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Antioxidative acylphloroglucinols from the roots of *Lysidice rhodostegia*

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ABSTRACT

Four new acylphloroglucinols, lysidicins I and J (**1** and **2**), and lysidisides V and W (**3** and **4**), were isolated from the roots of *Lysidice rhodostegia*. Among them, compound **1** is a novel acylphloroglucinol, and compound **2** is the first naturally occurring acylphloroglucinol derivative with an uncommon spiro(benzofuran-[2*H*]pyran) skeleton. Their structures were elucidated by spectroscopic and chemical methods. The absolute configuration of **1** was assigned by employing dimolybdenum tetraacetate-induced CD spectrum method, and that of **2** was determined by analysis of their experimental and theoretically calculated CD spectra. Compounds **3** and **4** exhibited potent antioxidative activity with IC_{50} values of 3.29 and 3.39 μM, respectively.

1. Introduction

Lysidice rhodostegia belongs to the genus *Lysidice* of the Leguminosae family, which is widely distributed in the Guangdong, Guangxi, Yunnan, and Guizhou Provinces in China. Its roots have been used in Chinese folk medicine for the treatment of ache, fracture, and hemorrhage.[1] Our previous investigations of the plant have led to the isolation of an array of structurally diverse compounds, including phloroglucinols, flavonoids, stilbenes, and triterpenoids, some of which displayed potent vasodilatory and antioxidative activities.[2–8] Recently, three phloroglucinols possessing an unprecedented benzo[*b*]furo[3,2-*d*]furan skeleton have been isolated from the roots of *L. rhodostegia*.[9] In ongoing research on bioactive constituents from this plant, two new acylphloroglucinols, lysidicins I and J (**1** and **2**), were isolated from the same sample. In addition, two new acylphloroglucinol glucosides, lysidisides V and W (**3** and **4**), were isolated from the recollected sample. In the present paper, we describe the isolation, structure elucidation, and antioxidative activity of these compounds, as well as a plausible biosynthetic pathway for compounds **1** and **2**.

2. Results and discussion

The dried roots of *L. rhodostegia* were extracted with 95% EtOH. This extract was suspended in water and then successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH. The crude EtOAc extract was subjected to repeated column chromatography to yield two novel acylphloroglucinols, lysidicins I and J (**1** and **2**) (Fig. 1). Two new acylphloroglucinol glucosides, lysidisides V and W (**3** and **4**), were isolated from the recollected sample via a similar procedure.

Lysidicin I (**1**) was assigned the molecular formula of $C_{27}H_{32}O_{10}$ on the basis of its HRESIMS (*m*/*z* 539.1902 $[M+Na]^+$, calcd for $C_{27}H_{32}O_{10}Na$, 539.1893). The ^{13}C NMR spectrum (Table 1) of **1** showed resonances for six oxygenated aromatic carbons at δ_C 154.3–163.7, six shielded aromatic carbons at δ_C 95.1–110.9, four methyls at δ_C 22.3–22.6, two methines at δ_C 24.71 and 24.74, two methylenes at δ_C 50.1 and 52.0, and two conjugated carbonyl carbons at δ_C 202.9 and 204.9. These are characteristic carbon resonances of two sets of isovalerylphloroglucinol moieties.[2,3,6,7] In addition, the ^{13}C NMR spectrum indicated the presence of two olefinic carbons (one of which is oxygenated) at δ_C 100.3 (C-1) and 155.6 (C-2). By comparing the above data with those in the reference,[6] a benzofuran moiety was established. The $^1H-^1H$ COSY NMR data showed the isolated spin-system of C-3–C-5 that was further supported by HMBC correlations from H-3 to C-4 and C-5, and from H-5 to C-4 and C-3. HMBC correlations from H-3 to C-1, C-2, C-6″, C-7″, and C-8″ enabled connection of C-3 to both C-2 and C-7″. The

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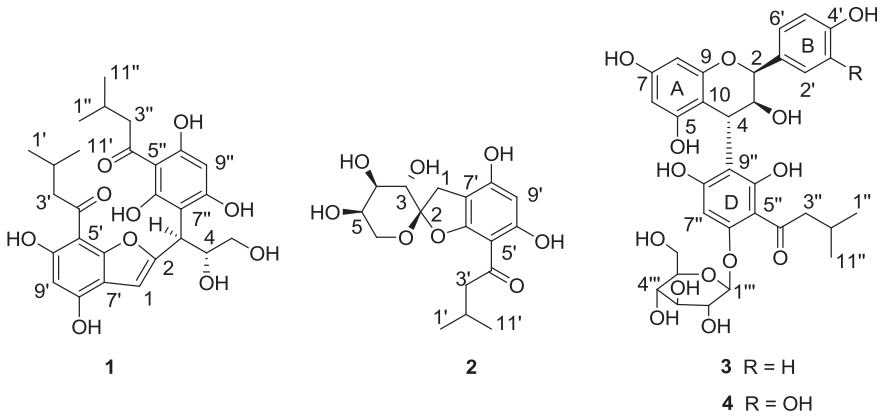


Fig. 1. Structures of compounds **1**–**4**.

Table 1
NMR spectroscopic data of compounds **1**–**4**

No.	**1**		**2**		**3**		**4**	
	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[c]	δ_H[d] (*J* in Hz)	δ_C[c]	δ_H[d] (*J* in Hz)	δ_C[c]	δ_H[d] (*J* in Hz)
1	100.3	6.57 s	36.1	2.82 d (15.0) 3.25 d (15.0)				
2	155.6		115.7		77.5	5.09 br s	77.4	5.02 br s
3	36.6	4.89 d (4.8)	71.8	3.86 overlap	72.9	3.84 br s	72.9	3.83 br s
4	72.0	4.48 d (5.6)	72.2	3.86 overlap	36.9	4.53 s	36.8	4.51 s
5	63.9	3.37 m	70.9	3.93 br s	157.7		157.8	
6			67.1	3.73 d (12.0) 4.00 d (12.0)	95.4	5.84 d (2.0)	95.6	5.82 d (2.0)
7					157.8		157.8	
8					95.4	5.92 d (2.0)	95.6	5.91 d (2.0)
9					158.3		158.3	
10					102.3		102.3	
1′	22.3	0.85 d (6.8)	23.3	0.92 d (6.0)	132.1		132.8	
2′	24.7	2.12 m	25.9	2.22 m	129.1	7.17 d (8.5)	115.3	6.84 br s
3′	50.1	2.86 m	52.4	2.84 dd (16.5, 7.5)	115.7	6.69 d (8.5)	145.9	
4′	204.9		204.8		157.7		145.6	
5′	100.9		102.7		115.7	6.69 d (8.5)	115.9	6.65 d (8.0)
6′	154.3		162.5		129.1	7.17 d (8.5)	119.2	6.62 br d (8.0)
7′	110.9		104.7					
8′	157.8		166.4					
9′	97.3	6.10 s	97.2	5.80 s				
10′	163.7		162.6					
11′	22.4	0.89 d (6.8)	23.2	0.94 d (6.0)				
1″	22.6	0.89 d (6.4)			22.9	0.90 d (6.5)	22.9	0.87 d (7.0)
2″	24.7	2.12 m			26.2	2.22 m	26.2	2.20 m
3″	52.0	2.86 m			54.3	3.15 dd (6.0, 15.5) 2.87 dd (7.5, 15.5)	54.3	3.14 dd (16.0, 6.0) 2.83 dd (16.0, 7.5)
4″	202.9				207.4		207.4	
5″	103.9				106.7		106.7	
6″	163.7				160.3		160.3	
7″	102.9				95.4	6.11 s	95.4	6.08 s
8″	163.1				164.0		164.1	
9″	95.1	5.92 s			110.7		110.7	
10″	161.5				165.9		165.9	
11″	22.6	0.90 d (6.4)			23.4	0.92 d (6.5)	23.4	0.91 d (6.5)
1‴					101.8	4.97 d (8.0)	101.8	4.94 d (8.0)
2‴					74.8	3.50	74.8	3.49
3‴					78.4	3.39	78.4	3.40
4‴					71.3	3.33	71.3	3.33
5‴					78.7	3.39	78.7	3.40
6‴					62.6	3.88 br d (11.5) 3.66 dd (11.5, 5.5)	62.5	3.86 br d (12.0) 3.65 dd (12.0, 6.0)

[a] 100 MHz, in DMSO-d_6.
[b] 400 MHz, in DMSO-d_6.
[c] 125 MHz, in CD_3OD.
[d] 500 MHz, in CD_3OD.

absolute configuration of C-3 was determined as *S*-configuration by the negative sign associated with the CD spectrum of the longest wavelength aromatic transition ^1L_b (280–290 nm).[10] The absolute configuration of C-4 was established by the induced CD spectrum method that employed $Mo_2(OAc)_4$, which is the simplest, most practical, and most reliable approach as far as absolute configurations of *vic*-diol is concerned.[11–14] Based on the empirical rule of this method, the energetically preferred conformation of *vic*-diol molecule in the chiral Mo-complexes is that the bulkier group points away from the rest of the complex, and the negative sign of

CD band at around 310 nm corresponds to a negative torsion angle of the O–C–C–O moiety in the preferred conformation of Mo-complex of **1** (Fig. 2). Thus, the absolute configuration at C-4 in **1** was determined as *R*.

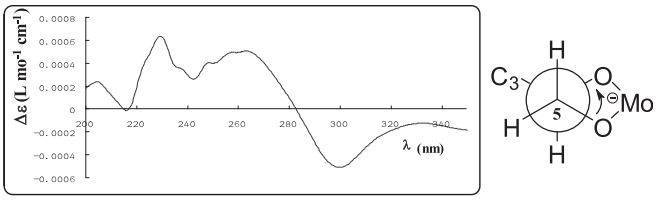


Fig. 2. CD spectrum (the inherent CD of **1** was subtracted) and preferred conformation of Mo-complex of **1**.

Lysidicin J (**2**) gave a pseudomolecular ion $[M+Na]^+$ peak at *m/z* 377.1219 in the HRESIMS, consistent with a molecular formula of $C_{17}H_{22}O_8$ (calcd for $C_{17}H_{22}O_8Na$, 377.1212), requiring seven degrees of unsaturation. Comparison of the ^1H and ^{13}C NMR chemical shifts between **1** and **2** indicated that **2** also contained an isovalerylphloroglucinol moiety. In addition, the ^{13}C NMR spectrum of **2** showed the presence of one ketal carbon at δ_C 115.7 (C-2), two methylene carbons at δ_C 36.1 (C-1), 67.1 (C-6), and three oxygen-bearing methine carbons at δ_C 71.8 (C-3), 72.2 (C-4), and 70.9 (C-5). These data, together with the HSQC and HMBC spectra, revealed the presence of a fructopyranose-like moiety existing in saffloquinoside A.[15] The HMBC correlations from H_2-1 to C-6′, C-7′, and C-8′, along with the degrees of unsaturation and the NOEds data, established the connection of fructopyranose-like and isovaleryphloroglucinol moieties. The relative configuration of **2** was determined according to NOEds of H-3 with H_2-1, H-2′ with H-4 and Hα-6, and H-4 with Hα-6 in the NOE and NOESY experiments.

The absolute configurations of the stereogenic centers, C-2, C-3, C-4, and C-5, of **2** were established by comparison of the experimental CD spectrum with electronic circular dichroism (ECD) spectra calculated by time-dependent density functional theory (TD-DFT).[16–20] Based on the relative configuration of **2**, two possible structures with absolute configurations (2*S*,3*S*,4*R*,5*R*) and (2*R*,3*R*,4*S*,5*S*) were considered. The ECD spectra of these two configurations were calculated and compared with the experimental CD spectrum of **2**. The results showed that the calculated ECD spectrum of (2*S*,3*S*,4*R*,5*R*) enantiomer exhibited a similar CD curve to that of the ECD spectrum, whereas the calculated ECD spectrum of the (2*R*,3*R*,4*S*,5*S*) enantiomer was opposite to that of the experimental CD spectrum (Fig. 3a) (see Supplementary data, for detailed information). Molecular orbitals (MO) analysis of (2*S*,3*S*,4*R*,5*R*)-enantiomer has been carried out at the B3LYP/6-31G(d) level of DFT theory with the PCM model in MeOH (Fig. 3b). The positive rotatory strength at 264 nm is mainly contributed by the electronic transition from MO93 to MO95, and the negative rotatory strength at 324 nm is attributed to the electronic transition from MO94 to MO95. These are the $\pi\rightarrow\pi^*$ transition from the filled CC orbital of phenyl group to antibonding orbital of CO group. Based on these results, the absolute configuration of **2** was determined to be 2*S*, 3*S*, 4*R*, and 5*R*, which is consistent with biogenetic considerations for the fructopyranose-like moiety in saffloquinoside A.[15]

The molecular formula of lysidiside V (**3**) was determined as $C_{32}H_{36}O_{14}$ based on HRESIMS (*m/z* 667.2015 $[M+Na]^+$, calcd for $C_{32}H_{36}O_{14}Na$, 667.2003). The ^1H and ^{13}C NMR spectra of **3** displayed resonances for one isovalerylphloroglucinol moiety. These data, together with the those for sugar moiety [δ_C 101.8 (C-1‴), 74.8 (C-2‴), 78.4 (C-3‴), 71.3 (C-4‴), 78.7 (C-5‴), and 62.6 (C-6‴)], suggested the presence of an isovalerylphloroglucinol glucoside unit.[2,8] Furthermore, the ^1H NMR spectrum displayed *meta*-coupling aromatic protons attributed to 1,2,3,5-tetrasubstiuted

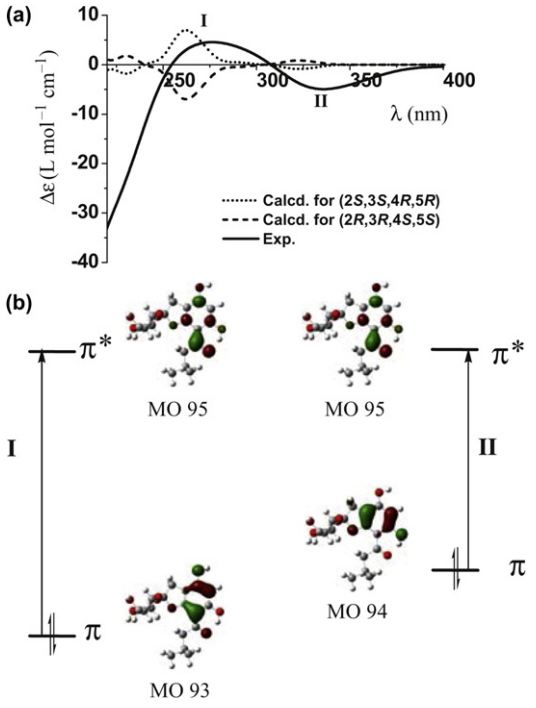


Fig. 3. (a) Calculated ECD spectra of (2*R*,3*R*,4*S*,5*S*), (2*S*,3*S*,4*R*,5*R*)-enantiomers and experimental ECD spectrum of **2**. (b) Electronic transitions and MO analysis.

aromatic ring protons at δ_H 5.84 (1H, d, *J*=2.0 Hz, H-6) and 5.92 (1H, d, *J*=2.0 Hz, H-8), and *ortho*-coupling aromatic protons assigned to *para*-disubstituted aromatic ring protons at δ_H 7.17 (2H, d, *J*=8.5 Hz, H-2′, 6′) and 6.69 (2H, d, *J*=8.5 Hz, H-3′, 5′). In the ^{13}C NMR spectrum of **3**, apart from the isovalerylphloroglucinol glucoside carbon signals, other signals were similar to those of epiafzelechin,[21] revealing the presence of one flavan-3-ol moiety. This was further confirmed by the HMBC spectrum. HMBC correlation from H-1‴ to C-6″ suggested that C-1‴ of the glucosyl unit was attached to C-6″ of aglycone, whereas those from H-3 to C-9″, H-4 to C-8″, C-9″, and C-10″ indicated the linkage positions between isovalerylphloroglucinol glucoside and flavan-3-ol moieties.

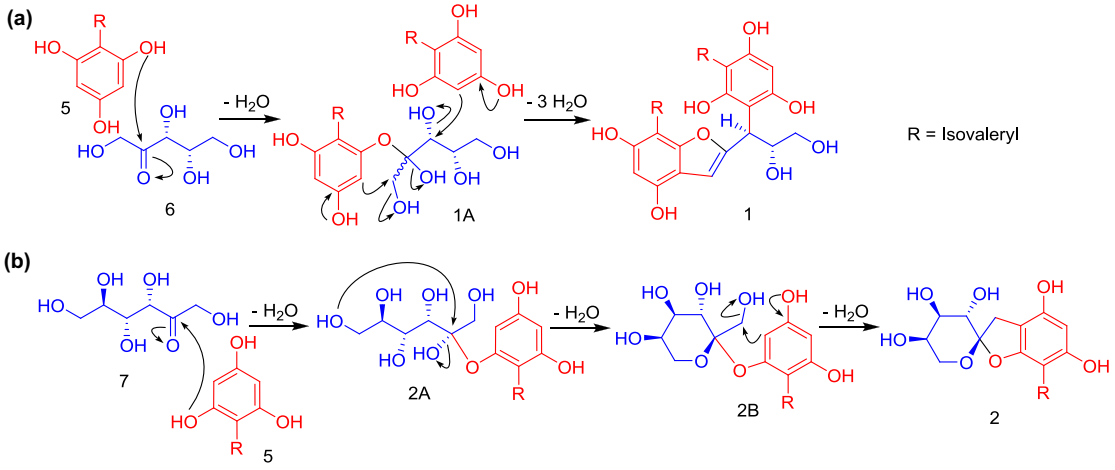
The molecular formula of lysidiside W (**4**) was determined to be $C_{32}H_{36}O_{15}$ by analysis of its HRESIMS data (*m/z* 661.2116 $[M+H]^+$, calcd for $C_{32}H_{37}O_{15}$, 661.2127). The ^1H NMR spectrum of **4** was very similar to that of **3**, except for the signals ascribed to the B-ring: an ABX system at δ_H 6.84 (1H, br s, H-2′), 6.65 (1H, d, *J*=8.0 Hz, H-5′), and 6.62 (1H, br d, *J*=8.0 Hz, H-6′), attributed to the 1,2,4-trisubstituted B-ring in **4** rather than the *para*-disubstituted B-ring in **3**. Thus, the only difference between **3** and **4** arose in B-ring, which was confirmed by the comparison of B-ring ^{13}C NMR chemical shifts [δ_C 132.8 (C-1′), 115.3 (C-2′), 145.9 (C-3′), 145.6 (C-4′), 115.9 (C-5′), 119.2 (C-6′)] of **4** with those [δ_C 132.1 (C-1′), 129.1 (C-2′, C-6′), 115.7 (C-3′, C-5′), 157.7 (C-4′)] of **3**.

The relative configurations of **3** and **4** were determined by analysis of the $^1H-^1H$ coupling constants. In contrast to the large coupling constant ($^3J_{2,3}$=6–10 Hz) observed in 2,3-*trans*-4-arylflavan-3-ols, the small coupling constants in compounds **3** and **4** suggested a cis relationship between these two protons. Similarly, the small vicinal coupling constant ($^3J_{3,4}$=1.2 Hz) between H-3 and H-4 in compounds **3** and **4** (^1H NMR in acetone-d_6, Supplementary data) indicated a 3,4-*trans* relative configuration ($^3J_{3,4}$=2–4 Hz observed in 3,4-*cis*-analogues).[22,23] The absolute configuration of C-4 in **3** and **4** were assigned by analysis of their CD spectra. The negative Cotton effect at the low wavelength (220–240 nm) indicated a 4*R*-configuration.[22,24] Therefore, the absolute configurations of C-2, C-3, and C-4 in **3** and **4** were

determined as 2*S*, 3*S*, and 4*R*. The large coupling constant (8.0 Hz) of the anomeric protons revealed the β-configuration for the glucoses.[25] Enzymatic hydrolysis of compounds **3** and **4** yielded the same monosaccharide D-glucopyranose, consistent with the stereochemistry of naturally occurring glucose, based on co-TLC with authentic sugar sample.

The antioxidative activity of the isolates was tested. Vitamin E was selected as the positive control for its well-known antioxidative activity. Lysidicins I and J (**1** and **2**) showed antioxidant activity with the 100% inhibitory rates at a concentration of 0.1 μmol/mL. At lower concentrations (0.01 and 0.001 μmol/mL), however, their antioxidative activity disappeared. Lysidisides V (**3**) and W (**4**) demonstrated significant antioxidative activity with IC_{50} values of 3.29 and 3.39 μM, respectively (Vitamin E, IC_{50}=33.4 μM).

A putative biosynthetic pathway of lysidicins I and J (**1** and **2**) was proposed as shown in Scheme 1. The isovalerylphloroglucinol moiety occurs in the structures of not only **1** and **2** but in all phloroglucinol derivatives isolated from *L. rhodostegia*. Therefore, the known natural product isovalerylphloroglucinol (**5**)[26] and ketose (L-ribulose (**6**) and D-fructose (**7**)) were supposed to be the biosynthetic precursors. The nucleophilic addition between **5** and **6** would give rise to the hemiketal intermediate **1A**, which could undergo intermolecular and intramolecular C-alkylation and dehydration to form lysidicin I (**1**). The nucleophilic addition product between **5** and **7**, intermediate **2A**, would undergo nucleophilic displacement reaction to produce intermediate **2B** with tetrahydropyran ring unit. Finally, through intramolecular C-alkylation, **2B** would generate lysidicin J (**2**).



Scheme 1. A plausible mechanism for formation of compounds **1** and **2**.

3. Conclusions

Four new acylphloroglucinols (**1**–**4**) were isolated from the roots of *L. rhodostegia*. Among them, compound **1** is a novel acylphloroglucinol, and compound **2** is the first naturally occurring acylphloroglucinol derivative with an uncommon spiro(benzofuran-[2*H*]pyran) skeleton. Compounds **3** and **4** belong to the 4-arylflavan-3-ol class; up to now, only one example belonging to this class of products has been isolated from natural sources.[27] The absolute configuration of **1** was assigned by employing dimolybdenum tetraacetate-induced CD spectrum method, and that of **2** was determined by analysis of their experimental and theoretically calculated CD spectra. The antioxidative activity of these compounds was investigated and compounds **3** and **4** showed potent antioxidative activity. Moreover, a putative biosynthetic pathway of compounds **1** and **2** was proposed.

4. Experimental

4.1. General procedures

Optical rotations were determined on a Perkin–Elmer 241 automatic digital polarimeter. CD spectrum was obtained from a JOUAN Mark II spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer. 1D and 2D NMR spectra were recorded on Varian SX-600, INOVA-500 and MP-400 spectrometers with TMS as internal standard. HRESIMS spectra were recorded on an Autospec-Ultima ETOF Spec mass spectrometer.

4.2. Computational details

All calculations were performed by the Gaussian03 program. For details of DFT calculations, see the Supplementary data.

4.3. Plant material

The roots of *L. rhodostegia* were collected from Guangxi Province of China in November 1999 and December 2006, respectively. The sample was identified by Professor Shou-Yang Liu (GuangXi College of Traditional Chinese Medicine), and a voucher specimen (No. 002775) is deposited in the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences.

4.4. Extraction and isolation

The air-dried roots of *L. rhodostegia* (14.0 kg) were extracted with 95% EtOH (15 L×3) and concentrated in vacuo to give the crude extract (1.1 kg), which was suspended in water and then successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc fraction (420 g) was subjected to repeated column chromatography on ODS (MeOH–H_2O (60:40 to 85:15)) and Sephadex LH-20 (MeOH) to yield **1** (6.3 mg) and **2** (16.6 mg). The air-dried roots of *L. rhodostegia* (4.7 kg) were extracted with 95% EtOH (10 L×3) and concentrated in vacuo to give the crude extract (563 g), which was suspended in water, and then successively extracted with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc extract (156 g) was chromatographed on ODS eluting with MeOH–H_2O (30:70 to 85:15), and

then purified by Sephadex LH-20 (MeOH–H_2O, 1:1), finally separated by ODS eluting with MeOH–H_2O (45:55 to 80:20) to afford compounds **3** (25.1 mg) and **4** (8.3 mg).

*4.4.1. Lysidicin I (**1**).* Pale yellow powder; $[\alpha]_D^{20}$ −14.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 233 (3.19), 292 (2.75) nm; CD (MeOH): λ ($\Delta\varepsilon$) 225 (+0.5), 254 (+19.5), 271 (0), 285 (−8.4), 314 (−0.3); IR (KBr): 3379, 1624, 1600, 1510, 1431, 1369, 1304, 1209 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS: *m*/*z* 539.1902 $[M+Na]^+$ (calcd for $C_{27}H_{32}O_{10}Na$, 539.1893).

*4.4.2. Lysidicin J (**2**).* Pale yellow powder; $[\alpha]_D^{20}$ −93.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.06), 290 (2.72) nm; CD (MeOH): λ ($\Delta\varepsilon$) 201 (−1.2), 254 (0), 279 (+0.5), 306 (0), 339 (−0.1), 385 (0); IR (KBr): 3319, 2952, 1639, 1620, 1523, 1464, 1117, 1087 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS: *m*/*z* 377.1219 $[M+Na]^+$ (calcd for $C_{17}H_{22}O_8Na$, 377.1212).

*4.4.3. Lysidiside V (**3**).* Pale yellow powder; $[\alpha]_D^{20}$ +67.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.23), 286 (3.75) nm; CD (MeOH): λ ($\Delta\varepsilon$) 200 (0), 210 (−3.5), 235 (0), 294 (−1.8), 387 (−0.5); IR (KBr):3371, 2958, 2874, 1607, 1516, 1428, 1145, 1078 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS: *m*/*z* 667.2015 $[M+Na]^+$ (calcd for $C_{32}H_{36}O_{14}Na$, 667.2003).

*4.4.4. Lysidiside W (**4**).* Pale yellow powder; $[\alpha]_D^{20}$ +52.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.19), 287 (3.71) nm; CD (MeOH): λ ($\Delta\varepsilon$) 200 (0), 209 (−6.9), 237 (0), 240 (+1.2), 300 (−3.8), 360 (−0.1); IR (KBr): 3313, 2957, 1605, 1520, 1434, 1149, 1072 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS: *m*/*z* 661.2116 $[M+H]^+$ (calcd for $C_{32}H_{37}O_{15}$, 661.2127).

4.5. Enzymatic hydrolysis and determination of absolute configuration of sugars

A solution of compound **3** (18 mg) in water (2 mL) was incubated with helicase (36 mg) for 12 h at 37 °C, then extracted with EtOAc. The extract of EtOAc was subjected to Sephadex LH-20 column eluted with MeOH to yield aglycone **3A** (4 mg). The aqueous phase was evaporated in vacuo to dryness to afford monosaccharide residue, which spotted on analytical silica gel TLC plate along with D-glucopyranose. The plate was developed in *n*-ButOH–Me_2CO–H_2O (4:5:1), air dried, sprayed with 10% aqueous H_2SO_4, and charred with a heat gun. The hydrolysate exhibited a black spot (R_f=0.37) that was identical with that observed for D-glucopyranose. The absolute configuration of sugar residue of **4** was assigned by the same method.

*4.5.1. Aglycone (**3A**).* Pale yellow powder; ^1H NMR (600 MHz, in CD_3OD): δ_H 7.16 (1H, d, *J*=8.4 Hz, H-2′/6′), 6.68 (1H, d, *J*=8.4 Hz, H-3′/5′), 5.92 (1H, d, *J*=2.0 Hz, H-8), 5.92 (1H, d, *J*=2.0 Hz, H-8), 5.84 (1H, d, *J*=2.0 Hz, H-6), 5.79 (1H, s, H-7″), 5.05 (1H, br s, H-2), 4.49 (1H, s, H-4), 3.83 (1H, br s, H-3), 2.86 (2H, d, *J*=5.4 Hz, H-3″), 2.17 (1H, m, H-2″), 0.93 (3H, d, *J*=6.6 Hz, H-11″), 0.90 (3H, d, *J*=6.6 Hz, H-1″); ^{13}C NMR (150 MHz, in CD_3OD): δ_C 207.2 (C-4″), 165.7 (C-10″), 164.3 (C-8″), 162.3 (C-6″), 158.5 (C-9),157.7 (C-5/7/4′), 132.0 (C-1′), 129.0 (C-2′/6′); 115.8 (C-3′/5′); 107.9 (C-9″); 105.6 (C-5″), 102.4 (C-10), 95.5 (C-7″), 95.2 (C-6/8), 77.5 (C-2), 72.9 (C-3), 53.9 (C-3″), 36.8 (C-4), 26.7 (C-2″), 23.2 (C-1″/11″); HRESIMS: *m*/*z* 505.1484 (calcd for $C_{26}H_{26}O_9Na$, 505.1474).

4.6. Bioassays[28]

The antioxidant activities of **1**–**4** were determined by the content of MDA (malondialdehyde), which was produced during microsomal lipid per-oxidation induced by ferrous-cysteine. MDA was detected by using the thiobarbituric acid (TBA) method. Microsomal protein (1 mg/mL), different concentration of compounds, cysteine (0.2 mM) in 0.1 M PBS were incubated for 15 min at 37 °C. Ferrous (0.5 mM) was added and the mixture was incubated for another 15 min at the same temperature. Equal volume of 20% TCA was added to terminate the reaction. The above solvent was centrifuged for 10 min at 3000 rpm. The supernatants reacted with 0.67% TBA for 10 min at 100 °C. After being cooled to room temperature, the MDA was determined by the absorbance at 532 nm, and then the inhibitory rates were calculated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.08.034.

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