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Four new flavonol glycosides from the leaves of *Ginkgo biloba*

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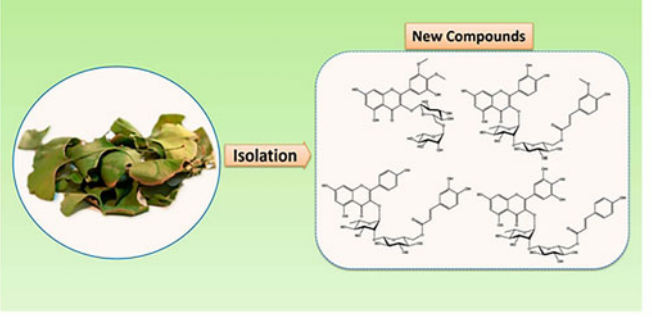
ABSTRACT

Four new flavonol glycosides, 5, 7, 5′-trihydroxy-3′, 4′-dimethoxy-flavonol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**1**), quercetin 3-*O*-(6-*trans*-feruloyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**2**), kaempferol 3-*O*-(6-*trans*-caffeoyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**3**), myricetin 3-*O*-(6-*trans*-*p*-coumaroyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**4**), together with nine known flavonoids and two known lignans, were isolated from the leaves of *Ginkgo biloba*. Their structures were determined by extensive spectroscopic analyses. Their cardioprotective effects against H_2O_2-induced apoptosis in H9c2 cells were also evaluated. The flavonol glycosides had stronger activity than the acylated flavonol glycosides at the concentration of 50 μM.



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Ginkgo biloba; cardioprotective activity; acylated flavonol glycoside

1. Introduction

The dried leaves of *Ginkgo biloba* L. (Ginkgoaceae) have been used as herbal remedies for centuries in China, and now their extracts are one of the most widely used herbal products and/or dietary supplements in the world (Lin et al. 2008). The *Ginkgo* leaves

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contain large numbers of active compounds, the most important of which are flavonoids and terpene trilactones. The extract of *Ginkgo* leaves is standardised for these two components (flavonol glycosides 24% and terpene trilactones 6%) (Mohanta et al. 2014). Active constituents in *Ginkgo* extract improve blood circulation, discourage clot formation, reinforce the walls of the capillaries, and protect nerve cells from harm when deprived of oxygen (Singh et al. 2008). Both *in vitro* and *in vivo* studies have demonstrated the protective effects of *Ginkgo* leaves extract on the response to oxidative stress (Varga et al. 1999).

In our investigation of constituents from *Ginkgo biloba*, we obtained thirteen flavonoids and two lignans. Compounds **1**, **2**, **3** and **4** were new compounds, and compound **5** was previously mentioned (Xie et al. 2006), but neither the isolation method nor the structural data was included. In this paper, we report the isolation and the structural elucidation of compounds **1**, **2**, **3**, **4** and **5**, as well as their protective effects on H_2O_2-induced H9c2 cells with the other ten compounds.

2. Results and discussion

Compound **1** was obtained as a pale yellow amorphous powder. Its molecular formula was suggested as $C_{29}H_{34}O_{17}$ from the basis of negative HR-ESI-MS (found *m/z* 653.1733 $[M-H]^-$) and positive HR-ESI-MS (found *m*/z 677.1704 $[M+Na]^+$) in combination with its NMR data. The ^{1}H NMR and ^{13}C NMR spectrum of **1** showed the presence of a flavone skeleton and two sugar moieties. The ^{1}H NMR spectrum showed one hydroxyl group at δ 12.51 (1H, s, 5-OH), meta-coupled signals of A ring at δ 6.21 (1H, d, $J = 1.8$ Hz, H-6) and 6.40 (1H, d, $J = 1.8$ Hz, H-8) and two meta-coupled aromatic protons at δ 7.45 (1H, d, $J = 2.0$ Hz, H-6′) and 7.12 (1H, d, $J = 2.0$ Hz, H-2′), suggested it to be a 3′, 4′, 5′-trisubstituted benzene in the B ring. It also indicated the presence of two methoxyl signals at δ 3.83 (3H, s, 4′-OCH_3) and 3.76 (3H, brs, 5′-OCH_3). Moreover, the spectrum exhibited two anomeric protons at δ 5.52 (1H, d, $J = 7.4$ Hz, H-1″) and δ 4.45 (1H, d, $J = 0.8$ Hz, H-1‴) and a methyl at δ 0.98 (3H, d, $J = 6.2$ Hz), suggesting a β-glucosyl and an α-rhamnosyl moieties. The ^{1}H-^{1}H COSY and TOCSY experiments permitted the full assignment of the sugar protons. The sugar moieties were detected as D-glucose and L-rhamnose after acid hydrolysis and TLC analysis. The ^{13}C NMR spectrum displayed signals for 29 carbons including flavonol disaccharide glycoside with two methoxy groups in aromatic ring (δ 56.3, 60.5). In the HMBC spectrum, the long-rang correlations from δ 5.52 (1H, d, $J = 7.4$ Hz, H-1″) to δ 134.1 (C-3), from δ 3.72 (1H, m, H-6″a) and 3.39 (1H, m, H-6″b) to δ 101.3 (C-1‴) confirmed the C-1″ (glc) and C-1‴ (rha) were attached to C-3 of aglycone and C-6″ (glc), respectively. The HMBC correlations between δ 7.45 (1H, d, $J = 2.0$ Hz, H-2′) and δ 156.1 (C-2), 110.2 (C-6′), 139.1 (C-4′); δ 7.12 (1H, d, $J = 2.0$ Hz, H-6′) and δ 156.1 (C-2), 139.1 (C-4′), 105.7 (C-2′); methoxyl proton δ 3.83 (3H, s, 3′-OCH_3) and δ 153.0 (C-3′), δ 3.76 (3H, s, 4′-OCH_3) and δ 139.1 (C-4′) were observed, indicating the presence of a 3′, 4′-dimethoxy-5′-hydroxybenzene of the B ring. Based on the evidence above, compound **1** was deduced as 5, 7, 5′-trihydroxy-3′, 4′-dimethoxyflavonol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (Figure 1).

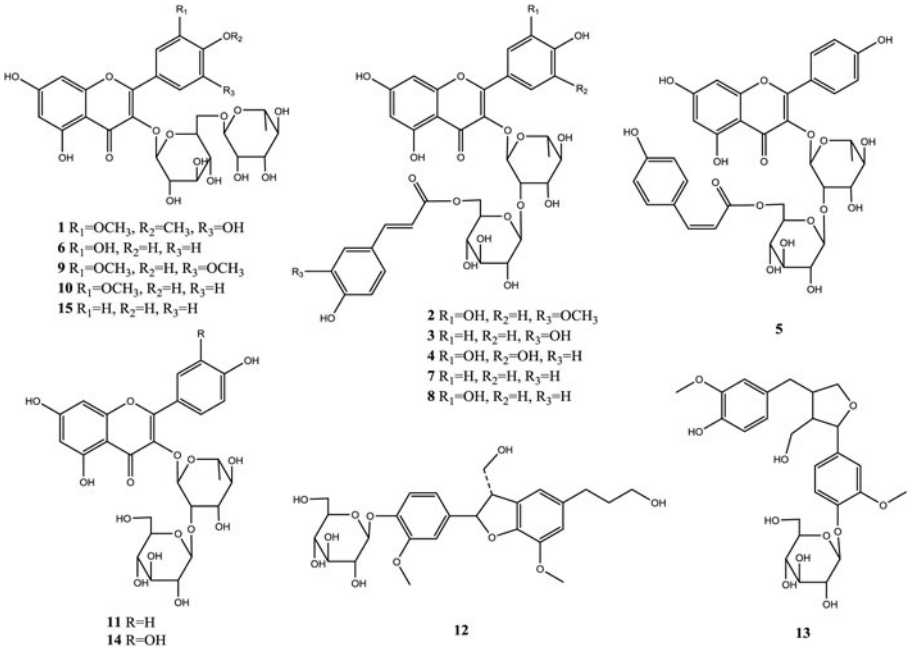

Figure 1. Structure of compounds **1–15**.

Compound **2** was obtained as a pale yellow amorphous powder. Its molecular formula was suggested as $C_{37}H_{38}O_{19}$ from the basis of negative HR-ESI-MS (found *m/z* 785.1944 $[M-H]^-$) and positive HR-ESI-MS (found *m/z* 809.1912 $[M+Na]^+$). The ^1H NMR spectrum showed one hydroxyl group at δ 12.67 (1H, s, 5-OH), two broad singlet signals of A ring at δ 6.18 (1H, s, H-6) and 6.33 (1H, s, H-8), as well as an ABX system at δ 6.90 (1H, d, $J = 8.4$ Hz, H-5′), 7.26 (1H, dd, $J = 8.4$, 2.0 Hz, H-6′) and 7.38 (H, d, $J = 2.0$ Hz, H-2′) of B ring, revealed the existence of a quercetin moiety. Moreover, the spectrum revealed signals of a methoxyl group at δ 3.75 (3H, s, 6‴-OCH_3), two olefinic doublets at δ 6.35 (1H, d, $J = 15.9$ Hz, H-2‴) and 7.45 (1H, d, $J = 15.9$ Hz, H-3‴), a second ABX system at δ 6.72 (1H, d, $J = 8.2$ Hz, H-8‴), 6.99 (1H, dd, $J = 8.2$, 1.7 Hz, H-9‴) and 7.21 (1H, d, $J = 1.7$ Hz, H-5‴) suggesting a substitution by an organic acid derivative of *trans*-ferulic acid. It also indicated the spectrum exhibited two anomeric protons at δ 4.29 (1H, d, $J = 7.8$ Hz, H-1‴) and δ 5.52 (1H, s, H-1″) and a methyl at δ 0.92 (3H, d, $J = 6.2$ Hz, H-6″), suggesting a β-glucosyl and an α-rhamnosyl moieties. The ^1H-^1H COSY experiments permitted the full assignment of the sugar protons. The configuration of sugar was confirm to be D-glucose and L-rhamnose by acid hydrolysis and TLC analysis. In the HMBC spectrum, the long-rang correlations from δ 5.52 (1H, s, H-1″) to δ 134.7 (C-3), from δ 4.29 (1H, d, $J = 7.8$ Hz, H-1‴) to δ 82.2 (C-2″) confirmed the C-1″ (rha) and C-1‴ (glc) were attached to C-3 of aglycone and C-2″ (rha), and the esterification of the glucose unit was confirmed by the correlations observed between δ 167.9 (C-1⁗) and both δ 4.04 (1H, d, $J = 11.8$ Hz, H-6a‴) and 4.22 (1H, dd, $J = 11.8$, 3.8 Hz, H-6b‴). Analysis of the ROESY spectrum showed a correlation between the methoxy protons δ 3.75 (3H, s, 6⁗-OCH_3) and proton δ 7.21 (1H, d, $J = 1.7$ Hz, H-5⁗), and allowed us to locate the methoxy group at C-6⁗. Based on the evidence above,

compound **2** was deduced as quercetin 3-*O*-(6-*trans*-feruloyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside.

Compound **3** was obtained as a pale yellow amorphous powder. Its molecular formula was suggested as $C_{36}H_{36}O_{18}$ from the basis of negative HR-ESI-MS (found *m/z* 755.1897 $[M-H]^-$) and positive HR-ESI-MS (found *m/z* 779.1835 $[M+Na]^+$). The ^1H NMR spectrum had two broad singlet signals at δ 6.19 (1H, s, H-6) and 6.37 (1H, s, H-8) of A ring, and an AA′BB′ system at δ 7.38 (2H, d, $J=8.8$ Hz, H-2′, 6′), 6.93 (2H, d, $J=8.8$ Hz, H-3′, 5′) of B ring, revealed the existence of a kaempferol moiety. Moreover, the spectrum revealed signals of two olefinic doublets at δ 6.11 (1H, d, $J=15.9$ Hz, H-2‴′) and 7.39 (1H, d, $J=15.9$ Hz, H-3‴′), a ABX system at δ 6.69 (1H, d, $J=8.2$ Hz, H-8‴′), 6.87 (1H,dd, $J=8.2$, 1.7 Hz, H-9‴′) and 6.96 (1H, d, $J=1.7$ Hz, H-5‴′) suggesting a substitution by a *trans*-caffeic acid. Compared ^1H and ^{13}C NMR spectroscopic data of **3** with those of **2** revealed that the structures of **3** had the same sugar moieties as **2**, D-glucose and L-rhamnose were identified by TLC upon acid hydrolysis of **3**. In the HMBC spectrum, the kaempferol was glycosylated at position 3, as observed by the long-rang correlations between δ 5.60 (1H, s, H-1″) and δ 134.8 (C-3); and the esterification of the glucose was confirmed by the correlations between δ 167.9 (C-1‴′) and δ 4.18 (2H, m, H-6‴). Based on the evidence above, compound **3** was deduced as kaempferol 3-*O*-(6-*trans*-caffeoyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside.

Compound **4** was obtained as a pale yellow amorphous powder. Its molecular formula was suggested as $C_{36}H_{36}O_{19}$ from the basis of negative HR-ESI-MS (found *m/z* 771.1788 $[M-H]^-$) and positive HR-ESI-MS (found *m/z* 795.1759 $[M+Na]^+$). The ^1H NMR spectrum showed one hydroxyl group at δ 12.67 (1H, s, 5-OH), two broad singlet signals of A ring at δ 6.17 (1H, s, $J=1.7$ Hz, H-6) and 6.33 (1H, s, $J=1.7$ Hz, H-8), as well as a singlet at δ 6.96 (2H, s, H-2′, 6′) of B ring, revealed the existence of a myricetin moiety. Moreover, the spectrum revealed signals of two doublets at δ 6.27 (1H, d, $J=15.9$ Hz, H-2‴′) and 7.46 (1H, d, $J=15.9$ Hz, H-3‴′) which identified *trans* olefinic double bond protons, and an AA′BB′ system at δ 6.72 (2H, d, $J=8.6$ Hz, H-6‴′, 8‴′) and 7.44 (2H, d, $J=8.6$ Hz, H-5‴′, 9‴′), which confirmed the presence of the *trans-p*-coumaroyl subunit. Compared ^1H NMR, ^{13}C NMR and HMBC spectroscopic data of **4** with those of **2** revealed that the structures of **4** had the same sugar components as **2**, and D-glucose and L-rhamnose were identified by TLC upon acid hydrolysis of **4**. In the HMBC spectrum, the myricetin was glycosylated at position 3, as observed by the long-rang correlations between δ 5.46 (1H, s, H-1″) and δ 133.3 (C-3); a cross peak between δ 165.3 (C-1‴′) and both δ 3.96 (1H, d, $J=11.5$ Hz, H-6a‴) and 4.22 (1H, d, $J=11.5$, 3.7 Hz, H-6b‴) established the linkage points between the two sugar and the *trans-p*-coumaroyl moieties. On the basis of these data, compound **4** was elucidated as myricetin 3-*O*-(6-*trans-p*-coumaroyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside.

Compound **5** was obtained as a yellow amorphous powder. Its molecular formula was suggested as $C_{36}H_{36}O_{17}$ from the basis of negative HR-ESI-MS (found *m/z* 739.1889 $[M-H]^-$) and positive HR-ESI-MS (found *m/z* 763.1856 $[M+Na]^+$). The ^1H NMR spectrum showed the characteristic proton signals at δ 6.35 (1H, d, $J=1.6$ Hz, H-8), 6.19 (1H, d, $J=1.6$ Hz, H-6), 7.66 (2H, d, $J=8.7$ Hz, H-2′, 6′), 6.91 (2H, d, $J=8.7$ Hz, H-3′, 5′), and 12.56 (1H, brs, 5-OH) revealed the existence of a kaempferol moiety. Moreover, the spectrum exhibited two doublets at δ 5.34 (1H, d, H-2‴′) and 6.39 (1H,

d, H-3′′′′) with coupling constants of $J = 12.8$ Hz which assigned *cis* olefinic double bond protons, and the other caused by the four aromatic ring proton signals at δ 7.48 (2H, d, $J = 8.7$ Hz, H-5′′′′, 9′′′′) and 6.69 (2H, d, $J = 8.7$ Hz, H-6′′′′, 8′′′′) (AA′BB′), which confirmed the presence of the *cis-p*-coumaroyl subunit (Lee et al. 2013). Compared ^{1}H NMR, ^{13}C NMR and HMBC spectroscopic data of **5** with those of **2** revealed that the structures of **5** had the same sugar components as **2**, D-glucose and L-rhamnose were identified by TLC upon acid hydrolysis of **5**. In the HMBC spectrum, a cross peak between δ 5.61 (1H, d, $J = 1.0$ Hz, H-1′′) and δ 137.4 (C-3), and between δ 4.28 (2H, m, H-6′′′) and δ 167.9 (C-1′′′′) established the linkage points between the two sugar and the *p-cis*-coumaroyl moieties. On the basis of these data, compound **5** was elucidated as kaempferol 3-*O*-(6-*cis-p*-coumaroyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside. This compound was previously reported (Xie et al. 2006) but neither the isolation method nor the structural data was included.

Additionally, the known compounds (**6–15**) were identified by comparing the spectroscopic data with those reported in the literature to be rutin (6) (Wang et al. 1999), kaempferol 3-*O*-(6-*p*-coumaroyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**7**) (Markham et al. 1992), quercetin 3-*O*-α-(6-*p*-coumaroyl)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**8**) (Markham et al. 1992), syringetin-3-*O*-rutnoside (9) (Victoire et al. 1988), isorhamnetin-3-*O*-rutinoside (**10**) (Victoire et al. 1988), kaempferol-3-*O*-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**11**) (Hasler et al. 1992), dihydrodehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside (**12**) (Matsuda et al. 1996), lariciresinol-4′-*O*-β-D-glucoside (**13**) (Sugiyama and Kikuchi 1993), quercetin-3-*O*-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**14**) (Hasler et al. 1992), kaempferol-3-*O*-rutinoside (**15**) (Markham et al. 1978). Among them, compounds **9** and **10** were isolated from the *Ginkgo* genus for the first time.

All the isolates were evaluated for their antioxidative effects against H_2O_2-induced cytotoxicity in cultured H9c2 cells. Among them, the inhibition rates of compound **9** was 50.34%, compounds **10** and **13** were more than 30%. The flavonol glycosides had stronger activity than the acylated flavonol glycosides at the concentration of 50 μM. None of the compounds showed a significant cytotoxicity on H9c2 cells at the concentration of 50 μM.

3. Conclusions

Thirteen flavonoids and two lignans were obtained, compounds **1**, **2**, **3** and **4** were new flavonoid glycosides, and the NMR data of compound **5** was unprecedented and fully assigned for the first time. All the isolates were evaluated for their antioxidative effects against H_2O_2-induced apoptosis in H9c2 cells. All the isolates were evaluated for their antioxidative effects against H_2O_2-induced cytotoxicity in cultured H9c2 cells. The flavonol glycosides had stronger activity than the acylated flavonol glycosides at the concentration of 50 μM

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Hasler A, Gross GA, Meier B, Sticher O. 1992. Complex flavonol glycosides from the leaves of Ginkgo biloba. Phytochemistry. 31(4):1391–1394.

Lee YG, Cho JY, Kim CM, Lee SH, Kim WS, Jeon TI, Park KH, Moon JH. 2013. Coumaroyl quinic acid derivatives and flavonoids from immature pear (*Pyrus pyrifolianakai*) fruit. Food Sci Biotechnol. 22(3):803–810.

Lin LZ, Chen P, Ozcan M, Harnly JM. 2008. Chromatographic profiles and identification of new phenolic components of Ginkgo biloba leaves and selected products. J Agric Food Chem. 56(15):6671–6679.

Markham KR, Geiger H, Jaggy H. 1992. Kaempferol-3-O-glucosyl(1-2)rhamnoside from Ginkgo biloba and reappraisal of other gluco(1-2, 1-3, and 1-4)rhamnoside structures. Phytochemistry. 31(3):1009–1011.

Markham KR, Ternai B, Stanley R, Geiger H, Mabry TJ. 1978. Carbon-13NMR studies of flavonoids-III: naturally occurring flavonoid glycosides and their acylated derivatives. Tetrahedron. 34(9):1389–1397.

Matsuda N, Sato H, Yaoita Y, Kikuchi M. 1996. Isolation and absolute structures of the neolignan glycosides with the enantiometric aglycones from the leaves of *Viburnum awabuki* K. KOCH. Chem Pharm Bull. 44(5):1122–1123.

Mohanta TK, Tamboli Y, Zubaidha PK. 2014. Phytochemical and medicinal importance of Ginkgo biloba L. Nat Prod Res. 28(10):746–752.

Singh B, Kaur P, Gopichand Singh RD, Ahuja PS. 2008. Biology and chemistry of Ginkgo biloba. Fitoterapia. 79:401–418.

Sugiyama M, Kikuchi M. 1993. Characterization of lariciresinol glucosides from *Osmanthus asiaticus*. Heterocycles. 36:117–121.

Varga E, Bodi A, Ferdinandy P, Droy-Lefaix MT, Blasig IE, Tosaki A. 1999. The protective effect of EGb 761 in isolated ischemic/reperfused rat hearts: a link between cardiac function and nitric oxide production. J Cardiovasc Pharmacol. 34(5):711–717.

Victoire C, Haag-Berrurier M, Lobstein-Guth A, Balz J, Anton R. 1988. Isolation of flavonol glycosides from *Ginkgo biloba* leaves. Planta Med. 54(03):245–247.

Wang M, Kikuzaki H, Csiszar K, Boyd CD, Maunakea A, Fong SFT, Ghai G, Rosen RT, Nakatani N, Ho C-T. 1999. Novel trisaccharide fatty acid ester identified from the fruits of *Morinda citrifolia* (noni). J Agric Food Chem. 47(12):4880–4882.

Xie P, Chen S, Liang YZ, Wang X, Tian R, Upton R. 2006. Chromatographic fingerprint analysis-a rational approach for quality assessment of traditional Chinese herbal medicine. J Chromatogr A. 1112(1–2):171–180.