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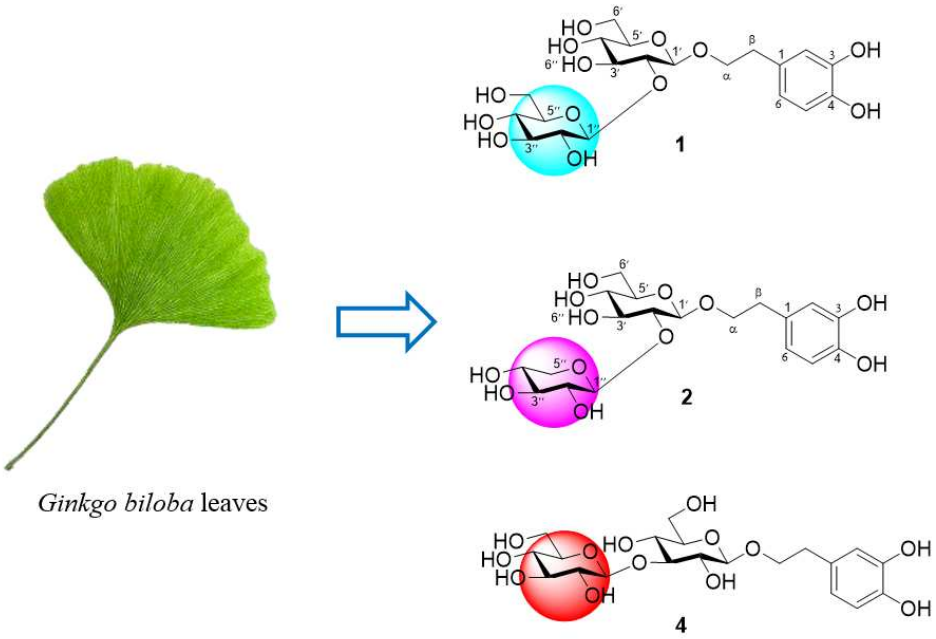
Graphical Abstract



Two New Phenylethanoid Glycosides from *Ginkgo biloba* Leaves and Their Tyrosinase Inhibitory Activities

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Abstract

Two undescribed phenylethanoid glycosides, Ginkgoside C (**1**) and D (**2**), together with ten known glycosides (**3**–**12**) were isolated from *Ginkgo biloba* leaves. Their structures were characterized by physical data analyses such as NMR, HRESIMS, as well as chemical hydrolysis. All compounds were tested for their tyrosinase inhibitory activities. At a concentration of 25 μM, compounds **2**, **4**, **5**, **6**, and **11** showed obvious mushroom tyrosinase inhibition activities, with %inhibition values of 19.12±2.59%, 25.79±1.83%, 16.07±1.07%, 24.46±1.10%, 18.64±3.62%, respectively, with kojic acid used as the positive control (27.50 ± 2.72%).

Key words

Ginkgo biloba, tyrosinase, antioxidants, hydrolysis, glycosides

Introduction

Ginkgo biloba leaves are rich in bioactive flavonoids, ginkgolic acids, terpene trilactones and phenolic compounds [1-6]. In China, the extract of *Ginkgo biloba* leaves (EGb) has been developed to be different dosage forms such as tablets, capsules and pills, which are widely used in the treatment of chest pain, stroke, hemiplegia, cerebral infarction, and coronary heart diseases [7]. In the past decades, a series of researches revealed that EGb could inhibite the activity of tyrosinase [8-11], an enzyme catalyzing the key steps of pigment melanin formation, indicating that EGb could be not only applied in the treatment of skin hyperpigmentation disorders, such as melasma, but also in the skin-whitening cosmetics production. As part of our continuous efforts to find novel tyrosinase inhibitors from natural medicines [12-13], the chemical constituents of EGb were investigated and their antityrosinase activities were evaluated by mushroom tyrosinase inhibition assay. A total of twelve glycosides were isolated, including two new phenylethanoid glycosides (**1**–**2**) (Figure 1). Five of them (**2**,**4**–**6**,**11**) showed obvious antityrosinase activities at a concentration of 25 μM. Herein, the isolation, structure elucidation and antityrosinase activities evaluation of the constituents from EGb were reported.

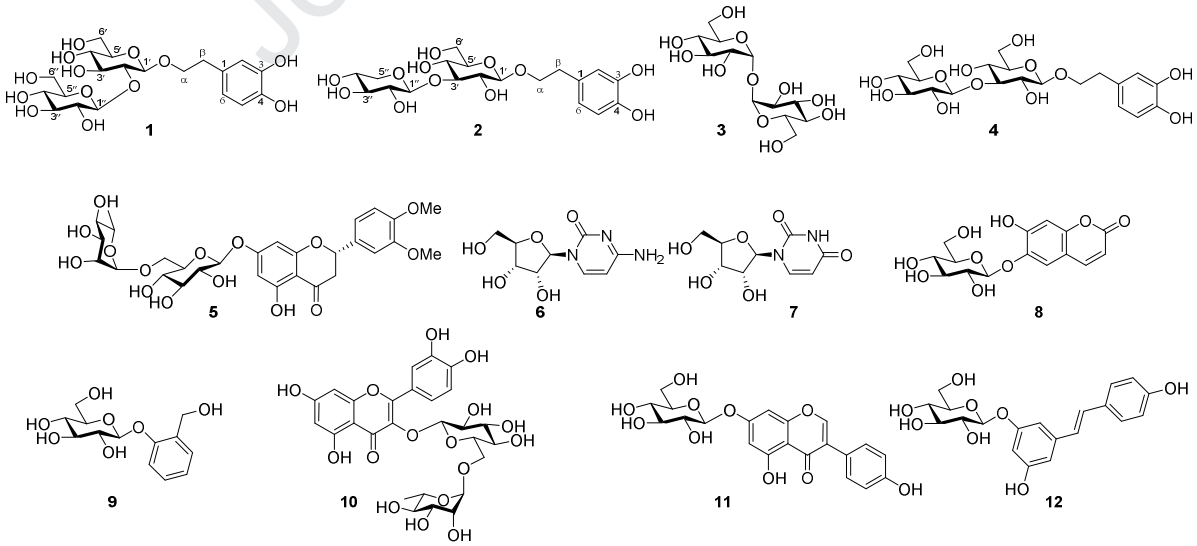

Fig. 1 Structures of compounds **1**–**12**.

Results and Discussion

Compound **1** was obtained as a colorless syrup. The molecular formula $C_{20}H_{30}O_{13}$, with six degrees of unsaturations, was established based on its quasi-molecular ion peak at *m/z* 501.1577 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_{13}Na$, 501.1584) in the HRESIMS spectrum. Its IR spectrum (KBr) showed absorption bands for hydroxyl (3374 cm^{-1}) and aromatic ring (1654, 1629, 1448 cm^{-1}). The ^{1}H-NMR spectrum of **1** displayed the signals of an ABX system ascribed to a 1,3,4-trisubstituted aromatic ring [δ 6.70 (d, J = 2.0 Hz, H-2, 1 H), 6.66 (d, J = 8.0 Hz, H-5, 1 H), 6.56 (dd, J = 8.0, 2.0 Hz, H-6, 1 H)], and an A_2B_2 system assigned to a hydroxyethyl group [δ 4.03 (m, H-αa, 1 H), 3.72 (m, H-αb, 1 H), 2.77 (m, H_2-β, 2 H)], indicating the presence of a 2-(3,4-dihydroxyphenyl)ethoxy moiety (Table 1). The two anomeric signals at δ 4.44 (d, J = 8.0 Hz, H-1', 1 H) and 4.61 (d, J = 8.0 Hz, H-1", 1 H) suggested that compound **1** should be a phenylethanoid glycoside (Table 1). The ^{13}C-NMR spectrum of **1** showed 20 carbons, of which eight were assigned to the aglycon moiety; the remaining twelve signals attributed to two hexose sugar residues (Table 1). Acid hydrolysis of **1** gave 3,4-dihydroxyphenylethyl alcohol (**1a**)[14] and d-glucose, which were identified by NMR and comparison with an authentic sample on TLC, respectively. The above evidences led to the assumption that the sugar portion of **1** was composed of two β-D-glucose residues. The NMR data of **1** were very similar to those of 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (**4**) [15], except that C-2' in **1** (δ 83.0) shifted downfield compared to **4**, while C-3' in **1** (δ 78.1) shifted upfield compared to **4** (Table 1). Thus, the *O*-glycosylation position between the two glucoses should be at C-2' in **1**, instead of at C-3' in **4**. The key HMBC correlations from H-1" to C-2', and H-2' to C-1", confirmed this deduction (Figure 2). Finally, compound **1** was elucidated to be 2-(3,4-

dihydroxyphenyl)ethyl 2-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranoside, named as Ginkgoside C (Figure 2).

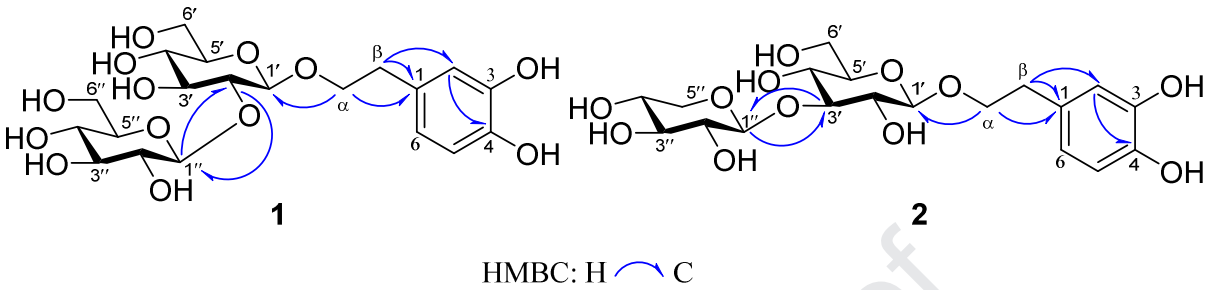

Fig. 2 Key HMBC correlations for compounds **1** and **2**.

Compound **2** was obtained as a colorless syrup. The molecular formula $C_{19}H_{28}O_{12}$, with six degrees of unsaturations, was established based on its quasi-molecular ion peak at *m/z* 471.1469 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478) in the HRESIMS spectrum. Its IR spectrum (KBr) showed absorption bands for hydroxyl (3372 cm^{-1}) and aromatic ring (1606, 1529, 1373 cm^{-1}). The ^{1}H-NMR spectrum of **1** was very similar to that of **2**, which also showed the signals of a 2-(3,4-dihydroxyphenyl)ethoxy moiety [*δ* 6.68 (d, *J* = 2.0 Hz, H-2, 1 H), 6.66 (d, *J* = 8.0 Hz, H-5, 1 H), 6.55 (dd, *J* = 8.0, 2.0 Hz, H-6, 1 H), 4.02 (m, H-*α*a, 1 H), 3.70 (m, H-*α*b, 1 H), 2.78 (m, H_2-*β*, 2 H)] and two anomeric protons (*δ* 4.34 (d, *J* = 8.0 Hz, H-1', 1 H) and 4.50 (d, *J* = 7.2 Hz, H-1", 1 H) (Table 1), indicating that **2** belonged to the phenylethanoid class of natural products. Analysis of ^{13}C-NMR data revealed that compound **2** had one carbon less than **1**. Acid hydrolysis of **2** gave 3,4-dihydroxyphenylethyl alcohol (**1a**), D-glucose and D-xylose. Therefore, the sugar portion of **2** consisted of one glucose and one xylose, rather than two glucoses in **1**. The NMR data of **2** were very similar to those of 2-(3,4-dihydroxyphenyl)ethyl 2-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside[16], except that C-2' in **2** (*δ* 74.5) shifted upfield, while C-3' in **2** (*δ* 88.1) shifted downfield compared to the latter compound (Table 1). Therefore, the *O*-glycosylation

position between the two sugars should be at C-3' in **2**, which was further confirmed by the key HMBC correlations from H-1" to C-3' and H-3' to C-1" (Figure 2). Hence compound **2** was elucidated as 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside, named as Ginkgoside D (Figure 2).

By comparing their NMR data with those published in literatures, the remaining ten known compounds (**3**–**12**) were identified as follows: trehalose (**3**) [17], 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranoside (**4**) [15], methyl hesperidin (**5**) [18], cytidine (**6**) [19], uridine (**7**) [20], esculin (**8**) [21], salicin (**9**) [22], rutin (**10**) [23], genistin (**11**) [24], and polydatin (**12**) [25], repectively. Compounds **5** and **8** were isolated from *Ginkgo biloba* for the first time.

All compounds (**1**–**12**) and kojic acid (positive control) were screened for their inhibitory activities against mushroom tyrosinase at a concentration of 25 μM. As shown in table 2, compounds **2**, **4**, **5**, **6**, and **11** showed strong mushroom tyrosinase inhibition activities, with %inhibition values close to kojic acid. It was interesting to find that phenylethanoid glycoside **1** showed weak antityrosinase activity while its analogues **2** and **4** revealed obvious activities. Perhaps the sugar part in C-2 position of compound **1** hindered the chelation of *ortho*-hydroxyls on the phenolic rings with the active site (metal ions) in the enzyme [26].

Materials and Methods

General Experimental Procedures. Optical rotations were determined on a Rudolph Autopol IV polarimeter. UV and FT-IR spectra were determined using Puxi TU-1950 and FTIR-650 instruments, respectively. NMR spectra were recorded on a Bruker AM-400 spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) were carried out on a Waters Xevo

G2-XS QTof spectrometer using electrospray ionization. Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Inc., China), ODS (50 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Semipreparative HPLC was carried out on a Persee L600-DP system using a reversed-phased C18 column (5 μm, 10 × 250 mm, H&E), with a UV detector (254 nm). TLC was performed with silica gel 60 F254 (Yantai Chemical Industry Research Institute).

Plant Material. The fresh leaves of *Ginkgo biloba* were collected in Xuchang, People's Republic of China, in September 2019, and identified by Prof. Lin Yang at School of Life Science and Engineering, Lanzhou University of Technology. A voucher specimen (SPH2019C) was deposited in School of Chemistry and Chemical Engineering, Xuchang University.

Extraction and Isolation. The air-dried leaves of *Ginkgo biloba* (3.7 kg) were extracted with 95% EtOH at room temperature (3 × 30 L), affording a crude extract of 256.3 g after evaporation of the solvent under reduced pressure. The extract was suspended in H_2O and partitioned with CH_2Cl_2, EtOAc and *n*-BuOH. The *n*-BuOH portion (29.4 g) was subjected to silica gel CC using CH_2Cl_2–MeOH (60:1 to 1:1) as eluent to give eight fractions F1–F8. Fraction F1 (eluted by CH_2Cl_2–MeOH 60:1) was subsequently purified using RP-C_{18} CC (MeOH–H_2O, 30:70 to 60:40) to afford compounds **8** (10.6 mg) and **9** (7.4 mg). Fraction F2 (eluted by CH_2Cl_2–MeOH 30:1) was further separated using RP-C_{18} CC (MeOH–H_2O, 20:80 to 60:40) to give compounds **7** (11.1 mg) and **11** (6.2 mg), and **12** (5.9 mg). Fraction F4 (eluted by CH_2Cl_2–MeOH 15:1) was chromatographed on a Sephadex LH-20 column (MeOH) to give compounds **3** (6.2 mg) and **6** (4.1 mg). Fraction F5 (eluted by CH_2Cl_2–MeOH 8:1) was purified by RP-C_{18} CC (MeOH–H_2O, 10:90) to give compound **5** (6.7 mg). Fraction F6 (eluted by CH_2Cl_2–MeOH 5:1) was purified on a Sephadex LH-20 column (MeOH) to give compounds **2** (11.2 mg) and **10** (7.7 mg). Fraction

F7 (eluted by CH_2Cl_2–MeOH 3:1) was purified by HPLC (CH_3CN-H_2O 10:90, 1.5 mL/min) to give compounds **1** (3.2 mg, t_r = 23.2 min) and **4** (10.5 mg, t_r = 28.3 min).

*Compound **1***. Colorless syrup. $[\alpha]_D^{20}$–12.0° (c 0.10, MeOH). IR (KBr) ν_{max} 3374, 2919, 1743, 1654, 1629, 1448, 1382, 1261, 1076 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 215 (4.2), 283 (3.7). HRESIMS *m/z* 501.1577 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_{13}Na$, 501.1584). ^1H NMR and ^{13}C NMR (CD_3OD), see Table 1.

*Compound **2***. Colorless syrup. $[\alpha]_D^{20}$–9.7° (c 0.31, MeOH). IR (KBr) ν_{max} 3372, 2923, 1606, 1529, 1373, 1286, 1247, 1157, 1081, 1043 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 211 (4.1), 284 (3.7). HRESIMS 471.1469 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478). ^1H NMR and ^{13}C NMR (CD_3OD), see Table 1.

Acid Hydrolysis of Compounds 1–2. The acid hydrolysis of compounds **1**–**2** was conducted according to the literature procedures.[12] The glycosides were separately dissolved in a mixture solvent of 8.0% HCl (0.5 mL) and MeOH (4 mL), then refluxed for 3 h. The reaction mixture was concentrated under vacuum to afford a residue, which was further purified by silica gel CC to give 3,4-dihydroxyphenylethyl alcohol (**1a**), D-glucose and D-xylose. The sugars were confirmed by co-TLC with authentic samples. TLC ($CHCl_3$/AcOH/H_2O 6:7:1): R_f value of D-glucose 0.24, of D-xylose 0.47.

3,4-Dihydroxyphenylethyl alcohol (**1a**) [14]: Colorless oil. ^1H-NMR(CD_3OD, 400 MHz): δ 6.67 (d, J = 8.0 Hz, H-5, 1 H), 6.65 (d, J = 2.0 Hz, H-2, 1 H), 6.52 (dd, J = 8.0, 2.0 Hz, H-6, 1 H), 3.67 (t, J = 7.2 Hz, H_2-α, 2 H), 2.66 (t, J = 7.2 Hz, H_2-β, 2 H); ^{13}C NMR (CD_3OD, 100 MHz): 146.3 (C-3), 144.8 (C-4), 131.9 (C-1), 121.4 (C-6), 117.2 (C-5), 116.4 (C-2), 64.7 (C-α), 39.8 (C-β).

Mushroom Tyrosinase Inhibition Assay. The mushroom tyrosinase inhibition assay was conducted according to the reported procedures [27]. Compounds (**1**–**12**, 10 μL, 25 μM) and mushroom tyrosinase (20 μL, 1000 U/mL) (Sigma-Aldrich) in a potassium phosphate buffer (50 mM, pH 6.5) were added to 170 μL of a mixture containing a 10 : 10 : 9 ratio of L-tyrosine solution (1 mM), potassium phosphate buffer (1 mM), and distilled water in a 96-well microplate. The plates were incubated at 37 °C for half an hour. Percentage tyrosinase inhibition was decided by measuring optical densities at 450 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Inc.). Three independent experiments were performed. Kojic acid (25 μM) was used as the positive control. The % inhibition was determined by [1 − (As/Ac)] × 100, where As is the absorbance of tested compound and Ac the non-treated control. Statistical analysis was determined using GraphPad Prism 5 software, and the results were expressed as means ± SEMs. The inhibitory rate > 5% is considered active.

Supporting Information

HRESIMS, 1D and 2D NMR, IR and UV spectra for compounds **1**–**2**, ^{1}H NMR and ^{13}C NMR spectra of **1a**. This material is available free of charge via the Internet at XXX.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] X.G. Liu, S.Q. Wu, P. Li, H. Yang, J. Pharm. Biomed. Anal. 113 (2015), 212−225.

[2] T.A. van Beek, P. Montoro, J. Chromatogr. A 1216 (2009), 2002−2032.

[3] B. Singh, P. Kaur, Gopichand, R.D. Singh, P.S. Ahuja, Fitoterapia 79 (2008), 401−418.

[4] P. Shu, M. Sun, J. Li, L. Zhang, H. Xu, Y. Lou, Z. Ju, X. Wei, W. Wu, N. Sun, J. Nat. Med. 74 (2020), 269−274.

[5] H.L. Dong, S. Lin, Q.L. Wu, R.X. Su, Z.L. Wu, H.Y. Dong, H.L. Li, W.D. Zhang, Fitoterapia 142 (2020), 104516.

[6] P. Shu, M. Sun, H. Xu, X. Yang, H. Niu, M. Geng, Z. Ju, Chem. Nat. Compd. 55 (2019), 1059−1061.

[7] Chinese Pharmacopoeia Commission, in 'Pharmacopoeia of the People's Republic of China', Chemical Industry Press, Beijing, 2015, Vol. 1, pp. 1491−1493.

[8] P. Fong, H.H.Y. Tong, Nat. Prod. Commun. 7 (2012), 1287−1294.

[9] Y. Ye, G.X. Chou, D.D. Mu, H. Wang, J.H. Chu, A.K.M. Leung, W.F. Fong, Z.L. Yu, J. Ethnopharmacol. 129 (2010), 387−390.

[10] J.H. Hwang, B.M. Lee, J. Toxicol. Environ. Health, Part A 70(2007), 393−407.

[11] P. Xu, Z. Qian, K. Zhang, Y. Wang, Tianran Chanwu Yanjiu Yu Kaifa, 18 (2006), 986−988.

[12] P. Shu, J. Li, Y. Fei, H. Zhu, M. Yu, A. Liu, H. Niu, S. Zou, X. Wei, Z. Ju, Z. Xu, J. Nat. Med. 74 (2020), 456−462.

[13] P. Shu, J. Li, Y. Fei, H. Zhu, L. Zhang, H. Niu, Y. Li, H. Liu, Z. Ju, X. Wei, F. Xiao, Z. Xu, Phytochem. Lett. 36 (2020), 32−36.

[14] H. Kırmızıbekmez, Y. İnan, R. Reis, H. Sipahi, A.C. Gören, E. Yeşilada, Nat. Prod. Res. 33 (2019), 2541−2544.

[15] H. Wang, Y. Sun, W.C. Ye, F. Xiong, H.J. Wu, C.H. Yang, S.X. Zhao, Chem. Pharm. Bull. 52 (2004), 615−617.

[16] L.N. Wang, D.X. Guo, S.Q. Wang, C.S. Wu, M.U. Rehman, H.X. Lou, Helv. Chim. Acta 94 (2011), 1146−1152.

[17] M.D. Brandao, L.S. Abreu, R. Geris, Chem. Biodiversity. 16 (2019), e1900353.

[18] B. Zhang, X. Ye, Z. Chen, B. Yao, P. Tan, X. Li, Yaoxue Xuebao 46 (2011), 811−817.

[19] K.I. Takatsuki, S. Ohgushi, S. Kohmoto, K. Kishikawa, M. Yamamoto, Nucleosides, Nucleotides Nucleic Acids 25 (2006), 719−734.

[20] H.H.F. Koolen, E.M.F. Pral, S.C. Alfieri, J.V.N. Marinho, A.F. Serain, A.J. Hernández-Tasco, N.L. Andreazza, M.J. Salvador, Phytochemistry 134 (2017), 106−113.

[21] S.A.L. Bayoumi, M.G. Rowan, J.R. Beeching, I.S. Blagbrough, Phytochemistry 71 (2010), 598−604.

[22] T. Ishikawa, K. Nishigaya, K. Takami, H. Uchikoshi, I.S. Chen, I.L. Tsai, J. Nat. Prod. 67 (2004), 659−663.

[23] Y.Z. Hou, K.K. Chen, X.L. Deng, Z.L. Fu, D.F. Chen, Q. Wang, Nat. Prod. Res. 31 (2017), 2572−2574.

[24] P. Parajuli, R.P. Pandey, T.T. Huyen Nguyen, B. Shrestha, T. Yamaguchi, J.K. Sohng, RSC Adv. 7 (2017) 16217−16231.

[25] J. Hu, T. Lin, J. Xu, R. Ding, G. Wang, R. Shen, Y.W. Zhang, H. Chen, Bioorg. Med. Chem. Lett. 26 (2016), 505−511.

[26] A. Karioti, A. Protopappa, N. Megoulas, H. Skaltsa, Bioorg. Med. Chem. 15 (2007), 2708−2714.

[27] S.K. Hyun, W.H. Lee, D.M. Jeong, Y. Kim, J.S. Choi, Biol. Pharm. Bull. 31 (2008), 154−158.

Table 1 ^{1}H and ^{13}C NMR data of **1** and **2**.[a]

Position	**1**		**2**	
	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	131.8		131.6	
2	116.5	6.70 (d, 2.0)	116.4	6.68 (d, 2.0)
3	146.2		146.3	
4	144.8		144.8	
5	117.5	6.66 (d, 8.0)	117.3	6.66 (d, 8.0)
6	121.5	6.56 (d, 8.0, 2.0)	121.4	6.55 (d, 8.0, 2.0)
β	36.7	2.77 (m)	36.7	2.78 (m)
α	72.3	4.03 (m)	72.2	4.02 (m)
		3.72 (m)		3.70 (m)
1'	103.1	4.44 (d, 8.0)	104.1	4.34 (d, 8.0)
2'	83.0	3.44 (dd, 8.8, 8.0)	74.5	3.37 (dd, 8.8, 8.0)
3'	78.1	3.55 (t, 8.8)	88.1	3.50 (t, 8.8)
4'	71.5	3.30 (m)	70.0	3.36 (m)
5'	77.9	3.26 (m)	77.8	3.26 (m)
6'	62.8	3.82 (dd, 12.0, 2.4)	62.7	3.86 (dd, 12.0, 1.6)
		3.72 (dd, 12.0, 4.8)		3.68 (dd, 12.0,4.2)
1"	105.0	4.61 (d, 8.0)	106.1	4.50 (d, 7.2)
2"	76.1	3.23 (m)	75.4	3.27 (m)
3"	78.3	3.22 (m)	77.8	3.35 (m)
4"	71.5	3.32 (m)	71.1	3.52 (m)
5"	77.9	3.25 (m)	67.2	3.91 (dd, 11.2, 4.2)
				3.24 (dd, 11.2, 10.8)
6"	62.8	3.86 (dd, 12.0, 2.0)		
		3.67 (dd, 12.0, 4.8)		

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C, recorded in CD_3OD.

Table 2 Inhibitory effects of compounds **1**–**12** and of kojic acid on mushroom tyrosinase.[a]

Compound	Tyrosinase inhibition (%)	Compound	Tyrosinase inhibition (%)
1	NI[b]	**8**	NI[b]
2	19.12±2.59	**9**	NI[b]
3	NI[b]	**10**	NI[b]
4	25.79±1.83	**11**	18.64±3.62
5	16.07±1.07	**12**	NI[b]
6	24.46±1.10	kojic acid (positive control)	27.50±2.72
7	NI[b]		

[a] Tyrosinase inhibitions were measured at a derivative concentration of 25 μM, with L-tyrosine as the substrate. Results were expressed as means ± SEMs. [b] NI: no inhibition.

Highlights

- Two new phenylethanoid glycosides (**1**–**2**) and ten known glycosides (**3**–**12**) were isolated from the leaves of *Ginkgo biloba*.
- The structures of new compounds (**1**–**2**) were determined by HRESIMS, NMR, acid hydrolysis, and comparisons with literatures.
- Compounds **2**, **4**–**6**, and **11** exhibited obvious mushroom tyrosinase inhibition activities.

Declaration of interests

☑The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: