JOURNAL OF NATURAL PRODUCTS

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Antimicrobial Constituents of the Mature Carpels of *Manglietiastrum sinicum*

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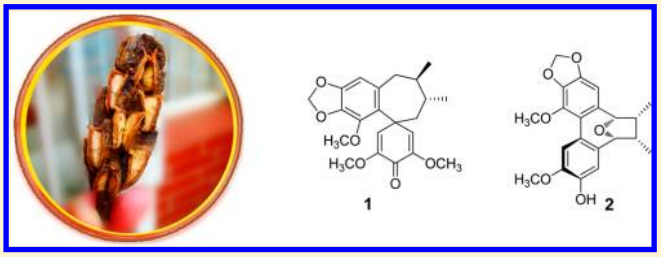
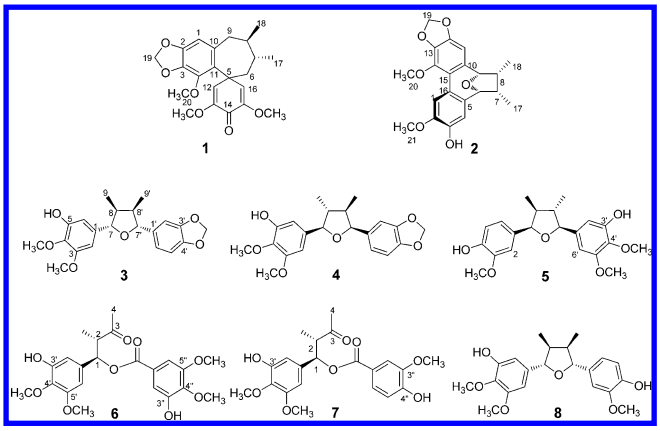
Supporting Information

ABSTRACT: Seven new compounds, including a eupodienone-type lignan (**1**), a dibenzocyclooctadiene-type lignan (**2**), three tetrahydrofuran-type lignans (**3**−**5**), and two 1-phenylbutyl benzoates (**6**, **7**), together with six known compounds, were isolated from the mature carpels of *Manglietiastrum sinicum*. The structures of new compounds **1**−**7** were defined by spectroscopic techniques, and the absolute configuration of manglisin A (**1**) was determined by X-ray crystallography. Compounds **1**−**4** exhibited moderate antimicrobial activities (MIC values: 0.016−0.14 μM) against *Staphylococcus aureus*, MRSA 82[#], MRSA 92[#], MRSA 98[#], and MRSA 331[#]. Compounds **2** and **3** showed weak cytotoxic activity against five human tumor cell lines.



Manglietiastrum sinicum Law ("hua-gai-mu" in Chinese), a rare and endangered tree found only in the southeast of Yunnan Province, is one of the oldest single-species-genera plants in the Magnoliaceae family.[1] The leaves and fruits of the plant have been used to treat rhinitis and indigestion in Chinese traditional medicine.[2] There has been little investigation into its chemical constituents. Only 14 compounds have been isolated from this species, of which the lignans exhibited significant inhibition against platelet aggregation induced by arachidonic acid (AA), adenosine diphosphate (ADP), and platelet-activating factor (PAF).[2] In the current study, seven new compounds, named manglisins A−G (**1**−**7**), as well as six known compounds, schinlignin B (**8**),[3] clemaphenol A (**9**),[4] (7*R*,8*R*)-4-hydroxy-3-methoxy-1′,2′,3′,4′,5′,6′,7′-heptanorlign-8′-one (**10**),[5] 7-methylesculeti (**11**),[6] 3-ketooleanolic acid (**12**),[7] and 7-oxositosterol (**13**),[8] were isolated from the mature carpels of *M. sinicum*. Herein, we report the isolation, structural identification, and biological properties of these new compounds, including their effects on bacteria, human cancer cell lines, and platelet aggregation.



RESULTS AND DISCUSSION

Manglisin A (**1**) was obtained as a colorless crystal, whose molecular formula was determined as $C_{22}H_{26}O_6$ by ^{13}C NMR spectroscopic data and an HREIMS ion at m/z 386.1739 $[M]^+$ (calcd for $C_{22}H_{26}O_6$, 386.1729), thus requiring 10 indices of hydrogen deficiency. A conjugated carbonyl and an aromatic group were evident from the IR absorption bands at 1664, 1614, and 1449 cm^{-1}, respectively. The ^1H NMR data (Table 1) indicated the presence of an aromatic proton (δ_H 6.34, s), two mutually coupled olefinic protons (δ_H 5.92, d, J = 1.8 Hz, and 6.21, d, J = 1.8 Hz), three methoxy groups (δ_H 3.59, 3.63, and 3.66, each s), one methylenedioxy group (δ_H 5.88, 2H, d, J = 8.8 Hz), and two methyl doublets (δ_H 0.96, d, J = 6.8 Hz, and 0.99, d, J = 6.7 Hz). In addition, the ^{13}C NMR and DEPT data (Table 1) revealed 22 carbon resonances, comprising five methyl (three *O*-methyl groups), five methine, three methylene, and nine quaternary carbons (three oxygenated carbons and one carbonyl carbon). The aforementioned information, combined with the characteristic spiro carbon resonance (δ_C 46.9), indicated that **1** was a eupodienone-type lignan.[9] Analysis of the 1D and 2D NMR data suggested a high similarity between **1** and eupodienone-5,[9] except for the absence of an acetoxy group at C-9 in **1**. In addition to the chemical shifts,

Received: February 27, 2014
Published: August 12, 2014

dx.doi.org/10.1021/np500187c | *J. Nat. Prod.* 2014, 77, 1800−1805

Table 1. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR Data of Compounds 1 and 2 in $CDCl_3$ (δ in ppm, J in Hz)

	1		2	
no.	δ_C	δ_H	δ_C	δ_H
1	107.4, d	6.34, s	117.7, d	7.48, s
2	147.5, s		145.6, s	
3	137.0, s		146.0, s	
4	143.7, s		113.6, d	6.64, s
5	46.9, s		137.7, s	
6α	47.6, t	2.64, dd (14.6, 9.6)	90.1, d	4.74, br s
6β		1.21,d (14.6)		
7	34.8, d	1.44, m	50.4, d	2.27, m
8	39.4, d	1.59, m	42.4, d	2.39, m
9α	40.2, t	3.48, dd (14.3, 5.6)	90.6, d	4.49, d (5.3)
9β		2.28, d (14.3)		
10	131.4, s		140.6, s	
11	123.2, s		104.0, d	6.53, s
12	122.8, d	5.92, d (1.8)	147.6, s	
13	149.2, s		139.6, s	
14	176.5, s		143.7, s	
15	149.1, s		124.6, s	
16	126.1, d	6.21, d (1.8)	124.2, s	
17	21.8, q	0.96, d (6.8)	14.0, q	1.04, d (6.4)
18	19.0, q	0.99, d (6.7)	13.7, q	1.02, d (6.8)
19	101.1, t	5.88, d (8.8)	102.3, t	5.99, 6.05, s
20	59.3, q	3.59, s	60.2, q	3.69, s
21	55.1, q	3.63, s	56.3, q	3.82, s
22	55.1, q	3.66, s		

HMBC correlations of H-1 (δ_H 6.34) and H_3-18 (δ_H 0.99) with C-9 (δ_C 40.2) and the ^{1}H−^{1}H COSY cross-peaks between H_2-9 (δ_H 3.48, 2.28) and H-8 (δ_H 1.59) further confirmed the general structure (Figure 1). In addition, three *O*-methyl

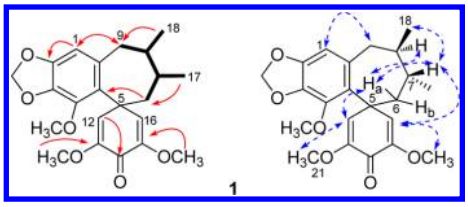


Figure 1. Key ^{1}H−^{1}H COSY (— in bold), HMBC (→ in red), and ROESY (↔ in blue) correlations of 1.

groups were located at C-4 (δ_C 143.7), C-13 (δ_C 149.2), and C-15 (δ_C 149.1) based on the HMBC correlations from H_3-20 (δ_H 3.59), H_3-21 (δ_H 3.63), and H_3-22 (δ_H 3.66) to the corresponding carbons. Thus, the planar structure of 1 was assigned as shown.

The relative configuration of 1 was difficult to assign due to the paucity of ROESY correlations. However, a single crystal of 1 was obtained; thus, the absolute configuration of 1 was assigned as 7*R*, 8*R* on the basis of X-ray diffraction using the Flack parameter [0.06(9)][10] and Cu Kα radiation (Figure 2).

Manglisin B (2) was obtained as a colorless oil, displaying the molecular formula $C_{21}H_{22}O_6$ by ^{13}C NMR data and the HREIMS ion at m/z 370.1407 (calcd for 370.1416). The ^{1}H and ^{13}C NMR data (Table 1) exhibited the presence of two benzene rings, two methyl doublets, two methoxy groups, and one methylenedioxy group. The 1D NMR data of 2 were similar to those of gymnothelignan L,[11] a dibenzocyclooctadiene-type lignan,[12] with the exception of the absence of the C-

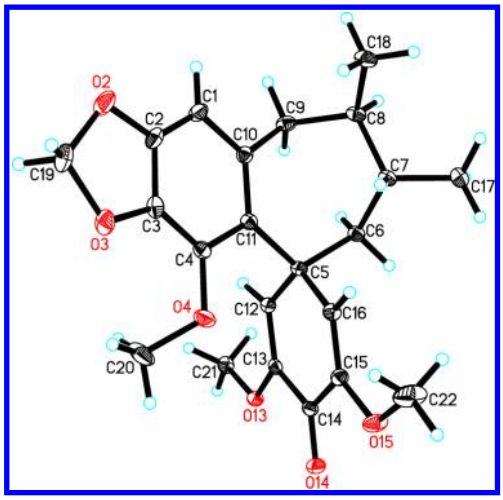


Figure 2. X-ray ORTEP drawing of 1.

4 methoxy group. This general structure was further confirmed by the HMBC correlations from H_3-20 (δ_H 3.69, s) to C-14 (δ_C 143.7), H_3-21 (δ_H 3.82, s) to C-2 (δ_C 145.6), and H-4 (δ_H 6.64, s) to C-6 (δ_C 90.1) (Figure 3). The ROESY cross-peaks of H-

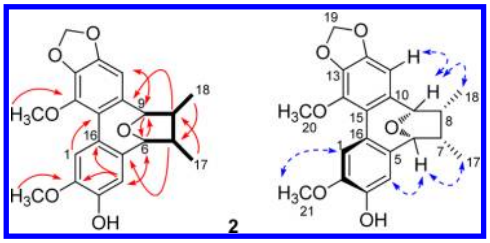


Figure 3. Key ^{1}H−^{1}H COSY (— in bold), HMBC (→ in red), and ROESY (↔ in blue) correlations of 2.

6/H_3-17 (δ_H 1.04) and H-9/H_3-18 (δ_H 1.02) as well as the coupling constants ($J_{8,9}$ = 5.3 Hz and $J_{6,7}$ = 0 Hz) indicated *cis*-7,8-dimethyl substitution.[13] Sequential positive and negative Cotton effects at approximately 240 and 220 nm evident in the electronic circular dichroism (ECD) spectrum indicated that 2 possessed *P*-helicity and an *S*-biphenyl configuration.[14] Thus, the absolute configuration of 2 was unambiguously established as 6*S*,7*S*,8*R*,9*R* and *S*-biphenyl.

Manglisin C (3), a colorless oil, gave the molecular formula $C_{21}H_{24}O_6$ in terms of ^{13}C NMR data and the molecular ion peak $[M]^+$ at m/z 372.1578 by HREIMS. Analysis of the 1D NMR data (Tables 2 and 3) suggested that 3 possessed a tetrahydrofuran moiety similar to schinlignin B (8).[3] The presence of a methylenedioxy group (δ_H 5.90, 2H, s; δ_C 100.9) instead of the 3′-OMe and 4′-OH groups in the latter was the main difference. The HMBC correlations of the protons at δ_H 5.90 (OCH_2O) with C-3′ (δ_C 147.7) and C-4′ (δ_C 147.0) as well as the ^{1}H−^{1}H COSY cross-peaks of H-5′/H-6′ confirmed that the methylenedioxy group was associated with C-3′ and C-4′ (Figure 4). The coupling constants of H-7 with H-8 (J = 7.0 Hz) and H-7′ with H-8′ (J = 7.0 Hz) implied the 7,8-*trans*-8,8′-*cis*-7′,8′-*trans* configuration for 3, which was consistent with those of schinlignin B and machilin-G.[15] The observed ROESY correlations of H-7/H_3-9 and H-7′/H_3-9′ further confirmed this configuration. Therefore, the structure of manglisin (3) was established as depicted.

dx.doi.org/10.1021/np500187c | J. Nat. Prod. 2014, 77, 1800−1805

Table 2. ^{1}H NMR Data of Compounds 3−5 and 8 in $CDCl_3$ (δ in ppm, J in Hz)

no.	3[a]	4[b]	5[b]	8[c]
2	6.59, d (1.6)	6.58, d (1.8)	6.87, d (1.8)	6.57, d (1.9)
5			6.89, d (8.4)	
6	6.67, d (1.6)	6.65, d (1.8)	6.82, dd (1.8, 8.4)	6.70, d (1.6)
7	4.43, d (7.0)	4.26, d (9.1)	5.11, d (8.4)	4.50, d (6.9)
8	2.29, m	1.68, m	2.23, m	2.25−2.34, m
9	1.04, d (6.8)	0.99, d (6.6)	0.65, d (6.6)	1.06, d (6.7)
2′	6.97, d (1.4)	6.80, br s	6.65, d (1.7)	6.98, s
5′	6.79, d (7.9)	6.71,d (8.4)		6.90, s
6′	6.88, dd (7.9, 1.4)	6.73, d (8.4)	6.77, d (1.7)	6.90, s
7′	4.44, d (7.0)	5.02, d (9.1)	4.36, d (9.0)	4.48, d (6.2)
8′	2.25, m	2.14, m	1.78, m	2.25−2.34, m
9′	0.99, d (6.8)	0.59, d (7.2)	1.08, d (6.6)	1.01, d (6.6)
3-$OCHH_3$	3.87, s	3.83, s	3.88, s	3.88, s
4-$OCHH_3$	3.88, s	3.83, s		3.88, s
3′-$OCHH_3$			3.88, s	3.85, s
4′-$OCHH_3$			3.90, s	
OCH_2O	5.95, s	5.89, s		

[a]Recorded at 500 MHz. [b]Recorded at 600 MHz. [c]Recorded at 400 MHz.

Table 3. ^{13}C NMR Data of Compounds 3−5 and 8 in $CDCl_3$ (δ in ppm)

no.	3[a]	4[b]	5[a]	8[a]
1	138.4, s	137.2, s	133.1, s	138.7, s
2	102.2, d	102.6, d	109.6, d	102.3, d
3	152.2, s	152.5, s	146.2, s	152.2, s
4	134.7, s	135.0, s	144.5, s	134.7, s
5	149.1, s	149.3, s	113.8, d	149.1, s
6	105.8, d	106.2, d	119.8, d	105.8, d
7	87.3, d	87.5, d	83.2, d	87.3, d
8	44.5, d	48.4, d	45.9, d	44.4, d
9	13.2, q	15.3, q	14.9, q	13.2, q
1′	135.8, s	135.2, s	137.2, s	133.8, s
2′	106.8, d	107.8, d	102.7, d	109.1, d
3′	147.7, s	146.7, s	152.3, s	145.0, s
4′	147.0, s	147.6, s	134.8, s	146.5, s
5′	108.0, d	108.0, d	149.2, s	114.1, d
6′	120.0, d	120.4, d	105.8, d	119.3, d
7′	87.3, d	83.3, d	87.2, d	87.2, d
8′	44.4, d	46.1 d	47.8, d	44.1, d
9′	12.6, q	15.3, q	15.1, q	12.6, q
3-$OCHH_3$	55.8, q	56.0, q	55.84, q	55.82, q
4-$OCHH_3$	60.9, q	61.2, q		60.9, q
3′-$OCHH_3$			55.79, q	55.76, q
4′-$OCHH_3$			60.9, q	
OCH_2O	100.9, t	101.1, t		

[a]Recorded at 100 MHz. [b]Recorded at 150 MHz.

Manglisin D (**4**), a colorless oil, had the same molecular formula as **3** by positive HREIMS and ^{13}C NMR spectroscopic data. The NMR data (Tables 2 and 3) indicated that **4** was a diastereoisomer of **3**. The ROESY (Figure S9, Supporting Information) correlations of H_3-9 (δ_H 0.99) with H-8′ (δ_H 2.14) and H-7 (δ_H 4.26), of H_3-9′ (δ_H 0.59) with H-8 (δ_H 1.68), and of H-7 with H-7′ and H-8′ indicated the 7,8-*trans*, 7′,8′-*cis*-8,8′-*trans* configuration of the tetrahydrofuran ring. The coupling constants of H-7/H-8 (J = 9.1 Hz), H-7′/H-8′ (J = 9.1 Hz), H_3-9/H-8 (J = 6.6 Hz), and H_3-9′/H-8′ (J = 7.2 Hz) confirmed the *rel*-(7*R*,8*R*,7′*S*,8′*R*)-configuration, similar to the configuration of futokadsurin B.[16] Therefore, the structure of compound **4** was defined as *rel*-(7*R*,8*R*,7′*S*,8′*R*)-3,4-dimethoxy-5-hydroxy-3′,4′-methylenedioxy-7,7′-epoxylignan.

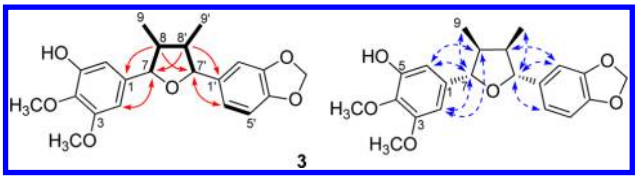


Figure 4. Key ^{1}H−^{1}H COSY (— in bold), HMBC (→ in red), and ROESY (↔ in blue) correlations of **3**.

Manglisin E (**5**) possessed the molecular formula $C_{21}H_{26}O_6$ from its HREIMS and ^{13}C NMR spectroscopic data. The 1D NMR data (Tables 2 and 3) resembled those of **8**. The connections established by the ^{1}H−^{1}H COSY and HMBC spectra (Figure S9, Supporting Information) indicated the same planar structure as schinlignin B (**8**).[3] The relative configuration of **5** was deduced to be 7,8-*cis*-8,8′-*trans*-7′,8′-*trans* on the basis of the coupling constants ($J_{7,8}$ = 8.4 and $J_{7',8'}$ = 9.0 Hz) observed for the benzylic protons (δ_H 5.11, H-7 and 4.36, H-7′, respectively) and the chemical shifts of C-7 (δ_C 83.2), C-8 (δ_C 45.9), C-7′ (δ_C 87.2), and C-8′ (δ_C 47.2), which was similar to those of *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4′-hydroxy-3,4,5,3′,5′-pentamethoxy-7,7′-epoxylignan.[17] The specific rotation of **5** showed a negative value ($[\alpha]_D^{23}$ −6) consistent with that of *rel*-(7*R*,8*S*,7′*S*,8′*S*)-4′-hydroxy-3,4,5,3′,5′-pentamethoxy-7,7′-epoxylignan ($[\alpha]_D^{21}$ −31). Therefore, the structure of manglisin E (**5**) was elucidated as *rel*-(7*R*,8*S*,7′*S*,8′*S*)-3,3′,4′-trimethoxy-4,5′-dihydroxy-7,7′-epoxylignan.

Manglisin F (**6**) was determined to possess the molecular formula $C_{22}H_{26}O_9$ by ^{13}C NMR data and positive HREIMS. Its UV spectrum suggested the presence of a benzenoid moiety based upon the absorption maxima at 265 and 207 nm.[18] The ^{1}H and ^{13}C NMR data (Table 4) were similar to those of meicocarpin.[19] The differences between the compounds were the presence of two hydroxy groups rather than two methoxy groups at C-3′ and C-3″ of meicocarpin, which was confirmed by the HMBC and ROESY data (Figure 5). In addition, the methyl (δ_H 0.96, d, J = 7.2 Hz, CH_3-2), acetyl (δ_H 2.29, s, H_3-4), methine (δ_H 3.39, dq, J = 10.2, 7.2 Hz, H-2), and benzylic oxymethine (δ_H 5.87, d, J = 10.2 Hz, H-1) protons were connected according to the ^{1}H−^{1}H COSY and HMBC data. Additionally, the ROESY correlation between H-1 and CH_3-2 and the coupling constants ($J_{1,2}$ = 10.2 Hz) indicated the 1,2-*trans*-configuration. Furthermore, compound **6** showed a positive Cotton effect at λ 272 nm ($\Delta\varepsilon$ +3.40) in the ECD spectrum, demonstrating the 1*R*-configuration.[20] On the basis of the above results, the structure of compound **6** was defined as (1*R*,2*S*)-1-(3-hydroxy-4,5-dimethoxyphenyl)-2-methyl-3-oxobutyl 3-hydroxy-4,5-dimethoxybenzoate.

Manglisin G (**7**) had the molecular formula $C_{21}H_{24}O_8$, as established by HREIMS and ^{13}C NMR spectroscopic data, which was 30 mass units greater than **6**. Comparison of the ^{1}H

dx.doi.org/10.1021/np500187c | J. Nat. Prod. 2014, 77, 1800−1805

Table 4. ^{1}H and ^{13}C NMR Data of Compounds 6 and 7 in Acetone-d_6 (δ in ppm, J in Hz)

	6[a]		7[b]	
no.	δ_C	δ_H	δ_C	δ_H
1	78.9, d	5.87, d (10.2)	78.7, d	5.86, d (10.0)
2	52.0, d	3.39, dq (10.2, 7.2)	52.1, d	3.33, dq (10.0, 7.0)
3	209.9, s		209.9, s	
4	30.6, q	2.29, s	29.7, q	2.24, s
1′	135.4, s		135.6, s	
2′	108.5, d	6.70, d (1.8)	108.5, d	6.66, d (1.8)
3′	151.1, s		151.2, s	
4′	137.0, s		137.0, s	
5′	154.0, s		154.0, s	
6′	103.9, d	6.73, d (1.8)	103.9, d	6.69, d (1.8)
1″	126.3, s		122.5, s	
2″	111.1, d	7.25, d (1.8)	113.2, d	7.52, d (1.5)
3″	151.2, s		148.1, s	
4″	141.4, s		152.2, s	
5″	153.9, s		115.6, d	6.89, d (8.3)
6″	105.7, d	7.17, d (1.8)	124.5, d	7.59, dd (8.3, 1.5)
RCOO′	165.0, s		165.2, s	
4′-OCHH_3	60.6, q	3.77, s	60.6, q	3.73, s
5′-OCHH_3	56.21, q	3.88, s	56.2, q	3.84, s
3″-OCHH_3			56.2, q	3.87, s
4″-OCHH_3	60.7, q	3.84, s		
2-CH_3	13.8, q	0.96, d (7.2)	13.9, q	0.93, d (7.0)

[a] ^{1}H and ^{13}C NMR data recorded at 600 and 150 MHz, respectively. [b] ^{1}H and ^{13}C NMR data recorded at 500 and 100 MHz, respectively.

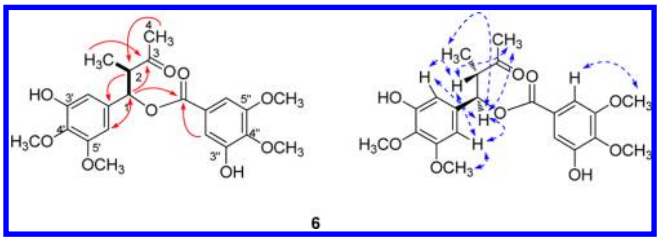


Figure 5. Key ^{1}H–^{1}H COSY (— in bold), HMBC (→ in red), and ROESY (↔ in blue) correlations of 6.

and ^{13}C NMR data (Table 4) with those of 6 indicated that 7 is a 4-hydroxy-3-methoxybenzoate. The ^{1}H–^{1}H COSY correlation between H-5″ (δ_H 6.89) and H-6″ (δ_H 7.59) and the HMBC correlation of the methoxy group (δ_H 3.87) to C-3″ (δ_C 148.1) indicated that the hydroxy group was located at C-4″ (δ_C 152.2) (Figure S9, Supporting Information). The ECD spectrum of 7 was the same as that of 6, with a positive Cotton effect at approximately 270 nm. This implied that they shared the same absolute configuration. As a consequence, the structure of 7 was defined as (1*R*,2*S*)-1-(3-hydroxy-4,5-dimethoxyphenyl)-2-methyl-3-oxobutyl 4-hydroxy-3-methoxybenzoate.

The new compounds were evaluated for their antimicrobial activity. Compounds 1–4 exhibited inhibitory activities against *Staph. aureus*, MRSA 82#, MRSA 92#, MRSA98#, and MRSA 331# with MIC values of 0.016–0.14 μM (Table 5).

Additionally, all new isolates were tested for their cytotoxicity against the human cancer cell lines HL-60 (leukemia), SMMC-7721 (hepatocellular carcinoma), A-549 (lung adenocarcinoma), MCF-7 (breast cancer), and SW480 (colon adenocarcinoma) using the MTT assay.[21] Compound 2 exhibited slightly selective cytotoxic activity, with IC_{50} values of 17.04 and 38.24 μM against HL-60 and SMMC-7721, respectively, while compound 3 showed weak cytotoxic activity against all the tested human cancer cell lines (IC_{50} values: 14.02–39.75 μM) (Table 6).

Table 5. Antimicrobial Activities of Compounds 1–5

	antimicrobial activities (MIC in μM)				
compound[a]	*Staph. aureus*	MASA 82	MRSA 92	MASA 98	MASA 331
1	0.13	0.065	0.065	0.016	0.032
2	0.068	0.14	0.068	0.034	0.14
3	0.067	0.067	0.067	0.034	0.13
4	0.067	0.067	0.067	0.067	0.034
5	0.13	0.067	0.13	0.13	>0.13
positive control[b]	0.0011	0.00054	0.00054	0.00054	0.00054

[a] Results of compounds 6 and 7 against the bacteria (a diameter of inhibition smaller than 10 mm in preliminary screening) were not listed. [b] Vancomycin hydrochloride as positive control.

Table 6. IC_{50} Values (μM) of Compounds 2 and 3 for Human Tumor Cell Lines

compound[a]	HL-60	SMMC-7721	A-549	MCF-7	SW-480
2	17.04	38.24	>40	>40	>40
3	39.75	>40	27.41	14.02	19.06
cisplatin[b]	3.08	10.20	9.08	17.48	11.99

[a] Results of compounds 1 and 4–7 against the five human tumor cell lines (IC_{50} > 40 μM) are not listed. [b] Cisplatin as positive control.

The inhibitory effect against platelet aggregation induced by AA, ADP, and PAF of compounds 1–7 was tested at 200 μg/mL (Table S1, Supporting Information). However, none of the compounds showed inhibitory activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained on a Jasco P-1020 polarimeter. UV spectroscopic data were measured on a Shimadzu-2401PC spectrophotometer. An Applied Photophysics Chirascan spectrometer was used for recording ECD spectra. IR spectra were obtained on a Bruker-Tensor-27 spectrometer with KBr pellets. 1D and 2D NMR spectra were acquired on a Bruker AM-400, a DRX-500, or an Avance III-600 spectrometer (Karlsruhe, Germany) with TMS as an internal standard. Mass spectra were recorded on a Waters HPLC-Thermo Finnigan LCQ Advantage ion trap mass spectrometer (Milford, PA, USA). HPLC preparation was performed on an Agilent 1100 series instrument equipped with a quaternary pump, a diode array detector, and an X-bridge column (10 × 250 mm). Column chromatography (CC) was performed with silica gel H (10–40 μm), silica gel G (100–200 mesh, 200–300 mesh, 300–400 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), or LiChroprep RP-18 gel (40–63 μm, Merck). Thin-layer chromatography was conducted on GF 254 silica gel plates (Qingdao Haiyang Chemical Co., Qingdao, China), and spots were visualized by heating the silica gel plates after being sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The mature carpels of *M. sinicum* were obtained from the Forestry Department of Yunnan Province, People's Republic of China, in August 2005, and were identified by Professor Gong Xun (Kunming Institute of Botany). A voucher specimen (H20050825) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

dx.doi.org/10.1021/np500187c | J. Nat. Prod. 2014, 77, 1800–1805

Extraction and Isolation. The air-dried and powdered carpels of *M. sinicum* (1.3 kg) were extracted with MeOH at room temperature (four times, 4 days each time). The combined organic layers were evaporated under reduced pressure to give a crude residue. The residue (80 g, 6.2%) was applied to silica gel chromatography, and 10 fractions (A–J) eluted with petroleum ether/EtOAc (1:0–0:1 gradient system) were collected.

Fraction F (3.35 g), showing a positive reaction to Dragendorff's reagent, was chromatographed on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, 3 × 120 cm) to afford five fractions (F1–F5). Fraction F2 (2.31 g) was chromatographed on silica gel (300–400 mesh), eluting with petroleum ether/acetone, to afford compound **1** (644 mg). Fraction F4 (0.69 g) was filtered over Sephadex LH-20 (MeOH) and then silica gel, eluting with petroleum ether/acetone (8:2). The residue was purified on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to give compound **11** (5.1 mg).

Fraction B (0.77 g) was also chromatographed on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to afford five fractions, B1–B5. Fraction B3 (0.18 g) was separated on an RP-18 column (30–100% MeOH/H_2O) to give fraction B3A (17.7 mg), which was purified by silica gel chromatography (petroleum ether/acetone) to afford compound **10** (1.5 mg). Fraction B4 (0.48 g) was subjected to a Sephadex LH-20 column (MeOH) and further purified by semipreparative HPLC using 60% MeOH/H_2O as the mobile phase (3 mL/min) to obtain compounds **2** (6.4 mg, t_R = 22.5 min), **3** (27.8 mg, t_R = 56 min), and **4** (6.6 mg, t_R = 53 min). After a similar chromatographic process, fraction E (1.23 g) was purified using a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1), a silica gel column (petroleum ether/acetone, 10:1–1:1), and semipreparative HPLC (55% MeOH/H_2O, 3 mL/min) to give compounds **5** (5.0 mg), **8** (14.0 mg), and **13** (8.5 mg).

Fraction G (1.59 g) was subjected to CC on silica gel (300–400 mesh) eluting with petroleum ether/acetone (85:15, 1000 mL) to give nine fractions (G1–G9). Compound **12** (1.0 mg) was obtained from fraction G4 (843.2 mg) by repeated chromatography with Sephadex LH-20 ($CHCl_3$/MeOH, 1:1). Fraction G7 (507.7 mg) was chromatographed on Sephadex LH-20 and then silica gel eluting with $CHCl_3$/MeOH (50:1, 500 mL). The residue was purified by semipreparative HPLC (28% MeCN/H_2O, 3 mL/min) to afford compounds **6** (3.7 mg, t_R = 32 min), **7** (26 mg, t_R = 24 min), and **9** (30.6 mg, t_R = 27.5 min).

*Manglisin A (**1**):* colorless monoclinic crystals from MeOH; mp 118–119 °C; $[\alpha]_D^{16}$ −64 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 280 (4.06), 245 (3.86), 216 (4.59) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+27.1), 320 (−4.98) nm; IR (KBr) ν_{max} 3428, 2933, 1664, 1614, 1478, 1449, 1461, 1220, 1118, 1047 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; ESIMS *m*/*z* 409 $[M + Na]^+$; HREIMS *m*/*z* 386.1739 $[M]^+$ (calcd for $C_{22}H_{26}O_6$, 386.1729).

*Manglisin B (**2**):* colorless oil; $[\alpha]_D^{16}$ +4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 291 (3.92), 219 (4.29), 195 (4.05) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−58.65), 242 (+71.12) nm; IR (KBr) ν_{max} 3430, 2961, 2927, 1624, 1249, 1080, 1040, 1014 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; ESIMS *m*/*z* 393 $[M + Na]^+$; HREIMS *m*/*z* 370.1407 $[M]^+$ (calcd for $C_{21}H_{22}O_6$, 370.1416).

*Manglisin C (**3**):* colorless oil; $[\alpha]_D^{16}$ +5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 283 (3.64), 203 (4.84) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 196 (−7.40), 208 (+10.70) nm; IR (KBr) ν_{max} 3433, 2962, 2934, 1596, 1491, 1445, 1248, 1201, 1164, 1105 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; ESIMS *m*/*z* 395 $[M + Na]^+$; HREIMS *m*/*z* 372.1578 $[M]^+$ (calcd for $C_{21}H_{24}O_6$, 372.1573).

*Manglisin D (**4**):* colorless oil; $[\alpha]_D^{16}$ +17 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 285 (3.49), 203 (4.64) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 196 (−12.08), 208 (+16.98) nm; IR (KBr) ν_{max} 3427, 1625, 1504, 1489, 1444, 1239, 1201, 1101, 1037, 1103 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; ESIMS *m*/*z* 395 $[M + Na]^+$; HREIMS *m*/*z* 372.1575 $[M]^+$ (calcd for $C_{21}H_{24}O_6$, 372.1573).

*Manglisin E (**5**):* yellow oil; $[\alpha]_D^{23}$ −6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 279 (2.63), 204 (3.91) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (−1.94), 209 (+2.07) nm; IR (KBr) ν_{max} 3441, 2959, 2927, 1629, 1620, 1515, 1463, 1274, 1235, 1202, 1103 cm^{-1}; ^1H and ^{13}C NMR data see Tables 2 and 3; ESIMS *m*/*z* 397 $[M + Na]^+$; HREIMS *m*/*z* 374.1722 $[M]^+$ (calcd for $C_{21}H_{26}O_6$, 374.1729).

*Manglisin F (**6**):* colorless oil; $[\alpha]_D^{26}$ +24 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 265 (3.84), 207 (4.60) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 204 (−5.76), 218 (+9.01), 272 (+3.40) nm; IR (KBr) ν_{max} 3441, 2926, 1711, 1629, 1384, 1358, 1222, 1107, 1001 cm^{-1}; ^1H and ^{13}C NMR data see Table 4; ESIMS *m*/*z* 457 $[M + Na]^+$; HREIMS *m*/*z* 434.1570 $[M]^+$ (calcd for $C_{22}H_{26}O_9$, 434.1577).

*Manglisin G (**7**):* colorless oil; $[\alpha]_D^{26}$ +43 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 291 (3.87), 264 (4.10), 204 (4.73) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (−7.80), 210 (+10.09), 265 (+4.62) nm; IR (KBr) ν_{max} 3425, 2969, 2937, 1712, 1629, 1514, 1463, 1283, 1217, 1103, 1029, 999 cm^{-1}; ^1H and ^{13}C NMR data see Table 4; negative ESIMS *m*/*z* 403 $[M - H]^-$; HREIMS *m*/*z* 404.1471 $[M]^+$ (calcd for $C_{21}H_{24}O_8$, 404.1471).

X-ray Crystal Structure Analysis. A colorless monoclinic crystal of **1** was obtained from MeOH. Intensity crystal data were collected at 100(2) K on a Bruker APEX DUO detector, employing graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å) and operating in the φ/ω scan mode. Bruker SAINT was used for cell refinement and data reduction. The crystal structure was solved by the direct method using the program SHELXS-97[22] and subsequent Fourier difference techniques. Refinement using SHELXL-97 was performed anisotropically by full-matrix least-squares on F^2 for all non-hydrogen atoms. The H atom positions were geometrically idealized using a riding model and allowed to ride on their parent atoms. Crystallographic data (excluding structure factor tables) for compound **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication (deposit number CCDC 987938). Copies of the data can be obtained free of charge upon application to the CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystallographic Data for Manglisin A (1). $C_{22}H_{26}O_6$, M = 386.43, monoclinic, space group $P21$, Z = 4, T = 100(2) K, with a = 9.85210(10) Å, b = 9.33940(10) Å, c = 21.7893(3) Å, α = 90.00°, β = 100.12°, γ = 90.00°, V = 1973.72(4) Å^3, μ(Cu Kα) = 0.774 mm^{-1}, 16 934 reflections measured, 6485 independent reflections (R_{int} = 0.0262). The final R_1 values were 0.0299 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0781 ($I > 2\sigma(I)$). The final R_1 values were 0.0299 (all data). The final $wR(F^2)$ values were 0.0781 (all data). The goodness of fit on F^2 was 1.088. Flack parameter = 0.06(9). The Hooft parameter is 0.00(3) for 2673 Bijvoet pairs.

Antimicrobial Assay. The MIC values of isolated compounds against *Staph. aureus*, MRSA 82#, MRSA 92#, MRSA 98#, and MASA 331# were determined by the agar plate punch assay,[23] followed by the 2-fold dilution method.[24] The test organisms were all grown on MH medium.[18] The assay was performed as described previously.[25]

Cytotoxicity Assays. The in vitro cytotoxicity against five human tumor cell lines of compounds **1**–**7** were assessed using the MTT assay.[21] Cytotoxicity evaluations were performed according to the previously described protocol.[26]

Platelet Aggregation Inhibition Assays.[2] The assays were carried out according to Born's method,[27] using ginkgolide B (GB, Sigma Chemical Company, 129 K1405 V) and acetylsalicylic acid (ASA), potent PAF and AA antagonists, respectively, as positive controls. AA and ADP were purchased from Chronolog Corporation, and PAF was purchased from Sigma Chemical Company. The compounds were dissolved with DMSO to 20 mg/mL, and rabbit's blood of the carotid artery was anticoagulated with 3.8% sodium citrate solution (9:1, v/v). After centrifuging the blood at 200 and 2400 rpm (for 20 min), respectively, platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained.[28] PPP was kept at 5 × 10^{11} $cell^{-1}$ as reference for platelet aggregation or adjustor for the platelet count in PRP. The maximal aggregation was recorded in plasma with an aggregometer (model Chronolog-700, Chronolog Corporation) at 37 °C to get the final concentration of inducers: AA 500 μM, ADP 10 μM, PAF 0.4 μg/mL. The compounds were incubated with PRP at 37 °C for 10 min before the addition of inducers. The percentage drug inhibition was calculated according to the previous formula.[2]

■ ASSOCIATED CONTENT

Supporting Information

The spectroscopic data (1D and 2D NMR spectra, UV, CD, IR, ESI, HREIMS for compounds **1**−**7** and the X-ray data for compound **1**) and the structures of **9**−**13** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the support of the analytical group of Analysis and Test Center of Kumming Institute of Botany for spectra analyses. We thank the group of Prof. Guo-ying Zuo at the Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command, and the groups of Prof. Yan Li and Prof. Jin-hua Zhao at Kumming Institute of Botany for their help with the bioassays. This research was financially supported by grants from the National Natural Science Foundation of China (No. 31270404).

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