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Lysidicins F−H, Three New Phloroglucinols from *Lysidice rhodostegia*

Xian-Fu Wu,[†] You-Cai Hu,[†] Shi-Shan Yu,*[,†] Nan Jiang,[‡] Jing Ma,[‡] Ren-Xiang Tan,[§] Yong Li,[†] Hai-Ning Lv,[†] Jing Liu,[†] and Shuang-Gang Ma[†]

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education), Beijing 100050, P. R. China, Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China, and State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, P. R. China

yushishan@imm.ac.cn

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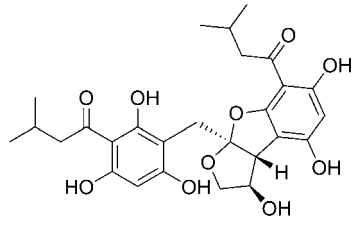
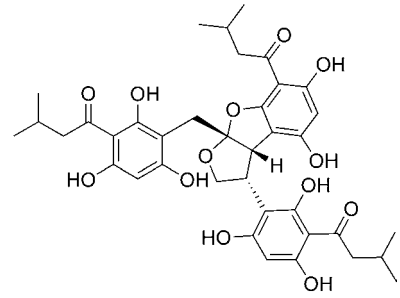
ABSTRACT



lysidicin F (1)



lysidicin G (2)

Three new phloroglucinols, named lysidicins F−H (1−3), were isolated from the roots of *Lysidice rhodostegia*. These compounds have a unprecedented benzyl benzo[*b*]furo[3,2-*d*]furan skeleton, and lysidicin F (1) is the first example of natural product with *trans*-fused furan rings. Their structures were established on the basis of extensive spectroscopic analysis, and the absolute configurations of them were determined by computational methods. A possible biosynthetic pathway for 1−3 was also postulated.

Phloroglucinol derivatives are a major class of secondary metabolites with fascinating chemical structures and intriguing biological activities. And they are widely distributed in land plants, marine plants, and microbes.[1] A large number of differently substituted and structurally diverse phloroglucinol derivatives have been isolated from natural sources; however, phloroglucinols with unique skeletons are seldom reported. Our prior work on *Lysidice rhodostegia*, a traditional medicine in China for the treatment of aches, fractures,

[†] Chinese Academy of Medical Sciences and Peking Union Medical College.
[‡] Nanjing University.
[§] Nanjing University.

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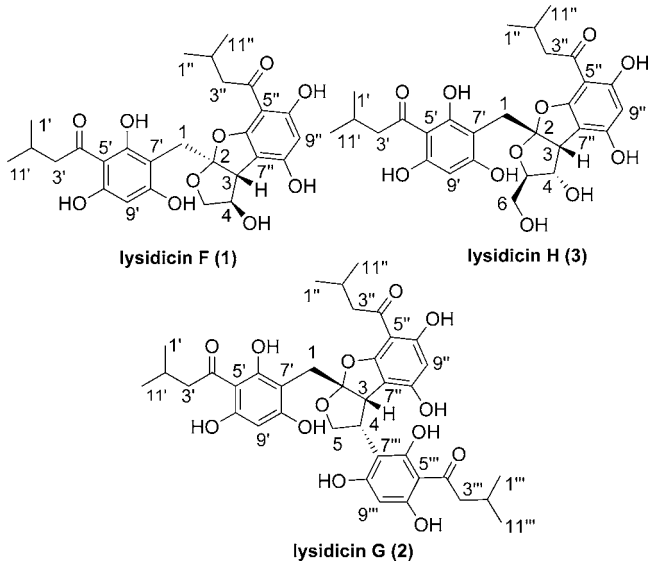
and hemorrhage,[2] led to the identification of bioactive metabolites with diverse structures including three novel phloroglucinols.[3]

During our continuing search for structurally unique and biologically significant phloroglucinol derivatives from this plant, we discovered three novel phloroglucinols named lysidicins F−H (**1**−**3**). All of them possess a unprecedented benzyl benzo[*b*]furo[3,2-*d*]furan skeleton, and lysidicin F (**1**) is the first example of natural product with *trans*-fused furan rings, which widen the knowledge of phloroglucinol derivatives. Moreover, their absolute configurations were deduced by computational methods, which provides an example of determining structurally complex natural products with novel skeletons using this method. Herein, we report the isolation and structural elucidations of **1**−**3** as well as their antioxidant activities.



Lysidicin F (**1**) was obtained as a pale yellow powder. Its molecular formula $C_{27}H_{32}O_{10}$ was determined by HRESIMS at *m*/*z* 539.1892 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_{10}Na$, 539.1893), implying 12 degrees of unsaturation. The ^{1}H NMR spectrum (Supporting Information, Table S1) of **1** displayed five exchangeable phenolic hydroxyl protons [δ_H 14.28 (1H, s, HO-6′), 13.29 (1H, s, HO-10″), 10.68 (1H, s, HO-8″), 10.56 (1H, s, HO-10′), and 10.30 (1H, s, HO-8′)] and two isolated aromatic protons [δ_H 5.91 (1H, s, H-9′) and 5.74 (1H, s, H-9″)]. The ^{13}C NMR and DEPT spectra exhibited two carbonyl carbon signals (δ_C 203.7 and 204.7), six oxygenated aromatic carbon signals (δ_C 164.8, 164.6, 163.1, 162.1, 160.4, and 160.3), six relative upfield aromatic carbon signals (δ_C 103.6, 103.5, 100.4, 99.7, 95.1, and 94.0), two methylene signals (δ_C 50.1 and 51.9), two methine signals (δ_C 24.3 and 24.9), as well as four methyl signals (δ_C 22.1, 22.54, 22.57, and 23.6). The signals of these carbons in combination with the protons described above suggested the presence of two isovalerylphloroglucinol units (**1a** and **1b**) (Figure 1), which are common in all phloroglucinol

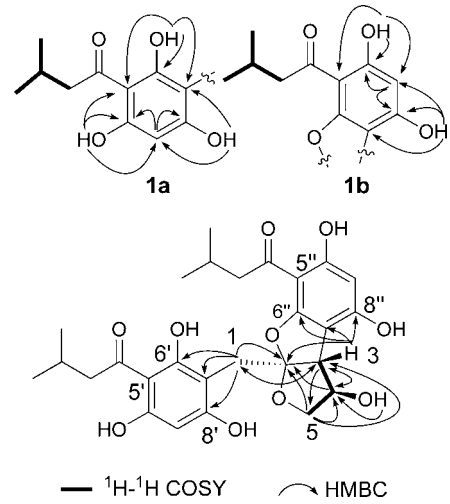


Figure 1. Structures of fragments **1a** and **1b** and key HMBC correlations of **1**.

derivatives isolated from this plant.[3] In addition to the signals of the aforementioned two units in the ^{13}C NMR spectrum of **1**, there were five carbon signals (δ_C 30.1, 54.5, 74.4, 74.5, and 123.8). Among them, the signal at δ_C 123.8 should be due to a ketal carbon[4] and the signals at δ_C 74.4 and 74.5 due to two oxygenated carbons. The fragments **1a** and **1b** only account for 10 degrees of unsaturation, which suggested the presence of another two rings. A series of HMBC correlations from H_2-1 to C-2, C-3, C-6′, C-7′, and C-8′; from H-3 to C-1, C-2, C-4, C-5, C-6″, C-7″, and C-8″; from H-4 to C-2 and C-3; from HO-4 to C-4; and from H-5β (δ_H 3.80) to C-2, C-3, and C-4, combined with spectroscopic data of fused furan moieties reported in the literature,[5] unequivocally established the structure of **1** as shown in Figure 1.

The relative configuration of **1** was deduced according to $^3J_{H,H}$ coupling constant values and NOE experiments. In the ^{1}H NMR spectrum, the singlet signal of H-3 indicated no coupling ($J = 0$ Hz) between H-3 and H-4, which is consistent with a *trans* orientation for these protons (in this case, the dihedral angle is near 90° in a five-membered ring).[6] A weak NOE between them further confirmed their *trans* orientation.[7] NOEs were observed between H-4 and H_2-1, while no NOEs were observed between H-3 and H_2-1, suggesting these protons (H-3 and H_2-1) were on different sides of the tetrahydrofuran

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ring. The absolute configuration of **1** was established by theoretical calculations of its electronic circular dichroism (ECD) using the time-dependent density functional theory (TD-DFT) method.[8] On the basis of the relative configuration of **1**, there were only two possible structures considered for it, with the absolute configurations (2*S*,3*R*,4*R*) or (2*R*,3*S*,4*S*). Their optimized geometries were obtained, and then the ECD spectra were calculated at the B3LYP/6-31G(d) level with the TD-DFT/PCM model in methanol solution (see Table S2 and Figure S1, Supporting Information, for detailed information). The results showed that the calculated ECD spectra of the (2*S*,3*R*,4*R*)- and (2*R*,3*S*,4*S*)-isomers exhibited a CD curve similar and opposite to that of the experimental spectrum, respectively (Figure 2).

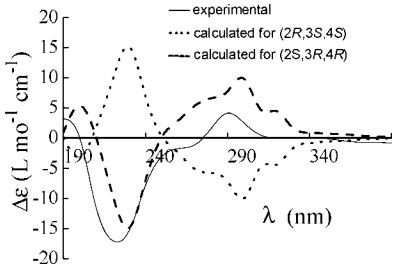


Figure 2. Calculated ECD spectra of the (2*S*,3*R*,4*R*)- and (2*R*,3*S*,4*S*)-isomers and the experimental ECD spectrum of **1**.

Accordingly, the calculated ECD spectrum of (2*S*,3*R*,4*R*)-isomer exhibited diagnostic positive and negative CEs around 310 and 230 nm, respectively, corresponding to the experimental CEs observed around 290 and 220 nm. Therefore, the absolute configuration of **1** was determined as 2*S*,3*R*,4*R*.

Molecular orbital (MO) analysis of the (2*S*,3*R*,4*R*)-isomer provided comprehension of the experimentally observed ECD spectrum of **1** at the molecular level (Figure 3). The electronic transitions from the filled CC orbital of phenyl group to antibonding orbital of CO group, $\pi_{CC} \rightarrow \pi^*_{CO}$, play a dominant role in the positive and negative CEs around 310 (such as MO136→MO138) and 230 nm (MO137→MO140), respectively.

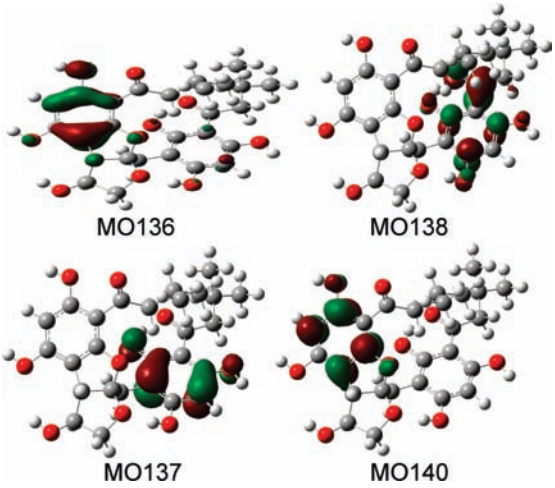


Figure 3. Some MOs involved in the key transitions in ECD of the (2*S*,3*R*,4*R*)-isomer at the B3LYP/6-31G(d) level of DFT theory with the PCM model in methanol solution.

The molecular formula of lysidicin G (**2**) was determined as $C_{38}H_{44}O_{13}$ by HRESIMS at *m*/*z* 709.2833 $[M + H]^+$ (calcd for $C_{38}H_{45}O_{13}$, 709.2860), suggesting 17 degrees of unsaturation, five more than **1**. Comparison of NMR data of **2** with those of **1** revealed **2** possessed a similar structural moiety to **1**, except for one more isovalerylphloroglucinol unit than **1**. The other obvious differences between the NMR spectral data of the two compounds were that the signals at δ_C 54.4 (C-3), 74.5 (C-4), and 74.4 (C-5) in **1** were shifted to δ_C 50.0 (C-3), 41.8 (C-4), and 71.5 (C-5) in **2**, respectively. Therefore, it could be concluded that the hydroxy group at C-4 (HO-4, δ_H 5.14) in **1** was replaced by one isovalerylphloroglucinol unit in **2**, which was confirmed by the correlations of H-4 with C-6‴, C-7‴, C-8‴, C-3, and C-5; H-3 and H_2-5 with C-7‴ in the HMBC experiment.

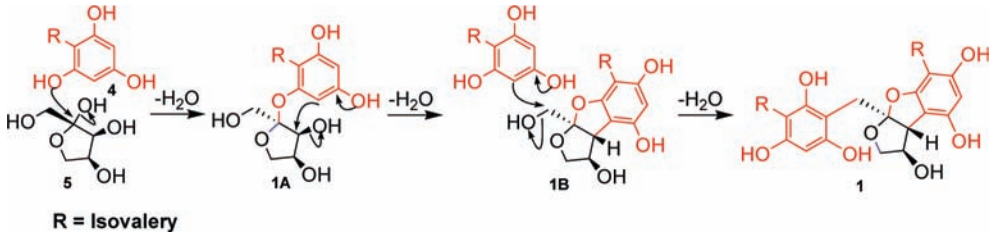
The assignment of the relative configuration of **2** was deterimined on the basis of the $^3J_{H,H}$ coupling constant values and NOE experiments. Different from compound **1**, the obvious coupling (J = 6.0 Hz) between H-3 and H-4 was observed in the ^{1}H NMR spectrum. Meanwhile, the NOE between H-3 and H-4 was strong. Thus, the relative configuration between these protons was established as the *cis* orientation.[6,7] Obvious NOEs between H-3 and H_2-1 supported that they were on the same side of the tetrahydrofuran ring. Similarly to **1**, the absolute configuration of **2** was determined by calculations of ECD spectra. Use of the TD-DFT method provided theoretically calculated ECD curves of a pair of enantiomers (2*R*,3*S*,4*R* and 2*S*,3*R*,4*S*). Comparison of experimental and theoretically simulated ECD curves of the enantiomers unambiguously demonstrated that **2** has the (2*R*,3*S*,4*R*) configuration.

HRESIMS of lysidicin H (**3**) gave a $[M + Na]^+$ peak at *m*/*z* 569.1977, corresponding to a molecular formula of $C_{28}H_{34}O_{11}$. The NMR data of **3** were similar to those of **1**, except the signals of one more hydroxymethyl group in **3**. The ^{1}H−^{1}H COSY correlations from H-5 to H-4 and H_2-6, as well as HMBC correlations from H_2-6 to C-5, and from H-4 to C-5 and C-6, established the connectivity of C4−C5−C6.

The relative configuration of **3** was established by analysis of NOEs and ^{1}H−^{1}H coupling constants. NOEs between H-3 and H_2-1, H-4, and H-1b (δ_H 2.98) suggested that H_2-1, H-3, and H-4 were on the same side of the tetrahydrofuran ring. Therefore, the relative configuration between H-3 and H-4 could be deduced as *cis* relationship, which was further confirmed by the magnitude of the $^3J_{H\text{-}3,H\text{-}4}$ coupling constant value (6.4 Hz).[6,7] The stereochemical relationship between H-4 and the hydroxymethyl group ($HOCH_2$-6) was established as *cis* because of observed NOEs between H-4 and H_2-6. Excellent agreement was observed between experimental and calculated ECD spectra for the (2*R*,3*R*,4*S*,5*R*) allowing the absolute configuration of **3** to be determined.

All lysidicins F−H (**1**−**3**) contain isovalerylphloroglucinol units, which occur in all phloroglucinol derivatives isolated

Scheme 1. Proposed Biogenetic Parthway for **1**



from *L. rhodostegia*. Thus, isovalerylphloroglucinol (**4**), a known natural product,[9] and ketose [L-arabinulose (**5**) and D-fructose (**6**)] were supposed to be the precursors (Scheme 1). Catalyzed by some enzymes, a S_N2 nucleophilic displacement reaction between **4** and β-anomer of **5** would produce glycosylation intermediate **1A**, which should further generate intermediate **1B** with *trans*-fused furan rings through intramolecular C-alkylation. Further intermolecular C-alkylation between **1B** and **4** would yield lysidicin F (**1**). Similarly, after a glycosylation and an intramolecular C-alkylation reacton, **4** and α-anomer of **5** could give an intermediate with *cis*-fused furan rings, the epimer diastereomer of **1B**, which finally generate lysidicin G (**2**) via intermolecular C-alkylation with two molecules of **4**. Lysidicins H (**3**) could be derived from **4** and **6** via a similar process described above.

The antioxidant activities of the isolates were tested according to reference procedures.[10] Vitamin E was selected as the positive control due to its well-known antioxiant activity. At a concentration of 0.1 μM/mL, lysidicins F−H (**1**−**3**) showed significant antioxidant activities with inhibitory rates of 100%, while vitamin E showed significant antioxidant activity with a rate of 81.54%. At lower concentrations, 0.01 and 0.001 μM/mL, however, antioxidant activities of **1**−**3** vanished.

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Supporting Information Available: 1D and 2D NMR spectra, characterization data, detailed computational method, isolation procedures, and ECD data of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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