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Antibacterial phenolic components from *Eriocaulon buergerianum*

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Abstract

Five phenolic components, 1,3,6-trihydroxy-2,5,7-trimethoxyxanthone (**1**), 7,3′-dihydroxy-5,4′,5′-trimethoxyisoflavone (**2**), toralactone-9-*O*-β-D-glucopyranoside (**3**), patuletin-3-*O*-[2-*O*-*E*-feruloyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] (**4**), patuletin-3-*O*-[β-D-glucopyranosyl-(1 → 3)-2-*O*-*E*-caffeoyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] (**5**), along with 19 known compounds were isolated from *Eriocaulon buergerianum* (Eriocaulaceae). Their structures were determined by spectroscopic and chemical methods. All 24 isolated compounds were tested against the pathogenic bacteria *Staphylococcus aureus* (ATCC 25923); as a result, 10 compounds were found to exhibit antibacterial activity with MICs ranging from 32 to 256 μg/ml.

Keywords: *Eriocaulon buergerianum*; Eriocaulaceae; Xanthone; Flavonoid; Naphthopyranone; Antibacterial activity; *Staphylococcus aureus* (ATCC 25-923)

1. Introduction

The Eriocaulaceae family embraces 13 genera, and the genus *Eriocaulon* (Eriocaulaceae) contains around 435 species distributed throughout the world. Previous phytochemical investigations of the genus *Eriocaulon* led to the identification of flavonoids, naphthopyranones, and γ-tocopheryl acetate (Bate-Smith and Harborne, 1969; Santos et al., 2005; Ho and Chen, 2002). *Ericaulon buergerianum* is a medicinal plant used in traditional Chinese medicine under the name "Gu-Jing-Cao" as an ophthalmic, anti-inflammatory and antimicrobial medicine (State Administration of Traditional Chinese Medicine, 1999; Ho and Chen, 2002). As part of our continuous efforts to find new bioactive natural products from traditional Chinese medicine, a systematic investigation of the constituents of *E. buergerianum* was carried out. As a result, five new phenolic components **1**–**5** and 19 known compounds **6**–**24** were isolated. Their structures were determined by spectroscopic and chemical methods. Antimicrobial activity of the 24 isolated compounds against the pathogenic bacteria *Staphylococcus aureus* (ATCC 25923) was evaluated. We herein report the isolation, structural identification, and antimicrobial activity of the components from *Ericaulon buergerianum*.

2. Results and discussion

The whole plants of *E. buergerianum* were extracted with 95% EtOH, and fractionated with petroleum ether, EtOAc and *n*-BuOH, successively. Five new compounds (**1**–**5**) were obtained from the EtOAc and *n*-BuOH extracts, respectively, by repeated chromatographic methods.

Compound **1** was obtained as yellow amorphous powder with the molecular formula $C_{16}H_{14}O_8$ deduced from HREIMS and NMR spectroscopic analyses. Its IR spectrum showed the presence of hydroxyl (3246 cm^{-1}) and carbonyl (1649 cm^{-1}) groups. The absorption maxima at 362, 324 and 242 nm in the UV spectrum of **1** indicated a xanthone skeleton (Harborne, 1984). Its ^{13}C NMR

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spectrum (Table 1) displayed 16 carbon signals separated by DEPT spectrum into three methoxyl, two methine and eleven quaternary carbons (one for a carbonyl carbon). The ^{1}H NMR spectrum of **1** (Table 1) showed a singlet hydroxyl proton signal at δ 13.10, two aromatic proton resonances at δ 6.49 (1H, *s*) and 7.23 (1H, *s*), and two singlets at δ 3.73 (3H) and 3.87 (6H) due to three methoxyl groups. From above data, the structure of **1** could be deduced to be a xanthone substituted by three hydroxyl and three methoxyl functions. The positions of these substitution functions were further established according to its HMBC spectrum, in which ^{13}C–^{1}H long-range correlation signals were observed between H-4 and C-2, C-3, C-4a, C-9a; between H-8 and C-5, C-6, C-8a, C-9, C-10a; between 2-OCH$_3$ and C-2; between 5-OCH$_3$ and C-5; and between 7-OCH$_3$ and C-7, respectively. Furthermore, NOE correlation signals between 7-OCH$_3$ and H-8; and between 5-OCH$_3$ and H-4 were also found in its ROESY spectrum. Therefore, the structure of **1** was deduced to be 1,3,6-trihydroxy-2,5,7-trimethoxyxanthone. The substitution functions at C-5, C-6 and C-7 of **1** were also confirmed by comparison of its ^{13}C NMR spectroscopic data with a similar compound 1,3,6-trihydroxy-5,7-dimethoyxanthone (Iinuma et al., 1996).

Compound **2** was obtained as a yellow amorphous powder with the molecular formula $C_{18}H_{16}O_7$ deduced from HREIMS and NMR spectroscopic analyses. The IR spectrum of **2** indicated hydroxyl (3327 cm^{-1}) and carbonyl (1630 cm^{-1}) groups in its structure. Its UV spectrum exhibited absorptions at 256 and 236 nm, typical bands for an isoflavone skeleton (Mabry et al., 1970). The ^{13}C NMR spectrum of **2** (Table 1) exhibited 18 signals separated by DEPT spectrum into three methoxyl, five methine and ten quaternary carbons (one for a carbonyl carbon). Its ^{1}H NMR spectrum (Table 1) showed a singlet proton resonance at δ 8.10 (1H, *s*), a typical proton signal of H-2 of an isoflavonoid, two meta substituted aromatic proton resonances at δ 6.37 (1H, *d*, $J = 2.1$ Hz) and 6.39 (1H, *d*, $J = 2.1$ Hz), two meta substituted aromatic proton signals at δ 6.60 (1H, *d*, $J = 1.8$ Hz) and 6.58 (1H, *d*, $J = 1.8$ Hz), and three methoxyl singlets at δ 3.66 (3H), 3.75 (3H), and 3.77 (3H). From above information, the structure of **2** was deduced to be an isoflavanoid substituted by two hydroxyl and three methoxyl groups. The substitution positions of these groups were elucidated according to the HMBC spectrum, in which ^{1}H–^{13}C long-range correlations were observed between H-2 and C-3, C-4, C-8a, C-1′; between H-6 and C-4a, C-5, C-8; between H-8 and C-4a, C-6, C-7, C-8a; between H-2′ and C-3, C-1′, C-4′, C-6′; between H-6′ and C-3, C-1′, C-2′, C-4′; and between the three methoxyl proton signals (4′-OCH$_3$, 5′-OCH$_3$, 5-OCH$_3$) and C-4′, C-5′, C-5, respectively. The structure of **2** was therefore established to be 7,3′-dihydroxy-5,4′,5′-trimethoxyisoflavone.

Compound **3** was obtained as yellow amorphous powder with the molecular formula $C_{21}H_{22}O_{10}$. Its IR spectrum showed the presence of hydroxyl (3423 cm^{-1}) and ester carbonyl (1680 cm^{-1}) functionalities in the structure. The UV spectrum of **3** presented absorption maxima at 278 and 380 nm, typical bands of naphthopyranone derivatives (Vilegas et al., 1998; Piacente et al., 2001). The ^{13}C NMR spectrum (Table 2) showed 21 signals including two methyl (one for a methoxy), one methylene, nine methine, and nine quaternary carbons (one for an ester carbonyl). Among them, six resonances could be assigned to a hexose moiety. The ^{1}H NMR spectrum displayed two meta-coupled proton signals at δ 6.89 (1H, *d*, $J = 1.6$ Hz) and 6.76 (1H, *d*, $J = 1.6$ Hz), two singlets at δ 6.45 (1H) and 7.09 (1H), one methyl group resonance at δ 2.18 (3H, *s*), and one methoxyl group at δ 3.85 (3H, *s*). The toralactone skeleton

Table 1
^{1}H, ^{13}C NMR spectroscopic data and ^{13}C–^{1}H long-range correlation signals in the HMBC spectra of **1** and **2** (DMSO-d_6)

No.	**1**			No.	**2**		
	^{13}C (δ)	^{1}H (δ) *J* (Hz)	HMBC (^{1}H → ^{13}C)		^{13}C (δ)	^{1}H (δ) *J* (Hz)	HMBC (^{1}H → ^{13}C)
1	153.6 (*s*)			2	151.3 (*d*)	8.09 (*s*)	C-3, C-4, C-8a, C-1′
2	130.6 (*s*)			3	124.8 (*s*)		
3	158.0 (*s*)			4	173.7 (*s*)		
4	94.1 (*d*)	6.49 (*s*)	C-2, C-3, C-4a, C-9a	4a	107.9 (*s*)		
4a	152.3 (*s*)			5	161.3 (*s*)		
5	134.9 (*s*)			6	96.6 (*d*)	6.37 (*d*, 2.1)	C-4a, C-5, C-8
6	147.0 (*s*)			7	162.4 (*s*)		
7	146.1 (*s*)			8	94.8 (*d*)	6.39 (*d*, 2.1)	C-4a, C-6, C-7, C-8a
8	99.4 (*d*)	7.23 (*s*)	C-5, C-6, C-8a, C-9, C-10a	8a	159.1 (*s*)		
8a	110.9 (*s*)			1′	127.8 (*s*)		
9	179.3 (*s*)			2′	110.3 (*d*)	6.60 (*d*, 1.8)	C-3, C-1′, C-4′, C-6′
9a	101.9 (*s*)			3′	150.0 (*s*)		
10a	145.9 (*s*)			4′	136.0 (*s*)		
2-OCH$_3$	60.1 (*q*)	3.73 (*s*)	C-2	5′	152.8 (*s*)		
5-OCH$_3$	61.0 (*q*)	3.87 (*s*)	C-5	6′	104.7 (*d*)	6.58 (d, 1.8)	C-3, C-1′, C-2′, C-4′
7-OCH$_3$	56.0 (*q*)	3.87 (*s*)	C-7	4′-OCH$_3$	60.0 (*q*)	3.66 (*s*)	C-4′
1-OH		13.10 (*s*)	C-1, C-2, C-9a	5′-OCH$_3$	55.8 (*q*)	3.75 (*s*)	C-5′
				5-OCH$_3$	56.0 (*q*)	3.77 (*s*)	C-5

Table 2
^{1}H, ^{13}C NMR spectroscopic data and ^{13}C–^{1}H long-range correlation signals in the HMBC spectrum of **3** (DMSO-d_6)

No.	^{13}C (δ)	^{1}H (δ) J (Hz)	HMBC ($^{1}H \rightarrow {}^{13}C$)
1	166.8 (*s*)		
3	152.8 (*s*)		
4	104.2 (*d*)	6.45 (*s*)	C-3, C-4a, C-5, C-10a, C-11
4a	132.6 (*s*)		
5	111.9 (*d*)	7.09 (*s*)	C-4, C-5a, C-6, C-9a, C-10a
5a	141.7 (*s*)		
6	100.5 (*d*)	6.89 (*d*, 1.6)	C-5, C-7, C-8, C-9a
7	161.4 (*s*)		
8	101.8 (*d*)	6.76 (*d*, 1.6)	C-6, C-7, C-9, C-9a
9	157.8 (*s*)		
9a	109.3 (*s*)		
10	162.6 (*s*)		
10a	98.6 (*s*)		
11	18.9 (*q*)	2.18 (*s*)	C-3, C-4
1′	101.3 (*d*)	5.01 (*d*, 7.7)	C-9
2′	73.4 (*d*)	3.41 (*m*)	C-3′
3′	77.4 (*d*)	3.30 (*t*-like, 9.1)	C-2′, C-4′
4′	69.8 (*d*)	3.17 (*t*-like, 9.0)	
5′	76.4 (*d*)	3.46 (*m*)	
6′	60.8 (*t*)	3.84 (*m*)	
		3.70 (*d*, 10.2)	C-4′
7-OCH_3	55.6 (*q*)	3.85 (*s*)	C-7

(Susumu and Michio, 1988) could be deduced from its HMBC spectrum (Table 2), in which ^{13}C-^{1}H long-range correlation signals were observed between H-4 and C-3, C-4a, C-5, C-10a, C-11; between H-5 and C-4, C-5a, C-6, C-9a, C-10a; between H-6 and C-5, C-7, C-8, C-9a; between H-8 and C-6, C-7, C-9, C-9a; between H-11 and C-3 and C-4; and between 7-OCH_3 and C-7. Furthermore, the hexose was proved to be glucose upon acidic hydrolysis and subsequent co-TLC analysis with authentic sugar samples. The anomeric proton signal at δ 5.01 (1H, *d*, $J = 7.7$ Hz) indicated the β-configuration of the glucose unit. ^{13}C–^{1}H long-range correlation resonance between H-1′ and C-9 in the HMBC spectrum indicated the linkage site of the glucose moiety to the aglycone. The structure of **3** was therefore deduced to be toralactone-9-*O*-β-D-glucopyranoside.

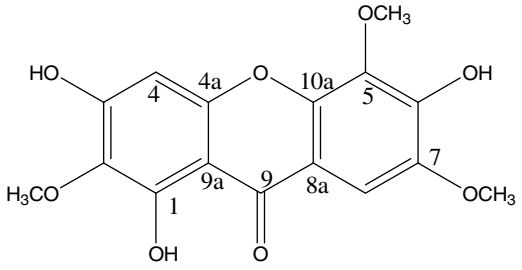
Compound **4** was obtained as a yellow amorphous powder with molecular formula $C_{38}H_{40}O_{21}$. Its IR spectrum showed the presence of hydroxyl (3406 cm^{-1}) and conjugated ester carbonyl (1701 cm^{-1}) groups. The UV spectrum exhibited absorptions at 332 and 282 nm, which were consistent with the presence of a flavonol skeleton (Mabry et al., 1970). The ^{1}H NMR spectrum (Table 3) showed signals at δ 7.54 (1H, *d*, $J = 2.3$ Hz), 7.52 (1H, dd, $J = 2.3$, 8.2 Hz), and 6.83 (1H, *d*, $J = 8.2$ Hz) due to one 1,3,4-trisubstituted benzene ring, an aromatic proton signal at δ 6.47 (1H, *s*), and a methoxyl group at δ 3.73 (3H, *s*). The confirmation of the skeleton and assignment of substitution functions were made on the basis of its HMBC spectrum, in which ^{1}H-^{13}C long-range correlation signals were observed between H-8 and C-4, C-4a, C-6, C-7, C-8a; between H-2′ and C-2, C-1′, C-4′, C-6′; between H-6′ and C-2, C-1′, C-2′, C-4′; between H-5′ and C-1′, C-3′, C-4′, C-6′; and between the methoxyl group of 6-OCH_3 and C-6. From the above evidence, the aglycone was established to be patuletin (Horie et al., 1997). In its ^{1}H NMR spectrum, signals due to an ABX coupling system at δ 7.34 (1H, *d*, $J = 1.8$ Hz), 7.09 (1H, dd, $J = 1.8$, 8.1 Hz) and 6.83 (1H, *d*, $J = 8.1$ Hz); a pair of olefinic proton signals at δ 6.21 (1H, *d*, $J = 15.7$ Hz) and 7.47 (1H, *d*, $J = 15.7$ Hz); and a methoxyl proton resonance at δ 3.88 (3H, *s*) suggested a feruloyl moiety in its structure, which was further confirmed by analysis of its HMBC spectrum. Acidic hydrolysis of **4** afforded glucose as its sugar component. The two anomeric proton signals at δ 5.54 (1H, *d*, $J = 7.9$ Hz) and 4.39 (1H, *d*, $J = 7.7$ Hz) indicated their β-glucosidic linkage. Furthermore, the interlinkage sites of the aglycone, two sugar moieties and the feruloyl group were established on the basis of the ^{13}C–^{1}H long-range correlation resonances between H-1″ and C-3; between H-1‴ and C-6″; and between H-2‴ and C-9⁗. The structure of **4** was finally established to be patuletin-3-*O*-[2-*O*-*E*-feruloyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside].

Compound **5** was obtained as a yellow amorphous powder with molecular formula $C_{43}H_{48}O_{26}$, deduced from HRESIMS and NMR analyses. Comparisons of its ^{1}H and ^{13}C NMR spectra with those of **4** indicated their structural similarity except for the existence of one more sugar moiety and one less methoxy group in the structure of **5.** Analyses of the ^{1}H–^{1}H COSY, HMQC and HMBC spectra of **5** confirmed the above deduction and indicated a caffeoyl group in the structure. Acidic hydrolysis of **5** yielded only glucose as its sugar component. The three anomeric proton signals at δ 5.53 (1H, $J = 7.5$ Hz), 4.39

Table 3
^{1}H, ^{13}C NMR spectroscopic data and ^{13}C–^{1}H long-range correlation signals in the HMBC spectra of **4** and **5** (DMSO-d_6)

No.	**4**			**5**		
	^{13}C (δ)	^{1}H (δ) J (Hz)	HMBC ($^{1}H \rightarrow {}^{13}C$)	^{13}C (δ)	^{1}H (δ) J (Hz)	HMBC ($^{1}H \rightarrow {}^{13}C$)
2	156.5 (*s*)			156.7 (*s*)		
3	132.9 (*s*)			132.8 (*s*)		
4	177.8 (*s*)			177.9 (*s*)		
4a	104.3 (*s*)			104.6 (*s*)		
5	152.2 (*s*)			152.4 (*s*)		
6	131.6 (*s*)			131.6 (*s*)		
7	158.1 (*s*)			157.4 (*s*)		
8	94.4 (*d*)	6.47 (*s*)	C-4, C-4a, C-6, C-7, C-8a	94.2 (*d*)	6.48 (*s*)	C-4, C-4a, C-6, C-7, C-8a
8a	151.8 (*s*)			151.6 (*s*)		
1′	121.2 (*s*)			121.1 (*s*)		
2′	116.2 (*d*)	7.54 (*d*, 2.3)	C-2, C-1′, C-4′, C-6′	116.2 (*d*)	7.54 (*d*, 2.2)	C-2, C-3′, C-4′, C-6′
3′	144.8 (*s*)			144.9 (*s*)		
4′	148.6 (*s*)			148.6 (*s*)		
5′	115.7 (*d*)	6.83 (*d*, 8.2)	C-1′, C-3′, C-4′, C-6′	115.4 (*d*)	6.84 (*d*, 8.5)	C-1′, C-3′, C-4′
6′	121.7 (*d*)	7.52 (*dd*, 8.2, 2.3)	C-2, C-1′, C-2′, C-4′	121.7 (*d*)	7.50 (*dd*, 8.5, 2.2)	C-2, C-2′, C-4′
6-OMe	60.1 (*q*)	3.73 (*s*)	C-6	60.4 (*q*)	3.70 (*s*)	C-6
5-OH		12.59 (*s*)			12.57 (*s*)	
Glucosyl						
1″	100.8 (*d*)	5.54 (*d*, 7.9)	C-3	100.3 (*d*)	5.53 (*d*, 7.5)	C-3
2″	73.7 (*d*)	3.22 (*t*-like, 8.6)	C-1″, C-3″	73.6 (*d*)	3.31 (*m*)	C-3″
3″	76.2 (*d*)	3.13 (*m*)	C-2″, C-4″	76.2 (*d*)	3.22 (*m*)	C-2″
4″	69.7 (*d*)	2.97 (*t*-like, 9.1)	C-3″, C-5″	69.8 (*d*)	3.00 (*m*)	C-3″
5″	78.4 (*d*)	3.08 (*m*)	C-4″	78.8 (*d*)	3.20 (*m*)	
6″	66.9 (*t*)	3.53 (*m*)	C-5″, C-1‴	65.9 (*t*)	3.66 (*m*)	C-1‴
		3.74 (*m*)	C-1‴		3.74 (*m*)	C-1‴
1‴	100.2 (*d*)	4.31 (*d*, 7.7)	C-6″	99.4 (*d*)	4.39 (*d*, 7.7)	C-6″
2‴	73.9 (*d*)	4.36 (*t*-like, 8.7)	C-1‴, C-3‴, C-9⁗	72.2 (*d*)	4.48 (*t*-like, 8.4)	C-1‴, C-3‴, C-9⁗′
3‴	74.3 (*d*)	2.76 (*t*-like, 8.7)	C-2‴, C-4‴	86.3 (*d*)	2.64 (*t*-like, 8.6)	C-2‴, C-4‴, C-1⁗
4‴	69.7 (*d*)	3.07 (*m*)	C-5‴	67.8 (*d*)	3.24 (*m*)	C-5‴
5‴	76.4 (*d*)	2.57 (*m*)		75.4 (*d*)	2.53 (*m*)	
6‴	60.1 (*t*)	3.38 (*m*)		59.5 (*t*)	3.27 (*m*)	C-5‴
		3.69 (*m*)			3.36 (*m*)	
1⁗				103.4 (*d*)	3.75 (*d*, 7.8)	C-3‴
2⁗				73.4 (*d*)	2.82 (*t*-like, 8.7)	C-1⁗, C-3⁗
3⁗				75.8 (*d*)	3.18 (*m*)	C-2⁗, C-4⁗
4⁗				70.0 (*d*)	2.95 (*m*)	C-3⁗
5⁗				76.4 (*d*)	3.40 (*m*)	C-6⁗
6⁗				61.1 (*t*)	3.46 (*m*)	C-5⁗
					3.81 (*m*)	
Feruloyl/Caffeoyl						
1⁗/1⁗′	125.9 (*s*)			125.9 (*s*)		
2⁗/2⁗′	111.1 (*d*)	7.34 (*d*, 1.8)	C-3⁗, C-4⁗, C-6⁗, C-7⁗	115.3 (*d*)	7.07 (*d*, 1.8)	C-3⁗′, C-4⁗′, C-6⁗′, C-7⁗′
3⁗/3⁗′	148.1 (*s*)			145.6 (*s*)		
4⁗/4⁗′	149.3 (*s*)			148.3 (*s*)		
5⁗/5⁗′	115.4 (*d*)	6.83 (*d*, 8.1)	C-1⁗, C-3⁗, C-4⁗	116.0 (*d*)	6.81 (*d*, 8.0)	C-1⁗′, C-3⁗′, C-4⁗′
6⁗/6⁗′	123.0 (*d*)	7.09 (*dd*, 8.1, 1.8)	C-2⁗, C-4⁗, C-7⁗	121.3 (*d*)	7.02 (*dd*, 8.0, 1.8)	C-2⁗′, C-4⁗′, C-7⁗′
7⁗/7⁗′	145.2 (*d*)	7.47 (*d*, 15.7)	C-1⁗, C-2⁗, C-6⁗, C-8⁗, C-9⁗	145.4 (*d*)	7.36 (*d*, 16.7)	C-1⁗′, C-2⁗′, C-6⁗′, C-8⁗′, C-9⁗′
8⁗/8⁗′	114.7 (*d*)	6.21 (*d*, 15.7)	C-1⁗, C-9⁗	113.9 (*d*)	6.07 (*d*, 16.7)	C-1⁗′, C-9⁗′
9⁗/9⁗′	165.9 (*s*)			166.1 (*s*)		
3⁗-OMe	55.9 (*q*)	3.88 (*s*)	C-3⁗			

(1H, $J = 7.7$ Hz), and 3.75 (1H, $J = 7.8$ Hz) indicated the β-configuration of the three glucose units. The interlinkage sites of the aglycone, three sugar moieties and the caffeoyl group of **5** were established on the basis of ^{13}C–^{1}H long-range correlation signals between H-1″ and C-3; between H-1‴ and C-6″; and between H-2‴ and C-9⁗′. The third glucose unit was deduced to connect to C-3‴ from the correlation signal between H-1⁗ with C-3‴. The downfield chemical shift of C-3‴ (~12.0 ppm), upfield chemical shift of C-2‴ (~1.7 ppm) and C-4‴ (~1.9 ppm) in comparison with those corresponding to carbons in **4** further confirmed such a substitution (Markham et al., 1978). The structure of **5** was finally identified to be patuletin-3-*O*-[β-D-glucopyranosyl-(1 → 6)-2-*O*-*E*-caffeoyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside].



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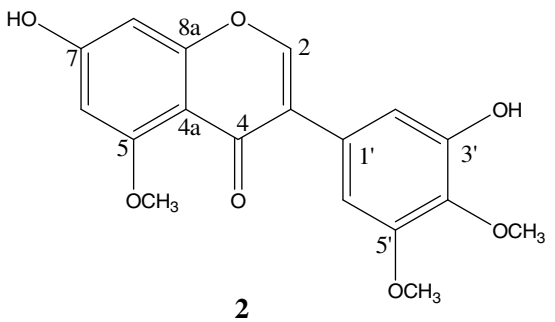

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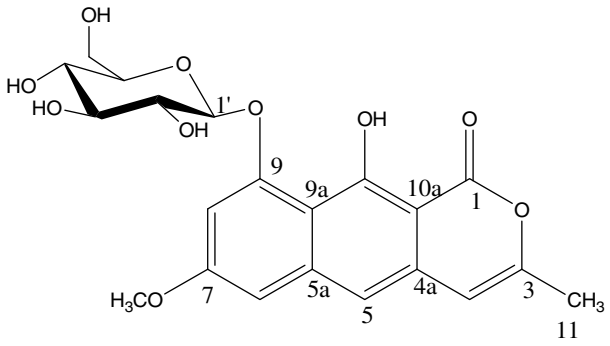

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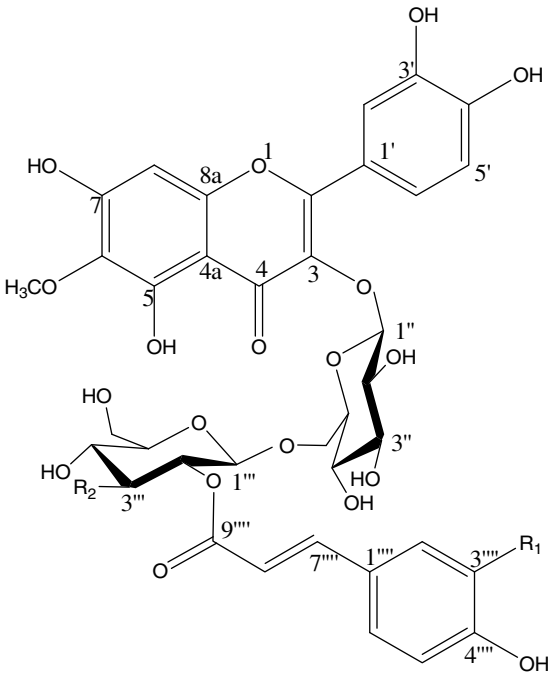
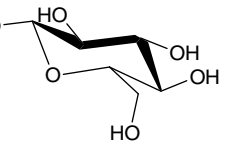


4 $R_1 = OCH_3$, $R_2 = OH$

5 $R_1 = OH$, $R_2 =$ 

The 19 known compounds were identified as (R)-semixanthomegnin (**6**) (Cotterill et al., 2003), 5,7,3′-trihydroxy-6,4′,5′-trimethoxyisoflavone (**7**) (Wollenweber et al., 2003), vanillic acid (**8**), ferulic acid (**9**) (Shen and Lin, 1996), 1,3,6,8-tetrahydroxy-2,7-dimethoxyxanthone (**10**) (Pinheiro et al., 1998), 1,3,6,8-tetrahydroxy-2-methoxyxanthone (**11**) (Iinuma et al., 1997), emodin (**12**) (Cohen and Towers, 1995), hispidulin (**13**), patuletin (**14**) (Horie et al., 1997), protocatechuic acid (**15**) (Sang et al., 2002), gerontoisoflavone A (**16**) (Chang et al., 1995), 3,4-dihydro-10-hydroxy-7-methoxy-3-(R)-methyl-1H-3,4-dihydronaphtho-[2,3c]-pyran-1-one-9-*O*-β-D-glucopyranoside (**17**), 5,4′-dihydroxy-6,3′-dimethoxyflavone-7-*O*-β-D-glucopyranoside (**18**) (Yuldashev et al., 1996), hispidulin-7-*O*-β-D-glucopyranoside (**19**) (Hase et al., 1995), patuletin-3-*O*-β-D-glucopyranoside (**20**) (Barron and Ibrahim, 1988), 3,4-dihydro-10-hydroxy-7-methoxy-3-(R)-methyl-1H-3,4-dihydronaphtho-[2,3c]-pyran-1-one-9-*O*-β-D-glucopyranosyl-

(1 → 6)-glucopyranoside (**21**), 3,4-dihydro-10-hydroxy-7-methoxy-3-(R)-methyl-1H-3,4-dihydronaphtho-[2,3c]-pyran-1-one-9-*O*-β-D-allopyranosyl-(1 → 6)-glucopyranoside (**22**) (Vilegas et al., 1999), patuletin-3-*O*-β-D-gentiobioside (**23**) (Aritomi et al., 1986), and patuletin-3-*O*-β-D-rutinoside (**24**) by comparison of their spectroscopic data with those reported in the literature. Among them, (R)-semiximthomegnin (**6**) was a synthetic compound, and was isolated from nature for the first time.

The whole plants of *E. buergerianum* were used in traditional Chinese medicine as an ophthalmic, anti-inflammatory and antimicrobial medicine. *S. aureus* is a virulent pathogen that is currently the most common cause of infections (Archer, 1998), including many ophthalmic diseases (Nakata et al., 2000; Shanmuganathan et al., 2005; Kiuchi et al., 1993; Ahmad et al., 2006). All compounds (**1-24**) purified from *E. buergerianum* were therefore evaluated against a standard strain of *S. aureus* (ATCC 25923). As a result, ten components were found to exhibit antibacterial activity with minimum inhibitory concentrations (MICs) ranging from 32 to 256 μg/ml (Table 4).

The Eriocaulaceae is a natural group of herbaceous monocotyledons, characterized by small flowers densely distributed in the capitula. The family embraces 13 genera occurred predominantly in tropical region. The infrafamilial taxonomy of the Eriocaulaceae is complex, the isolated compounds from *E. buergerianum* show taxonomic signification for the delimitation of the genera in the Eriocaulaceae.

Paepalanthus is the largest genus in the family Eriocaulaceae, and naphthopyranones isolated from many species of *Paepalanthus* (Vilegas et al., 1990, 1999, 1998; Andrade et al., 1999, 2002; Santos et al., 2001; Piacente et al., 2001; Dokkedal et al., 2004) have been used as the chemical markers for *Paepalanthus*. The naphthopyranones were also isolated from the *E. buergerianum* in the present study and in *E. ligulatum* (Santos et al., 2005), which supported the approximation of the two genera in evolution (Santos et al., 2005; Ricci et al., 1996). The presence of flavones in *E. buergerianum* (Ho and Chen, 2002) and *E. ligulatum* (Santos et al., 2005) is thus in disagreement to a previous study (Ricci et al., 1996) that reported *Eriocaulon* bearing only flavonols. They concluded that these species can then be sharply distinguished from *Leiothrix* and *Syngonanthus*, of which, only flavone derivatives were found. Until recently, all the flavanones and flavanols isolated from *Eriocaulon* bear a 6-methoxy functionality except for quercetagetin, which may be supposed to be evidence to distinguish *Eriocaulon* from *Leiothrix* (Dokkedal and Salatino, 1992) and *Syngonanthus*.

Table 4
The MIC values of antibacterial compounds from *Eriocaulon buergerianum*

Compounds and control	MICs (μg/ml)
12, **15**	32
6, **8**, **14**	64
11	128
9, **10**, **16**, **20**	256
Methicillin	1

3. Conclusions

E. buergerianum is used in traditional Chinese medicine as ophthalmic, anti-inflammatory and antimicrobial agent. The 10 antibacterial components found in the investigation may, therefore, contribute in part to the therapeutic effects of this medicinal plant. The newly identified components from *E. buergerianum* possess chemtaxonomic signification in the family Eriocaulaceae, which supports the approximation of *Eriocaulon* and *Paepalanthus* in evolution; and distinguished *Eriocaulon* from *Leiothrix* and *Syngonanthus*.

4. Experiment

4.1. General methods

Melting points were determined on a Shengguang apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM400 spectrometer. Chemical shifts were expressed in δ (ppm) units relative to TMS as an internal standard and coupling constants were given in Hertz. Optical rotations were measured with Perkin–Elmer 241MC polarimeter. UV spectra were recorded using a Shimadzu UV-2550 spectrometer with MeOH as solvent. IR spectra were recorded with a Perkin–Elmer 577 spectrometer. LR-ESIMS was measured using a Finnigan LCQ-DECA instrument; HR-ESIMS was measured using a Waters Q-TOF spectrometer. LR- and HR-EIMS were obtained on a MAT-95 spectrometer.

4.2. Plant material

The whole plants of *E. buergerianum* were purchased from Pan-an county, Zhejiang province of China, and identified by Professor Jingui Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (No. 20040707) has been deposited in the Herbarium of Shanghai Institute of Materia Medica.

4.3. Extraction and isolation

Chopped and air-dried whole plants of *E. buergerianum* (20.0 kg) were extracted at room temperature with 95% EtOH (24 h × 3) and concentrated under reduced pressure to give the crude extract (155.7 g). The crude extract was dissolved in 5.5 l H_2O, filtered and then fractionated with petroleum ether, EtOAc, and *n*-BuOH (each, 5.5 l × 3), successively. The EtOAc extract (62.6 g) was purified using a silica gel column, eluted with a $CHCl_3$–MeOH gradient (20:1–2:1) to give eight fractions (E1–E8). Fraction E1

(9.6 g) was successively subjected to polyamide chromatography with a H_2O–EtOH gradient (1:0–1:4), and silica gel with petroleum ether–Me_2CO (10:1–1:1) to give **1** (36 mg), **6** (21 mg), **7** (10 mg), **8** (190 mg), **9** (20 mg), **10** (52 mg), **11** (60 mg), and **12** (8 mg). Fraction E2 (1.3 g) was subjected to a silica gel column, eluted with a $CHCl_3$–MeOH gradient (50:1–20:1) to afford **13** (108 mg). Fraction E3 (11.0 g) was purified over polyamide with a H_2O–EtOH gradient (1:0–1:4) to give four subfractions ($E3_1$–$E3_4$). Subfraction $E3_1$ was separated on silica gel column eluted with $CHCl_3$–MeOH (25:1) to yield **15** (140 mg). Sediment from the MeOH solution of $E3_2$ afforded **2** (70 mg). Yellow needle crystals from $E3_3$ were **16** (90 mg). $E3_4$ was purified on a silica gel column, eluted with $CHCl_3$–MeOH (20:1) to yield **14** (270 mg). Fraction E4 (6.9 g) was successively purified using polyamide with a H_2O–EtOH gradient (1:0–1:4), and using silica gel with $CHCl_3$–MeOH (15:1) to give **3** (19 mg) and **17** (18 mg). Fraction E5 (5.0 g) was subjected to a polyamide column eluted with a H_2O–EtOH gradient (1:0–1:4) to give **18** (110 mg). Fraction E6 (3.4 g) was purified by silica gel eluted with a $CHCl_3$–MeOH gradient (10:1–4:1) to afford **19** (38 mg) and **20** (40 mg). Fraction E7 (1.0 g) was purified using silica gel column with $CHCl_3$–MeOH (6:1) as eluent to give **21** (72 mg). Fraction E8 (9.2 g) was subjected to polyamide column eluted with a H_2O–EtOH gradient (1:0–1:4) to give subfractions $E8_1$ and $E8_2$. Subfraction $E8_1$ (1.1 g) was further purified using a silica gel column with $CHCl_3$–MeOH (5:1) as eluent to give **22** (15 mg). $E8_2$ (3.0 g) was successively purified on a silica gel column with a $CHCl_3$-MeOH gradient (6:1–3:1) and on Sephadex LH-20 with MeOH to yield **4** (17 mg) and **23** (30 mg). The *n*-BuOH extract (72.0 g) was purified using a polyamide column with a H_2O–EtOH gradient (1:0–1:4) as eluent to give fractions B1 and B2. Fraction B1 (19.8 g) was successively subjected to silica gel column eluted with a $CHCl_3$–MeOH gradient (10:1–4:1) and Sephadex LH-20 eluted with MeOH to afford **22** (340 mg), **23** (350 mg) and **24** (80 mg). Fraction B2 (40.1 g) was repeatedly purified on silica gel with $CHCl_3$-MeOH (7:2) and Sephadex LH-20 eluted with MeOH to yield **5** (65 mg).

4.3.1. 1,3,6-Trihydroxy-2,5,7-trimethoxyxanthone (*1*)

Yellow needle crystals (H_2O–EtOH), m.p. 220–222 °C. UV (MeOH) λ_{max} nm (log ε): 362 (3.92), 352 (3.91), 324 (4.16), 294 (3.89), 242 (4.36), 222 (4.25). IR (KBr) ν (cm^{-1}): 3246, 1649, 1578, 1473, 1300, 1121, 1036, 766. EIMS *m/z* (% rel. int.): 335 (19), 334 (100), 319 (98), 316 (32), 291 (89), 275 (12), 258 (13), 230 (8), 69 (7); HREIMS *m/z* 334.0695 (Calc. for $C_{16}H_{14}O_8$ 334.0689). For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1.

4.3.2. 7,3′-Dihydroxy-5,4′,5′-trimethoxyisoflavone (*2*)

Yellow amorphous powder, m.p. 262–265 °C. UV (MeOH) λ_{max} nm (log ε): 256 (4.45), 236 (4.26). IR (KBr) ν (cm^{-1}): 3327, 3217, 2937, 1630, 1579, 1466, 1309, 1205, 1155, 1105, 1088, 999, 835. EIMS *m/z* (% rel. int.): 344 (9), 329 (9), 149 (83), 112 (24), 97 (20), 85 (22), 83 (27), 71 (46), 60 (51), 57 (100), 55 (69); HREIMS *m/z* 344.0902 (Calc. for $C_{18}H_{16}O_7$ 344.0896). For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1.

4.3.3. Toralactone-9-O-β-D-glucopyranoside (*3*)

Yellow amorphous powder, m.p. 197–198 °C. $[\alpha]_D^{25}$ −92.0 (c 0.18, MeOH). UV (MeOH) λ_{max} nm (log ε): 380 (4.61), 332 (4.71), 278 (3.50), 200 (4.16). IR (KBr) ν (cm^{-1}): 3423, 2922, 1680, 1626, 1579, 1371, 1236, 1163, 1074, 1043. ESIMS *m/z*: 457 $[M+23]^+$, 433 $[M-1]^-$, 271 [M-162−1]$^-$; HRESIMS *m/z* 457.1123 (Calc. for $C_{21}H_{22}O_{10}Na$ 457.1111). For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2.

4.3.4. Patuletin-3-O-[2-O-E-feruloyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] (*4*)

Yellow amorphous powder, m.p. 226-228 °C. $[\alpha]_D^{25}$ −1.0 (c 0.28, MeOH). UV (MeOH) λ_{max} nm (log ε): 332 (4.12), 282 (4.39), 212 (3.30). IR (KBr) ν (cm^{-1}): 3406, 2928, 1701, 1603, 1516, 1471, 1369, 1275, 1202, 1072, 820. ESIMS *m/z*: 855 $[M+23]^+$, 831 $[M-1]^-$; HRESIMS *m/z* 855.1973 (Calc. for $C_{38}H_{40}O_{21}Na$ 855.1960). For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3.

4.3.5. Patuletin-3-O-[β-D-glucopyranosyl-(1 → 3)-2-O-E-caffeoyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] (*5*)

Yellow amorphous powder, m.p. 232–235 °C. $[\alpha]_D^{25}$ −10.0 (c 0.22, MeOH). UV (MeOH) λ_{max} nm (log ε): 340 (4.37), 282 (4.65), 254 (4.47), 236 (4.55), 212 (4.40). IR (KBr) ν (cm^{-1}): 3396, 2924, 1701, 1605, 1473, 1369, 1284, 1202, 1074, 818. ESIMS *m/z* 1983 $[2M+23]^+$, 1003 $[M+23]^+$, 979 $[M-1]^-$, 817 [M-162−1]$^-$; HRESIMS *m/z* 1003.2347 (Calc. for $C_{43}H_{48}O_{26}Na$ 1003.2332). For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3.

4.3.6. Acid hydrolysis of compounds *3*–*5*

A solution of each compound (10 mg) in 5% HCl (4 ml) was heated in a boiling water bath for 3 h, and then cooled and filtered. Each filtrate was neutralized with Ag_2CO_3, and the ppt. was removed. The filtrate was checked by co-TLC with authentic sugar samples, with *n*-BuOH–pyridue–H_2O (9:5:4) as developing solvent.

4.3.7. Antibacterial assays

The standard *S. aureus* strain (ATCC 25923) was obtained from a Mueller–Hinton broth culture of 24 h at 37 °C, and then diluted in a sterile 9% NaCl solution to reach the turbidity of 0.5 McFarland Standard. All isolated phenolic compounds from *E. buergerianum* were dissolved in dimethylsulfoxide (DMSO) and diluted in sterile distilled H_2O by 2-fold serial dilutions. One ml of dilution was incorporated into 9 ml of Mueller–Hinton agar so that plates contained concentrations of compounds ranging from 128 to 1 μg/ml for compounds **3**, **4**, **7** and **12,** from 256 to 1 μg/ml for the other compounds. Diluted bacterial

suspensions (2 μl) were delivered with a final inoculum of approximatively 1.5×10^4 CFU/ml per spot of inoculation on the agar plates. After incubation for 24 h at 37°C, the MIC values were defined as the lowest concentration of compound to prevent visible growth. The experiments were run in triplicate. An agar plate containing 2% DMSO without any test compound served as a negative control and no inhibition was observed. The antibiotic methicillin was tested as a positive control.

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