Sarmentosumols A to F, New Mono- and Dimeric Alkenylphenols from *Piper sarmentosum*

Shi-Xian Yang[1], Qian-Yun Sun[2], Fu-Mei Yang[2], Guang-Wan Hu[1], Ji-Feng Luo[1], Yue-Hu Wang[1], Chun-Lin Long[1,3]

[1] Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, People's Republic of China

[2] Key Laboratory of Chemistry for Natural Products, Guizhou Province and Chinese Academy of Sciences, Guiyang, People's Republic of China

[3] College of Life and Environmental Sciences, Minzu University of China, Beijing, People's Republic of China

Abstract

Two new mono- and four new dimeric alkenylphenols, namely sarmentosumols A to F (**1**–**6**), were isolated from the aerial parts of *Piper sarmentosum*. The structures of these compounds were determined through a detailed analysis of NMR and MS data. Their antimicrobial activity against *Escherichia coli, Staphyloccocus aureus*, and *Candida albicans*, and their cytotoxic activity against human myeloid leukemia (K562) and human lung adenocarcinoma (A549) cell lines were also evaluated. Except for sarmentosumol A (**1**), whose MIC on *S. aureus* was reported to be 7.0 µg/mL, none of the other newly discovered compounds exhibited antimicrobial property. The studied compounds did not possess any cytotoxic property.

Key words

Piperaceae · *Piper sarmentosum* · alkenylphenols · antimicrobial activity

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

The genus *Piper* (Piperaceae) consists of approximately 2000 species. These are mainly distributed in the tropical areas, and approximately 60 species are found in China [1]. Phytochemical investigations of *Piper* species have led to the isolation of compounds such as amide alkaloids, phenylpropanoids, lignans, terpenoids, kawapyrones, piperolides, flavonoids, and alkenylphenols [2–4]. *P. sarmentosum* Roxb. is a perennial herb commonly found in China including Fujian, Guangdong, Guangxi, Guizhou, Hainan, Tibet, and Yunnan. This plant is also found in other countries, including Cambodia, India, Indonesia, Laos, Malaysia, Philippines, and Vietnam [1]. The fruits, leaves, stems, and/or roots of this plant are used for the treatment of toothache, stomachache, cough, asthma, fractures, malaria, and postpartum edema of the feet [5]. In previous investigations, amide alkaloids, phenylpropanoids, lignans, and *C*-benzylated dihydroflavones have been isolated from the different parts of *P. sarmentosum* [6–14]. Interestingly, the leaves of *P. sarmentosum* are used for wrapping food in Thailand and in the Philippines. Furthermore, researchers have found three antimicrobial phenylpropanoids from the plant, proving its indigenous applications [11]. Continuing with the search for bioactive constituents from *Piper* [15], six new alkenylphenols (**1**–**6**) (**Fig. 1**) were isolated from the aerial parts of *P. sarmentosum*. The antimicrobial and cytotoxic activities of the isolates were also evaluated. In the current paper, the structural elucidation and bioassay results of the new compounds are reported.

Compound **1** was obtained in the form of pale yellow oil. The molecular formula $C_{16}H_{24}O$ assigned to compound **1** was determined by the HREIMS at *m*/*z* 232.1835 $[M]^+$ (calcd. 232.1827). The IR spectrum showed typical absorption bands for the OH (3384 cm^{-1}) and phenyl (1613, 1513, and 1455 cm^{-1}) groups. The ^1H NMR spectrum of **1** (**Table 1**) clearly showed signals for a *p*-disubstituted phenyl ring [δ_H 7.06 (2H, d, J = 8.3 Hz, H-3,5) and 6.76 (2H, d, J = 8.3 Hz, H-2,6)], and an alkenyl fragment. By comparison of the NMR data with those of the known compounds [4], compound **1** was identified as an alkenylphenol. According to the molecular formula of **1** obtained by HREIMS, the length of the side chain was 10 carbon atoms. Based on the EIMS fragments (**Fig. 2**) of **1** and its HMBC correlations (**Fig. 3**) from H_2-1′ to C-3 and C-5, and H-2′ to C-4, the double bond was located at C-2′. The geometry of the double bond was also determined by comparing the ^{13}C NMR chemical shifts of the allylic carbons [δ_C 32.5 (C-1′) and 27.2 (C-4′)] with the chemical shifts in the *cis* analogue climacostol [δ_C 33.2 (C-1′) and 27.3 (C-4′)] [16], as well as the *trans* analogue marginatine [δ_C 39.0 (C-1′) and 32.7 (C-4′)]

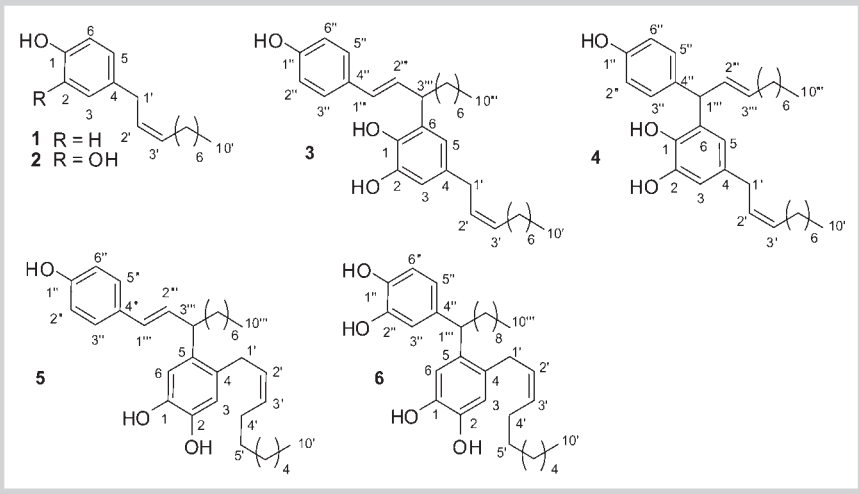

Fig. 1 Structures of compounds **1**–**6**.

Position	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	153.5 s		141.5 s	
2	115.2 d	6.76 (d, 8.3)	143.5 s	
3	129.4 d	7.06 (d, 8.3)	115.4 d	6.70 (s)
4	133.5 s		134.4 s	
5	129.4 d	7.06 (d, 8.3)	120.6 d	6.62 (d, 7.6)
6	115.2 d	6.76 (d, 8.3)	115.3 d	6.77 (d, 7.6)
1′	32.5 t	3.33 (d, 5.7)	32.7 t	3.27 (d, 5.3)
2′	128.3 d	5.51 (m)	128.1 d	5.49 (m)
3′	130.8 d	5.51 (m)	130.8 d	5.49 (m)
4′	27.2 t	2.14 (q like, 6.5)	27.2 t	2.11 (q like, 6.5)
5′	29.7 t	1.39 (m)	29.7 t	1.38 (m)
6′	29.2 t[a]	1.31 (m)	29.2 t[b]	1.30 (m)
7′	29.3 t[a]	1.31 (m)	29.3 t[b]	1.30 (m)
8′	31.9 t	1.28 (m)	31.8 t	1.27 (m)
9′	22.7 t	1.30 (m)	22.6 t	1.29 (m)
10′	14.1 q	0.89 (t, 6.6)	14.1 q	0.88 (t, 6.7)

Table 1 ^1H ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data of compounds **1** and **2**.

[a,b] Data under the same entry are interchangeable

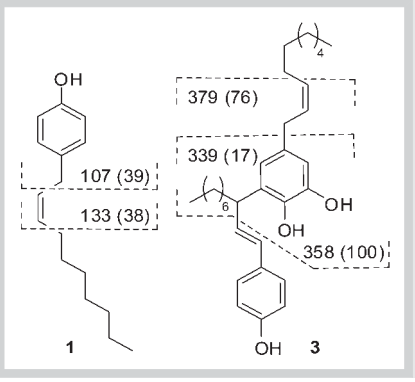

Fig. 2 EIMS fragmentation of **1** and **3** with relative intensity values in parentheses.

[17]. The values found in **1** indicated a *cis* double bond. Accordingly, the structure of **1** was elucidated as (*Z*)-4-(dec-2-enyl)phenol and was given the common name sarmentosumol A.

Based on the $[M]^+$ at *m*/*z* 248.1774 (HRESIMS), the molecular formula of compound **2** was identified to be $C_{16}H_{24}O_2$. The ^1H NMR spectrum of **2** (**Table 1**) showed the presence of a 1,2,4-trisubstituted phenyl ring [δ_H 6.77 (1H, d, *J* = 7.6 Hz, H-6), 6.70 (1H, s, H-3) and 6.62 (1H, d, *J* = 7.6 Hz, H-5)], as well as an alkenyl fragment in **2**. The NMR data of **2** were very close to those of **1**. Based on the HMBC spectrum (Supporting Information) of **2**, the difference between the two compounds depended on the substituted mode of the phenyl ring. Therefore, the structure of **2** was elucidated as (*Z*)-4-(dec-2-enyl)benzene-1,2-diol and then given the common name sarmentosumol B.

The molecular formula of compound **3** was confirmed to be $C_{32}H_{46}O_3$ by HREIMS. The ^1H NMR spectrum of **3** (**Table 2**) showed signals for a *p*-disubstituted [δ_H 7.23 (2H, d, *J* = 8.6 Hz, H-3″,5″) and 6.76 (2H, d, *J* = 8.6 Hz, H-2″,6″)] and a tetrasubstituted [δ_H 6.61 (1H, d, *J* = 1.7 Hz, H-3) and 6.56 (1H, d, *J* = 1.7 Hz, H-5)] phenyl rings, a *trans* double bond [δ_H 6.42 (1H, d, *J* = 15.9 Hz, H-1‴) and 6.19 (1H, d, *J* = 15.9, 7.4 Hz, H-2‴)], and two methyl groups [δ_H 0.88 (3H, t, *J* = 7.0 Hz, 10‴) and 0.87 (3H, t, *J* = 7.0 Hz, H-10′)]. According to the molecular formula of compound **3** and its NMR characters, it was identified as a dimer of alkenylphenols. Based on the ^1H–^1H COSY and HMBC correlations (**Fig. 3**) of **3** and its EIMS fragments (**Fig. 2**), the two monomers were determined as (*Z*)-4-(dec-2-enyl)benzene-1,2-diol and (*E*)-4-decenylphenol. In the HMBC spectrum of **3**, the correlations from H-5 to C-3‴ and H-3‴ to C-1 and C-5 were also observed. Therefore, both fragments were linked through C-6-C-3‴. However, the configuration of C-3‴ remains unclear. Thus, the structure of **3** was elucidated (**Fig. 1**) and then given the common name sarmentosumol C.

According to their HREIMS, the molecular formulae of compounds **4**–**6** were assigned to be $C_{32}H_{46}O_3$, $C_{32}H_{46}O_3$, and $C_{32}H_{48}O_4$, respectively. Their NMR data (**Tables 2** and **3**) were very similar to those of **3**, implying that these compounds might also be dimers of alkenylphenols. Based on their EIMS fragments, and ^1H–^1H COSY and HMBC correlations (Supporting Information), the structures of **4**–**6** were elucidated (**Fig. 1**) and then given the common names sarmentosumols D–F.

All of the compounds were evaluated for their cytotoxic activity against human myeloid leukemia (K562) and human lung adenocarcinoma (A549) cell lines, and antimicrobial activity against *Escherichia coli, Staphyloccocus aureus*, and *Candida albicans*. Sarmentosumol A (**1**) exhibited antimicrobial activity by inhibiting the growth of *S. aureus* with an MIC of 7.0 µg/mL. It did not exhibit any cytotoxic activity nor could it inhibit the growth of *E. coli* and *C. albicans*. Other compounds were inactive against all tested cell lines and microorganisms up to the 250 µg/mL concentration used. The inactive sarmentosumol B (**2**) only possesses one more hydroxy group at the phenyl ring than the active sarmentosumol A (**1**). Previous results showed that alkenylphenols gibbilimbols A–D with double bonds at C-3′ or C-4′ showed good inhibitory effects on *S. epidermidis* and *Bacillus cereus* with MIC values of 2.0–8.0 µg/mL [4]. These implied that the substituted modes of the benzene ring and the location of the double bonds at the side chain of alkenylphenols play significant roles on the antimicrobial activity. It is worth further clarifying the structure-activity relationship between alkenylphenols and their bioactivity.

Materials and Methods

The aerial parts of *P. sarmentosum* were collected from Xishuangbanna in Yunnan Province, PR China, in May 2011. The plant material was identified by Dr. Guang-Wan Hu at Kunming Institute

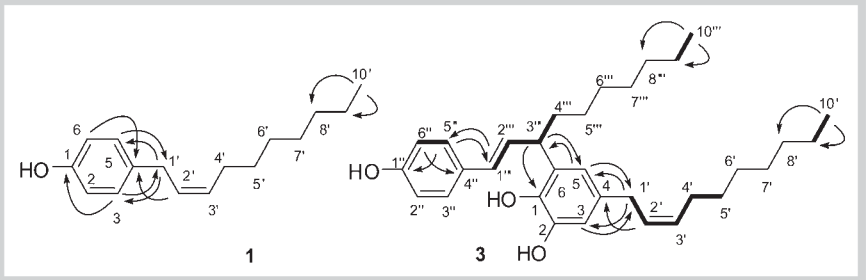

Fig. 3 Key ^{1}H–^{1}H COSY (bold) and HMBC (arrows, H→C) correlations of **1** and **3**.

Table 2 ^{1}H NMR ($CDCl_3$) data of compounds **3**–**6**, *J* in Hz.

Position	3[a]	4[a]	5[a]	6[b]
3	6.61 (d, 1.7)	6.65 (d, 1.6)	6.68 (s)	6.65 (overlapped)
5	6.56 (d, 1.7)	6.42 (d, 1.6)		
6			6.76 (s)	6.72 (overlapped)
1′	3.28 (d, 5.8)	3.24 (d, 5.8)	3.34 (dd, 15.7, 6.4)	3.31 (dd, 15.6, 7.1)
			3.29 (dd, 15.7, 6.4)	3.16 (dd, 15.6, 7.1)
2′	5.51 (m)	5.48 (m)	5.44 (m)	5.36 (m)
3′	5.50 (m)	5.47 (m)	5.46 (m)	5.45 (m)
4′	2.12 (q like, 6.4)	2.09 (m)	2.14 (q like, 6.9)	2.08 (q like, 7.0)
5′	1.39 (m)	1.36 (m)	1.42 (m)	1.37 (m)
6′–9′	1.29 (m)	1.29 (m)	1.28 (m)	1.27 (m)
10′	0.87 (t, 7.0)[c]	0.88 (t, 6.5)	0.87 (t, 6.5)[d]	0.87 (t, 6.8)[e]
2′′	6.76 (d, 8.6)	6.78 (d, 8.4)	6.74 (d, 8.4)	
3′′	7.23 (d, 8.6)	7.08 (d, 8.4)	7.19 (d, 8.4)	6.63 (overlapped)
5′′	7.23 (d, 8.6)	7.08 (d, 8.4)	7.19 (d, 8.4)	6.63 (overlapped)
6′′	6.76 (d, 8.6)	6.78 (d, 8.4)	6.74 (d, 8.4)	6.72 (overlapped)
1′′′	6.42 (d, 15.9)	4.72 (d, 7.1)	6.22 (d, 16.0)	3.91 (t, 6.7)
2′′′	6.19 (dd, 15.9, 7.3)	5.87 (dd, 15.4, 7.1)	6.08 (dd, 16.0, 7.4)	1.79 (m)
3′′′	3.57 (q like, 7.3)	5.47 (m)	3.54 (q like, 7.4)	1.22 (m)
4′′′	1.80 (m)	2.08 (m)	1.69 (m)	1.27 (m)[g]
5′′′	1.32 (m)	1.36 (m)	1.28 (m)[f]	
6′′′–9′′′	1.29 (m)	1.29 (m)		
10′′′	0.88 (t, 7.0)[c]	0.88 (t, 6.5)	0.88 (t, 6.5)[d]	0.88 (t, 6.8)[e]

[a] Measured at 500 MHz; [b] at 400 MHz; [c–e] data under the same entry are interchangeable; [f] signals for H-5′′′–H-9′′′; [g] signals for H-4′′′–H-9′′′

of Botany, and a voucher specimen (No. BN1101) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

The air-dried, powdered plants of *P. sarmentosum* (8 kg) were extracted with MeOH (3 × 15 L) under reflux (4 h, 3 h, and 3 h, respectively). The MeOH extracts were evaporated under reduced pressure. The residue was suspended in H_2O and extracted with petroleum ether and $CHCl_3$ to give two corresponding portions. According to the preliminary results on TLC, the two parts (220 g) were combined and subjected to column chromatography over silica gel G (80–100 mesh, 10 × 120 cm, 1 kg) using petroleum ether–AcOEt (10 : 1, 5 : 1, 2 : 1, and 0 : 1, each 5 L) as the eluent to yield four fractions (A–D). Fractions A–C were subjected to column chromatography over silica gel C_{18}, silica gel H, and/or Sephadex LH-20, semipreparative HPLC, and/or prep. TLC to yield **1** (13.3 mg), **2** (7.0 mg), **3** (9.8 mg), **4** (3.0 mg), **5** (10.3 mg), and **6** (6.9 mg). The details on the isolation of compounds **1**–**6** and the bioassay methods for the antimicrobial and cytotoxic activities of compounds **1**–**6** are shown in Supporting Information.

Supporting information

A detailed Materials and Methods section as well as 1D NMR and 2D NMR, UV, IR, MS, and HRMS spectra of compounds **1**–**6** are available as Supporting Information.

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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Table 3 ^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **3–6**.

Position	3	4	5	6
1	139.5 s	139.0 s	141.2 s	141.4 s
2	144.1 s	144.7 s	141.8 s	143.6 s
3	113.1 d	113.4 d	116.4 d	116.4 d
4	133.8 s	134.0 s	131.8 s	131.9 s
5	119.6 d	120.6 d	135.7 s	136.0 s
6	130.4 s	130.2 s	114.1 d	114.4 d
1′	32.9 t	32.5 t	30.2 t	30.0 t
2′	128.2 d	128.1 d	128.5 d	128.3 d
3′	130.8 d	130.8 d	130.6 d	130.7 d
4′	27.2 t	27.2 t	27.4 t	27.4 t
5′–7′	29.2–29.7 t	29.1–29.7 t	29.3–29.7 t	29.2–29.7 t
8′	31.8 t	31.8 t	31.89 t[c]	31.9 t
9′	22.65 t[a]	22.6 t	22.7 t	22.6 t
10′	14.08 q[b]	14.1 q	14.10 q[d]	14.1 q
1′′	154.9 s	154.3 s	154.4 s	141.2 s
2′′	115.4 d	115.5 d	115.4 d	143.2 s
3′′	127.6 d	129.6 d	127.4 d	114.9 d
4′′	154.9 s	134.0 s	130.7 s	138.5 s
5′′	127.6 d	129.6 d	127.4 d	120.7 d
6′′	115.4 d	115.5 d	115.4 d	115.1 d
1′′′	129.3 d	48.4 d	128.2 d	44.7 d
2′′′	130.9 d	131.0 d	132.4 d	36.7 t
3′′′	43.4 d	133.4 d	43.0 d	28.0 t
4′′′	33.7 t	32.5 t	36.1 t	29.2–29.7 t[f]
5′′′	27.6 t	29.1–29.7 t[e]	27.7 t	
6′′′,7′′′	29.2–29.7 t		29.3–29.7 t	
8′′′	31.8 t	31.8 t	31.88 t[c]	31.9 t
9′′′	22.63 t[a]	22.7 t	22.7 t	22.6 t
10′′′	14.09 q[b]	14.1 q	14.11 q[d]	14.1 q

[a–d] Data under the same entry are interchangeable, [e] Signals for C-5′′′–C-7′′′; [f] signals for C-4′′′–C-7′′′

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Correspondence
Prof. Chun-Lin Long
Kunming Institute of Botany, Chinese Academy of Sciences
132# Lanhei Road, Heilongtan
Kunming 650201
PR China
Phone: + 86 10 68 93 03 81
Fax: + 86 10 68 93 03 81
long@mail.kib.ac.cn

Dr. Yue-Hu Wang
Kunming Institute of Botany, Chinese Academy of Sciences
132# Lanhei Road, Heilongtan
Kunming 650201
PR China
Phone: + 86 87 15 22 33 18
Fax: + 86 87 15 22 33 18
wangyuehu@mail.kib.ac.cn