JOURNAL OF NATURAL PRODUCTS

Note

Cite This: J. Nat. Prod. 2018, 81, 1098−1102

pubs.acs.org/jnp

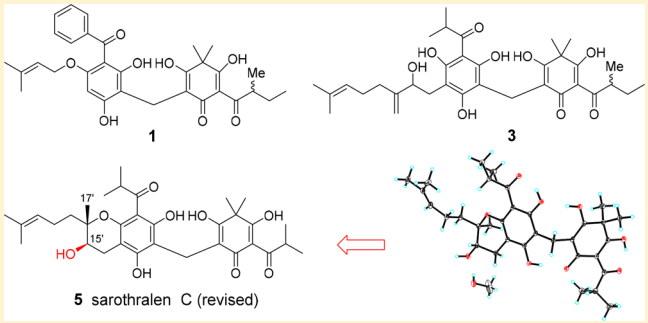
Antibacterial Dimeric Acylphloroglucinols from *Hypericum japonicum*

Yan-Ping Li, Kun Hu, Xing-Wei Yang,* and Gang Xu*

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, and Yunnan Key Laboratory of Natural Medicinal Chemistry, Kunming 650201, People's Republic of China

Supporting Information

ABSTRACT: Nine dimeric acylphloroglucinols, including the new hyperjaponicols A−D (**1**−**4**), were isolated from the whole plant of *Hypericum japonicum*. The new structures were determined by the interpretation of NMR and MS data, and the relative configuration of the known compound, sarothralen C (**5**), was reassigned via NMR spectroscopic analysis and single-crystal X-ray diffraction data. The inhibitory activities of the isolates against four bacterial strains were evaluated, and compounds **1**−**4**, **6**, and **7** exhibited significant antibacterial activity with MIC values of 0.8−3.4 μM. In addition, compound **3** showed moderate lipase inhibitory activity (IC_{50} 8.3 μM).



Hypericum, the largest genus within the Hypericaceae,[1] is a prolific source of acylphloroglucinol derivatives.[2,3] These metabolites showed considerable structural diversity varying from simple acylphloroglucinols to monocyclic and polycyclic polyprenylated acylphloroglucinols (MPAPs and PPAPs).[2,3] Unlike the MPAPs and PPAPs, dimeric acylphloroglucinols consisting of filicinic acid and a phloroglucinol moiety linked by a methylene bridge are a special type of acylphloroglucinols isolated from some *Hypericum* plants.[1] Although less than 40 members of these types of metabolites have been reported thus far, many of them have valuable biological activities, such as antibacterial, antidepressant, antiproliferative, and antinociceptive activities.[1,4,5] *Hypericum japonicum* Thunb. is a traditional Chinese medicinal plant used for treating hepatitis and "dampness-heat" disease,[6] and it is a rich source of dimeric acylphloroglucinols.[7−10] Flavonoids, xanthones, and other acylphloroglucinol derivatives including the filicinic acid-containing meroterpenoids have previously been isolated from this plant.[11−15]

In a continuous search for new and bioactive acylphloroglucinols from *H. japonicum* Thunb.,[12,15] nine dimeric acylphloroglucinols, the new hyperjaponicols A−D (**1**−**4**) and five known analogues (**5**−**9**), were isolated. The new structures were elucidated via interpretation of the NMR and MS data, and the relative configuration of the known sarothralen C (**5**)[10] was reassigned via NMR spectroscopic analysis and single-crystal X-ray diffraction data. This Note describes the isolation and structural determination of the new compounds **1**−**4**, the structural revision of sarothralen C (**5**), and the antibacterial and antilipase activities of the isolates.

The crude MeOH extract of the whole plant of *H. japonicum* was subjected to purification, and four new dimeric acylphloroglucinols, hyperjaponicols A−D (**1**−**4**), and five known analogues, sarothralen C (**5**),[10] sarothralin/japonicin C (**6**),[7,16] sarothralen A (**7**),[8] and saroaspidins A (**8**) and B (**9**),[8] were obtained.

Hyperjaponicol A (**1**) was obtained as a yellow gum. Its molecular formula $C_{32}H_{36}O_8$ was established by the ^{13}C NMR and HRESIMS data (*m*/*z* 547.2337 $[M - H]^-$, calcd 547.2332). The UV spectrum confirmed conjugated groups with maximum absorptions at 241 and 331 nm, while the FTIR spectrum showed absorption bands due to hydroxy (3427 cm^{-1}) and aromatic (1599 and 1447 cm^{-1}) functionalities. The ^{13}C and DEPT-NMR spectroscopic data (Table 1) showed a total of 32 carbon resonances, including a shielded sp^2 carbon at δ_C 108.3 (C-1) and three deshielded carbons at δ_C 188.1 (C-2), 200.5 (C-6), and 211.1 (C-7), and are indicative of the presence of an enol-β-triketo system.[12,15] The HMBC correlations from the *gem*-dimethyl protons at δ_H 1.50 (Me-13 and Me-14) to a quaternary carbon at δ_C 45.0 (C-5) and two oxygenated sp^2 carbons at δ_C 172.2 (C-4) and 200.5 (C-6) indicated the connectivity of C-4/C-5/C-6. The correlations of the methylene protons at δ_H 3.59 (H_2-12) with δ_C 112.1 (C-3), 188.1 (C-2), and C-4 suggested the connectivity of C-2/C-3/C-4 (Figure 1). A *sec*-butyl group linked to C-7 was indicated by the $^1H-^1H$ COSY correlations of Me-9/H-8/H_2-10/Me-11, together with the HMBC correlations of Me-9 (δ_H 1.15) with C-7. These fragments, in combination with the enol-β-triketo system, established a filicinic acid moiety of **1** with a C_5 side chain at C-1 and a methylene at C-3.

Besides the aforementioned 14 carbon signals, the remaining 18 resonances of **1**, assignable to a carbonyl at δ_C 200.3, 14 sp^2 carbons (including three oxygenated ones at δ_C 161.4, 161.9, and 164.7), an oxymethylene (δ_C 65.9), and two methyls (δ_C 18.0 and 25.6), indicated an acylphloroglucinol moiety with an

Received: January 4, 2018
Published: April 18, 2018

DOI: 10.1021/acs.jnatprod.8b00017
J. Nat. Prod. 2018, 81, 1098−1102

Table 1. ^{13}C NMR Data for Compounds 1−5 (δ in ppm, 150 MHz)

no.	1[a]	2[a]	3[a]	4[a]	5[a]	5[b]	sarothralen C[b]
1	108.3, C	108.3, C	108.3, C	107.8, C	107.9, C	107.1, C	107.2, C
2	188.1, C	188.1, C	188.2, C	188.1, C	188.1, C	187.3, C	187.4, C
3	112.1, C	112.4, C	112.2, C	112.2, C	112.0, C	111.2, C	111.3, C
4	172.2, C	172.1, C	172.3, C	172.3, C	172.3, C	171.7, C	171.8, C
5	45.0, C	45.0, C	44.9, C	44.9, C	44.9, C	44.3, C	44.4, C
6	200.5	200.4	200.4	200.3	199.1	199.5, C	199.5, C
7	211.1, C	211.1, C	211.1, C	211.5, C	211.2, C	210.8, C	210.8, C
8	43.7, CH	43.7, CH	43.7, CH	37.3, CH	37.2, CH	36.6, CH	36.6, CH
9	16.6, CH_3	16.6, CH_3	16.6, CH_3	19.6, CH_3	20.1, CH_3	19.3, CH_3	19.2, CH_3
10	27.4, CH_2	27.4, CH_2	27.4, CH_2	19.2, CH_3	19.4, CH_3	19.2, CH_3	19.2, CH_3
11	12.1, CH_3	12.1, CH_3	12.1, CH_3				
12	17.2, CH_2	17.8, CH_2	17.8, CH_2	17.8, CH_2	17.3, CH_2	16.9, CH_2	17.0, CH_2
13	25.1, CH_3	25.0, CH_3	25.1, CH_3	25.1, CH_3	25.1, CH_3	25.4, CH_3	25.6, CH_3
14	25.0, CH_3	25.0, CH_3	24.9, CH_3	24.9, CH_3	25.0, CH_3	24.3, CH_3	24.4, CH_3
1′	106.7, C	106.5, C	106.3, C	106.2, C	106.1, C	106.0, C	106.1, C
2′	161.9, C	161.4, C	162.1, C	162.0, C	161.7, C	161.9, C	162.0, C
3′	105.7, C	104.9, C	105.0, C	105.0, C	104.2, C	103.6, C	103.7, C
4′	161.4, C	161.2, C	161.8, C	161.7, C	156.0, C	154.7, C	154.6, C
5′	94.9, CH	108.8, C	107.4, C	107.4, C	102.2, C	100.2, C	100.1, C
6′	164.7, C	160.4, C	161.4, C	161.3, C	162.5, C	161.9, C	162.1, C
7′	200.3, C	212.1, C	212.2, C	212.3, C	211.6, C	210.8, C	210.8, C
8′	142.4, C	39.7, CH	39.7, CH	39.7, CH	39.7, CH	39.1, CH	39.1, CH
9′ (13′)	128.5, CH	19.7, CH_3	19.6, CH_3	19.7, CH_3	19.2, CH_3	19.7, CH_3	19.8, CH_3
10′ (12′)	128.4, CH	19.6, CH_3	19.6, CH_3	19.3, CH_3	19.2, CH_3	19.3, CH_3	19.3, CH_3
11′	131.5, CH						
14′	65.9, CH_2	22.3, CH_2	30.6, CH_2	30.6, CH_2	26.9, CH_2	26.1, CH_2	26.2, CH_2
15′	119.6, CH	123.1, CH	77.0, CH	77.0, CH	66.0, CH	66.6, CH	66.7, CH
16′	137.6, C	136.6, C	151.7, C	151.7, C	81.9, C	80.6, C	80.7, C
17′	25.6, CH_3	16.3, CH_3	109.2, CH_2	109.2, CH_2	18.6, CH_3	18.8, CH_3	18.9, CH_3
18′	18.0, CH_3	40.4, CH_2	32.8, CH_2	32.8, CH_2	38.6, CH_2	37.5, CH_2	37.6, CH_2
19′		27.2, CH_2	27.2, CH_2	27.2, CH_2	22.6, CH_2	22.1, CH_2	22.1, CH_2
20′		124.9, CH	124.9, CH	124.9, CH	124.9, CH	123.5, CH	123.6, CH
21′		131.7, C	132.1, C	132.1, C	132.1, C	132.4, C	132.4, C
22′		25.8, CH_3	25.8, CH_3	25.8, CH_3	25.8, CH_3	25.6, CH_3	25.7, CH_3
23′		17.7, CH_3	17.7, CH_3	17.7, CH_3	17.6, CH_3	17.6, CH_3	17.6, CH_3

[a]Recorded in acetone-d_6/0.1% TFA. [b]Recorded in $CDCl_3$.

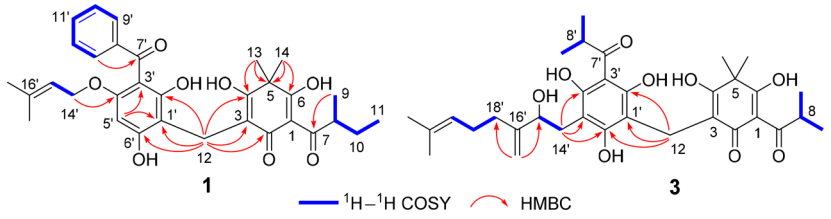


Figure 1. Key HMBC and ^{1}H–^{1}H COSY correlations of 1 and 3.

O-isoprenyl side chain. This assumption was confirmed by the HMBC correlations from H_2-12 to C-1′ (δ_C 106.7), C-2′ (δ_C 161.9), and C-6′ (δ_C 164.7), from H-5′ (δ_H 6.12) to C-3′ (δ_C 105.7) and C-1′, and from H-9′ and H-13′ (δ_H 7.52) of the monosubstituted benzene ring to C-7′ (δ_C 200.3). The *O*-isoprenyl group was assigned to C-4′ on the basis of the HMBC correlation of H_2-14′ (δ_H 4.31) with C-4′ (δ_C 161.4) (Figure 1). Thus, the structure of **1** was elucidated to be a homologue of sarothralin (**6**),[7,16] a known dimeric acylphloroglucinol with an isopropyl group at C-7.

Hyperjaponicol B (**2**) had the molecular formula $C_{34}H_{46}O_8$ as deduced by the ^{13}C NMR and HRESIMS data. Comparison of the 1D NMR spectroscopic data of **1** and **2** (Tables 1 and 2) revealed that the structure of **2** also possessed a filicinic acid moiety with a *sec*-butyl group at C-7. The signals of the acylphloroglucinol moiety with isobutyryl (δ_C 212.1, C-7′; 39.7, C-8′; δ_C 19.7, C-9′; and δ_C 19.6, C-10′) and geranyl (C-14′−C-23′) moieties at C-3′ and C-5′, respectively, were identical to those of sarothralen A (**7**). In the HMBC spectrum of **2**, the correlations from H_2-12 (δ_H 3.55) to C-1′ (δ_C 106.5), C-2′ (δ_C 161.4), and C-6′ (δ_C 160.4) and from H_2-14′ (δ_H 3.40) to C-4′ (δ_C 161.2), C-5′ (δ_C 108.8), and C-6′ confirmed the assignment. Hence, the structure of **2**, an analogue of sarothralen A (**7**),[8] was assigned as shown.

The molecular formula of hyperjaponicol C (**3**) was assigned as $C_{34}H_{46}O_9$ by the HRESIMS (*m*/*z* 597.3068, $[M - H]^-$) and

DOI: 10.1021/acs.jnatprod.8b00017
J. Nat. Prod. 2018, 81, 1098−1102

Table 2. ^{1}H NMR Data for Compounds 1−5 (600 MHz, δ in ppm, J in Hz)

no.	1[a]	2[a]	3[a]	4[a]	5[a]	5[b]	sarothralen C[b]
8	4.10, m	4.08, m	4.11, m	4.18, sept (6.6)	4.19, sept (6.6)	4.21, sept (6.6)	4.21, sept (6.7)
9	1.15, d (6.8)	1.15, d (6.6)	1.13, d (6.4)	1.15, d (6.6)	1.17, d (6.6)	1.18, d (6.6)	1.18, d (6.7)
10	1.78, m	1.75, overlap	1.76, m	1.15, d (6.6)	1.15, d (6.6)	1.18, d (6.6)	1.18, d (6.7)
	1.43, m	1.42, m	1.41, m				
11	0.92, t (7.4)	0.92, t (7.6)	0.91, t (6.8)				
12	3.59, s	3.55, s	3.54, s	3.54, s	3.55, s	3.56, s	3.57, s
13	1.50, s	1.50, s	1.48, s	1.49, s	1.48, s	1.44, s	1.48, s
14	1.50, s	1.50, s	1.49, s	1.48, s	1.48, s	1.54, s	1.55, s
5′	6.12, s						
8′		4.05, m	4.07, m	4.09, sept (6.6)	4.00, sept (6.6)	3.92, sept (6.7)	3.91, sept (6.7)
9′ (13′)	7.52, m	1.14, d (6.6)	1.15, d (6.6)	1.15, d (6.6)	1.15, d (6.6)	1.19, d (6.7)	1.19, d (6.7)
10′ (12′)	7.42, t (7.8)	1.14, d (6.6)	1.15, d (6.6)	1.15, d (6.6)	1.15, d (6.6)	1.19, d (6.7)	1.19, d (6.7)
11′	7.52, overlap						
14′	4.31, d (6.6)	3.40, d (6.6)	3.14, brd (14.8)	3.13, brd (14.8)	2.91, dd (16.5, 5.6)	2.94, dd (16.8, 5.2)	2.94, dd (17.1, 5.5)
			2.76, m	2.76, m	2.53, dd (16.5, 7.9)	2.64, dd (16.8, 6.2)	2.65, dd (17.1, 6.4)
15′	4.72, t (6.6)	5.05, t (6.6)	4.39, brd (8.0)	4.38, brd (8.2)	3.94, dd (7.9, 5.6)	3.93, m	3.94, brt
17′	1.56, s	1.76, s	5.12, brs	5.12, brs	1.32, s	1.37, s	1.38, s
			4.86, brs	4.86, brs			
18′	1.54, s	2.04, m	2.22, m	2.21, m	1.83, m	1.74, m	1.75, m
		1.95, m	2.16, m	2.14, m	1.73, m		
19′		2.04, overlap	2.22, overlap	2.21, overlap	2.20, m	2.12, m	2.13, m
		1.95, overlap	2.16, overlap	2.14, overlap			
20′		5.16, t (6.8)	5.15, t (6.2)	5.15, t (7.0)	5.12, t (6.6)	5.07, t (6.8)	5.08, t (6.7)
22′		1.58, s	1.65, s	1.65, s	1.64, s	1.67, s	1.67, s
23′		1.53, s	1.61, s	1.61, s	1.59, s	1.59, s	1.59, s
4-OH						10.09, s	10.08, s
6-OH						18.75, s	18.70, s
2′-OH						16.35, s	16.32, s
6′-OH						11.51, s	11.47, s

[a]Recorded in acetone-d_6/0.1% TFA. [b]Recorded in $CDCl_3$.

^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data of **3** (Tables 1 and 2) resembled those of **2**. Instead of an olefinic methine at δ_C 123.1 (C-15′) and a methyl at δ_C 16.3 (C-17′) in **2**, an oxymethine (δ_C 77.0) and an olefinic methylene (δ_C 109.2) were present in **3**, indicating the hydroxylation of C-15′ and formation of a $\Delta^{16'(17')}$ double bond. This was evidenced by the HMBC correlations from the terminal double-bond protons (H_2-17′, δ_H 4.86 and 5.12, brs) to C-15′ (δ_C 77.0) and C-18′ (32.8) of **3** (Figure 1). The relative configurations of C-8 and C-15′ for **3** remain to be determined. The structure of hyperjaponicol D (**4**) was determined as an analogue of **3**, with the *sec*-butyl group in **3** being replaced by an isopropyl group in **4**. Analysis of the 2D NMR data of **4** suggested that the other structural units of **3** and **4** are identical.

Since compounds **1**−**4** exhibited small optical rotation values, compounds **1** and **2** were examined by a CHIRALCEL OJ-RH column (Figure S1, Supporting Information). The results suggested that compounds **1**−**4** are scalemic mixtures. However, efforts of chiral-phase HPLC separation of these enantiomers failed.

Compound **5** was initially identified as sarothralen C,[10] a known dimeric acylphloroglucinol possessing a benzopyran moiety, by their highly similar ^{1}H and ^{13}C NMR data in $CDCl_3$ (Tables 1 and 2). In 1994, the authors claimed that they had observed an NOE correlation between H-15′ (δ_H 3.94) and Me-17′ (δ_H 1.38) and assigned a *trans* relationship between HO-15′ and Me-17′ in sarothralen C. However, the ^{1}H NMR signals of H-15′ and H-8′ (δ_H 3.91) in $CDCl_3$ nearly overlapped in the ^{1}H NMR spectrum, thus casting doubt on the earlier configurational determination. Therefore, the NMR spectra of **5** were recorded in acetone-d_6 (0.1% trifluoroacetic acid, TFA), and the signals of H-15′ (δ_H 3.94) and H-8′ (δ_H 4.00) were clearly separated. The NOE correlations of H-15′ with 14′a (δ_H 2.91) and of H-14′b (δ_H 2.53) with Me-17′ (δ_H 1.37) indicated the *cis* relationship of HO-15′ and Me-17′ (Figure 2). Subsequent X-ray diffraction study of **5** (CCDC 1814249) confirmed its relative configuration (Figure 3) and suggested that the structure of sarothralen C (**5**) should be reassigned as shown in Chart 1.

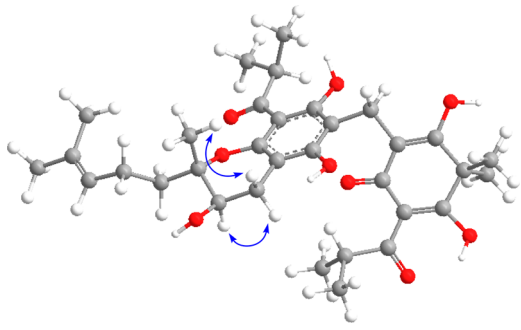


Figure 2. Key ROESY correlations of sarothralen C (**5**).

Interestingly, low-amplitude Cotton effects and the space group ($P\bar{1}$, $Z = 2$) of the crystal structure indicated that **5** might be a scalemic mixture. The subsequent chiral-phase HPLC resolution of **5** with a CHIRALCEL OJ-RH column afforded

DOI: 10.1021/acs.jnatprod.8b00017
J. Nat. Prod. 2018, 81, 1098−1102

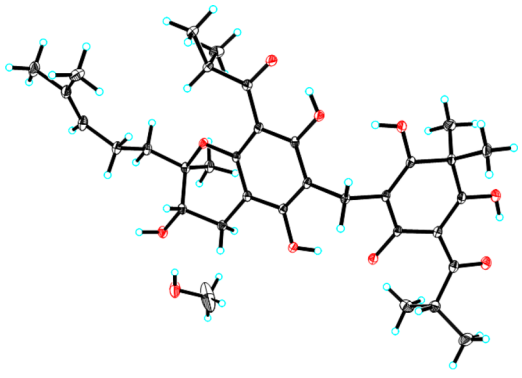
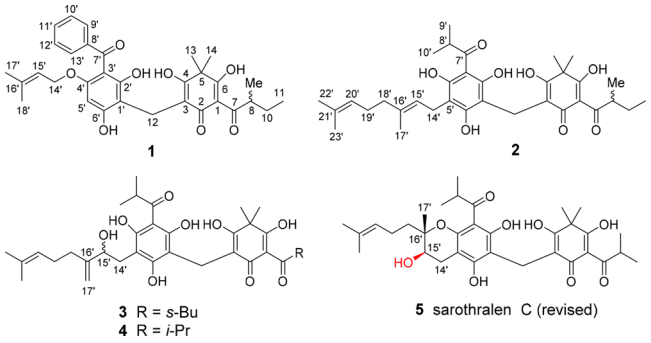


Figure 3. ORTEP drawing of sarothralen C (**5**).

Chart 1



enantiomers (+)-**5** and (−)-**5**, whose electronic circular dichroism (ECD) curves were mirror images. Efforts toward absolute configuration determination of (+)-**5** and (−)-**5** were undertaken using TDDFT ECD calculations at the B3LYP-SCRF/6-31+G(d,p)//B3LYP/6-31G(d) level in MeCN with the polarizable continuum model. However, these efforts failed to produce reconcilable experimental and calculated ECD curves. The failure of the simulation may be attributed to the flexibility of the molecule, which led to a large ensemble of conformations.

Since some dimeric acylphloroglucinols are reported to be potent antibiotics,[8−10,17] the inhibitory activity of compounds **1**−**7** was evaluated against four bacterial strains, *Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 6539, *Staphylococcus aureus* ATCC 25922, and *Enterococcus faecalis* ATCC 10541. Compounds **1**−**4**, **6**, and **7** exhibited significant antibacterial activity with MIC values of 0.8−3.4 μM, some of which showed stronger inhibitory activity than the positive control, cefotaxime sodium (Table 3). The MIC values are the same order of magnitude as those of known analogues.[10,17] Compound **5** was less active than compounds **2**−**4** and **7**, which suggested that 4′-OH or a long lipophilic chain at C-5′ might be important for the antibacterial activity of this class of acylphloroglucinols.

In addition, the inhibitory activity of the isolates against lipase was also evaluated in vitro, and compounds **2** and **3** exhibited moderate lipase inhibitory activity with IC_{50} values of 8.3 and 14.5 μM, respectively. This is the first report of lipase inhibitory activity of dimeric acylphloroglucinols.

EXPERIMENTAL SECTION

General Experimental Procedures. The general experiments were the same as the reported procedures with minor modification (General Experimental Procedures, Supporting Information).[18,19]

Table 3. MIC (μM) Values of Compounds 1−7 against Four Bacterial Strains

compound	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Enterococcus faecalis*
1	1.8	1.8	0.9	1.8
2	0.9	3.4	1.7	1.7
3	0.8	3.3	0.8	0.8
4	0.9	1.7	0.9	0.9
5	21.4	85.6	5.4	10.7
6	1.9	1.9	0.9	1.9
7	0.9	1.8	1.8	1.8
cefotaxime sodium[a]	0.4	3.3	3.3	0.4

[a]Positive control.

Plant Material. The whole plants were bought in Kunming, China, in March 2014. The materials were collected in Jingxi County, Guangxi Province, China. The plant species was identified by Dr. W. Fang, and a voucher specimen (no. 201403H01) has been deposited at the Kunming Institute of Botany, Kunming Institute of Botany.

Extraction and Isolation. The dried whole plants of *H. japonicum* (14.0 kg) were extracted with MeOH (2 × 16 L, rt), and the crude extract (2.5 kg) was subjected to silica gel column chromatography (CC) eluted with $CHCl_3$ to obtain an acylphloroglucinol-rich fraction (164.2 g).[12,15,18] This fraction was chromatographed over MCI gel CC ($MeOH/H_2O$, from 7:3 to 10:0) to afford four major fractions (A−E). Fraction B (25.5 g) was separated on silica gel CC with petroleum ether/acetone (from 1:0 to 10:1), to give four subfractions (B1−B4). Fraction B3 (3.0 g) and fraction B4 (4.6 g) were purified by preparative HPLC ($MeOH/H_2O$, 92:8, 0.1% v/v TFA) to yield compounds **1** (180 mg, t_R = 9.8 min), **3** (220 mg, t_R = 18.6 min), **4** (360 mg, t_R = 16.3 min), **5** (6 mg, t_R = 11.2 min), and **6** (540 mg, t_R = 8.5 min). Fr. C (19.3 g) was subjected to silica gel CC with petroleum ether/acetone (from 1:0 to 10:1), to obtain five fractions (C1−C5). Fraction C1 (3.3 g) was separated by preparative HPLC ($MeCN/H_2O$, 90:10, 0.1% v/v TFA) to yield **2** (1.0 g, t_R = 14.1 min) and **7** (2.1 g, t_R = 12.7 min). Similarly, compounds **8** (35 mg, t_R = 10.9 min) and **9** (54 mg, t_R = 12.2 min) were obtained from fraction D (29.6 g) by a combination of silica gel CC and semipreparative HPLC ($MeOH/H_2O$, 85:15, 0.1% v/v TFA).

*Hyperjaponicol A (**1**):* colorless gum; $[\alpha]^{20}_D$ −8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.39), 241 (4.11), 331 (3.89) nm; IR (KBr) ν_{max} 3427, 2968, 2930, 1620, 1599, 1447, 1383, 1323, 1287, 1189, 700, 612 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESI *m*/*z* 547 $[M - H]^-$; HRESIMS *m*/*z* 547.2337 $[M - H]^-$ (calcd for $C_{32}H_{35}O_8$, 547.2332).

*Hyperjaponicol B (**2**):* colorless gum; $[\alpha]^{20}_D$ −2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.45), 225 (4.32), 305 (4.27), 340 (4.11) nm; IR (KBr) ν_{max} 3428, 2973, 2931, 1615, 1471, 1434, 1385, 1243, 1192, 1140, 1038, 907 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 581 $[M - H]^-$; HRESIMS *m*/*z* 581.3117 $[M - H]^-$ (calcd for $C_{34}H_{45}O_8$, 581.3114).

*Hyperjaponicol C (**3**):* colorless gum; $[\alpha]^{20}_D$ −2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.41), 224 (4.32), 304 (4.26), 338 (4.11) nm; IR (KBr) ν_{max} 3428, 2973, 2931, 1613, 1474, 1436, 1384, 1243, 1194, 1139, 1038, 903 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; TOFMS *m*/*z* 597 $[M - H]^-$; HRESIMS *m*/*z* 597.3068 $[M - H]^-$ (calcd for $C_{34}H_{45}O_9$, 597.3064).

*Hyperjaponicol D (**4**):* colorless gum; $[\alpha]^{20}_D$ −8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.43), 225 (4.34), 304 (4.28), 339 (4.12) nm; IR (KBr) ν_{max} 3427, 2972, 2932, 1613, 1472, 1435, 1384, 1243, 1193, 1139, 1036, 909 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 583 $[M - H]^-$; HRESIMS *m*/*z* 583.2903 $[M - H]^-$ (calcd for $C_{33}H_{43}O_9$, 583.2907).

*Sarothralen C (**5**):* colorless needles ($MeOH/H_2O$); mp 127−128 °C; $[\alpha]^{20}_D$ +7 (*c* 0.1, MeOH) for (+)-**5**; $[\alpha]^{20}_D$ −9 (*c* 0.1, MeOH) for (−)-**5**; UV (MeOH) λ_{max} (log ε) 206 (4.43), 226 (4.33), 305 (4.25), 339 (4.10) nm; IR (KBr) ν_{max} 3435, 2978, 2939, 1616, 1478, 1435,

DOI: 10.1021/acs.jnatprod.8b00017
J. Nat. Prod. 2018, 81, 1098−1102

1384, 1242, 1196, 1142, 1041, 890 cm^{-1}; ECD (0.0006 M, MeCN) λ_{max} ($\Delta\varepsilon$) 220 (−2.1), 311 (+0.45) nm for (+)-**5**; CD (0.0008 M, MeCN) λ_{max} ($\Delta\varepsilon$) 219 (+1.9), 309 (−0.55) nm for (−)-**5**; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS m/z 583 $[M - H]^-$; HRESIMS m/z 583.2912 $[M - H]^-$ (calcd for $C_{33}H_{43}O_9$, 583.2907).

Crystallographic data of **5**: $C_{33}H_{44}O_9 \cdot CH_4O$, M = 616.72, a = 9.8858(2) Å, b = 12.0145(2) Å, c = 14.3049(2) Å, α = 89.4420(10)°, β = 76.0430(10)°, γ = 85.2500(10)°, V = 1643.12(5) Å^3, T = 100(2) K, space group $P\bar{1}$, Z = 2, μ(Cu Kα) = 0.746 mm^{-1}, 20 606 reflections measured, 5796 independent reflections (R_{int} = 0.0309). The final R_1 values were 0.0572 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1708 ($I > 2\sigma(I)$). The final R_1 values were 0.0580 (all data). The final $wR(F^2)$ values were 0.1719 (all data). The goodness of fit on F^2 was 1.046. Crystallographic data for **5** were deposited at the Cambridge Crystallographic Data Center as supplementary publication (deposit number CCDC 1814249). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk.

Antibacterial Assay. The antibacterial activity of compounds **1**−**7** against *E. coli* ATCC 11775, *S. typhimurium* ATCC 6539, *S. aureus* ATCC 25922, and *E. faecalis* ATCC 10541 was evaluated in a 24 h growth assay with minor modifications.[20] The final concentration range was 50−3.13 μg/mL for compound **5** but 2.0−0.05 μg/mL for compounds **1**−**4**, **6**, and **7**. Each experiment was repeated three times, and cefotaxime sodium was used as a positive control.

Lipase Inhibition Assay. For antilipase tests of the compounds, a porcine pancreatic lipase was used, and *p*-nitrophenyl butyrate (*p*-NPB) was used as a substrate. A 5 μL amount of the lipase solution (40 U/mL) in Tris-HCl buffer (100 mM Tris-HCl, 5 mM $CaCl_2$; pH 7.0) was added in a 96-well microtiter plate. A 1 μL sample of each compound in DMSO and 184 μL of the same Tris-HCl buffer were added and mixed with the enzyme-buffer to start the reaction. After incubation at 37 °C for 15 min, 10 μL of the substrate solution (10 mM *p*-NPB in DMF) was added. Enzymatic reactions were carried out for 15 min at 37 °C. The hydrolysis of *p*-NPB to *p*-nitrophenol was monitored at 400 nm using a spectrophotometer. The IC_{50} value of each compound was calculated by Reed and Muench's method.[21] Each experiment was repeated three times, and the known lipase inhibitor orlistat was used as a positive control (IC_{50} 0.004 μM).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00017.

General experimental procedures, original MS and NMR spectra of **1**−**5**, ECD calculations (PDF)

Crystallographic file for **5** (CIF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: yangxingwei@mail.kib.ac.cn.

*E-mail: xugang008@mail.kib.ac.cn.

ORCID

Xing-Wei Yang: 0000-0002-9578-2986

Gang Xu: 0000-0001-7561-104X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The work was financially supported by the Natural Sciences Foundation of Yunnan Province (Nos. 2015FA032, 2016FB017), the foundation from Youth Innovation Promotion Association CAS to X.W.Y.(No. 2016350), the West Light Foundation of the Chinese Academy of Sciences to X.W.Y., and foundations from Kunming Institute of Botany (KIB2017001) and Southeast Asia Biodiversity Research Institute (2017CAS-SEABRIQG003), Chinese Academy of Sciences.

■ REFERENCES

(1) Ccana-Ccapatinta, G. V.; Correa de Barros, F. M.; Bridi, H.; Von Poser, G. L. *Phytochem. Rev.* **2015**, *14*, 25−50.

(2) (a) Ciochina, R.; Grossman, R. B. *Chem. Rev.* **2006**, *106*, 3963−3986. Yang, X. W.; Grossman, R. B.; Xu, G. *Chem. Rev.* 10.1021/acs.chemrev.7b00551.

(3) Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558−591.

(4) Bridi, H.; Ccana-Ccapatinta, G. V.; Stolz, E. D.; Meirelles, G. C.; Bordignon, S. A. L.; Rates, S. M. K.; Von Poser, G. L. *Phytochemistry* **2016**, *122*, 178−183.

(5) Ccana-Ccapatinta, G. V.; Stolz, E. D.; Da Costa, P. F.; Rates, S. M. K.; Von Poser, G. L. J. *J. Nat. Prod.* **2014**, *77*, 2321−2325.

(6) Li, Y. H. In *Flora of China*; Wu, Z. Y., Ed.; Science Press: Beijing, 1990; Vol. *50*, pp 47−48.

(7) Ishiguro, K.; Yamaki, M.; Takagi, S.; Yamagata, Y.; Tomita, K. *J. Chem. Soc., Chem. Commun.* **1985**, 26−27.

(8) Ishiguro, K.; Yamaki, M.; Kashihara, M.; Takagi, S. *Planta Med.* **1986**, *52*, 288−290.

(9) Ishiguro, K.; Yamaki, M.; Kashihara, M.; Takagi, S.; Isoi, K. *Planta Med.* **1990**, *56*, 274−276.

(10) Ishiguro, K.; Nagata, S.; Fukumoto, H.; Yamaki, M.; Isoi, K. *Phytochemistry* **1994**, *35*, 469−471.

(11) Liu, L. S.; Liu, M. H.; He, J. Y. *Molecules* **2014**, *19*, 10733−10754.

(12) Yang, X. W.; Li, Y. P.; Su, J.; Ma, W. G.; Xu, G. *Org. Lett.* **2016**, *18*, 1876−1879.

(13) Hu, L.; Zhang, Y.; Zhu, H.; Liu, J.; Li, H.; Li, X. N.; Sun, W.; Zeng, J.; Xue, Y.; Zhang, Y. *Org. Lett.* **2016**, *18*, 2272−2275.

(14) Hu, L.; Xue, Y.; Zhang, J.; Zhu, H.; Chen, C.; Li, X. N.; Liu, J.; Wang, Z.; Zhang, Y.; Zhang, Y. *J. Nat. Prod.* **2016**, *79*, 1322−1328.

(15) Li, Y. P.; Yang, X. W.; Xia, F.; Yan, H.; Ma, W. G.; Xu, G. *Tetrahedron Lett.* **2016**, *57*, 5868−5871.

(16) Gu, G. M.; Feng, S. Z.; Wang, X. Y. *Acta Chim. Sin.* **1988**, *46*, 246−251.

(17) (a) Rocha, L.; Marston, A.; Potterat, O.; Kaplan, M. A. C.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1995**, *40*, 1447−1452. (b) Rocha, L.; Marston, A.; Potterat, O.; Kaplan, M. A. C.; Hostettmann, K. *Phytochemistry* **1996**, *42*, 185−188.

(18) Yang, X. W.; Li, M. M.; Liu, X.; Ferreira, D.; Ding, Y.; Zhang, J. J.; Liao, Y.; Qin, H. B.; Xu, G. *J. Nat. Prod.* **2015**, *78*, 885−895.

(19) Yang, X. W.; Yang, J.; Xu, G. *J. Nat. Prod.* **2017**, *80*, 108−113.

(20) Yang, X. W.; Luo, X. D.; Lunga, P. K.; Zhao, Y. L.; Qin, X. J.; Chen, Y. Y.; Liu, L.; Li, X. N.; Liu, Y. P. *Tetrahedron* **2015**, *71*, 3694−3698.

(21) Kim, J. H.; Kim, H. J.; Park, H. W.; Youn, S. H.; Choi, D.-Y.; Shin, C. S. *FEMS Microbiol. Lett.* **2007**, *276*, 93−98.

DOI: 10.1021/acs.jnatprod.8b00017
J. Nat. Prod. 2018, 81, 1098−1102