ORGANIC LETTERS

2006
Vol. 8, No. 11
2269−2272

Novel Phloroglucinol Derivatives from the Roots of *Lysidice rhodostegia*

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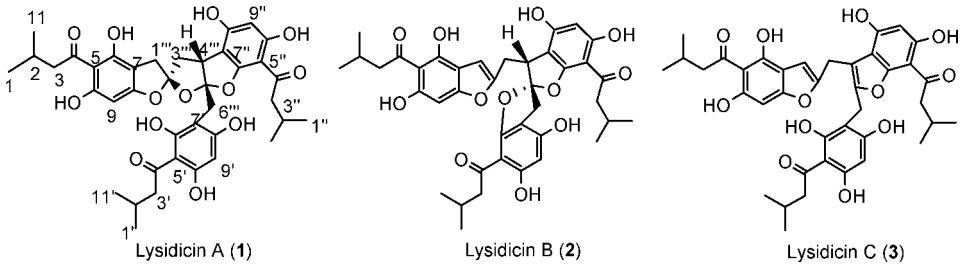
Received March 2, 2006

ABSTRACT



Three novel phloroglucinol derivatives of lysidicins A–C (1–3) have been isolated from the roots of *Lysidice rhodostegia* and structures were elucidated by comprehensive NMR and MS spectroscopic analysis. 1 and 2 possess spirocyclic benzodihydrofuran skeleton. Their relative stereochemistries were assigned by NOE or NOESY experiment. A plausible pathway for the biosynthesis of 1–3 from 4 and a ketose derivative was postulated.

As part of a program aimed at searching for bioactive substances from *Lysidice rhodostegia* Hance (Fabaceae), a shrubbery plant that has been used for the treatment of ache, fractures, and hemorrhage for a long time in China,[1] several new phloroglucinol glycosides, flavanols, and stilbenes which showed vasodilator activities have been isolated from the EtOAc and *n*-BuOH extracts.[2] Interestingly, all those phloroglucinol derivatives isolated from this plant contain a (3-methylbutyryl) phloroglucinol moiety (**4**) that is also a major ingredient in this plant. Continuing study on the *n*-BuOH extract led to the isolation of three novel phloroglucinol derivatives. In this paper, the isolation, structure elucidation, and postulated biogenetic formation of **1**−**3** are described.

The *n*-BuOH extract (190 g) of the roots of *Lysidice rhodostegia* (7.0 kg) was subjected to column chromatography on silica gel to obtain eight major fractions (B_1−B_8). The fraction B_4 (6.06 g) was further separated by repeating chromatographies over ODS and Sephadex LH-20 to provide 8.5 mg of lysidicin A (**1**), 10.5 mg of lysidicin B (**2**), and 12.6 mg of lysidicin C (**3**), **1** and **2** of which possess the spirocyclic benzodihydrofuran skeleton.

Lysidicin A (**1**)[3] was obtained as yellow powder. It showed the molecular ion peak at *m*/*z* 743.4 $(M + Na)^+$ in positive

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(2) (a) Gao, S.; Feng, N.; Yu, S. S.; Yu, D. Q.; Wang, X. L. *Planta Med.* **2004**, 70, 1128. (b) Gao, S.; Fu, G. M.; Fan, L. H.; Yu, S. S.; Yu, D, Q. *J. Integ. Plant. Biol.* **2005**, 47, 759. (c) Gao, S.; Yu, S. S.; Yu, D. Q. *Chin. Chem. Lett.* **2004**, 15, 313. (d) Gao, S.; Fu, G. M.; Fan, L. H.; Yu, S. S.; Yu, D. Q. *Chin. J. Nat. Med.* **2005**, 3, 144.

(3) Lysidicin A (**1**): yellow powder; $[\alpha]^{23}_D$ 6 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3311, 2958, 2870, 1701, 1622, 1508, 1431, 1369, 1306, 1163, 1009, 976, 820, 694 cm^{-1}; UV (MeOH) λ_{max} 335, 285, 231.

10.1021/ol060514o CCC: $33.50
Published on Web 04/27/2006

ESIMS, and the molecular formula of $C_{39}H_{44}O_{13}$ was established on the basis of HRESIMS [$(M + Na)^+$, *m*/*z* 743.2694, calcd 743.2680, indicating 18 degrees of unsaturation]. The ^{1}H NMR spectrum of **1** (Table 1) displayed

Table 1. ^{1}H (500 MHz), ^{13}C NMR (125 MHz), and HMBC Data of **1** in DMSO-d_6

position	δH (mult; *J* in Hz)	δ**C**	HMBC
1	0.61 (d; 6.5)	22.5	2, 3, 11
2	1.72 (overlap)	25.9	1, 3, 4. 11
3	2.42 (dd; 8.5, 14.0)	49.6	1, 2, 4. 11
	1.72 (d; 14.0)		4
4		204.2	
5		101.6	
6		161.2	
7		103.4	
8		160.5	
9	5.84 (s)	96.4	4, 5, 7, 8, 10, 1‴
10		164.8	
11	0.58 (d; 6.5)	22.7	1, 2, 3
1′	0.88 (d; 6.5)	23.29	2′, 3′, 11′
2′	2.10 (m)	25.6	1′/11′, 3′, 4′
3′	2.85 (d; 7.0)	52.6	1′/11′, 2′, 4′, 5′
4′		204.7	
5′		104.4	
6′		165.5	
7′		100.3	
8′		164.0	
9′	5.98 (s)	94.8	4′, 5′, 7′, 8′, 10′
10′		161.5	
11′	0.88 (d; 6.5)	23.29	1′, 2′, 3′
1″	0.79 (d; 7.0)	22.5	2″, 3″, 4″, 11″
2″	2.00 (m,7.0)	25.9	1″, 3″, 4″, 11″
3″	2.91 (dd; 6.5, 15.0)	49.6	1″, 2″, 4″, 11″
	2.54 (dd; 6.5, 15.0)		4″
4″		204.3	
5″		101.1	
6″		161.0	
7″		107.0	
8″		160.9	
9″	5.83 (s)	96.8	5″, 7″, 8″, 10″
10″		165.7	
11″	0.58 (d; 6.5)	22.7	1″, 2″, 3″, 4″
1‴	3.10, 2.94 (2 × d; 16.0)	36.2	6, 7, 2‴, 3‴
2‴		121.3	
3‴-α	2.62 (d; 14.0)	41.4	1‴, 2‴, 4‴, 5‴, 7″
3‴-*β*	2.44 (dd; 9.0, 14.0)		7″
4‴	4.16 (d; 9.0)	45.3	2‴, 3‴, 6‴, 6″, 7″
5‴		124.8	
6‴	3.16, 3.14 (2 × d; 14.0)	30.6	4‴, 5‴, 6′, 7′, 8′
OH-6′	14.37 (s)		4′, 5′, 6′, 7′
OH-10″	13.26 (s)		4″, 5″, 8″, 9″, 10″
OH-10	13.03(s)		4, 5, 8, 9, 10
OH-8″	10.79 (s)		7″,8″, 9″
OH-6	10.70 (br s)		6, 7
OH-10′	10.70 (br s)		5′, 9′, 10′
OH-8′	10.47 (s)		7′, 8′, 9′

seven exchangeable phenolic hydroxyl protons at δ 14.37, 13.26, 13.03, 10.79, 10.70, 10.70, and 10.47 (s, each, HO-6′, HO-10″, HO-10, HO-8″, HO-6, HO-10′, and HO-8′) and three uncoupled aromatic protons at δ 5.83, 5.84, and 5.98 (s, each, H-9″, H-9, and H-9′) in the downfield region. In the ^{13}C NMR and DEPT spectra of **1**, nine oxygen-substituted aromatic carbon signals (δ 160.5−165.7), nine relative upfield aromatic carbon signals (δ 94.8−107.0), as well as six methyl signals at δ 22.5−23.3, three methylene signals at δ 49.6−52.6, three methine signals at δ 25.5−25.9, and three carbonyl signals at δ 204.2−205.7 were observed. Considering the fact that most major chemical consitituents isolated from this plant possess a (3-methylbutyryl) phloroglucinol unit,[2] it is reasonable to presume that **1** contained three (3-methylbutyryl) phloroglucinol moieties which were proved by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments (Figure 1). Those fragments combined with the remaining

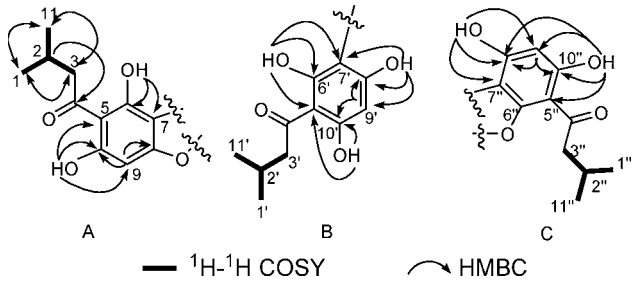

Figure 1. Structural fragments of **1** illustrating key ^{1}H−^{1}H COSY and HMBC correlations.

units (three methylene groups at δ 30.6, 36.2, and 41.4, one methine group at δ 45.3, and two quaternary carbons at δ 121.3, 124.8) accounted for a partial molecular formula of $C_{39}H_{44}O_{12}$, which revealed that no other hydroxyl group is present in **1**. Thus, a remaining oxygen atom and the two unconnected oxygen atoms in fragments A and C required the presence of at least two carbon−oxygen bonds except for the assigned phloroglucinol units. Taking into account the chemical shift values of the remaining six unassigned carbons, it is resonable only that the two relative downfield carbons [δ 121.3 (C-2‴), 124.8 (C-5‴)] were connected to oxygen atoms. The relative downfield signals of this two carbons required the presence of two ketal units[4] and the absence of olefinic carbons or peroxides, which in turn required the presence of three rings to satisfy the degrees of unsaturation.

The ^{1}H NMR data (Table 1) disclosed that C-1‴ (δ 36.2) and C-6‴ (δ 30.6) were each connected to quaternary carbons while C-3‴ (δ 41.4) was linked to C-4‴ (δ 45.3) directly. In the HMBC spectrum, the correlations from H-1‴ to C-2‴, C-3‴, C-7, and C-6, from H-3‴ to C-1‴, C-2‴, C-4‴, C-5‴, and C-7″, from H-4‴ to C-2‴, C-3‴, C-6‴, C-6″, and C-7″, and from H-6‴ to C-4‴, C-5‴, C-6′, C-7′ and C-8′ combined with the signals of ^{1}H NMR and ^{13}C NMR demonstrated that C-1‴, C-4‴, and C-6‴ were directly connected to C-7, C-7″, and 7′, respectively (Figure 2). Summarizing all these data, the structure of **1** was elucidated as shown in Figure 2.

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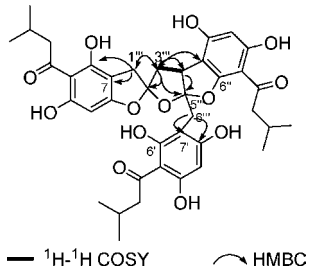


Figure 2. Structure of **1** illustrating partial ^{1}H−^{1}H COSY and HMBC correlations.

The relative stereochemistry of **1** was elucidated on the basis of ^{1}H−^{1}H coupling constants and NOESY experiment (Figure 3). The chirality at C-4‴ was proposed as R*

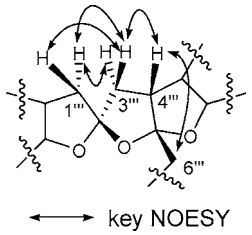


Figure 3. Key NOESY correlations for **1**.

according to the correlation between H-4‴ and H-6‴ observed in NOESY. The signals at δ 4.16 (d, J = 9.0 Hz, H-4‴), 2.62 (d, J = 14.0 Hz, H_1-3‴), and 2.44 (dd, J = 9.0, 14.0 Hz, H_2-3‴) showed the absence of coupling between H-4‴ and one H_1-3‴, which required a dihedral angle of ~90° [5] between them. Thus the signal at δ 2.62 was due to H_α-3‴ while the other proton signal at δ 2.44 should be due to H_β-3‴, which was supported by the NOESY experiment where the correlation between H-4‴ and H_β-3‴ was observed. In addition, H_β-3‴ was correlated with both H_α-1‴ and H_β-1‴, while H_α-3‴ was correlated only with H_α-1‴; similarly, H_α-1‴ was correlated with both H_α-3‴ and H_β-3‴, while H_β-1‴ was correlated only with H_β-3‴. These results allowed us to assign the relative configuration at C-2‴ as R*. Thus, the relative stereochemistry of **1** was determined as 2‴R*4‴R*5‴R*.

Comparison of the NMR data for lysidicin B (**2**)[6] with those of **1** revealed structural similarities, including the presence of three (3-methylbutyryl) phloroglucinol units as exhibited by all other phloroglucinol derivatives isolated from this plant before. The NMR and HRESIMS data for **2** revealed its molecular formula as $C_{39}H_{42}O_{12}$, which had one more degree of unsaturation and one H_2O fragment less than **1**. The obvious difference of ^{13}C NMR between **1** and **2** (Table 2) was that the signals at δ 36.2 (C-1‴), 121.3 (C-2‴), and 41.4 (C-3‴) in compound **1** were replaced by δ 102.0, 152.2, and 27.7 in compound **2**, respectively, which

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data of **2** and **3** in DMSO-d_6

	lysidicin B		lysidicin C	
position	δH (mult; J in Hz)	δ**C**	δH (mult; J in Hz)	δ**C**
1	0.92 (d; 6.8)	22.3	0.86 (d; 6.4)	22.2
2	2.10 (m; 6.8)	24.6	2.07 (overlap)	
3	2.81(dd; 6.8, 13.6)	50.0	2.83 (d; 6.4)	50.1
	2.74 (dd; 6.8, 13.6)			
4		202.0		202.1
5		100.7		100.9
6		154.8		154.5
7		111.1		111.2
8		158.0		157.7
9	6.10 (s)	97.4	6.10 (s)	97.4
10		164.0		163.6
11	0.91(d; 6.8)	22.3	0.86 (d; 6.4)	22.2
1′	0.43 (d; 6.8)	21.6	0.76 (d; 6.4)	22.8
2′	1.46 (m; 6.8)	26.4	2.07 (overlap)	
3′	2.38 (dd; 6.8)	51.0	2.81 (d; 6.4)	51.9
4′		203.2		204.8
5′		100.3		103.7
6′		159.1		164.1
7′		102.6		101.8
8′		160.6		162.5
9′	5.96 (s)	97.1	6.00 (s)	94.0
10′		164.9		160.6
11′	0.55 (d; 6.8)	22.0	0.76 (d; 6.4)	22.8
1″	0.48 (d; 6.4)	22.0	0.86 (d; 6.4)	22.3
2″	1.76 (m; 6.8)	26.3	2.07 (overlap)	
3″	2.68 (dd; 6.0, 12)	51.0	2.89 (d; 6.4)	49.9
	2.20 (dd; 8.0, 12)			
4″		203.2		202.3
5″		100.3		101.6
6″		159.3		154.1
7″		106.6		110.6
8″		161.0		158.9
9″	5.95 (s)	96.6	6.05 (s)	97.3
10″		164.7		163.5
11″	0.64 (d; 6.8)	21.7	0.86 (d; 6.4)	22.3
1‴	6.53 (s)	102.0	6.49 (s)	99.5
2‴		152.2		154.7
3‴	3.34 (dd; 5.6, 15.2)	27.7	4.29 (s)	30.7
	3.20 (dd; 6.0, 15.2)			
4‴	3.96 (dd; 6.0, 5.6)	44.8		109.2
5‴		124.3		151.7
6‴	3.70 (d; 16.4)	31.1	3.96 (s)	18.9
6‴	3.29 (d; 16.4)			
OH-6′			14.35 (s)	
OH-10″	13.10 (s)		13.45 (s)	
OH-10	13.48 (s)		13.42 (s)	
OH-8″	11.05 (br s)		11.08 (br s)	
OH-6	11.05 (br s)		11.08 (br s)	
OH-10′	13.04 (s)		10.59 (s)	
OH-8′	11.05 (br s)		10.49 (s)	

(5) (a) Chang, L. C.; Gerhäuser, C.; Song, L.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1997**, *60*, 869. (b) Buechi, G.; Fowler, K. W.; Nadzan, A. M. *J. Am. Chem. Soc.* **1982**, *104*, 544.

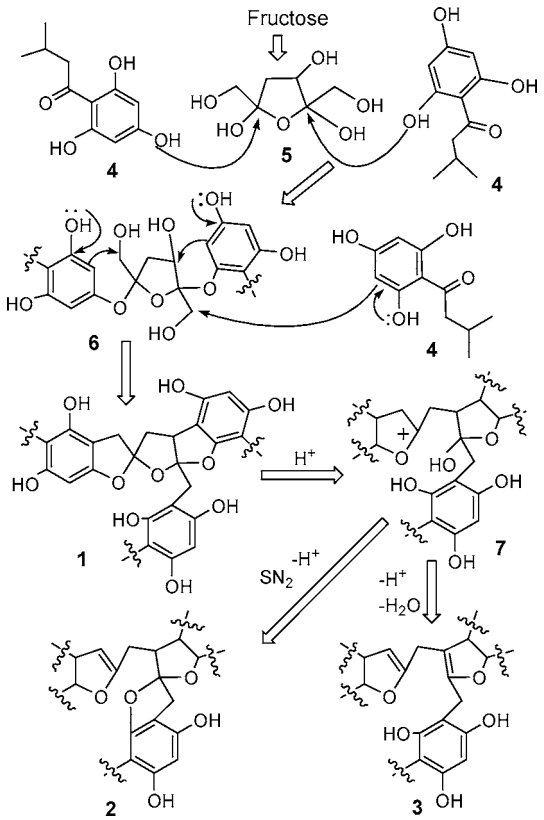
(6) Lysidicin B (**2**): yellow powder; $[\alpha]^{23}_D$ 16 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3377, 2958, 2870, 1637, 1622, 1576, 1508, 1425, 1369, 1308, 1163, 1009, 982, 820, 669 cm^{-1}; UV (MeOH) λ_{max} 330, 281, 232.

indicated the presence of monooxygenated olefinic carbons. Key HMBC correlations of H-1‴ with C-6, C-7, and C-2‴; H-3‴ with C-2‴, C-4‴, C-5‴, and C-7‴; H-4‴ with C-2‴, C-3‴, C-5‴, C-6″, and C-7″; and H-6‴ with C-6′, C-7′, and C-5‴ permitted assignment of the structure of lysidicin B as shown in **2**. The relative stereochemstry of **2** was determined as 4‴R*5‴S* since the NOE between H-4‴ and one H-6‴ was observed experimentally, which suggested the cis relationship between them.

Similarly, the NMR data for lysidicin C (**3**)[7] were comparable to those of **1** and **2** (Table 2), and this suggested that they had analogical skeletons. Importantly, the major difference between **3** and **1/2** was the presence of two series of monooxygenated olefinic carbons (δ 99.5, 154.7 and δ 109.2, 151.7). Those data, combined with the HRESIMS information (molecular formula $C_{39}H_{42}O_{12}$, the same as that of **2**), indicated the formation of a double bond between C-4‴ and C-5‴, which was supported by DEPT, HSQC, and HMBC analyses. Key HMBC correlations of H-3‴ with C-1‴, C-2‴, C-4‴, C-5‴, and C-7″; and H-6‴ with C-6′, C-7′, C-8′, C-4‴, and C-5‴ permitted assignment of the structure of lysidicin C as shown in **3**.

A plausible biosynthetic pathway for **1**, **2**, and **3** from **4** and 3-deoxy-2,5-hexodiulose (**5**) was proposed as illustrated in Scheme 1. Compounds **4** and **5** (**5** could be derived from fructose[8]) were presumed to be precursors for these metabolites. It seems that the nucleophilic displacement reaction between two molecules of **4** and the active sugar form (uridine diphosphosugar) of **5** led to the formation of biosynthetic intermediate **6**, which further underwent an internal and intermolecular C-alkylation process to form **1** since there were suitable nucleophic carbons activated by phenol groups in the phloroglucinol unit. Theoretically, compound **1** could form carbenium ion in acidic condition, and further undergo deprotonation and SN_2 nucleophilic displacement or deprotonation and dehydration processes to form **2** or **3**, which were further supported experimentally. [9] Since these biosynthetic pathways (Scheme 1) are highly speculative, other possible mechanisms for the formation of these three compounds have been considered, for example, the alkylation step precedes the formation of the C−O bonds.

Scheme 1. Proposed Biogenesis of Lysidicins A−C (**1**−**3**) from **4** and Fructose Derivative



Acknowledgment. The research project was supported by the Key Project of National Natural Science Foundations of China (No. 20432030), Beijing Natural Science Foundations (No. 7062046), '973' Project (No. 2004CB518906), and PCSIRT (No. IRT0514). The authors are grateful to the colleagues of our institute for NMR and MS spectra measurements.

Supporting Information Available: IR, MS, 1D and 2D NMR spectra, as well as another proposed biogenesis of lysidicin A−C (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Lysidicin C (**3**): yellow powder; $[\alpha]^{23}_{D}$ 0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3309, 2958, 2871, 1618, 1514, 1425, 1304, 1159, 1078, 1014, 825 cm^{-1}; UV (MeOH) λ_{max} 290, 224.

(8) Zegota, H.; Sonntag, C. V. *Z. Naturforsch., B*: *Anorg. Chem. Org. Chem.* **1981**, 36, 1331.

(9) Lysidicin A (1.5 mg) was dissolved in 95% EtOH (2 mL) containing 3 mg of tartaric acid and refluxed for 2 h. Then, the resulting mixture was concentrated and diluted to 0.5 mL with methanol. TLC and HPLC showed three main spots/peaks corresponding to those of **1**, **2**, and **3**; LC-MS analysis of the reaction mixture in negative ion ESI mode also showed three main peaks at 3.0 (*m*/*z* 719), 3.5 (*m*/*z* 701), and 6.4 min (*m*/*z* 701), which correspond to those of **1**, **2**, and **3**.