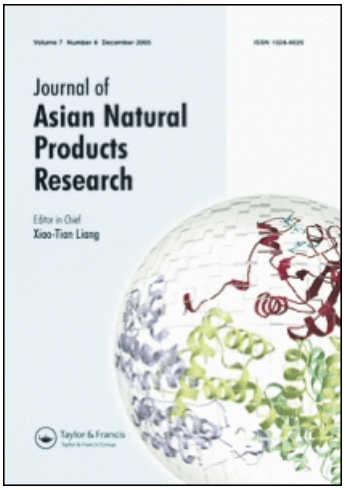


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# Two new compounds from the roots of *Lysidice rhodostegia*

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# Two new compounds from the roots of *Lysidice rhodostegia*

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Two new compounds, lysidicin D (**1**) and lysidicin E (**2**), were isolated from the roots of *Lysidice rhodostegia*. Their structures were elucidated by means of spectroscopic methods. Among them compound **1** showed potent anti-oxidant activity on *in vitro*.

*Keywords*: *Lysidice rhodostegia*; Leguminosae; Lysidicin D; Lysidicin E; Anti-oxidant activity

## 1. Introduction

*Lysidice rhodostegia* belongs to the genus *Lysidice* of the family Leguminosae, widely distributed in Guangxi, Guangdong, Yunnan, and Guizhou provinces of China, and its roots have been used for the treatment of ache, fractures and hemorrhage by local folks in China [1]. Flavonoids, phloroglucinols, stilbenes, lignans and triterpenoids have been isolated from the title plant [2–6]. Further investigation on the title plant led to the isolation of two new compounds, lysidicin D (**1**) and lysidicin E (**2**). In this paper, we report the isolation, structural elucidation, and plausible biogenetic pathways of them, along with the anti-oxidant activity of **1**.

## 2. Results and discussion

Lysidicin D (**1**) was obtained as yellow powder, and the HRESIMS exhibited a quasimolecular ion peak at *m/z* 443.1452 $[M + Na]^+$, suggesting the molecular formula to be $C_{25}H_{24}O_6$. In the IR spectrum, absorption bands attributable to hydroxyl groups (3342 $cm^{-1}$), conjugated carbonyl groups (1620 $cm^{-1}$) and benzene rings (1608 and 1510 $cm^{-1}$) were observed. The $^1H$ NMR spectrum of **1** (table 1) showed 1, 2, 3, 5-tetrasubstituted aromatic protons at $\delta$ 6.30

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Table 1. $^{1}$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) data for **1** in acetone-$d_6$.

| *Position* | $\delta_C$ | $\delta_H$ *(mult.; J in Hz)* | *HMBC* |
|---|---|---|---|
| 1 | 161.5 | | |
| 2 | 108.6 | | |
| 3 | 158.7 | | |
| 4 | 106.9 | 7.73 (1H, s) | C-2, C-3, C-4a, C-4b, C-10a |
| 4a | 142.2 | | |
| 4b | 113.7 | | |
| 5 | 157.7 | | |
| 6 | 102.8 | 6.30 (1H, br s) | C-4b, C-5, C-7, C-8 |
| 7 | 159.4 | | |
| 8 | 109.4 | 6.19 (1H, br s) | C-4b, C-6, C-7, C-9 |
| 8a | 141.4 | | |
| 9 | 38.3 | 2.97 (1H, br d, 15.2) | C-4b, C-8, C-8a, C-10, C-10a, C-1′ |
| | | 3.11 (1H, dd, 15.2, 5.2) | C-4b, C-8, C-8a, C-10, C-10a, C-1′ |
| 10 | 34.8 | 4.50 (1H, d, 5.2) | C-1, C-10a, C-4a, C-8a, C-9, C-1′, C-2′ (C-6′) |
| 10a | 118.6 | | |
| 1′ | 135.0 | | |
| 2′ (6′) | 129.4 | 6.87 (2H, d, 8.4) | C-10, C-3′, C-4′ |
| 3′ (5′) | 115.3 | 6.53 (2H, d, 8.4) | C-1′, C-4′ |
| 4′ | 156.2 | | |
| 1″ | 207.4 | | |
| 2″ | 53.8 | 3.10 (2H, d, 6.4) | C-1″, C-3″, C-4″ (C-5″) |
| 3″ | 25.8 | 2.30 (1H, m) | C-1″, C-2″, C-4″ (C-5″) |
| 4″ (5″) | 23.1 | 0.96 (6H, d, 6.8) | C-2″, C-3″ |
| HO-1 | | 13.63 (1H, s) | C-1, C-2, C-10a |
| HO-3 | | 9.42 (1H, s) | C-2, C-3, C-4 |
| HO-5 | | 8.72 (1H, s) | C-4b, C-5, C-6 |
| HO-7 | | 8.36 (1H, s) | C-6, C-7, C-8 |
| HO-4′ | | 7.87 (1H, s) | C-3′, C-4′, C-5′ |



(1H, br s, H-6), 6.19 (1H, br s, H-8), pentasubstituted aromatic proton at $\delta$ 7.73 (1H, s, H-4), aliphatic protons of ABX system at $\delta$ 2.97 (1H, br d, $J = 15.2$ Hz, $H_1$-9), 3.11 (1H, dd, $J = 15.2, 5.2$ Hz, $H_2$-9) and 4.50 (1H, d, $J = 5.2$ Hz, H-10). These data in combination with 12 aromatic carbons ($\delta$ 102.8–161.5) and two aliphatic carbons ($\delta$ 34.8, 38.3) in the $^{13}$C NMR spectrum of **1** indicated the presence of dihydrophenanthrene moiety, which was confirmed by the HMBC correlations from H-4 to C-2, C-3, C-4a, C-4b, and C-10a; H-6 to C-4b, C-5, C-7, and C-8; H-8 to C-4b, C-6, C-7, and C-9; H-9 to C-4b, C-8a, C-8, C-10, and C-10a; H-10 to C-1, C-10a, C-4a, C-8a, and C-9. The proton signals at $\delta$ 6.87 (2H, $J = 8.4$ Hz, H-2′, 6′), 6.53 (2H, $J = 8.4$ Hz, H-3′, H-5′) suggested the existence of 4-hydroxyphenyl moiety, which was attached to C-10 by the HMBC correlations from H-9 to C-1′, H-10 to C-1′ and C-2′ (C-6′), H-2′ (H-6′) to C-10. The placements of phenolic hydroxyls were determined by the HMBC correlations of their protons with carbons (figure 1). The above data indicated the presence of structure unit A shown in figure 1. In the $^{13}$C NMR and DEPT spectra of **1**, two methyl signals at $\delta$ 23.1, a methylene signal at $\delta$ 53.8, a methine signal at $\delta$ 25.8, and a carbonyl signal at $\delta$ 207.4 together with the corresponding protons showed the presence of a 3-methylbutyryl segment. Apparently, it should be linked to C-2 of structure unit A. The absolute configuration at C-10 was determined as R by CD spectrum for its positive Cotton effect in the 270–290 nm range [7]. Thus the structure of compound **1** was established as (10R)-3-methyl-1-[1,3,5,7-tetrahydroxy-10-(4-hydroxyphenyl)-9,10-dihydro-2-phenanthrenyl]-1-butanone, named as lysidicin D (figure 2).

Lysidicin E (**2**) was obtained as yellow powder, and the molecular formula $C_{28}H_{32}O_{10}$ was determined by HRESIMS. Its IR spectrum displayed absorption bands of hydroxyl groups (3213 cm$^{-1}$), γ-lactone carbonyl group (1793 cm$^{-1}$), carbonyl groups (1707 cm$^{-1}$),

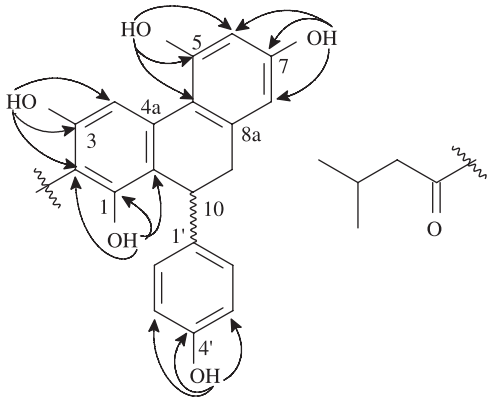
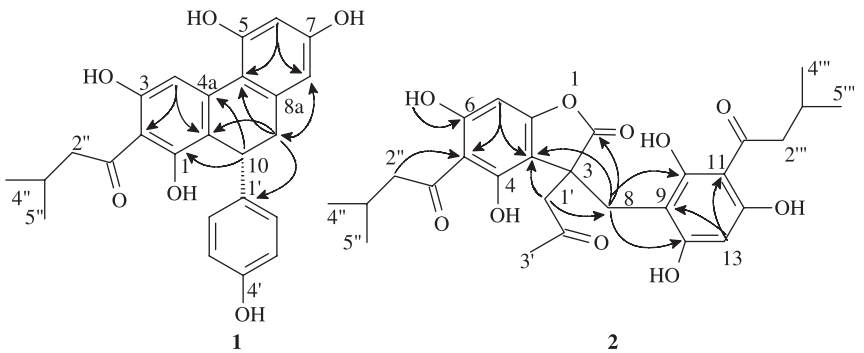



Figure 1. Structure unit A, 3-methylbutyryl segment and partial HMBC correlations of **1**.



conjugated carbonyl groups (1620 $cm^{-1}$), and benzene rings (1600 and 1510 $cm^{-1}$). The $^{13}C$ NMR (table 2) and DEPT spectra suggested the presence of four methyl groups at $\delta$ 22.3 (2C), 22.5 (2C), two methylene groups at $\delta$ 50.7, 51.8, two methine groups at $\delta$ 25.3, 24.8, two carbonyl groups at $\delta$ 202.8, 204.6 and six *O*-bearing aromatic carbons at $\delta$ 154.7, 160.4, 160.8, 163.4, 163.7, 164.9 and six upfield-shifted aromatic carbons at $\delta$ 93.8, 98.0, 99.8, 100.1, 103.4, 108.5, so two 3-methylbutyryl-phloroglucinol moieties were inferred [6,8], which was confirmed by the $^1H$ NMR (table 2) and HMBC spectra (figure 2). Resonances for the prontons at $\delta$ 1.99 (3H, s) and carbons at $\delta$ 29.3 and 205.9 suggested the presence of the fragment —CO—$CH_3$. Besides above carbon signals, the $^{13}C$ NMR spectrum also revealed the presence of two methylene groups at $\delta$ 29.1 (C-8) and 47.4 (C-1′), one quaternary carbon at $\delta$ 47.0 (C-3) and one ester carbonyl group at $\delta$ 176.8 (C-2). In the HMBC spectrum, H-8 ($\delta$ 3.10, 2.80) and H-1′ ($\delta$ 3.68, 3.12) correlated with the quaternary carbon at $\delta$ 47.0 (C-3) and the ester carbonyl carbon at $\delta$ 176.8 (C-2), indicating the presence of the fragment. —$CH_2$—C(COO—)—$CH_2$— · H-1′ also correlated with the carbonyl carbon at $\delta$ 205.9 (C-2′) and the methyl carbon at $\delta$ 29.3 (C-3′) of the fragment —CO—$CH_3$, suggesting the presence of the fragment —$CH_2$—CO—$CH_3$, which was further confirmed by the correlations of H-3′ with C-1′ and C-2′. So the segment —$CH_2$—C(COO—)—$CH_2$—CO—$CH_3$ was established. H-8 correlated with C-9, C-10, C-14 and H-1′ correlated with C-3a, which suggested C-8 and C-3 were directly connected to C-9 and C-3a, respectively. Therefore, compound **2** was




**1** **2**

Figure 2. Structures and key HMBC correlations of **1** and **2**.

Table 2. $^{1}$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) data for **2** in DMSO-$d_6$.

| *Position* | $\delta_C$ | $\delta_H$ *(mult.; J in Hz)* | *HMBC* |
|---|---|---|---|
| 2 | 176.8 | | |
| 3 | 47.0 | | |
| 3a | 108.5 | | |
| 4 | 154.7 | | |
| 5 | 100.1 | | |
| 6 | 163.7 | | |
| 7 | 98.0 | 5.95 (1H, s) | C-3, C-3a, C-5, C-6, C-7a |
| 7a | 160.4 | | |
| 8 | 29.1 | 3.10 (1H, d, 14.0) | C-2, C-3, C-3a, C-9, C-10, C-14 |
| | | 2.80 (1H, d, 14.0) | C-2, C-3, C-3a, C-9, C-10, C-14 |
| 9 | 99.8 | | |
| 10 | 164.9 | | |
| 11 | 103.4 | | |
| 12 | 160.8 | | |
| 13 | 93.8 | 5.89 (1H, s) | C-8, C-9, C-11, C-12, C-14, C-1″ |
| 14 | 163.4 | | |
| 1′ | 47.4 | 3.68 (1H, d, 19.0) | C-2′, C-2, C-3a, C-3, C-8 |
| | | 3.12 (1H, d, 19.0) | C-2′, C-2, C-3a, C-3, C-8 |
| 2′ | 205.9 | | |
| 3′ | 29.3 | 1.99 (3H, s) | C-1′, C-2′ |
| 1″ | 202.8 | | |
| 2″ | 50.7 | 2.81 (1H, dd, 7.0, 14.0) | C-5, C-1″, C-3″, C-4″ (C-5″) |
| | | 2.63 (1H, dd, 7.0, 14.0) | C-5, C-1″, C-3″, C-4″ (C-5″) |
| 3″ | 25.3 | 1.95 (1H, m) | C-1″, C-2″, C-4″ (C-5″) |
| 4″ (5″) | 22.3 | 0.85 (6H, d, 7.0) | C-2″, C-3″ |
| 1‴ | 204.6 | | |
| 2‴ | 51.8 | 2.86 (1H, dd, 7.0, 14.0) | C-1‴, C-3‴, C-4‴ (C-5‴) |
| | | 2.76 (1H, dd, 7.0, 14.0) | C-1‴, C-3‴, C-4‴ (C-5‴) |
| 3‴ | 24.8 | 2.10 (1H, m) | C-1‴, C-2‴, C-4‴ (C-5‴) |
| 4‴ (5‴) | 22.5 | 0.86 (6H, d, 7.0) | C-2‴, C-3‴ |
| HO-6 | | 12.97 (1H, s) | C-5, C-6, C-7 |



deduced to be 4,6-dihydroxy-5-(3-methylbutyryl)-3-(2-oxopropyl)-3-[2,4,6-trihydroxy-3-(3-methylbutyryl)benzyl]-2,3-dihydrobenzo[b]furan-2-one, named as lysidicin E (figure 2).

The plausible biogenetic pathways for lysidice D (**1**) and lysidice E (**2**) were proposed as shown in figures 3 and 4. The one-electron oxidation of phenol group in 3-methylbutyryl-phloroglucinol (**3**) gave free radical **3a**. The free-radical addition between the resonance form

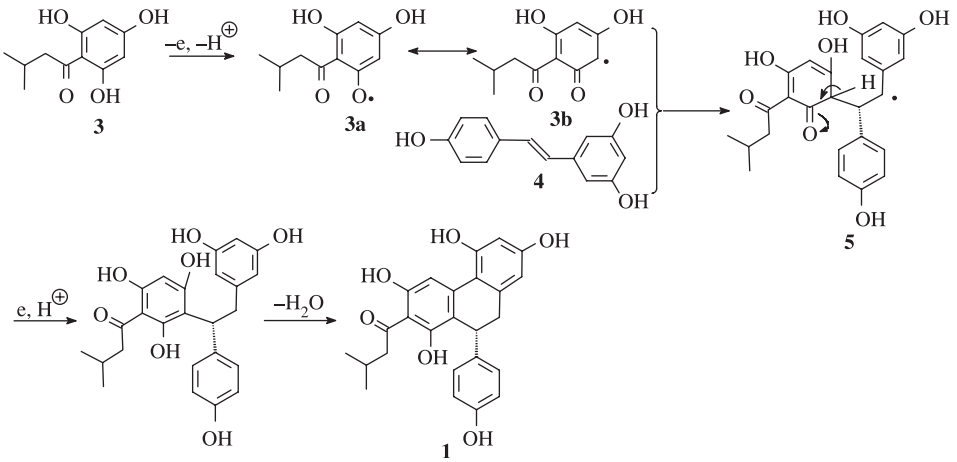



Figure 3. Proposed biogenetic pathway for **1**.

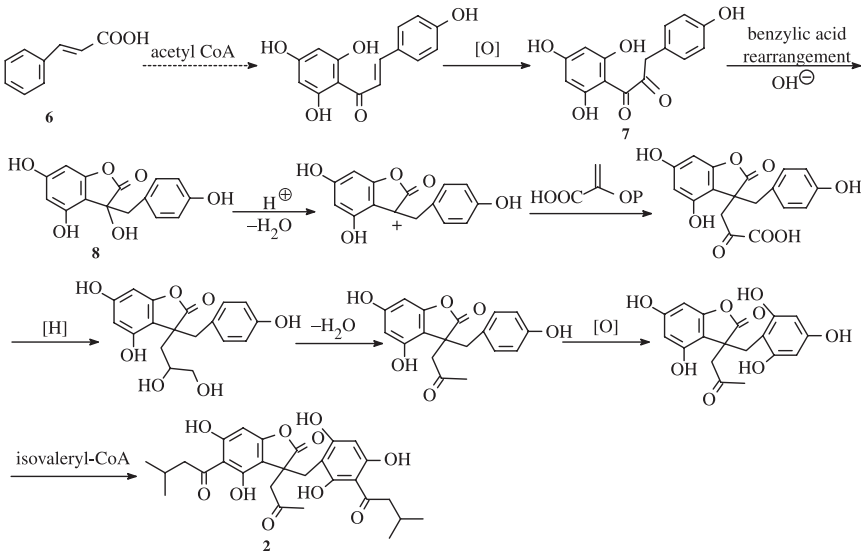



Figure 4. Proposed biogenetic pathway for **2**.

**3b** of **3a** and resveratrol (**4**) could generate the intermediate product **5**, which was subsequently restored to aromaticity by keto-enol tautomerism. Then the intramolecular dehydration led to the formation of compound **1**; On the basis of biogenesis for flavones, the condensation between cinnamic acid (**6**) and acetyl CoA followed by oxidation resulted in 3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-1,2-propanedione (**7**), which then gave 3,4,6-trihydroxy-3-(4-hydroxybenzyl)-2,3-dihydrobenzo[*b*]furan-2-one (**8**) through benzylic acid rearrangement [9,10]. Then the intermediate (**8**) underwent dehydration, electrophilic addition, reduction, acylation, oxygenation, and so on, to form compound **2** [11].

The anti-oxidant activity of compound **1** was tested according to the process of the reference [12]. Vitamin E was selsected as the positive control. Compound **1** showed stronger anti-oxidant activity than vitamin E at the concentrations of $10^{-4}$, $10^{-5}$, $10^{-6}$ mol/L (table 3).

## 3. Experimental

### *3.1 General experimental 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 Perkin-Elmer 683 FT infrared spectrometer. UV spectra were obtained on a Shimadzu

Table 3. Inhibitory rates of MDA.

| *Compound* | *Concentration (M)* | *Inhibitory rate (%)* |
|---|---|---|
| **1** | $10^{-4}$ | 87.8 |
| | $10^{-5}$ | 70.1 |
| | $10^{-6}$ | 43.8 |
| Vit E | $10^{-4}$ | 81.5 |
| | $10^{-5}$ | 33.5 |
| | $10^{-6}$ | −4.7 |

UV-240 instrument. NMR spectra were run on a Varian INOVA-500 FT-NMR spectrometer with TMS as internal standard. Mass spectra were recorded on an Autospec-Ultima ETOF Spec mass spectrometer. Silica $GF_{254}$ for TLC and silica gel (160–200 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company, China. RpC-18 silica gel (50μ) and Sephadex LH-20 were purchased from Fuji Silysica Chemical Ltd.

### *3.2 Plant material*

The roots of *Lysidice rhodostega* were collected from Guangxi Province, China, in November 1999 and identified by Professor Shou-yang Liu. A voucher specimen (No.002775) is deposited in the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences.

### *3.3 Extraction and isolation*

The air-dried roots of *L. rhodostega* (7.0 kg) were extracted with 95% EtOH (20 L × 3) and the extract were concentrated *in vacuo* to yield a dark brown residue (930 g), which was suspended in water and then successively partitioned with EtOAc (6 L) and n-BuOH (6 L). The n-BuOH fraction (210 g) was subjected to column chromatography on silica gel, eluting with a gradient of $CH_2Cl_2$—$CH_3OH$ (9:1 → 7:3) to obtain 12 fractions (Fr.1–12). Fraction 1 (40.0 g) was further subjected to RpC-18 silica gel eluting with $CH_3OH$—$H_2O$ (3:7 → 4:6) to obtain 10 subfractions (B1–B10). The fraction B4 (6.06 g) was further separated by Sephadex LH-20 column chromatography, eluting with MeOH to provide lysidicin D (**1**, 32.6 mg) and lysidicin E (**2**, 10.5 mg).

**3.3.1 Lysidicin D (1)**. Yellow powder (32.6 mg); $[\alpha]_D^{25}$ + 24 (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ (nm): 219, 344; CD (MeOH) $[\theta]_{269}$ 0, $[\theta]_{280}$ + 2760, $[\theta]_{294}$ + 21725, $[\theta]_{302}$ 0; IR (KBr) $V_{max}$ ($cm^{-1}$): 3342, 1620, 1608, 1510; $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR spectral data see table 1; HRESIMS *m/z* 443.1452 $[M + Na]^+$ (calcd for $C_{25}H_{24}O_6Na$, 443.1471).

**3.3.2 Lysidicin E (2)**. Yellow powder (10.5 mg); $[\alpha]_D^{25}$ + 40 (*c* 0.01, MeOH); UV (MeOH) $\lambda_{max}$ (nm): 206, 231, 286; IR (KBr) $V_{max}$ ($cm^{-1}$): 3213, 1793, 1707, 1620, 1600, 1510; $^1H$ (500 MHz) and $^{13}C$ (125 MHz) NMR spectral data see table 2; HRESIMS *m/z* 551.1918 $[M + Na]^+$ (calcd for $C_{28}H_{32}O_{10}Na$, 551.1893).

### *3.4 Anti-oxidant bioassays*

The antioxidant activities of **1** and Vit E were determined by the content of MDA (malondialhyde), 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 [12].

## Acknowledgements

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