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Frutescone A−G, Tasmanone-Based Meroterpenoids from the aerial parts of *Baeckea frutescens*

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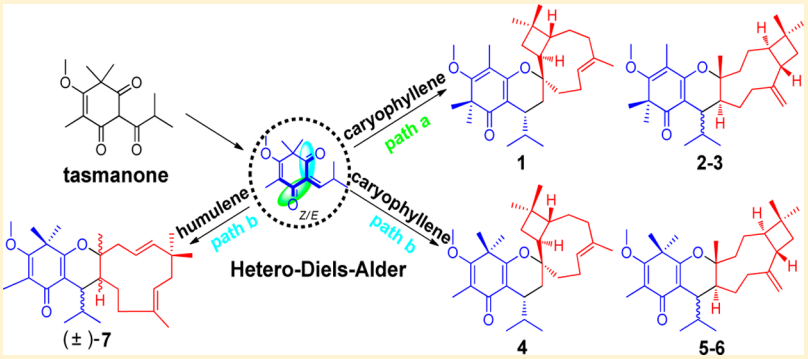
Supporting Information



ABSTRACT: Frutescone A−G [(**1**−**6**), (+)-7, (−)-7], a new group of naturally occurring tasmanone-based meroterpenoids, were isolated from the aerial parts of *Baeckea frutescens* L. Compounds **1** and **4** featured a rare carbon skeleton with an unprecedented oxa-spiro[5.8] tetradecadiene ring system, existing as two favored equilibrating conformers in $CDCl_3$ solution, identified by variable-temperature NMR. The regioselective syntheses of **4**−**7** were achieved in a concise manner by a biomimetically inspired key hetero-Diels−Alder reaction "on water". Compounds **1**, **4**, and **5** exhibited moderate cytotoxicities in vitro.

INTRODUCTION

Baeckea frutescens L. (Myrtaceae), an aromatic shrub, widely distributed in south China, southeast Asia, and northern Australia, has long been used in folk medicine for treating fever, rheumatism, and snake bites.[1] The aerial parts of *B. frutescens* as the major herb of "Compound Huangsong Lotion" is used clinically in China for the external treatment of gynecological infectious diseases.[2] Previous phytochemical studies of this plant led to the isolation of phloroglucinols,[3] sesquiterpenoids,[4] flavonoids,[5,6] and chromones[7] with anti-inflammatory, antibacterial, and cytotoxic activities.

As parts of our ongoing efforts to investigate the structurally attractive and bioactive constituents, eight unusual tasmanone-based meroterpenoids, frutescone A−G [**1**−**6**, (+)-7, (−)-7] (Figure 1), were isolated from the aerial parts of *B. frutescens* L. Compounds **1** and **4** featured a rare carbon skeleton with an unprecedented oxa-spiro[5.8] tetradecadiene ring system, existing as two favored equilibrating conformers in $CDCl_3$ solution, identified by variable-temperature NMR. Meroterpenoid hybrids are characterized by the connection of phloroglucinol to various terpenoid moieties.[12] In this paper, phytochemical studies of a series of new naturally occurring tasmanone-coupled caryophyllene or humulene involving extensive regio- and stereoselectivity would provide positive clues and evidence for the biomimetic synthesis of the meroterpenoids. The regioselective syntheses of 4−7 were achieved in moderate to low yield in a concise manner using a biomimetic hetero-Diels−Alder (HDA) reaction "on water" (Scheme 2). Furthermore, compounds **1**, **4**, and **5** exhibited moderate antitumor activities in vitro. Herein, we report the isolation, structural elucidation, possible biosynthesis, and biological activities of **1**−**7**.

RESULTS AND DISCUSSION

Frutescone A (**1**) was obtained as yellowish crystals. Its molecular formula was determined to be $C_{29}H_{44}O_3$ by HRESIMS at m/z 441.3365 $[M + H]^+$ (calcd for $C_{29}H_{45}O_3$, 441.3363). ^{1}H NMR spectra recorded at room temperature presented broad and incomplete signals (Figure 2), and most of the ^{13}C NMR signals were extremely low or invisible (Table 1). Variable-temperature NMR studies of **1** indicated that two equilibrating conformers, in a ratio of approximately 5:6 (^{1}H NMR integration), existed in $CDCl_3$ solution at 242 K.

The ^{1}H NMR spectrum for the major isomer set 1 (Table 2) showed one olefinic proton [δ_H 5.35 (1H, t, J = 7.2 Hz)], six methyl groups [δ_H 1.23, 1.22, 0.80, 0.89 (each 3H, s); 1.13 (3H, d, J = 6.4 Hz), 0.75 (3H, d, J = 6.9 Hz)], two vinyl methyl groups [δ_H 1.71, 1.69 (each 3H, s)], and a methoxyl group [δ_H 3.74 (3H, s)]. The ^{13}C NMR and DEPT spectra displayed 29

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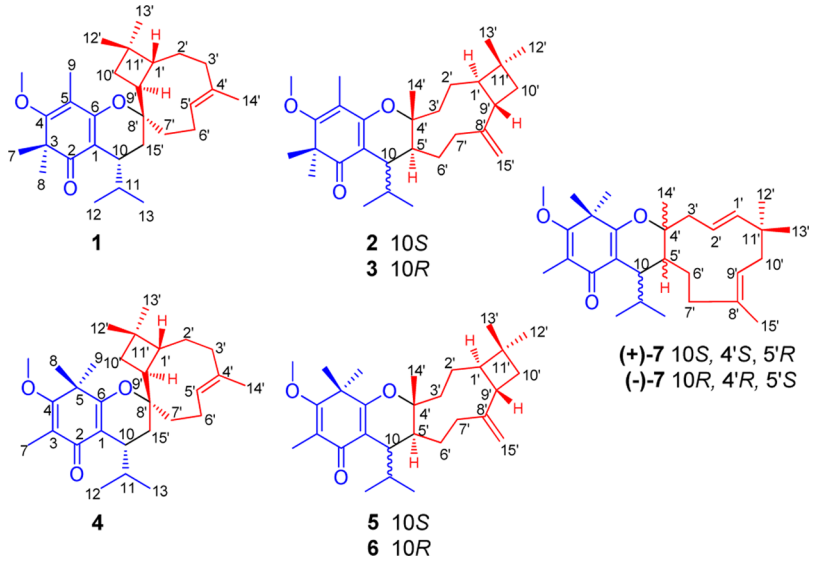

Figure 1. Structures of compounds **1**−**7**.

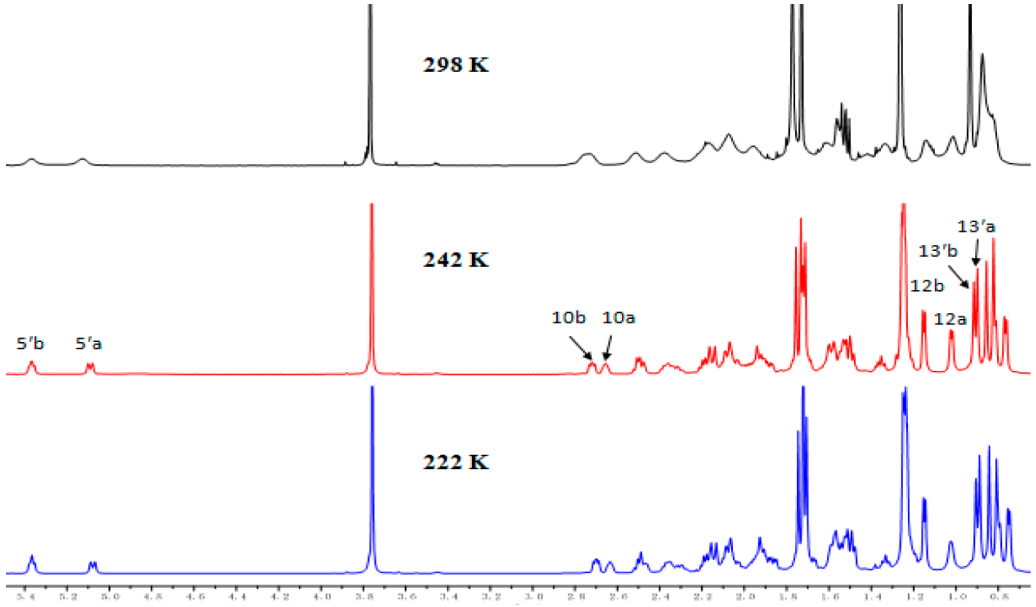

Figure 2. Variable-temperature ^{1}H NMR spectra of **1** in $CDCl_3$.

carbon resonances categorized into nine methyls, six methylenes, five methines, and nine quaternary carbons. The HMBC correlations from Me-9 to C-4 and C-6, from OMe to C-4, from Me-7 and Me-8 to C-2 and C-4, from both Me-12 and Me-13 to C-10, from H-10 to C-6, and from H-11 to C-1 (Figure 3) established the partial structure of isobutyryl phloroglucinol moiety (**1a**). Careful comparison of the NMR data of **1** with those of the literature[8,12b] indicated the presence of a caryophyllene moiety (**1b**), which was further confirmed by HMBC correlations from Me-12′ and Me-13′ to C-1′ and C-10′, from Me-14′ to C-3′ and C-5′, and from H-1′ and H_2-6′ to C-8′, as well as the ^{1}H−^{1}H COSY correlations (Figure 3). Furthermore, the HMBC correlations from H-10 and H_2-15′ to C-8′ and from H_2-15′ to C-7′ revealed the substructures **1a** and **1b** were connected via a C_{10}−$C_{15'}$ bond. Thus, the planar structure of **1** was therefore determined.

In the NOESY spectrum, the cross peak between H_2-3′ and H-5′ and between H_2-6′ and Me-14′ indicated an *E*- geometry for the double bond ($\Delta^{4',5'}$). Due to the flexible nine-membered ring of caryophyllene, four conformational isomers existed with different dispositions of the exocyclic methylene and olefinic methyl groups.[8] *ββ* (set 1) and *βα* (set 2), as being the two lowest energy conformations, were detected in **1**, similar to the caryophyllene-based meroterpenoid isolated from *Myrtus communis*.[12b] The 2D NMR correlations found in NMR signals of set 2 were almost the same as those recorded in set 1. With the aid of ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY experiments recorded at 242 K, all ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in Table 2.

A single crystal of **1** (Figure 3, CCDC 1495749) was obtained from methanol, and X-ray crystallographic analysis was carried out by using the anomalous dispersion of Cu K*α* radiation. The crystallized conformer was the preferable *ββ* conformer with the $C_{3'}$−$C_{4'}$−$C_{5'}$−$C_{6'}$ torsional angle of 152.76°. The absolute configuration of **1** was determined as

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Table 1. ^{1}H and ^{13}C NMR Data of 1 and 4 in $CDCl_3$ (298 K)

	1[a]		4[a]	
no.	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C
1		c		108.6
2		200.3		188.5
3		49.1		117.4
4		167.7		171.3
5		111.9		43.1
6		c		169.2
7	1.25, s	25.8	1.86, s	10.2
8	1.24, s	c	b	c
9	1.75, s	9.9	1.19, s	23.3
10	2.71, brs	c	b	c
11	b	c	b	c
12	b	21.0	0.84, d (6.8)	21.0
13	b	c	b	c
1′	2.49, brs	c	2.48, brs	c
2′	b	c	b	c
3′	b	c	b	c
4′		c		c
5′	5.35, brs	c	5.35, brs	c
	5.11, brs		5.10, brs	
6′	b	c	b	c
7′	b	c	b	c
8′		c		c
9′	b	c	1.34, m	c
10′	b	c	b	c
11′		32.7		32.3
12′	b	29.9	b	c
13′	0.91, s	24.4	0.90, s	25.0
14′	1.71, s	16.0	1.70, s	c
15′	b	c	b	c
OMe	3.75, s	61.9	3.81, s	61.7

[a]Recorded at 600 (^{1}H) and 150 (^{13}C) MHz. [b]Signals unassigned. [c]Signals invisible.

10*R*, 1′*R*, 8′*R*, 9′*S* by refinement of the Flack parameter [0.06 (5)].

Frutescone B (**2**) was obtained as yellowish crystals. It shared the same molecular formula of $C_{29}H_{44}O_3$ as **1** based on the HRESIMS data. A comparison of the NMR data of **2** (Table 3) with those of **1** suggested the presence of the same isobutyryl phloroglucinol moiety (**2a**), which was further confirmed by the HMBC correlations (Figure 4). The two spin systems (H_2-3′/H_2-2′/H-1′/H-9′/H_2-10′ and H_2-7′/H_2-6′/H-5′/H-10/H-11/Me-12 and Me-13) in the $^{1}H-^{1}H$ COSY spectrum, together with the HMBC correlations from H_2-15′ to C-7′ and C-9′, from H-1′ to C-8′, from Me-12′ and Me-13′ to C-1′ and C-10′, from H_2-2′ and H_2-6′ to C-4′, and from H_2-3′ and Me-14′ to C-5′ indicated the presence of a caryophyllene unit (**2b**). Furthermore, the HMBC correlations from H-10 to C-4′ and C-6′ and from H-5′ to C-11 confirmed that substructure **2a** was coupled with **2b** through the $C_{10}-C_{5'}$ bond.

The relative stereochemistry of **2** could be elucidated by a ROESY experiment. The NOE correlations of Me-14′/H-10, H-5′/Me-12, and Me-13 indicated that Me-14′ and H-10 were β-oriented, while H-5′ was α-oriented.

Interestingly, HPLC analyses of **2** using different chiral columns showed one sharp signal, but the crystal exhibited twinning (Figure 5, CCDC 1495761), including two identical units. The final refinement on the Cu Kα data resulted in the Flack parameter 0.05 (4), allowing unambiguous assignment of the absolute structure of **2** (10*S*, 1′*R*, 4′*R*, 5′*S*, 9′*S*).

2 and **3** shared the same planar structures, as deduced by the detailed analysis of HRESIMS and NMR spectroscopic data. The relative stereochemistry of **3** was assigned by the ROESY spectrum (Figure 4). The correlations of Me-14′/H-11 together with the absence of Me-14′/H-10 and H-5′/H-11, Me-12 revealed α orientations of H-10 and H-5′ and β orientation of Me-14′. In addition, the experimental CD curves of **2** and **3** were almost reverse, suggesting the chiral difference of C-10 near the chromophore moiety. The stereochemistry of **3** was designated as 10*R*, 1′*R*, 4′*R*, 5′*S*, 9′*S* by X-ray crystallographic analysis (Figure 6, CCDC 1495762).

Frutescone D (**4**) was obtained as colorless needle crystals with the molecular formula of $C_{29}H_{44}O_3$ established by the HRESIMS data. Interestly, the variable-temperature NMR data of **4** were very similar to those of **1** (Table 2), except for the different ratio of the existing two atropisomers [approximately 6:5 of $\beta\beta$ (set 1):$\beta\alpha$ (set 2)], which indicated that **4** shared the same oxa-spiro[5.8] tetradecadiene skeleton. Moreover, the UV absorption maxima at 204, 248, and 298 nm in **4** were dramatically distinguished from those of **1** (217, 339 nm) (see the Supporting Information), implying a different phloroglucinol moiety (**4a**), which was further confirmed by the HMBC correlations from Me-7 to C-2 and C-4, from OMe to C-4, from both Me-8 and Me-9 to C-4 and C-6, from Me-12 and Me-13 to C-10, from H-10 to C-6, and from H-11 to C-1 (Figure 4). The linkage of $C_{10}-C_{15'}$ between **4a** and **4b** was supported by the HMBC cross peaks from H-10 to C-8′ and from H_2-15′ to C-7′.

The relative stereochemistry of **4** was deduced as shown in Figure 1 by a single-crystal X-ray Mo Kα diffraction study (Figure 6, CCDC 1495774). Combining the comparison of experimental CD curve (Figure 7) with those of **5** and **6** allowed the absolute configuration of **4** as 10*R*, 1′*R*, 8′*R*, 9′*S*.

Frutescone E (**5**) and frutescone F (**6**) were isolated as a pair of C-10 epimers of tasmanone-coupled caryophyllene meroterpenoids characterized with the almost reverse experimental CD spectra (Figure 7). The structure and relative configurations of **5** and **6** were elucidated by unambiguous analyses of $^{1}H-^{1}H$ COSY, HMBC, and NOESY spectra (Figure 8) as well as comparison of NMR data with those of **2**–**4**. The absolute configurations were determined to be 10*S*, 1′*R*, 4′*R*, 5′*S*, 9′*S* (**5**) and 10*R*, 1′*R*, 4′*R*, 5′*S*, 9′*S* (**6**) by ECD calculation using the TDDFT method at the B3LYP/6-31G(d) level (Figure 9).

Frutescone G (**7**) consists of a pair of enantiomers (see the Supporting Information) with the molecular formula of $C_{29}H_{44}O_3$ determined by the HRESIMS data. Comparison of the NMR data of **7** (Table 3) with those of **4**–**6** suggested the presence of substructure **7a**. Three spin systems (H_2-7′/H_2-6′/H-5′/H-10/H-11/Me-12 and Me-13, H-1′/H-2′/H_2-3′, and H-9′/H_2-10′) in the $^{1}H-^{1}H$ COSY spectrum and the HMBC correlations from Me-12′ and Me-13′ to C-1′ and C-10′, from Me-15′ to C-7′ and C-9′, and from H-3′ and Me-14′ to C-5′ led to construction of a humulene unit (**7b**) (Figure 8).[12b] The HMBC correlations from H-10 to C-4′ and C-6′ and from H-5′ to C-11 indicated that **7a** and **7b** were connected via a $C_{10}-C_{5'}$ bond.

The NOESY correlations of H-1′/H_2-3′, H_2-7′/H-9′, and Me-15′/H_2-10′ suggested *E* geometry of the double bonds ($\Delta^{1',2'}$, $\Delta^{8',9'}$). Me-14′ had obvious cross-peaks with H-11 and Me-12 but had no NOE correlation with H-10. Combined with

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Table 2. ^{1}H and ^{13}C NMR Data of 1 and 4 in $CDCl_3$ (242 K)

	1[a,b]				4[a,b]			
	set 1 ($\beta\beta$)		set 2 ($\beta\alpha$)		set 1 ($\beta\beta$)		set 2 ($\beta\alpha$)	
no.	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C
1		106.8		107.3		108.1		108.1
2		200.5		200.4		188.7		188.8
3		48.8		48.7		117.2		117.2
4		167.2		167.5		171.3		171.2
5		112.1		111.9		42.9		42.9
6		166.4		166.4		169.4		168.7
7	1.24, s	25.7	1.23, s	25.9	1.84, s	10.3	1.85, s	10.2
8	1.21, s	22.8	1.22, s	22.7	1.25, s	24.7	1.23, s	24.8
9	1.74, s	10.1	1.71, s	10.0	1.18, s	23.0	1.15, s	23.1
10	2.64, dt (9.0, 4.2)	32.3	2.70, dd (10.4, 6.7)	32.2	2.64, dq (7.6, 4.2)	31.8	2.70, dd (10.4, 6.2)	31.7
11	2.05	32.2	1.60	32.4	2.05, m	31.8	1.59, m	32.6
12	1.00, d (6.4)	20.8	1.13, d (6.4)	22.5	0.81, d (6.4)	20.8	1.12, d (6.4)	21.4
13	0.80, d (6.9)	20.9	0.75, d (6.9)	21.0	0.97, d (6.8)	20.9	0.77, d (6.8)	22.3
1′	2.50	54.9	2.17	53.3	2.45	54.5	2.13	52.4
2′a	1.60	29.6	1.95	30.3	2.01	30.5	1.71	29.7
2′b	1.47		1.51		1.52		1.43	
3′a	2.46	36.8	2.07	39.9	2.45	39.8	2.05	37.3
3′b	1.70		1.95		1.60		1.95	
4′		133.2		137.8		133.2		137.7
5′	5.07, d (12.1)	125.6	5.35, t (7.2)	120.0	5.06, d (12.1)	125.6	5.34, t (8.1)	119.9
6′	2.30	23.7	2.40	22.3	2.26	23.7	2.32	22.4
7′a	2.05	40.9	2.01	43.2	2.05	40.3	1.93	42.8
7′b	1.72		1.90		1.75		1.75	
8′		85.8		87.9		83.7		85.3
9′	1.35	47.8	1.57	46.4	1.37, t (9.4)	47.7	1.61	46.2
10′	1.28	35.9	2.50	35.1	1.22	36.6	2.45	35.2
11′		31.1		31.1		30.9		32.1
12′	0.84, s	29.9	0.80, s	29.7	0.85, s	29.9	0.80, s	29.7
13′	0.88, s	24.7	0.89, s	23.1	0.87, s	24.9	0.88, s	22.8
14′	1.70, s	16.2	1.69, s	16.2	1.68, s	16.2	1.69, s	16.2
15′	1.71	24.6	1.90	23.9	1.71	24.7	1.89	23.9
OMe	3.74, s	62.1	3.74, s	62.1	3.79, s	62.0	3.79, s	62.0

[a]Recorded at 600 (^{1}H) and 150 (^{13}C) MHz. [b]Overlapped signals without designating multiplicity.

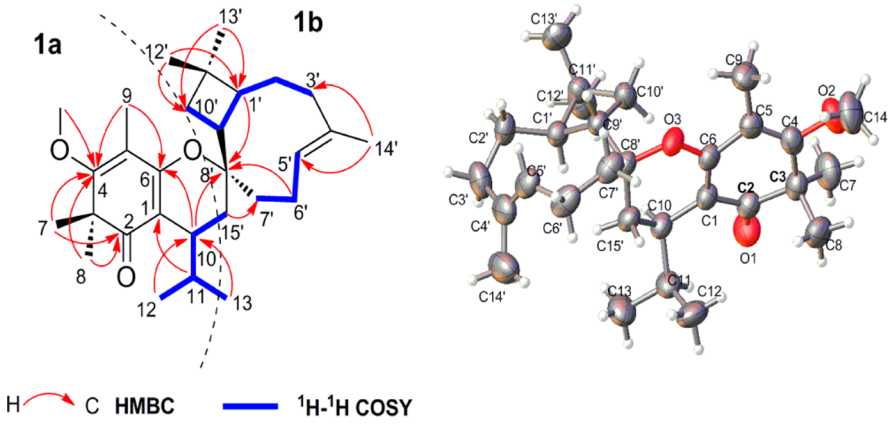

Figure 3. Key 2D NMR correlations and X-ray structure of 1 (30% probability level).

the absence of NOE correlation from H-5′ to H-11 and Me-12, it was suggested that Me-14′, H-11, and Me-12 were β orientations while H-10 and H-5′ were α orientations, which were found to be the same with those of **3** and **6**, as reported in the literature.[12b,d] Thus, the relative stereochemistry of **7** was determined as in Figure 1. The chiral stereochemistries of (+)-**7** and (−)-**7** were designated as 10*S*, 4′*S*, 5′*R* and 10*R*, 4′*R*, 5′*S*, respectively, by the calculated ECD spectra (Figure 9).

The plausible biosynthetic pathway of triketone (flavesone, leptospermone)-coupled sesquiterpenoids has been previously proposed.[12b,c] Herein, a different biogenetic precursor, tasmanone (**8**),[9] is believed to be involved in the biosynthesis of **1**−**7**. As illustrated in Scheme 1, the key intermediate **8**, β-

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Table 3. ^{1}H and ^{13}C NMR Data of 2, 3, and 5−7 in $CDCl_3$

	2[a]		3[a]		5[a]		6[b]		7[a]	
no.	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C	δ_H (J, in Hz)	δ_C
1		111.8		110.4		114.8		112.6		115.2
2		199.8		200.2		187.8		188.2		188.0
3		49.6		49.1		118.0		117.4		117.8
4		167.6		167.9		172.1		171.9		172.2
5		112.3		111.7		42.5		42.7		42.7
6		167.4		165.8		169.7		168.9		170.6
7	1.26, s	25.2	1.26, s	25.8	1.88, s	10.0	1.89, s	10.1	1.34, s	23.8
8	1.23, s	23.4	1.25, s	23.6	1.32, s	23.6	1.30, s	24.4	1.24, s	23.7
9	1.83, s	9.7	1.81, s	9.9	1.23, s	23.9	1.28, s	23.5	1.90, s	10.2
10	2.36, m	40.7	2.68, t (4.2)	36.0	2.35, d (7.1)	40.4	2.70, m	35.8	2.67, t (3.7)	41.5
11	2.02, m[c]	32.2	2.02, m[c]	26.6	2.05, m[c]	31.9	2.03, m[c]	26.0	2.10, m[c]	35.5
12	0.88, d (7.1)	20.5	1.15, d (6.9)	26.8	0.89, d (7.2)	20.8	1.17, d (6.8)	26.8	0.75, d (6.6)	18.4
13	0.80, d (7.1)	20.0	0.65, d (6.9)	19.9	0.75, d (7.2)	19.3	0.64, d (7.0)	19.7	0.91, d (7.0)	22.7
1′	2.03, m[c]	54.3	1.60, m[c]	57.0	2.10, m[c]	54.2	1.56, m[c]	57.1	5.19, m[c]	143.3
2′a	1.75, m[c]	22.2	1.54, m[c]	23.7	1.83, m[c]	21.8	1.59, m[c]	23.7	5.19, m[c]	121.1
2′b	1.42, m[c]		1.39, m[c]		1.42, m[c]		1.33, m[c]			
3′a	2.02, m[c]	39.3	2.06, m[c]	44.1	2.05, m[c]	39.2	2.03, m[c]	44.5	2.51, m	45.0
3′b	1.84, m[c]		1.55, m[c]		1.90, m[c]		1.47, m		2.28, m[c]	
4′		82.7		85.6		82.7		84.2		84.6
5′	2.10, m[c]	38.9	1.77, m[c]	39.3	2.14, m[c]	38.4	1.78, m[c]	39.6	2.30, m[c]	40.6
6′	1.53, m[c]	36.3	1.76, m[c]	25.2	1.56, m	36.2	1.71−1.81, m[c]	25.1	1.33, m[c]	33.4
7′a	2.44, m	35.5	2.40, m	35.6	2.45, m[c]	35.5	2.41, m	35.7	2.12, m[c]	36.4
7′b	2.01, m[c]		2.16, m[c]		2.03, m[c]		2.16, m			
8′		152.8		151.4		152.8		151.3		136.1
9′	2.40, m	42.3	2.45, m	41.1	2.40, m[c]	42.2	2.43, m[c]	41.7	5.10, t (8.5)	124.0
10′a	1.62, t (9.5)	35.5	1.69, m[c]	36.8	1.76, t (10.2)	36.0	1.70, m	36.6	2.10, m[c]	41.6
10′b			1.58, m[c]				1.58, m[c]		1.90, m[c]	
11′		33.8		34.4		33.7		34.4		38.5
12′	1.03, s	30.7	0.93, s	29.9	1.02, s	30.7	0.93, s	29.9	1.11, s	25.9
13′	1.01, s	22.6	0.96, s	21.9	0.99, s	22.6	0.96, s	21.9	1.09, s	28.5
14′	0.99, s	21.6	1.32, s	23.3	0.99, s	21.3	1.29, s	23.1	1.02, s	21.2
15′	4.94, brs	111.2	4.90, brs	111.1	4.94, brs	111.2	4.88, brs	110.9	1.62, s	18.1
	4.87, brs				4.88, brs					
OMe	3.78, s	61.8	3.76, s	61.8	3.83, s	61.8	3.83, s	61.8	3.85, s	61.8

[a]Recorded at 300 (^{1}H) and 75 (^{13}C) MHz. [b]Recorded at 500 (^{1}H) and 125 (^{13}C) MHz. [c]Overlapped signals without designating multiplicity.

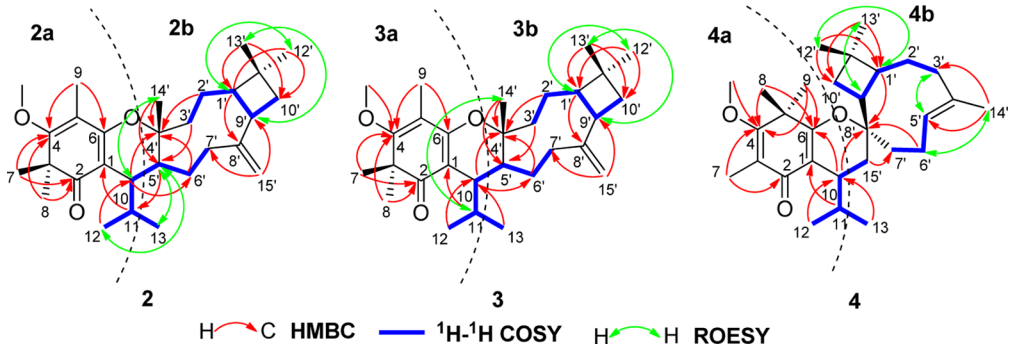

Figure 4. ^{1}H−^{1}H COSY, HMBC, and ROESY correlations of 2−4.

caryophyllene (9), and α-humulene (10) were reported as the major constituents of *B. frutescens* essential oil.[4] Selective reduction and dehydration of 8 generated the active intermediate **A1**, which could undergo a HDA reaction with 9 or 10 to afford 1−7 in a regio- and stereoselective manner.

Further exploration on the biosynthetic origin led to the regioselective syntheses of 4−7 initiated with commercially available phloroglucinol (11), 9, or 10 as substrates, as presented in Scheme 2. First, acetylation of 11 would give acetyl phloroglucinol (12)[10] and trimethylation of 12 would provide 13,[10c] which could undergo acid-induced retro-Claisen condensation and Knoevenagel condensation to afford the active intermediate 15. Hetero-Diels−Alder reaction in toluene/water (3:1) of 15 with 9 or 10, respectively, was followed by methylation. Notably, the HDA reaction carried out by stirring a toluene solution of the substrates "on water"[11]

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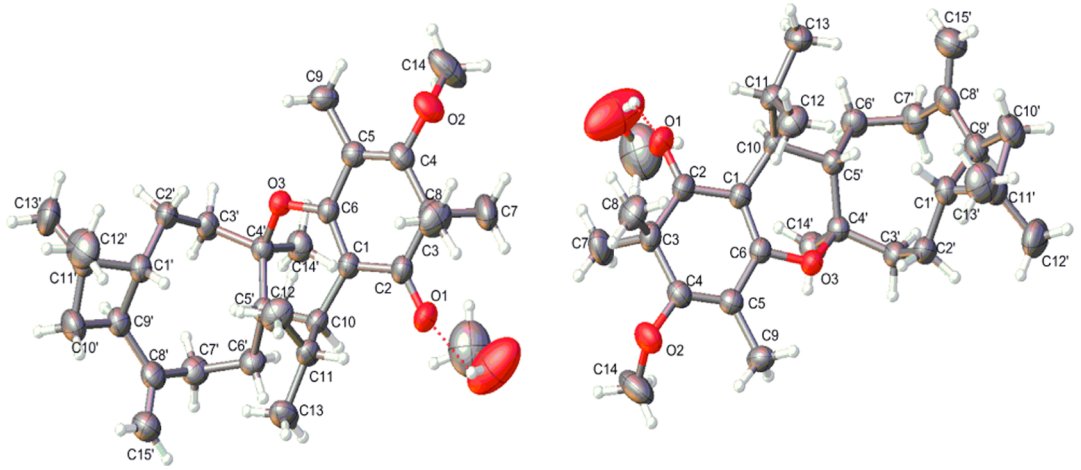
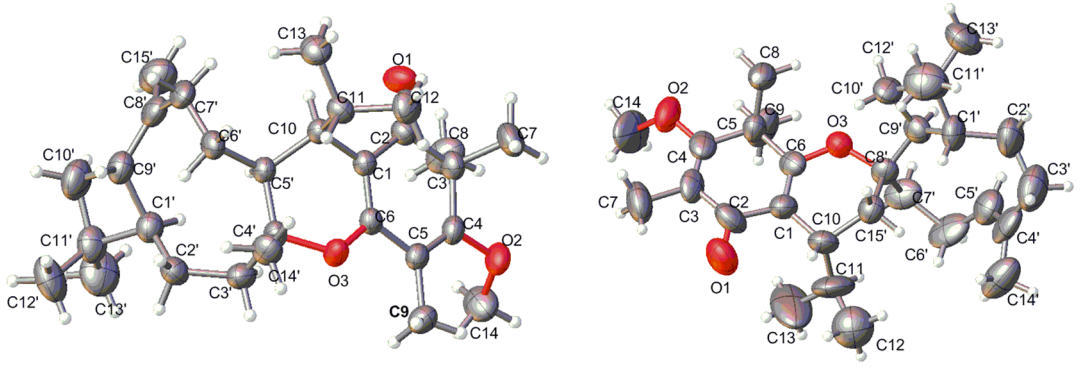


Figure 5. X-ray crystallographic twinning structures of **2** (30% probability level).



3 **4**

Figure 6. X-ray crystallographic structures of **3** and **4** (30% probability level).

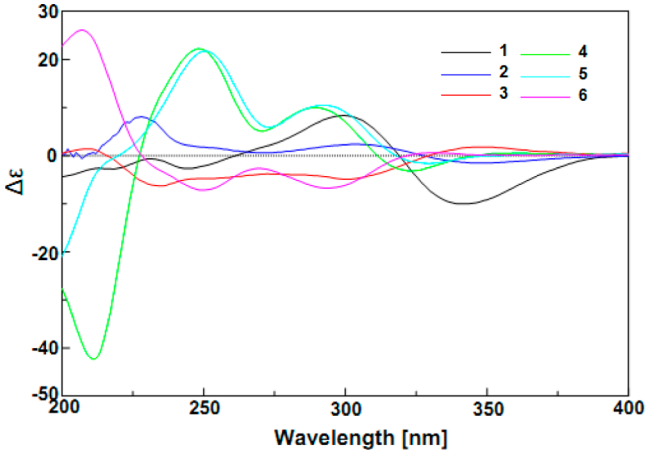


Figure 7. Experimental CD spectra of **1**–**6**.

proceeded at a considerably higher rate and yield than in toluene alone or solvent-free conditions. After two-step sequences, regiospecific cyclization adducts **4**–**7** were isolated in 60% (**4**:**5**:**6** = 1.1:1.0:1.1) or 13.5% (**7**) yield, while the products **1**–**3** were in extremely trace amounts due to unfavorable regioselectivity. The acceleration of HDA reaction "on water" was presumably ascribed to hydrophobic collapse and/or an hydrogen-bond mediated stabilization of the transition state.[11] Consequently, the NMR spectra and chiral HPLC analyses of synthetic compounds **4**–**7** were compared to those of natural frutescone D–G (**4**–**7**) isolated from *B. frutescens* and found being identical.

The isolates were evaluated for cytotoxicities against three human cancer cell lines Caco-2, A549, and HepG2 (Table 4). Compounds **1** and **4** exhibited moderate cytotoxic activities against Caco-2 with IC_{50} values of 8.08 and 10.20 μM, respectively. Compound **5** showed activities against Caco-2 and A549 with IC_{50} values of 7.96 and 12.14 μM.

In order to verify that the reported compounds **1**–**7** are indeed natural products, the fresh aerial parts of *B. frutescens* were percolated with methanol at room temperature or extracted with CH_2Cl_2 directly by ultrasonator, respectively. The two crude extracts were analyzed by HPLC-Q/TOF-MS or LC-MSD Trap methods, respectively (see the Supporting Information). Compounds **1**–**7** were detected in the above two different crude extracts by comparsion of the HPLC retention times, MS^n spectra, and UV absorptions with those of isolates. Thus, compounds **1**–**7** are proved to be naturally occurring products in *B. frutescens*, not artifacts produced during the isolation and purification procedure.

■ CONCLUSION

Frutescone A–G (**1**–**7**), a new group of naturally occurring unusual tasmanone-based meroterpenoids, were first isolated from *B. frutescens* L. Biogenetically, compounds **1**–**7** possessed extensive regio- and stereoselective [4 + 2] cycloaddition

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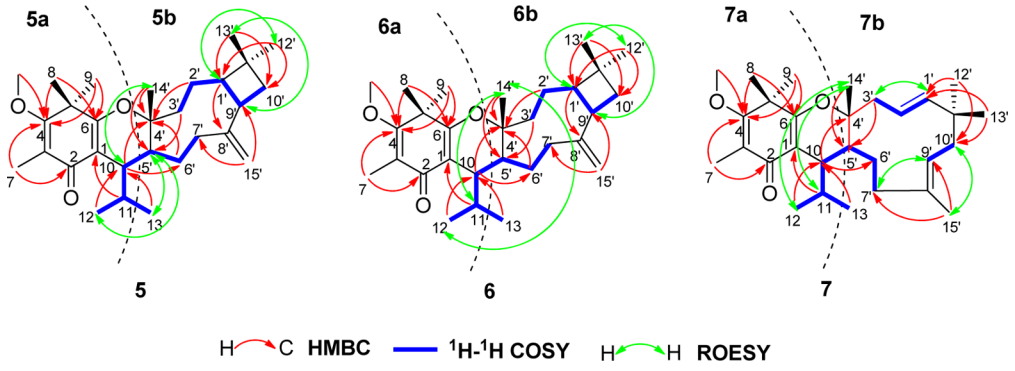


Figure 8. ^{1}H−^{1}H COSY, HMBC, and ROESY correlations of **5**−**7**.

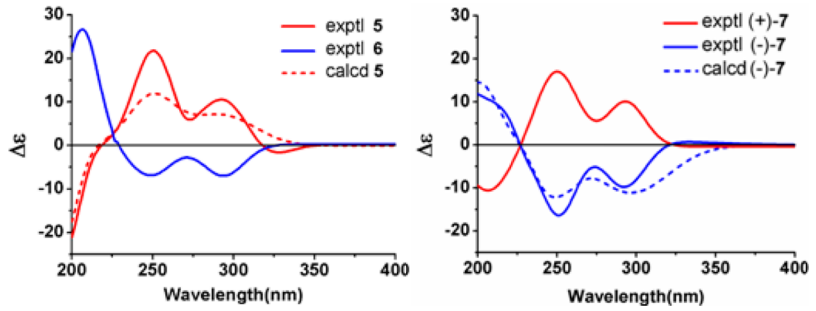
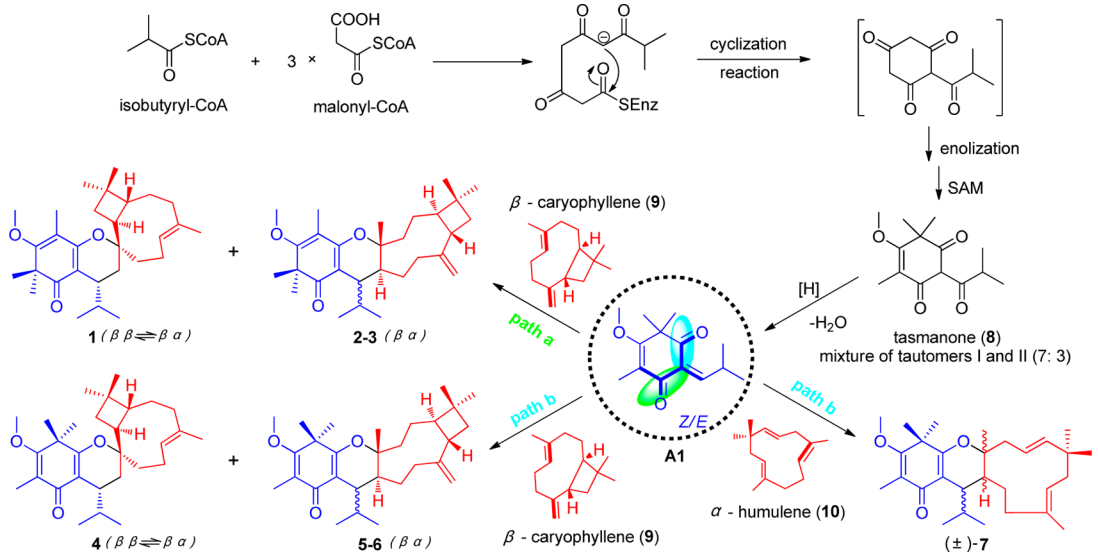


Figure 9. Calculated and experimental ECD spectra of **5** and (−)-7.

Scheme 1. Plausible Biosynthetic Pathways of 1−7



architectures,[12] which largely enriched the chemodiversity of phloroglucinol−terpene derivatives. Compounds **4**−**7** as the regioselectively favored [4 + 2]-cycloaddition adducts were achieved by a biomimetic HDA reaction "on water", which not only enabled the structural assignments but also established the superior role of water in aspects of rate, yield, and selectivity and could provide valuable inspiration for eco-friendly organic synthesis in aqueous suspension. The broadened applicability of this strategy for construction of bioactive tasmanone-based phloroglucinols to diverse terpenoids is currently under research in our laboratory.

EXPERIMENTAL SECTION

General Experimental Procedures. Column chromatography was performed using silica gel (100−200 and 200−300 mesh), MCI gel (CHP20P, 75−150 μm), Sephadex LH-20, and ODS (50 μm). Optical rotation was measured on a polarimeter. UV spectra were recoreded on a UV−vis spectrophotometer using MeOH as the solvent. IR spectra were carried on a FT-IR spectrometer with KBr disks. CD spectra were obtained on a spectropolarimeter. 1D and 2D NMR spectra were recorded in $CDCl_3$ on 600, 500, and 300 MHz (^{1}H) NMR spectrometers with TMS as internal standard. All NMR assignments were based on ^{1}H−^{1}H COSY, HSQC, and HMBC spectroscopic data. Diffraction data were collected on diffractometers using Cu Kα and Mo Kα radiation. Preparative HPLC and chiral HPLC separations were performed using a C8 column (250 × 20 mm,

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Scheme 2. Regioselective Synthesis of 4−7 via Hetero-Diels−Alder Reaction "on Water"

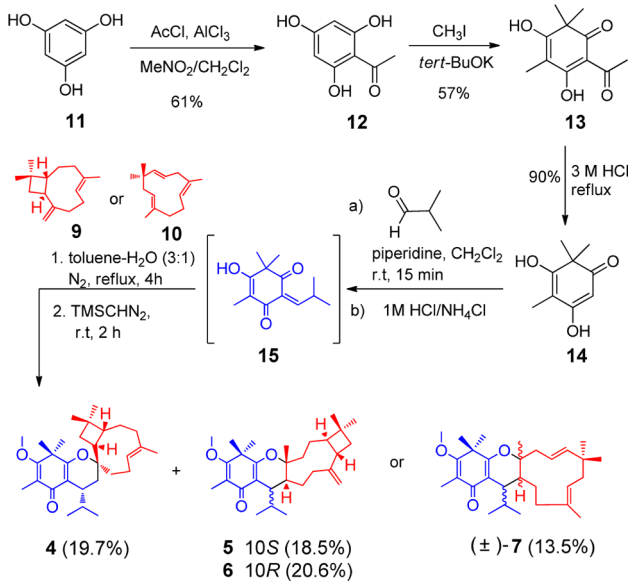

Table 4. Cytotoxicity Data of 1−7 (IC_{50}, μM)

compound	Caco-2	A549	HepG2
1	8.08 ± 2.79	20.07 ± 5.77	>50
2	23.25 ± 3.91	41.33 ± 4.42	>50
3	14.83 ± 5.97	27.74 ± 4.76	>50
4	10.20 ± 3.44	26.25 ± 6.10	>50
5	7.96 ± 2.56	12.14 ± 5.55	>50
6	16.51 ± 2.52	39.02 ± 6.02	>50
7	14.31 ± 4.07	25.71 ± 5.89	>50
doxorubicin[a]	6.03 ± 4.31	6.31 ± 2.89	8.23 ± 2.64

[a]Doxorubicin was used as a positive control.

5 μm) and Chiral OD-RH column (250 × 10 mm, 5 μm), respectively. Both HRESIMS and LC-MS analyses were performed on an analytical HPLC system coupled with a Q/TOF mass spectrometer equipped with an ESI source in positive mode.

Plant Material. The aerial parts of *Baeckea frutescens* (Myrtaceae) were collected from Guangxi province of P. R. China in October of 2014 and authenticated by Prof. Min-Jian Qin (Department of Medicinal Plants, China Pharmaceutical University, Nanjing). A voucher specimen (No. BF201410) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The dry aerial parts of *B. frutescens* (18 kg) were extracted with 95% EtOH (100 L) under reflux (3 × 4 h) at 83 °C. The combined extracts were concentrated under reduced pressure to afford a dark green residue extract (2.0 kg), which was suspended in water (3 L) and successively extracted with petroleum ether (4 × 3 L), chloroform (3 × 3 L), and *n*-butanol (4 × 3 L).

The petroleum ether extract (500 g) was subjected to silica gel column chromatography with PE−EtOAc (100:0 → 40:60, v/v) as eluent to afford eight fractions (Fr. A−H). Fr. C (104 g) was further separated into five fractions (Fr. C1−C5) on a silica gel column (PE−EtOAc, 100:0→30:70, v/v) according to their TLC profiles. Fr. C2 (10.5 g) was subjected to a MCI gel column with a gradient mixture of MeOH−H_2O (60:40 → 100:0, v/v) as eluent to give Fr. C2.1−6. Then Fr. C2.4 (310 mg) was run on an ODS column (MeCN−H_2O, 60:40 → 100:0, v/v) to afford Fr. C2.4.1−5. Then Fr. C2.4.4 (183 mg) was applied onto Sephadex LH-20 ($CHCl_3$−MeOH, 50:50) to yield Fr. C2.4.4.1−3. Then Fr. C2.4.4.3 was further purified by recycled preparative HPLC (MeCN−H_2O, 90:10, 12 mL/min) to afford **1** (7 mg), **3** (10 mg), and **4** (24 mg). Fr. D (73 g) was chromatographed on a silica gel column with PE−EtOAc (100:0 → 40:60, v/v) as eluent to yield six fractions (Fr. D1−D6). Fr. D4 was further subjected to an ODS column using a gradient elution of MeOH−H_2O (65:35 → 100:0, v/v), Sephadex LH-20 gel column ($CHCl_3$−MeOH, 50:50), and recycled preparative HPLC (MeCN-H_2O, 85:15, 12 mL/min), respectively, to yield **5** (31 mg), **7** (10 mg), **2** (6 mg), and **6** (27 mg). Compound **7** was further separated into enantiomers (+)-7 (1.0 mg, t_R 13.1 min) and (−)-7 (1.0 mg, t_R 15.9 min) by chiral HPLC using MeCN−H_2O (85:15, 4 mL/min) as the mobile phase.

*Frutescone A (**1**).* Yellowish crystals (MeOH), mp 198−200 °C; $[\alpha]_D^{20}$ −129.0 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data see Tables 1 and 2; IR (KBr)ν_{max} 2951, 2921, 2849, 1657, 1648, 1624, 1557, 1468, 1388, 1128 cm^{-1}; UV (MeOH) λ_{max} (log ε) 217 (4.45), 339 (3.79) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 231 (−0.6), 299 (+8.3), 342 (−10.0) nm; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3365 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

*Frutescone B (**2**).* Yellowish crystals (MeOH), mp 202−204 °C; $[\alpha]_D^{20}$ +28.6 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data see Table 3; IR (KBr) ν_{max} 2962, 2930, 2870, 1662, 1636, 1570, 1459, 1384, 1128 cm^{-1}; UV (MeOH) λ_{max} (log ε) 202 (3.94), 348 (2.87) nm; CD (MeOH): λ_{max} ($\Delta\varepsilon$) 228 (+8.1), 303 (+2.4), 350 (−1.5) nm; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3367 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

*Frutescone C (**3**).* Yellowish crystals (MeOH), mp 193−197 °C; $[\alpha]_D^{20}$ +74.7 (*c* 0.03, MeOH); ^{1}H and ^{13}C NMR data see Table 3; IR (KBr) ν_{max} 2962, 2926, 2873, 1660, 1628, 1568, 1397, 1385, 1130 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.13), 342 (3.38) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 235 (−6.2), 301 (−4.9), 349 (+1.8) nm; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3359 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

*Frutescone D (**4**).* Colorless needle crystals (MeOH); mp 175−177 °C; $[\alpha]_D^{20}$ +110.0 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data see Tables 1 and 2; IR (KBr) ν_{max} 2950, 2935, 2869, 1664, 1610, 1400, 1363, 1130 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.41), 248 (4.20), 298 (3.90) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−42.3), 248 (+22.2), 290 (+10.0) nm; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3357 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

*Frutescone E (**5**).* Light yellow oil; $[\alpha]_D^{20}$ +96.7 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data see Table 3; IR (KBr) ν_{max} 2955, 2962, 2869, 1670, 1622, 1465, 1400, 1110 cm^{-1}; UV (MeOH) λ_{max} (log ε) 203 (4.45), 250 (4.16), 296 (3.81) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (−10.5), 251 (+21.8), 292 (+10.5) nm; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3363 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

*Frutescone F (**6**).* Light yellow oil; $[\alpha]_D^{20}$ −51.6 (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data see Table 3; IR (KBr) ν_{max} 2956, 2931, 1747, 1662, 1616, 1469, 1389, 1130 cm^{-1}; UV (MeOH) λ_{max} (log ε) 203 (4.38), 249 (4.20), 298 (3.87) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (+26.1), 250 (−7.2), 294 (−6.8) nm; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3363 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

*Frutescone G (**7**).* Light yellow oil; $[\alpha]_D^{20}$ +180.0 (*c* 0.1, MeOH) for (+)-7, $[\alpha]_D^{20}$ −160.0 (*c* 0.1, MeOH) for (−)-7; ^{1}H and ^{13}C NMR data see Table 3; IR (KBr)ν_{max} 2958, 2928, 1664, 1624, 1458, 1384, 1121 cm^{-1}; UV (MeOH) λ_{max} (log ε) 204 (4.06), 252 (3.85), 297 (3.46) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (−9.7), 251 (+17.1), 294 (+9.6) nm for (+)-7; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (+9.7), 251 (−16.4), 294 (−9.5) nm for (−)-7; ESIMS *m*/*z* 441 $[M + H]^+$; HRESIMS *m*/*z* 441.3366 $[M + H]^+$ (calcd $C_{29}H_{45}O_3$ 441.3363).

X-ray Crystallographic Analyses. *Crystallographic Data of **1**.* $C_{29}H_{44}O_3$, *M* = 440.64, monoclinic, space group $P2_1$ (no. 4), *a* = 9.11100(10) Å, *b* = 9.88290(10) Å, *c* = 14.9752(2) Å, α = 90°, β = 100.1270(10)°, γ = 90°, *V* = 1327.41(3) Å^3, *Z* = 2, *T* = 291(2) K, μ(Cu Kα) = 0.592 mm^{-1}, D_{calcd} = 1.102 g/cm^3, 11 309 reflections measured (14.994° ≤ 2Θ ≤ 139.392°), 4688 unique (R_{int} = 0.0152, R_{sigma} = 0.0172) which were used in all calculations. The final R_1 was 0.0355 ($I > 2\sigma(I)$), and wR_2 was 0.1040 (all data). The goodness-of-fit on F^2 was 1.060. Flack parameter = 0.06 (5). (CCDC1495749).

*Crystallographic Data of **2**.* $C_{29}H_{44}O_3$, *M* = 440.00, monoclinic, space group $P2_1$ (no. 4), *a* = 8.78970(10) Å, *b* = 12.3070(2) Å, *c* = 26.0231(3) Å, α = 90°, β = 93.9320(10)°, γ = 90°, *V* = 2808.42(6) Å^3, *Z* = 4, *T* = 288(2) K, μ(Cu Kα) = 0.562 mm^{-1}, D_{calcd} = 1.118 g/cm^3,

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26 234 reflections measured (7.95° ≤ 2Θ ≤ 141.348°), 10 061 unique (R_{int} = 0.0163, R_{sigma} = 0.0177) which were used in all calculations. The final R_1 was 0.0402 ($I > 2\sigma(I)$), and wR_2 was 0.1269 (all data). The goodness-of-fit on F^2 was 1.031. Flack parameter = 0.05 (4). (CCDC 1495761)

Crystallographic Data of ***3***. $C_{29}H_{44}O_3$, M = 440.64, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 8.41900(10) Å, b = 17.21920(10) Å, c = 18.20720(10) Å, α = 90°, β = 90°, γ = 90°, V = 2639.47(4) Å^3, Z = 4, T = 290(2) K, μ(Cu Kα) = 0.538 mm^{-1}, D_{calcd} = 1.109 g/cm^3, 23 773 reflections measured (7.066° ≤ 2Θ ≤ 142.604°), 5034 unique (R_{int} = 0.0194, R_{sigma} = 0.0142) which were used in all calculations. The final R_1 was 0.0307 ($I > 2\sigma(I)$), and wR_2 was 0.0862 (all data). The goodness-of-fit on F^2 was 1.039. Flack parameter = −0.05 (5). (CCDC 1495762)

Crystallographic data of ***4***. $C_{29}H_{44}O_3$, M = 440.64, monoclinic, space group $P2_1$ (no. 4), a = 10.928(2) Å, b = 6.4320 (13) Å, c = 19.345 (4) Å, α = 90°, β = 95.38(3)°, γ = 90°, V = 1353.85(5) Å^3, Z = 2, T = 298(2) K, μ(Mo Kα) = 0.068 mm^{-1}, D_{calcd} = 1.08093 g/cm^3. The R (reflections) was 0.0742 (1121), and wR_2 (reflections) was 0.0978 (2724). The goodness-of-fit on F^2 was 1.001. (CCDC 1495774)

Synthetic Experimental Part. *Acylphloroglucinol (**12**).*[10] To a stirred suspension of phloroglucinol (**11**) (10.0 g, 0.079 mol) in a mixture of CH_2Cl_2 (80 mL) and nitromethane (80 mL) was added aluminum trichloride (42.3 g, 0.317 mol, 4 equiv), and the mixture was stirred at room temperature for 30 min. To this dark suspension acetyl chloride (5.7 mL, 0.079 mol, 1 equiv) was added slowly by syringe and refluxed for 3 h until completion of the reaction checked by TLC. Thereafter, the mixture was cooled to room temperature and poured into ice−water followed by evaporation of the volatiles under reduced pressure. Then the water phase was extracted by EtOAc (3 × 160 mL). The combined organic layers were washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the residue by silica gel chromatography (PE−EtOAc, 2:1) provided acylphloroglucinol (**12**) (8.1 g, 61%) as yellowish crystals. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 12.20 (2H, br s, OH), 10.33 (1H, brs, OH), 5.80 (2H, s, Ar), 2.54 (3H, s, Me). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 202.4 (C = O), 164.7 (C-4), 164.3 (C-2, C-6), 104.0 (C-1), 94.6 (C-3, C-5), 32.3 (COMe). ESIMS m/z 169 [M + H]$^+$, 167 [M − H]$^-$.

*2-Acetyl-3,5-dihydroxy-4,6,6-trimethylcyclohexa-2,4-dienone (**13**).*[10c] To a solution of acylphloroglucinol (**12**) (5 g, 29.8 mmol) and *tert*-BuOK (12.4 g, 110 mmol, 3.7 equiv) in anhydrous MeOH (70 mL) was added methyl iodide (5.6 mL, 89.6 mmol, 3 equiv). The resulting mixture was heated to reflux for 7 h and then cooled to room temperature. The mixture was concentrated under reduced pressure, and the residue was redissolved in water and acidified with 1 M HCl aqueous solution before it was extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on a silica gel colunm and eluted with PE−EtOAc (9:1 to 4:1) to provide **13** (3.56 g, 57%). Light yellow powder, ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 18.94 (1H, brs, chelated−OH), 2.49 [3H, s, C(O)Me], 1.79 (3H, s, Me), 1.30 (6H, s, Me × 2). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 199.2 (C = O), 196.0 (C = O), 188.7 (C−OH), 176.1 (C−OH), 105.0, 101.8, 48.2 [C(Me)$_2$], 27.7 [C(O)Me], 24.3 (Me × 2), 7.2 (Me). ESIMS m/z 211 [M + H]$^+$, 209 [M − H]$^-$.

*3,5-Dihydroxy-4,6,6-trimethylcyclohexa-2,4-dienone (**14**).* A suspension of **13** (200 mg, 0.95 mol) in 3 M HCl (20 mL) was refluxed at 110 °C for 2 h until the starting material had disappeared (TLC control). Thereafter, the mixture was cooled to room temperature and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed twice with water, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was passed through a silica gel column using PE−EtOAc (2:1) as eluent to provide **14** (144 mg, 90%) as a light yellow oil. **14** was prone to isomerization, so it was used immediately for the next step.

*(Z/E)-5-Hydroxy-4,6,6-trimethyl-2-(2-methylpropylidene)-cyclohex-4-ene-1,3-dione (**15**).* To a suspension of **14** (200 mg, 1.19 mmol) in CH_2Cl_2 (3.6 mL) was added isobutyraldehyde (164 μL, 1.8 mmol, 1.5 equiv) followed by dropwise addition of piperidine (68 μL, 2.4 mol, 2 equiv) at room temperature. After being stirred for 15 min, the reaction mixture was quenched with 1 M HCl-saturated NH_4Cl solution (2 mL) and stirred vigorously for 1 h. This mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was passed through a short flash silica gel column using CH_2Cl_2 as eluent to afford **15** (79 mg, 30%) as yellow oil. **15** was rather unstable and used immediately for the next step.

*Frutescone D−F (**4**−**6**).* A solution of **15** (60 mg, 0.27 mmol) and β-caryophyllene (184 μL, 0.81 mmol, 3 equiv) in toluene−H_2O (3:1, 7.2 mL) was refluxed at 110 °C for 4 h under a nitrogen atmosphere. The reaction mixture was concentrated to dryness in vacuo and dissolved in anhydrous EtOAc−MeOH (5:1, 3 mL). Then a solution of $TMSCHN_2$ in diethyl ether (0.5 mL, 1 mmol, 2 M in hexane) was added slowly at room temperature under nitrogen atmosphere, and the mixture was stirred for 2 h. The mixture was concentrated, and the residue was subjected to silica gel column chromatography with PE−EtOAc (100:0 to 95:5, v/v) as eluent to yield four fractions (A−D). Then fraction B was purified by recycled preparative HPLC (MeCN−H_2O, 80:20, 12 mL/min) to afford **4** (23.4 mg, 19.7%), **5** (22.0 mg, 18.5%), and **6** (24.5 mg, 20.6%). The synthetic compounds **4**−**6** were identical by TLC, NMR, and chiral HPLC comparison in with the natural products **4**−**6** (see the Supporting Information). Compound **4**, light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ_H 1.87 (3H, s, Me-7), 1.27 (3H, s, Me-8), 1.20 (3H, s, Me-9), 2.73 (1H, brs, H-10), 0.84 (3H, d, J = 7.1 Hz, Me-12), 2.47 (1H, brs, H-1′), 5.34 (1H, brs, H-5′), 5.13 (1H, brs, H-5′), 1.37 (1H, m, H-9′), 0.87 (3H, s, Me-12′), 0.91 (3H, s, Me-13′), 1.71 (3H, s, Me-14′), 3.81 (3H, s, OMe). ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ_C 108.7 (C-1), 188.5 (C-2), 117.4 (C-3), 171.3 (C-4), 43.1 (C-5), 169.3 (C-6), 10.1 (C-7), 23.3 (C-9), 21.0 (C-12), 54.8 (C-1′), 125.9 (C-5′), 32.3 (C-11′), 30.0 (C-12′), 25.1 (C-13′), 61.8 (OMe). Compound **5**, light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.87 (3H, s, Me-7), 1.32 (3H, s, Me-8), 1.23 (3H, s, Me-9), 2.35 (1H, dd, J = 7.0, 2.4 Hz, H-10), 2.10−1.95 (1H, m, H-11), 0.89 (3H, d, J = 7.1 Hz, Me-12), 0.75 (3H, d, J = 7.1 Hz, Me-13), 2.15−2.05 (1H, m, H-1′), 1.86−1.75 (1H, m, H-2′a), 1.40 (1H, m, H-2′b), 2.10−1.95 (1H, m, H-3′a), 1.89 (1H, m, H-3′b), 2.14 (1H, m, H-5′), 1.64−1.49 (2H, m, H-6′), 2.50−2.37 (1H, m, H-7′a), 2.10−1.95 (1H, m, H-7′b), 2.47−2.36 (1H, m, H-9′), 1.80−1.65 (2H, m, H-10′), 1.02 (3H, s, Me-12′), 0.99 (3H, s, Me-13′), 0.99 (3H, s, Me-14′), 4.94 (1H, brs, H-15′a), 4.87 (1H, brs, H-15′b), 3.83 (3H, s, OMe). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 114.8 (C-1), 187.8 (C-2), 118.0 (C-3), 172.1 (C-4), 42.5 (C-5), 169.8 (C-6), 10.0 (C-7), 23.6 (C-8), 23.9 (C-9), 40.4 (C-10), 31.9 (C-11), 20.8 (C-12), 19.3 (C-13), 54.2 (C-1′), 21.8 (C-2′), 39.2 (C-3′), 82.6 (C-4′), 38.5 (C-5′), 36.2 (C-6′), 35.5 (C-7′), 152.8 (C-8′), 42.2 (C-9′), 36.0 (C-10′), 33.7 (C-11′), 30.7 (C-12′), 22.6 (C-13′), 21.3 (C-14′), 111.2 (C-15′), 61.8 (OMe). Compound **6**, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.90 (3H, s, Me-7), 1.31 (3H, s, Me-8), 1.29 (3H, s, Me-9), 2.70 (1H, m, H-10), 2.08−2.00 (1H, m, H-11), 1.17 (3H, d, J = 6.9 Hz, Me-12), 0.64 (3H, d, J = 6.9 Hz, Me-13), 1.60−1.53 (1H, m, H-1′), 1.63−1.56 (1H, m, H-2′a), 1.36 (1H, m, H-2′b), 2.08−2.00 (1H, m, H-3′a), 1.48 (1H, m, H-3′b), 1.79 (1H, m, H-5′), 1.82−1.70 (2H, m, H-6′), 2.48−2.39 (2H, m, H-7′), 2.48−2.39 (1H, m, H-9′), 1.70 (1H, m, H-10′a), 1.63−1.56 (1H, m, H-10′b), 0.94 (3H, s, Me-12′), 0.97 (3H, s, Me-13′), 1.30 (3H, s, Me-14′), 4.89 (2H, brs, H-15′), 3.84 (3H, s, OMe). ^{13}C NMR (75 MHz, $CDCl_3$) δ 112.7 (C-1), 188.3 (C-2), 117.5 (C-3), 171.9 (C-4), 42.7 (C-5), 168.9 (C-6), 10.1 (C-7), 24.4 (C-8), 23.5 (C-9), 35.8 (C-10), 26.0 (C-11), 26.8 (C-12), 19.7 (C-13), 57.1 (C-1′), 23.7 (C-2′), 44.5 (C-3′), 84.2 (C-4′), 39.7 (C-5′), 25.1 (C-6′), 35.8 (C-7′), 151.4 (C-8′), 41.8 (C-9′), 36.6 (C-10′), 34.4 (C-11′), 29.9 (C-12′), 21.9 (C-13′), 23.1 (C-14′), 110.9 (C-15′), 61.8 (OMe).

*(±)-Frutescone G (**7**).* A solution of **15** (50 mg, 0.23 mmol) and α-humulene (**10**) (155 μL, 0.68 mmol, 3 equiv) was reacted in toluene−H_2O (3:1, 6 mL) and refluxed at 110 °C for 4 h under a nitrogen atmosphere. The reaction mixture was concentrated to dryness in vacuo and redissolved in anhydrous EtOAc−MeOH (5:1, 2.5 mL). Then a solution of $TMSCHN_2$ in diethyl ether (0.35 mL, 0.7 mmol, 2 M in hexane) was added slowly at room temperature under nitrogen

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atmosphere, and the mixture was stirred for 2 h. The mixture was concentrated in vacuo, and the residue was subjected to silica gel column chromatography with PE−EtOAc (100:0 to 95:5, v/v) as eluent to yield four fractions (A−F). Then fraction D was purified by preparative HPLC (MeCN−H_2O, 85:15, 12 mL/min) to afford (±)-7 (13.3 mg, 13.5%). The synthetic racemic mixture (±)-7 was identical by TLC, NMR, and chiral HPLC in comparison with the natural product (±)-frutescone G (7) (see the Supporting Information). Compound 7, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.34 (3H, s, Me-7), 1.24 (3H, s, Me-8), 1.90 (3H, s, Me-9), 2.67 (1H, t, J = 4.0 Hz, H-10), 2.17−2.00 (1H, m, H-11), 0.75 (3H, d, J = 6.8 Hz, Me-12), 0.92 (3H, d, J = 6.8 Hz, Me-13), 5.18 (1H, m, H-1′), 5.18 (1H, m, H-2′), 2.54−2.49 (1H, m, H-3′a), 2.30−2.23 (1H, m, H-3′b), 2.35−2.25 (1H, m, H-5′), 1.37−1.25 (2H, m, H-6′), 2.17−2.00 (2H, m, H-7′), 5.10 (1H, m, H-9′), 2.17−2.00 (1H, m, H-10′a), 1.90 (1H, m, H-10′b), 1.10 (3H, s, Me-12′), 1.09 (3H, s, Me-13′), 1.01 (3H, s, Me-14′), 1.62 (3H, s, Me-15′), 3.85 (3H, s, OMe). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 115.2 (C-1), 188.0 (C-2), 117.9 (C-3), 172.1 (C-4), 42.7 (C-5), 170.6 (C-6), 23.8 (C-7), 23.7 (C-8), 10.2 (C-9), 41.5 (C-10), 35.5 (C-11), 18.4 (C-12), 22.8 (C-13), 143.3 (C-1′), 121.1 (C-2′), 45.0 (C-3′), 84.6 (C-4′), 40.6 (C-5′), 33.4 (C-6′), 36.4 (C-7′), 136.1 (C-8′), 124.0 (C-9′), 41.6 (C-10′), 38.5 (C-11′), 25.9 (C-12′), 28.6 (C-13′), 21.2 (C-14′), 18.1 (C-15′), 61.8 (OMe).

Cell Viability Assay. The effects of the isolates on cell viability were measured by the MTT reduction assay. Caco-2, A549, and HepG2 human cancer cells were seeded onto 96-well plates (1×10^5 cells/mL in 100 μL of medium) and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Each tumor cell line was exposed to the test compounds at various concentrations in triplicate for 24 h, with doxorubicin as positive control. Then 20 μL of MTT solution (5 mg/mL) was added to each well and incubated continuously for another 4 h at 37 °C. After the supernatant was removed, the formed formazan crystals were dissolved in DMSO (150 μL/well) by constant shaking for 10 min. The absorbance was measured on an Infinite M200 Pro (Tecan) microplate reader at a test wavelength of 490 nm. IC_{50} was determined as the concentration that inhibited cell growth by 50% using the MTT assay. The data represent the mean of three experiments performed in triplicate and are expressed as means ± SD.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02643.

ECD calculations and MS and NMR spectra for **1**−**7** (PDF)
Crystallographic data for **1** (CIF)
Crystallographic data for **2** (CIF)
Crystallographic data for **3** (CIF)
Crystallographic data for **4** (CIF)

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Notes

The authors declare no competing financial interest.

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