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FULL PAPER

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New Phenylpropanoid Glycosides from *Illicium majus* and Their Radical Scavenging Activities

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Chemical investigation of the ethanol extract of the branch and leaves of *Illicium majus* resulted in the isolation of four new phenylpropanoid glycosides (**1**–**4**) and one new phenolic glycoside (**9**), along with 13 known ones. Spectroscopic techniques were used to elucidate the structures of the new isolates such as 3-[(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-1-benzofuran-5-yl]propyl β-D-glucopyranoside (**1**), [(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-2,3-dihydro-1-benzofuran-3-yl]methyl 2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**2**), [(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-2,3-dihydro-1-benzofuran-3-yl]methyl 2-*O*-α-L-rhamnopyranosyl-β-D-xylopyranoside (**3**), 3-[(2*R*,3*S*)-3-({[2-*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-xylopyranosyl]oxy}methyl)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydro-1-benzofuran-5-yl]propyl acetate (**4**), and 4-(2-hydroxyethyl)phenyl 3-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (**9**). Free radical scavenging activities of the isolates were elucidated through the DPPH assay method. The most active compounds, 1-*O*-caffeoyl-*β*-D-glucopyranose (**17**) and soulieana acid 1 (**18**), exhibited moderate radical scavenging activities ($IC_{50} = 37.7 \pm 4.4$ μM and $IC_{50} = 97.2 \pm 3.4$ μM, respectively). The antibacterial activities of the isolates against *Staphylococcus aureus* and *Escherichia coli* were also assessed, and no activity was shown at the measured concentration (< 32 μg/mL).

Keywords: Illiciaceae, *Illicium majus*, phenylpropanoid, DPPH, free radical scavenging activities.

Introduction

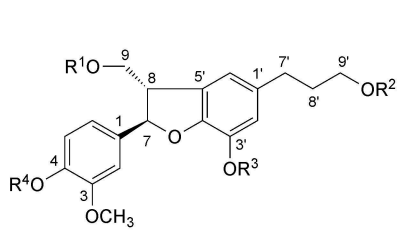
Illicium majus Hook. f. & Thomson (Illiciaceae) is also known as '*Shen xian guo*' and is a tree that grows up to 20 m and has similarities to magnolia with seed capsules that resemble those of star anise. Its distribution includes southern China, Myanmar, and Vietnam. Even though all parts of the tree have been classified as toxic by botanists, the roots, leaves and bark of *I. majus* are used in folk medicine to promote blood circulation, relive pain, dispel wind, and treat rheumatic arthritis.[1,2] To date, various seco-prezizaane-type sesquiterpenes, diterpenes, prenylated C_6–C_3 compounds, and lignans have been isolated from the different parts of this plant,[3,4] and among them, only three glycosides have currently been reported from this plant.[1–6] In our continuing work to discover new bioactive constituents from the *Illicium* genus, we carefully investigated the high-polarity components (BuOH fraction) of the ethanol extract of *I. majus*, expecting to separate more natural active products. As a result, 4 new phenylpropanoid glycosides (**1**–**4**), 1 new phenolic glycoside (**9**), and 13 known compounds (**5**–**8** and **10**–**18**) were isolated and elucidated (*Fig-*

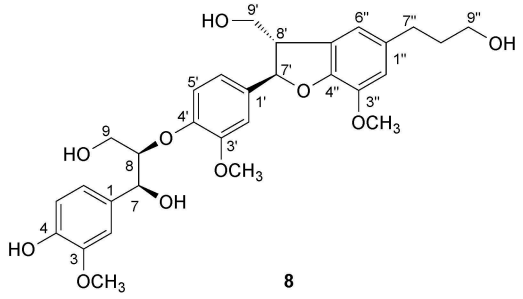
Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202001012

ure 1), together with their free radical scavenging activities.

Results and Discussion

Compound **1** was obtained as a white powder and assigned the molecular formula $C_{25}H_{32}O_{11}$ by high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) *m/z* 507.1872 $[M-H]^-$ (calc. for $C_{25}H_{31}O_{11}$: 507.1845). The infrared (IR) spectrum demonstrated the hydroxy absorption at 3414 cm^{-1} and aromatic rings bands at 1630, 1610 and 1517 cm^{-1}. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum displayed characteristic ABX spin-system signals at δ 6.97 (1H, d, $J=1.8$, H-2), 6.84 (1H, dd, $J=8.1$, 1.8, H-6), and 6.76 (1H, d, $J=8.1$, H-5), aromatic protons at δ 6.62 (1H, s, H-6′) and 6.60 (1H, s, H-2′), one of the C_6-C_3 moiety protons at δ 5.48 (1H, d, $J=6.1$, H-7), 3.44 (1H, dd, $J=12.5$, 6.1, H-8), 3.81–3.83 (1H, m, H-9a), and 3.74 (1H, dd, $J=11.0$, 7.4, H-9b), and the other C_6-C_3 protons at δ 2.60 (2H, t, $J=7.4$, H-7′), 1.85–1.89 (2H, m, H-8′), 3.91–3.94 (1H, m, H-9′a), and 3.52–3.55 (1H, m, H-9′b). These NMR data were closely related to those of compound **7**,[6,7] except for the difference of the chemical shift of C-9′ (δ 68.6) and the presence of one sugar signal [δ 4.25 (1H, d, $J=7.8$) and δ 103.0, 73.8, 76.5, 70.2, 76.7, and 61.4] due to the β-glucopyranosyl unit.[8] The above analysis revealed that the *O*-β-D-glucopyranosyl group was located at C-9′, and additional proof to support the conclusion was obtained by heteronuclear multiple bond correlations (HMBCs) from H-1″ to C-9′ (*Figure 2*). In addition, $J_{7,8}=6.1$ in the ^{1}H-NMR spectrum indicated the *trans*-configuration between C-7 and C-8, which was further confirmed by the correlations H-7/H-9 and H-2/H-8 in the rotating





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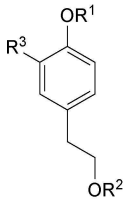
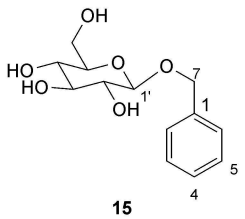
1 R^1 = H, R^2 = β-D-glucopyranosyl, R^3 = H, R^4 = H
2 R^1 = α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl, R^2 = H, R^3 = H, R^4 = H
3 R^1 = α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl, R^2 = H, R^3 = H, R^4 = H
4 R^1 = α-(4′-*O*-acetyl)-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl, R^2 = Ac, R^3 = H, R^4 = H
5 R^1 = H, R^2 = H, R^3 = β-D-glucopyranosyl, R^4 = H
6 R^1 = H, R^2 = H, R^3 = H, R^4 = β-D-glucopyranosyl
7 R^1 = H, R^2 = H, R^3 = H, R^4 = H



9 R^1 = β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl, R^2 = H, R^3 = H
10 R^1 = β-D-glucopyranosyl, R^2 = H, R^3 = H
11 R^1 = H, R^2 = H, R^3 = β-D-glucopyranosyl
12 R^1 = β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl, R^2 = H, R^3 = H
13 R^1 = H, R^2 = β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl, R^3 = H
14 R^1 = H, R^2 = H, R^3 = H



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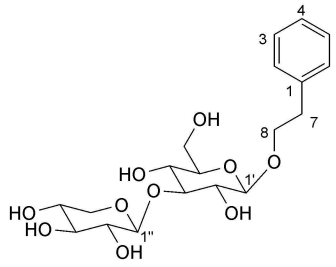

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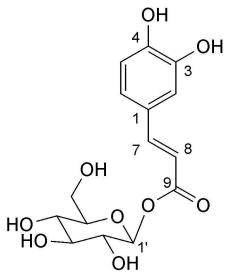

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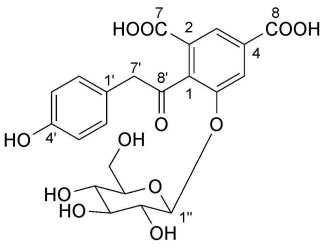

18

Figure 1. The structures of compounds **1**–**18**.

frame Overhauser effect spectroscopy (ROESY) spectrum.[8,9] The absolute configurations of C-7 and C-8 were established by the circular dichroism (CD) spectral comparison with dihydrobenzofuran-type neolignans according to the positive or negative Cotton effects at 235–245 and 285–300 nm.[10,11] The CD spectrum of compound **1** showed positive Cotton effects at 235–245 and 285–300 nm, indicating that the configurations at C-7 and C-8 were (7*R*) and (8*S*), respectively. Thus, compound **1** was determined as 3-[(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-1-benzofuran-5-yl]propyl β-D-glucopyranoside.

Compound **2**, a white gum, has the molecular formula $C_{31}H_{42}O_{15}$ as deduced from the quasi-molecular ion peak at *m/z* 653.2451 $[M-H]^-$ (calc. for $C_{31}H_{41}O_{15}$: 653.2438) in the HR-ESI-MS mass spectra. Its IR absorption bands at 3413, 1606, and 1517 cm^{-1} exhibited the presence of hydroxy and aromatic groups. There were 31 carbon signals in the ^{1}H- and ^{13}C-NMR spectrum, of which 19 were attributed to the aglycone and the remainder to the sugar moieties. Comparison of **2** with those of compound **7** indicated that they had the same skeleton,[8,9] except for the presence of sugar moieties. Comparison of two anomeric carbon signals [δ 4.40 (1H, d, *J* = 7.5, H-1″) and 101.5 (C-1″); δ 5.20 (1H, d, *J* = 1.1, H-1‴) and 100.8 (C-1‴)] and other sugar unit signals in the NMR spectra with those previously reported,[12] the disaccharide chain was determined as α-L-rhamnopyranosyl-(1→2)-β-glucopyranoside. Moreover, the sites of attachment of sugar moieties on the aglycone at C-9 of **2** and the position of the inter-sugar linkage were further determined by HMBCs from H-1″ (δ 4.40) to C-9 (δ 71.4) and H-1‴ (δ 5.20) to C-2″ (δ 77.9) (*Figure 2*). The CD data for **2** showed Cotton effects similar to those of compound **1**. Through the same methods as those used for determination of the structure of compound **1**, the absolute configuration of **2** was also confirmed. Thus, compound **2** was elucidated as [(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-2,3-dihydro-1-benzofuran-3-yl] methyl 2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside.

Compound **3** was assigned the molecular formula $C_{30}H_{40}O_{14}$ by HR-ESI-MS (*m/z* 623.2345 $[M-H]^-$, calc. for $C_{30}H_{39}O_{14}$: 623.2345). The NMR spectrum of **3** exhibited characteristic signals due to the dihydrobenzofuran skeleton and sugar moieties. A comparison of the NMR spectral data of **3** with those of **2** (*Tables 1* and *2*) indicated that both compounds **3** and **2** were similar, except for the sugar residue linked to C-9 of

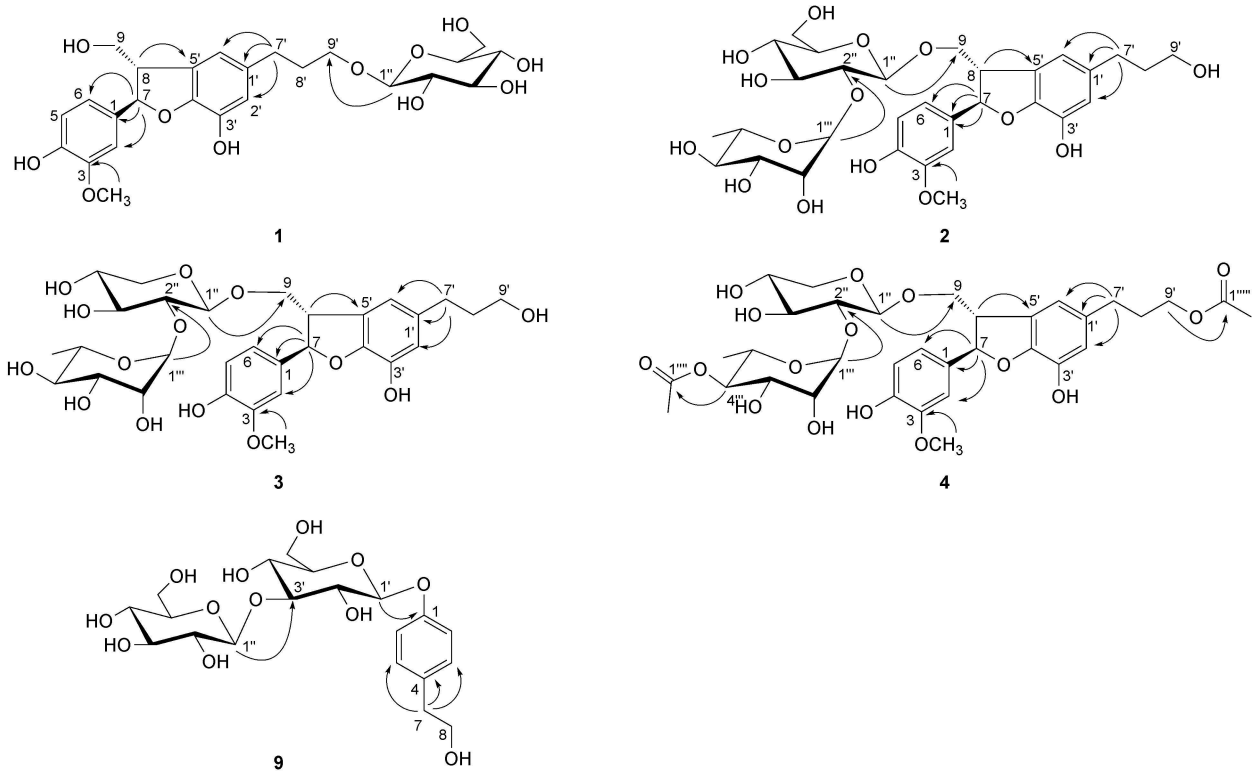

Figure 2. The key HMBCs of compounds **1**–**4** and **9**.

Table 1. ^{13}C-NMR spectroscopic data for compounds **1**–**4** and **9** in CD_3OD.

No.	**1**[b]	**2**[a]	**3**[b]	**4**[b]	**9**[b]
1	133.7	133.7	133.7	133.2	156.1
2	109.1	109.5	109.3	109.4	116.4
3	147.6	147.9	147.6	147.7	129.5
4	146.0	145.7	145.8	146.1	132.9
5	114.7	114.6	114.7	114.7	129.5
6	118.3	118.4	118.3	118.5	116.4
7	87.3	88.1	88.1	87.9	38.0
8	54.4	51.8	51.9	51.9	63.0
9	63.7	71.4	71.4	70.2	
1′	135.3	135.3	135.4	134.5	100.7
2′	115.8	115.7	115.7	115.7	72.9
3′	140.4	140.5	140.5	140.7	86.3
4′	145.1	145.1	145.1	145.2	68.4
5′	128.4	127.8	127.6	127.9	76.4
6′	115.4	115.4	115.4	115.1	61.0
7′	31.2	31.3	31.3	31.3	
8′	31.6	34.3	34.4	34.3	
9′	68.6	60.9	60.9	63.7	
1″	103.0	101.5	102.2	102.1	103.9
2″	73.8	77.9	77.8	76.6	74.1
3″	76.5	77.9	77.5	77.9	76.3
4″	70.2	70.4	70.1	70.1	70.2
5″	76.7	76.5	65.5	65.6	76.8
6″	61.4	61.4			61.2
1‴		100.8	100.9	100.2	
2‴		71.0	70.9	70.7	
3‴		70.8	70.8	68.9	
4‴		72.7	72.6	74.4	
5‴		68.5	68.5	66.2	
6‴		16.5	16.5	16.2	
OMe	55.0	55.1	55.0	55.0	
1⁗				171.4	
2⁗				19.8	
1⁗′				171.8	
2⁗′				19.5	

[a] Recorded in 100 MHz, δ, ppm. [b] Recorded in 150 MHz, δ, ppm.

the aglycone. The signals from the sugar moiety, including 2 anomeric signals at δ 4.38 (d, $J=7.1$) and δ 5.20 (d, $J=1.2$) in the ^{1}H-NMR spectrum and 11 carbons signals at δ 102.2, 77.8, 77.5, 70.1, 65.5 and δ100.9, 70.9, 70.8, 72.6, 68.5, 16.5 in the ^{13}C-NMR spectra were in agreement with those of published work.[13–15] Thus, the sugar unit was identified as α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl. Furthermore, the sequence of the sugars and linkage site in the aglycone of **3** were further confirmed by the HMBCs between H-1‴ (δ 5.20) and C-2″ (δ 77.8), H-1″ (δ 4.38) and C-9 (δ 71.4) (*Figure 2*).

The CD spectrum of **3** showed two similar positive Cotton effects in compounds **1** and **2**, suggesting that C-7 and C-8 have (*R*) and (*S*) configurations, respectively. From the above evidence, the structure of **3** was determined to be [(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-2,3-dihydro-1-benzofuran-3-yl]methyl 2-*O*-α-L-rhamnopyranosyl-β-D-xylopyranoside.

Compound **4**, a white amorphous solid, provided an HR-ESI-MS spectrum with a quasi-molecular ion peak at *m/z* 707.2557 $[M-H]^-$, (calc. for $C_{34}H_{43}O_{16}$: 707.2534), indicating the molecule to be $C_{34}H_{44}O_{16}$. The IR spectrum exhibited absorption bands attributable to hydroxy (3412 cm^{-1}), carbonyl (1726 cm^{-1}), and aromatic groups (1611, 1517 cm^{-1}). The NMR also showed signals for D-xylopyranosyl (δ 102.1, 76.6, 77.9, 70.1, 65.6) and L-rhamnopyranosyl groups (δ 100.2, 70.7, 68.9, 74.4, 66.2, 16.2) that were similar to those of **3**, except for the presence of two additional acetyl groups [δ 171.4 (C-1⁗), 1.87 (3H, s, H-2⁗); δ 171.8 (C-1⁗′), 1.93 (3H, s, H-2⁗′)]. Two acetyl groups were inferred to be linked at C-9′ and C-4‴, based on the downfield shift of C-9′ in 3 from δ 60.9 to 63.7 and C-4‴ from δ 72.6 to 74.4. The above assignments were confirmed by means of HMBC experiments, in which the correlations between H-9′ (δ 3.96) and C-1⁗′ (δ 171.8), between H-4‴ (δ 4.75) and C-1⁗ (δ 171.4) were observed (*Figure 2*). The CD data for **4** and **3** exhibited similar Cotton effects. Consequently, compound **4** was elucidated as 3-[(2*R*,3*S*)-3-({[2-*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-xylopyranosyl]oxy}methyl)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydro-1-benzofuran-5-yl]propyl acetate.

Compound **9**, a white amorphous solid, was analyzed and yielded the molecule to be $C_{20}H_{30}O_{12}$ from its negative HR-ESI-MS spectrum (*m/z* 461.1665 $[M-H]^-$). The ^{1}H-NMR spectra revealed an aromatic AB system at δ 7.14 (2H, d, $J=8.6$) and δ 7.01 (2H, d, $J=8.6$), two methylene protons at δ 2.76 (2H, t, $J=7.1$) and δ 3.70 (2H, t, $J=7.1$), and two anomeric protons at δ 4.92 (d, $J=7.5$, H-1′) and 4.60 (1H, d, $J=7.9$, H-1″) belonging to two β-D-glucose moieties. Comparison of the spectroscopic data with known compound **12** revealed that compound **9** has a different connection between the two glucopyranosyl units. The chemical shift at δ 86.3 was the characteristic resonance of the (1→3) connection between the two β-D-glucopyranosyl moieties in **9**.[16] The assignment was further confirmed by significant correlations from H-1″ (δ 4.60) to C-3′ (δ 86.3) in the HMBC spectrum (*Figure 2*). Therefore, compound **9** was determined to be 4-(2-hydroxyethyl)phenyl 3-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

Table 2. ^{1}H-NMR spectroscopic data for compounds **1**–**4** and **9** in CD_3OD.

No.	**1**[b]	**2**[a]	**3**[b]	**4**[b]	**9**[b]
1	–	–	–	–	–
2	6.97 (d, J=1.8)	7.00 (d, J=1.7)	7.00 (d, J=1.7)	6.89 (d, J=1.8)	7.14 (d, J=8.6)
3	–	–	–	–	7.01 (d, J=8.6)
4	–	–	–	–	–
5	6.76 (d, J=8.1)	6.74 (d, J=8.2)	6.77 (d, J=7.8)	6.67 (d, J=8.1)	7.01 (d, J=8.6)
6	6.84 (dd, J=8.1, 1.8)	6.86 (dd, J=8.2, 1.8)	6.87 (dd, J=8.2, 1.7)	6.76 (dd, J=8.1, 1.8)	7.14 (d, J=8.6)
7	5.48 (d, J=6.1)	5.62 (d, J=5.6)	5.58 (d, J=5.6)	5.44 (d, J=6.8)	2.76 (t, J=7.1)
8	3.44 (dd, J=12.5, 6.1)	3.59–3.64 (m)	3.59–3.63 (m)	3.45–3.49 (m)	3.70 (t, J=7.1)
9	3.81–3.83 (m)	4.04 (dd, J=9.3, 9.3)	3.93–3.95 (overlap)	3.95–3.97 (overlap)	
	3.74 (dd, J=11.0, 7.4)	3.89–3.94 (overlap)		3.70–3.72 (m)	
1′	–	–	–	–	4.92 (d, J=7.5)
2′	6.60 (s)	6.56 (s)	6.58 (d, J=1.0)	6.46 (s)	3.62–3.64 (m)
3′	–	–	–	–	3.64–3.66 (overlap)
4′	–	–	–	–	3.50–3.52 (m)
5′	–	–	–	–	3.39 (dd, J=9.1, 8.9)
6′	6.62 (s)	6.63 (s)	6.63 (s)	6.49 (s)	3.88 (dd, J=12.0, 2.1)
					3.63–3.65 (overlap)
7′	2.60 (t, J=7.4)	2.56 (t, J=7.4)	2.58 (t, J=7.6)	2.48 (t, J=7.8)	
8′	1.85–1.89 (m)	1.77–1.79 (m)	1.80–1.82 (m)	1.78–1.80 (m)	
9′	3.91–3.94 (m)	3.55 (t, J=6.5)	3.58 (t, J=6.5)	3.96 (t, J=6.5)	
	3.52–3.55 (m)				
1″	4.25 (d, J=7.8)	4.40 (d, J=7.5)	4.38 (d, J=7.1)	4.27 (d, J=7.3)	4.60 (d, J=7.9)
2″	3.18–3.20 (m)	3.41 (dd, J=9.0, 7.6)	3.42 (dd, J=8.9, 7.0)	3.32 (dd, J=8.9, 7.4)	3.29–3.31 (m)
3″	3.23–3.26 (m)	3.47 (dd, J=11.8, 8.6)	3.45 (dd, J=8.9, 8.5)	3.36 (dd, J=8.8, 8.8)	3.45–3.47 (m)
4″	3.27–3.29 (m)	3.27–3.29 (overlap)	3.51–3.55 (m)	3.39–3.42 (m)	3.27–3.29 (m)
5″	3.33–3.36 (m)	3.22–3.26 (m)	3.88–3.90 (m)	3.77–3.79 (overlap)	3.33 (dd, J=6.4, 3.2)
			3.21 (dd, J=11.4, 10.1)	3.10 (dd, J=11.3, 10.1)	
6″	3.86 (dd, J=11.9, 2.1)	3.86 (dd, J=12.0, 2.0)			3.88 (dd, J=12.0, 2.1)
	3.67 (dd, J=11.9, 5.6)	3.67–3.70 (overlap)			3.63–3.65 (overlap)
1‴		5.20 (d, J=1.1)	5.20 (d, J=1.2)	5.20 (d, J=1.4)	
2‴		3.89–3.94 (overlap)	3.93–3.95 (overlap)	3.84–3.85 (m)	
3‴		3.67–3.70 (overlap)	3.70 (dd, J=9.5, 3.3)	3.76–3.78 (overlap)	
4‴		3.32–3.34 (m)	3.35–3.38 (overlap)	4.74–4.76 (overlap)	
5‴		3.89–3.94 (overlap)	3.90–3.93 (m)	3.99–4.02 (m)	
6‴		0.96 (d, J=6.2)	1.02 (d, J=6.2)	0.72 (d, J=6.2)	
OMe	3.82 (s)	3.83 (s)	3.85 (s)	3.73 (s)	
2⁗				1.87 (s)	
2⁗′				1.93 (s)	

[a] Recorded in 400 MHz, δ, ppm. [b] Recorded in 600 MHz, δ, ppm.

Thirteen known compounds were identified as (7*R*,8*S*)-4,9,9′-trihydroxy-3-methoxyl-7,8-dihydrobenzofuran-1′-propylneolignan-3′-*O*-β-D-glucopyranoside (**5**),[7] (2*R*,3*S*)-2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-β-D-glucopyranoside (**6**),[17] (7*R*,8*S*)-4,3′,9,9′-tetrahydroxy-3-methoxyl-7,8-dihydrobenzofuran-1′-propylneolignan (**7**),[6,7] xanthiumnolic C (**8**),[18] icariside D_2 (**10**),[19] cimidahurinine (**11**),[20] 4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy] phenylethanol (**12**),[21] 2-(4-hydroxyphenyl)ethanol-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**13**),[22] *p*-hydroxyphenethyl alcohol (**14**),[23] benzyl-β-D-glucopyranoside (**15**),[24] artselaeroside A (**16**),[25] 1-*O*-caffeoyl-β-D-glucopyranose (**17**),[26] and soulieana acid 1 (**18**).[27] (The NMR data were shown in the *Supporting Information*).

The radical scavenging activities of compounds **1**–**18** were investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays at a concentration of 0.16 mM, with L-ascorbic acid as the positive control. The results are shown in *Table 3*. New compounds **1**–**3** exhibited considerable DPPH radical scavenging activities: 66.01 ± 0.38 %, IC_{50} = 94.4 ± 4.2 μM for **1**; 53.90 ± 1.61 %, IC_{50} = 147.3 ± 4.1 μM for **2**; 55.17 ± 1.47 %, IC_{50} = 128.7 ± 4.5 μM for **3**; and 86.81 ± 0.26 %, IC_{50} =

Table 3. DPPH radical scavenging activities of compounds **1**–**18**.[a]

Compounds	DPPH radical scavenging activity (%)	Compounds	DPPH radical scavenging activity (%)
1	66.01 ± 0.38	10	< 5.00
2	53.90 ± 1.61	11	16.49 ± 2.42
3	55.17 ± 1.47	12	< 5.00
4	33.80 ± 0.58	13	< 5.00
5	29.19 ± 0.89	14	< 5.00
6	47.69 ± 0.67	15	< 5.00
7	34.12 ± 0.96	16	16.55 ± 0.40
8	22.38 ± 0.67	17	84.84 ± 0.29
9	13.19 ± 0.97	18	80.28 ± 2.15
		L-ascorbic acid	86.81 ± 0.26

[a] The radical scavenging effects of compounds **1**–**18** and L-ascorbic acid from three independent experiments at a concentration of 0.16 mM. The results were expressed as mean ± SEMs.

31.3 ± 4.0 μM for L-ascorbic acid. Compounds **17** and **18** were the most active, exhibiting moderate radical scavenging activities (84.84 ± 0.29 %, IC_{50} = 37.7 ± 4.4 μM and 80.28 ± 2.15 %, IC_{50} = 97.2 ± 3.4 μM, respectively). Compound **17**, was a previously reported compound possessing a 3,4-diphenol moiety, and it exhibited obvious DPPH radical scavenging activity.[28] The introduction of sugar fragments in a compound can enhance the free radical scavenging activity,[29] and therefore, compounds **1**–**8** exhibited stronger DPPH radical-scavenging activity than compounds **9**–**16**. As previously reported, the carboxy groups on the aromatic ring in compound **18** may be responsible for its stronger activity.[27] These results suggested that the hydroxy groups, carboxy groups, and the sugar moiety perhaps played a significant role in the process of radical scavenging activities.

Conclusions

Herein, we described the isolation and structural identification of compounds **1**–**18**, including the evaluation of their activity. Among them, we have identified 4 new phenylpropanoid glycosides, 1 new phenolic glycoside, and 13 known compounds from the branch and leaves of *I. majus*. The antioxidant effects of compounds **1**–**18** were evaluated by the DPPH radical scavenging experiments, and most of the compounds that exhibited radical scavenging activities possessed a phenolic hydroxy group and a sugar moiety. This indicated that it is likely that the phenolic hydroxy group and the sugar moiety will enhance free radical scavenging activity. Because compounds **17** and **18** exhibited radical scavenging activities, they might be developed as promising natural antioxidants. Our data will enrich the known enriched the chemical constituents of *I. majus*, and also provide a theoretical basis for further development and utilization of this plant.

Experimental Section

General

Ultraviolet (UV) spectra and Infrared (IR) spectra (KBr) were evaluated on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan) and Bio-Rad FTS-135 spectrometer (Hercules, California, USA), respectively. NMR were obtained on DRX-400 or Advance III-600 spectrometers (Bruker, Bremerhaven, Germany) with TMS as the internal standard. Optical rotations were recorded on a JASCO P-1020 polarimeter (Horiba, Tokyo, Japan). The high-resolution mass spectra A were conducted on Shimadzu LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Meigao Chemical Company, Qingdao, China). Sephadex LH-20 (20–150 μm, Pharmacia Fine Chemical Co., Ltd., Sweden.). Semi-preparative RP-HPLC purification (LC-20AT Shimadzu liquid chromatography system with ChromCoreTMC18 semi-preparative column, 250 mm × 10 mm, 5 μm) was used. Thin-layer chromatography (TLC) were conducted and detected on by heating after spraying with 10 % H_2SO_4.

Plant Material

A branch and leaves of the plant were collected in Yunnan Province, China, during September 2015 and identified as *Illicium majus* Hook. f. & Thomson by Prof. Mengqi Liu (School of Pharmaceutical Science, Henan University of Chinese Medicine), School of Pharmaceutical Science, Zhengzhou University, where a voucher specimen of *I. majus* (2015-09-03) was deposited.

The air-dried and powdered branch and leaves of *I. majus* (10.0 kg) were refluxed with 95 % EtOH (30 L × 3) for 2 h each time. After removal of solvent, the EtOH extract was dissolved in H_2O and then fractionated with $CHCl_3$ (3.5 L × 3) and BuOH (3 L × 3) to give a $CHCl_3$ fraction and BuOH fraction, respectively. The

BuOH fraction (150 g) was loaded onto a microporous resin (D101, 120×12 cm) and sequentially eluted with the EtOH/H_2O mixtures (0:100, 20:80, 40:60, 60:40, 80:20 and 100:0) and yielded six fractions (Frs. 1–6). Fraction 5 (20 g) was further fractionated with silica gel column chromatography (CC, 9×41 cm, 1000 g) by gradient elution with CH_2Cl_2/MeOH (CH_2Cl_2/MeOH, 100:0, 90:10, 80:20, 70:30, each in 10 L) to produce seven fractions (Frs. 5.1–5.7). Frs. 5.2, 5.4, and 5.6 were loaded onto MCI gel® to yield subfractions (Frs. 5.2.1–5.2.4; Frs. 5.4.1–5.4.6; and Frs.5.6.1–5.6.5) with a step gradient composed of ethanol and water (20, 40, 60, 80, 100% ethanol). Fr. 5.2.2 was further chromatographically separated on Sephadex LH-20 to yield three portions (Frs. 5.2.2.1–5.2.2.3), which were further purified by semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC) (C_{18}, 20×250 mm, MeOH/H_2O = 40:60 or 50:50, flow rate 2.0 mL/min) to furnish compounds **2** (11 mg), **3** (7 mg), **12** (6 mg), and **16** (13 mg). Fr. 5.4.2 underwent chromatography on a silica gel (CH_2Cl_2: MeOH = 90:10) column and was purified by semi-preparative RP-HPLC (C_{18}, 20×250 mm, flow rate 2.5 mL/min) using MeOH/H_2O (40:60 and 50:50) to yield compounds **4** (9 mg), **9** (20 mg), **13** (7 mg), and **17** (mg). Fr. 5.4.6 and Fr. 5.6.2 were separated by silica gel column chromatography using solvent mixtures of increasing polarity (CH_2Cl_2/MeOH, v/v = 95:5–80:20), both of which were purified by semi-preparative RP-HPLC (C_{18}, 20×250 mm, flow rate 2.0 mL/min) using MeOH/H_2O (40:60 and 50:50) to afford compounds **1** (8 mg), **5** (11 mg), **6** (5 mg), **10** (23 mg), **11** (6 mg), **15** (9 mg), and **16** (13 mg). Fr. 6 (10 g) was also separated by silica gel CC (5×30 cm, 200 g) and eluted with CH_2Cl_2/Me_2CO (100:0, 95:5, 80:20, v/v, each in 2.5 L) in gradient system to obtain five fractions (Frs. 6.1–6.5). Fr. 6.2 (1.0 g) was further subjected to column chromatography on silica gel (2.0×30 cm, 40 g) to obtain compounds **14** (15 mg) and **7** (30 mg), which were eluted with petroleum ether/AcOEt (80:20, 70:30, v/v). Fr. 6.3 (200 mg) was subjected to Sephadex LH-20 CC (MeOH) to furnish compounds **8** (15 mg) and **18** (11 mg). Each of the purified compounds had a degree of purity > 90%.

3-[(2*R*,3*S*)-7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-1-benzofuran-5-yl]propyl β-D-glucopyranoside (**1**). White powder. $[\alpha]_D^{23.9} = +2.70$ (c = 0.103, MeOH). UV (MeOH): λ_{max} (log ε) = 283 (5.47), 203 (6.40) nm. CD ($c = 2.64\times10^{-4}$ M, MeOH), $\Delta\varepsilon$ (nm): −3.26 (202), +6.58 (211), +0.95 (237), +0.79 (293). (IR (KBr): ν_{max} = 3414, 2919, 1630, 1610, 1517, 1277, 1033 cm^{-1}. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. HR-ESI-MS: *m/z* 507.1872 $[M-H]^-$ (calc. for $C_{25}H_{31}O_{11}$: 507.1845).

[(2*R*,3*S*)-7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-2,3-dihydro-1-benzofuran-3-yl]methyl 2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (**2**). White gum. $[\alpha]_D^{24.2} = -5.09$ (c = 0.103, MeOH). UV (MeOH): λ_{max} (log ε) = 283 (5.81), 203 (6.87) nm. CD ($c = 1.89\times10^{-4}$ M, MeOH), $\Delta\varepsilon$ (nm): −1.83 (201), +6.35 (210), +0.93 (240), +1.15 (292). IR (KBr): ν_{max} = 3413, 2920, 1606, 1517, 1384, 1051 cm^{-1}. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. HR-ESI-MS: *m/z* 653.2451 $[M-H]^-$ (calc. for $C_{31}H_{41}O_{15}$: 653.2438).

[(2*R*,3*S*)-7-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-2,3-dihydro-1-benzofuran-3-yl]methyl 2-*O*-α-L-rhamnopyranosyl-β-D-xylopyranoside (**3**). White powder. $[\alpha]_D^{24.2} = -23.27$ (c = 0.168, MeOH). UV (MeOH): λ_{max} (log ε) = 283 (5.87), 202 (6.92) nm. CD ($c = 1.42\times10^{-4}$ M, MeOH), $\Delta\varepsilon$ (nm): −3.02 (202), +7.17 (210), +1.05 (239), +1.32 (293). IR (KBr): ν_{max} = 3423, 2924, 1612, 1517, 1277, 1040 cm^{-1}. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. HR-ESI-MS: *m/z* 623.2345 $[M-H]^-$ (calc. for $C_{30}H_{39}O_{14}$: 623.2345).

3-[(2*R*,3*S*)-3-({[2-*O*-(4-*O*-Acetyl-α-L-rhamnopyranosyl)-β-D-xylopyranosyl]oxy}methyl)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydro-1-benzofuran-5-yl]propyl acetate (**4**). White powder. $[\alpha]_D^{23.5} = -29.13$ (c = 0.103, MeOH). UV (MeOH): λ_{max} (log ε) = 283 (5.87), 202 (6.92) nm. CD ($c = 1.82\times10^{-4}$ M, MeOH), $\Delta\varepsilon$ (nm): −0.55 (202), +5.33 (210), +0.82 (238), +0.92 (292). IR (KBr): ν_{max} = 3412, 2925, 1726, 1611, 1517, 1258, 1039 cm^{-1}. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. HR-ESI-MS: *m/z* 707.2557 $[M-H]^-$ (calc. for $C_{34}H_{43}O_{16}$: 707.2534).

4-(2-Hydroxyethyl)phenyl 3-*O*-β-D-Glucopyranosyl-β-D-glucopyranoside (**9**). White amorphous solid. $[\alpha]_D^{19.0} = -24.5$ (c = 0.103, MeOH). UV (MeOH): λ_{max} (log ε) = 273 (5.23), 220 (5.99) nm. IR (KBr): ν_{max} = 3399, 2919, 1611, 1511, 1231, 1076 cm^{-1}. ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. HR-ESI-MS: *m/z* 461.1665 $[M-H]^-$ (calc. for $C_{20}H_{29}O_{12}$: 461.1662).

Acid Hydrolysis of Compounds **2**–**4** *and* **9**

A sample (2 mg each) of compounds **2**–**4** and **9** in 10% HCl (5 mL) was refluxed at 90 °C for 4 h. After cooling to room temperature, the mixture was extracted with $CHCl_3$, and the remaining aqueous

phase was neutralized to pH = 6 with saturated $NaHCO_3$. After evaporating to dryness, the residue was analyzed for sugar by thin-layer chromatography (TLC) over silica gel (BuOH/acetic acid/water 5:1:2.5, v/v/v). The D-glucose, D-xylose, and L-rhamnose as sugar moieties in compounds **2**–**4** and **9** were confirmed, respectively (see *Supporting Information*).

DPPH Radical Scavenging Assay

The DPPH radical scavenging evaluation was carried out on the basis of the reported literature.[29] Briefly, 20 μL of each compound solution [in dimethyl sulfoxide (DMSO)] at 0.16 mM concentrations was mixed with 180 μL of 0.1 mM DPPH methanol solution in a 96-well microplate. Mixtures were shaken well, after a 0.5 h incubation period at 25 °C in the dark, the scavenging of DPPH radicals were determined according to the measured absorbance at 517 nm by a Multiskan FC microplate reader. The DPPH radical scavenging activity (%) was determined by the following equation: Radical scavenging activity (%) = $[1-(A_s/A_c)]\times 100$, where A_c denotes the absorbance of the control without samples and A_s denotes the absorbance of the tested samples. L-Ascorbic acid acted as a positive control. The experiment was repeated three times.

Antimicrobial Activity Evaluation

The antibacterial activity (against *Staphylococcus aureus* and *Escherichia coli*) of isolates **1**–**18** was also assessed by the broth microdilution method according to the CLSI guidelines with vancomycin and meropenem, respectively, as positive control drugs.[30] Neither antibiotic exhibited antimicrobial activity at the measured concentration (< 32 μg/mL).

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Author Contribution Statement

Ji-Feng Liu, Juan Du and Yan-Bing Zhang designed the research. Fang Li and Ying-Ying Fu performed the research. Fang Li, Lei Wang, and Ting-Ting Yan also performed part of activities DPPH radical scavenging activities. Fang Li, Ji-Feng Liu and Nen-Ling Zhang wrote and polished the article.

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