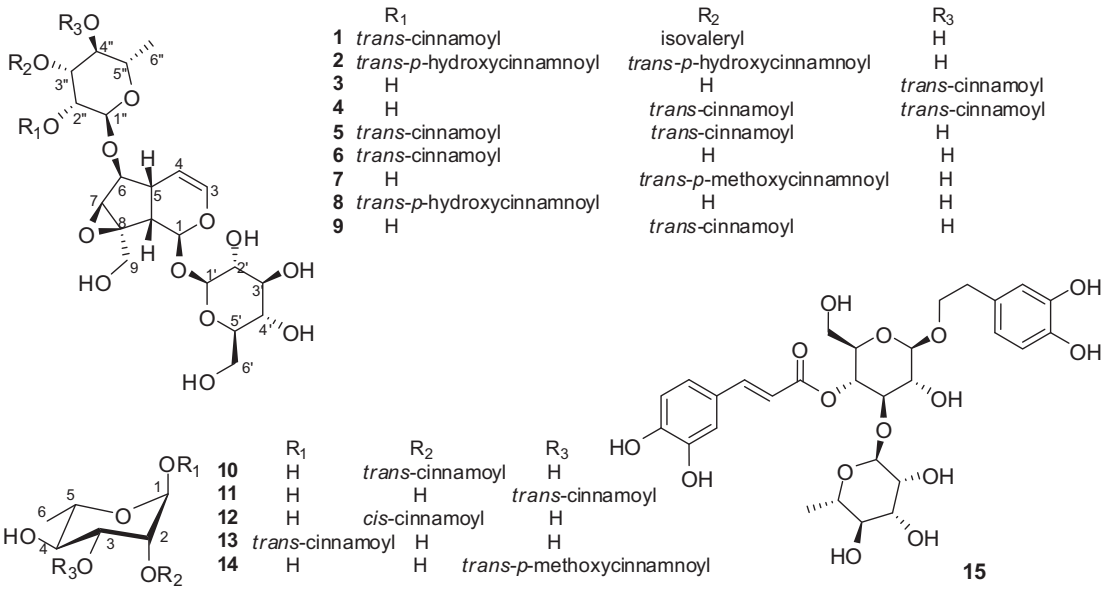

Fig. 1. The structures of compounds **1**–**15**.

(HPLC). Fifteen compounds (Fig. 1) were identified, including 2 new iridoid glycosides (**1** and **2**), 3 new acylated rhamnosides (**10**–**12**), and 10 known compounds.

Compound **1** was found to have the elemental composition $C_{35}H_{46}O_{16}$ by HR-ESI-MS (*m*/*z* 757.2476 $[M+Cl]^-$. Its IR spectrum indicated the presence of hydroxy (3412 cm^{-1}) and conjugated carbonyl (1723 and 1636 cm^{-1}) groups. The NMR data (Table 1) demonstrated the signals of two carbonyl groups (δ_C 174.1 and 167.4), a monosubstituted phenyl ring, a *trans* double bond [δ_H 7.71 (d, *J* = 16.0) and 6.58 (d, *J* = 16.0)], a β-glucosyl group [δ_H 4.75 (d, *J* = 7.9)], and an α-rhamnosyl group [δ_H 5.03 (br s) and 1.31 (d, *J* = 6.1)]. By comparing its NMR data with those of the known compounds, **3**–**9** (Taskova et al., 2006; Tatli et al., 2003; Otsuka et al., 1990, 1991b), compound **1** was deduced to be an acylated iridoid glycoside containing a characteristic fragment of 6-*O*-α-L-rhamnopyranosylcatalpol. The NMR data for compound **1** was very similar to those of 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**6**) except for the presence of additional signals from an isovaleryl group in **1** (Taskova et al., 2006; Tatli et al., 2003). The *trans*-cinnamoyl and isovaleryl groups were located at C-2″ and C-3″ by the HMBC correlations (Fig. 2) from H-2″ to C-9‴ and H-3″ to C-1‴', respectively. These deductions were supported by the ^1H–^1H COSY spectra (Fig. 2 and Supporting Information) of compound **1**. Accordingly, compound **1** was identified as 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl-3″-*O*-isovaleryl)rhamnopyranosylcatalpol, for which we give the trivial name gmelinoside M.

The molecular formula of compound **2** was determined to be $C_{39}H_{44}O_{18}$ by HRESIMS, indicating 18 degrees of unsaturation. The NMR data (Table 1) for compound **2** was very similar to those of 6-*O*-α-L-(2″-*O*-*trans*-*p*-hydroxycinnamoyl)rhamnopyranosylcatalpol (**8**) except that there were signals for two *trans*-*p*-hydroxycinnamoyl groups in compound **2,** whereas there was only one *trans*-*p*-hydroxycinnamoyl group in compound **8** (Otsuka et al., 1990). The two *trans*-*p*-hydroxycinnamoyl groups in compound **2** were located at C-2″ and C-3″ by the HMBC correlations (Supporting Information) from H-2″ to C-9‴ and H-3″ to C-1‴', respectively. Therefore, compound **2** was identified as 6-*O*-α-L-(2″, 3″-di-*O*-*trans*-*p*-hydroxycinnamoyl)rhamnopyranosylcatalpol, and it was named gmelinoside N.

The MS, HRMS and NMR data (Table 2) for **10**–**12** indicated these compounds to be isomers containing two fragments, namely a *trans*- (compounds **10** and **11**) or a *cis*-cinnamoyl (compound **12**) moieties and a rhamnopyranosyl group in their molecular structures, like those known to be present in 1-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose (**13**) (Salib et al., 2008). The downfield chemical shifts were observed for H-2 (δ_H 5.07, br s) in **10**, H-3 (δ_H 5.12, dd, *J* = 9.5, 2.5 Hz) in **11** and H-2 (δ_H 5.03, br s) in **12**, which implied that the cinnamoyl groups should be attached at C-2 of compounds **10** and **12**, and C-3 of compound **11**, respectively. This deduction was further confirmed by their HMBC spectra (Fig. 2 and Supporting Information). Thus, compounds **10**–**12** were identified as 2-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose, 3-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose and 2-*O*-*cis*-cinnamoyl-α-L-rhamnopyranose, respectively.

The known glycosides were determined to be 6-*O*-α-L-(4″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**3**) (Taskova et al., 2006), 6-*O*-α-L-(3″,4″-di-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**4**) (Taskova et al., 2006), 6-*O*-α-L-(2″,3″-di-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**5**) (Taskova et al., 2006), 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (verbaspinoside, **6**) (Taskova et al., 2006; Tatli et al., 2003), 6-*O*-α-L-(3″-*O*-*trans*-*p*-methoxycinnamoyl)rhamnopyranosylcatalpol (**7**) (Otsuka et al., 1991a), 6-*O*-α-L-(2″-*O*-*trans*-*p*-coumaroyl)rhamnopyranosylcatalpol, (**8**) (Otsuka et al., 1990), 6-*O*-α-L-(3″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**9**) (Tatli et al., 2003), 1-*O*-*trans*-cinnamoyl-α-L-rhamnopyranose (**13**) (Salib et al., 2008), 3-*O*-*trans*-*p*-methoxycinnamoyl-α-L-rhamnopyranose (**14**) (Otsuka et al., 1991a), and verbascoside (**15**) (Miyase et al., 1982), by comparing their spectroscopic data with those reported in the literature.

2.2. Hepatoprotective activity

The cytoprotective activity against CCl_4-induced cytotoxicity of 11 selected compounds (**1**–**9**, **10**, **11** and **15**) that were isolated from *G. arborea* flowers was investigated. The preliminary screening results revealed that compounds **1**, **2**, **4**, **7**, **8** and **15** displayed significant activity against CCl_4-induced cytotoxicity, whereas the other compounds did not. Therefore compounds **1**, **2**, **4**, **7**, **8** and **15** were selected for determinations of their EC_{50} and CC_{50} values (Table 3). Among the compounds tested, compounds **2** exhibited the most potent suppressing effects, with EC_{50} values of