

Chemical Constituents from the Stems of *Manihot esculenta*

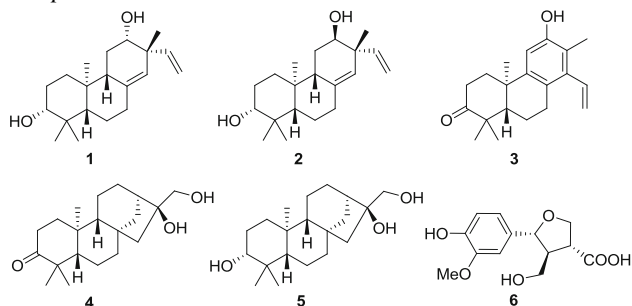


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Abstract Two new compounds, maniesculentins A (**1**) and B (**6**), together with four known ones were isolated from the stems of *Manihot esculenta* Crantz. The structures of the new compounds were elucidated by extensive spectroscopic methods including NMR spectroscopy and mass spectrometry. The two new compounds (**1**, **6**) were assayed for antibacterial activity against four tested bacteria lines.

Graphical Abstract



Keywords Euphorbiaceae · *Manihot esculenta* · Chemical constituents · Diterpenoids

1 Introduction

The Euphorbiaceae produce a diverse range of secondary metabolites. Recently, a series of secondary metabolites

with fascinating structural features and significant biological activities from Euphorbiaceae family were reported. The main constituents of Euphorbiaceae are diterpenoids with different skeletons such as jatrophanes, lathyrane, tiglanes, ingenanes, and myrsinanes with a wide array of pharmaceutical activities, such as antiproliferative, cytotoxic, antimicrobial and anti-inflammatory, anticancer and

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antioxidant activities [1–7]. *Manihot* (Euphorbiaceae), a shrub, has about 60 species and is widely cultivated in tropical regions. Two species were introduced in China, which are *M. esculenta* Crantz and *M. glaziovii*. *M. esculenta* is widely cultivated in Fujian, Guangdong, Guangxi, Guizhou, Hainan, Taiwan and Yunnan provinces of China [8]. Previous study on *M. esculenta* roots reported various types of stress metabolites, which are predominantly steroids and diterpenoids produced in the damaged cassava root tissue by cutting and fungal-infection [9]. Recently investigations in China mainly focused on the roots distributed in Hainan [10]. However, the study on the plant distributed in Yunnan has not yet reported. Therefore, searching for novel structural and bioactive natural products from its stems led to the isolation of two new compounds **1** and **6** (Fig. 1), and four known diterpenoids, yucalexin P–21 (**2**) [10, 11], cleistanthene-type sonderianol (**3**) [12], calliterpenone (**4**) [13], *ent*-kauran-3 α ,16 α ,17-triol (**5**) [14]. Herein, this paper describes the isolation, structure elucidation and antimicrobial activities of these compounds.

2 Results and Discussion

Maniesculentin A (**1**) was isolated as a white, amorphous powder. Its molecular formula $C_{20}H_{32}O_2$ was determined by HREIMS ($[M]^+$ m/z 304.2408, calcd. 304.2402), indicating five degrees of unsaturation. The IR absorption signals revealed the presence of hydroxyl (3430 cm^{-1}) and olefinic (1634 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) of **1** exhibited four angular methyl group (δ_H 0.74, 0.82, 1.01 and 1.07), two oxygenated methines [δ_H 3.26 (1H, dd, $J = 11.4$ and 4.1 Hz), 3.63 (1H, s); δ_C 78.8, 72.7], one trisubstituted double bond [δ_H (5.07, s); δ_C 138.1, 125.7], and one terminal double bond [δ_H 5.01 (1H, d, $J = 17.6$ Hz), 5.00 (1H, d, $J = 10.0$ Hz), 5.74 (1H, dd, $J = 17.6, 10.0$ Hz); δ_C 146.1, 114.2], which were supported by HSQC and HMBC

experiments (Fig. 2). The above information suggested that compound **1** should be an *ent*-pimarane-type diterpenoid. Detailed analysis of NMR data indicated that **1** was an isomer of **2** [10, 11], as indicated by the significant variation of ^{13}C NMR signals at δ_C 25.9 for C-11, 43.7 for C-13 and 23.5 for Me-17 in **1** instead of δ_C 36.8 for C-11, 47.5 for C-13 and 26.0 for Me-17 in **2**, suggesting the β -orientation for 12-OH. In addition, the ROESY correlation (Fig. 3) of H-9/H-12 further indicated β -orientation for 12-OH. The above elucidation was further confirmed by 2D NMR (HSQC, ^1H - ^1H COSY, and HMBC) (Fig. 2). Firstly, the ^1H - ^1H COSY (Fig. 2) revealed the presence of partial structures of $-\text{CH}_2-\text{CH}_2-\text{CH}$ (O)-, $-\text{CH}-\text{CH}_2-\text{CH}_2-$ and $-\text{CH}_2-\text{CH}$ (O)-, as shown with bold lines in Fig. 2. Secondly, the HMBC correlations (Fig. 2) of both two methyl protons H₃-19 (δ_H 0.82) and H₃-18 (δ_H 1.02) with C-3 (δ_C)/C-5 (δ_C 54.1)/C-4 (δ_C 38.9); the other two methyl protons H₃-20 (δ_H 0.74), and H₃-17 (δ_H 1.07) with C-1 (δ_C 36.7)/C-5 (δ_C 54.1)/C-9 (δ_C 46.3)/C-10 (δ_C 38.2), and C-12 (δ_C 72.7)/C-13 (δ_C 43.7)/C-14 (δ_C 125.7), respectively; the H-7 (δ_H 2.37 and 3.10) with C-5 (δ_C 54.1)/C-10 (δ_C 38.2)/C-14 (δ_C 125.7); and the H-11 (δ_H 1.65) with C-8 (δ_C 138.1)/C-13 (δ_C 43.7) constructed the typical fused A/B/C- ring system of the *ent*-pimarane-type diterpenoid core. The relative configuration of 3-OH was α -orientation determined by the ROESY correlation (Fig. 3) from H-3 to H-5 and the large coupling constant (dd, $J = 11.4$ and 4.1 Hz) of H-3 with H₂-2 [15]. Thus, the structure of **1** was determined as 3 α ,12 α -dihydroxy-pimara-8(14),15-dien.

Maniesculentin B (**6**) was obtained as colorless oil. Its molecular formula was deduced to be $C_{13}H_{16}O_6$ from the HREIMS at m/z 268.0943 (calcd. 268.0947), corresponding to six degrees of unsaturation. The IR spectrum showed absorption bands at 3442, 1764, 1631 and 1517 cm^{-1} , indicating the presence of hydroxyl, carbonyl and aromatic groups, respectively. The ^{13}C NMR and DEPT spectroscopic data (Table 1) exhibited 13 carbon signals,

Fig. 1 Chemical structures of compounds **1–6**

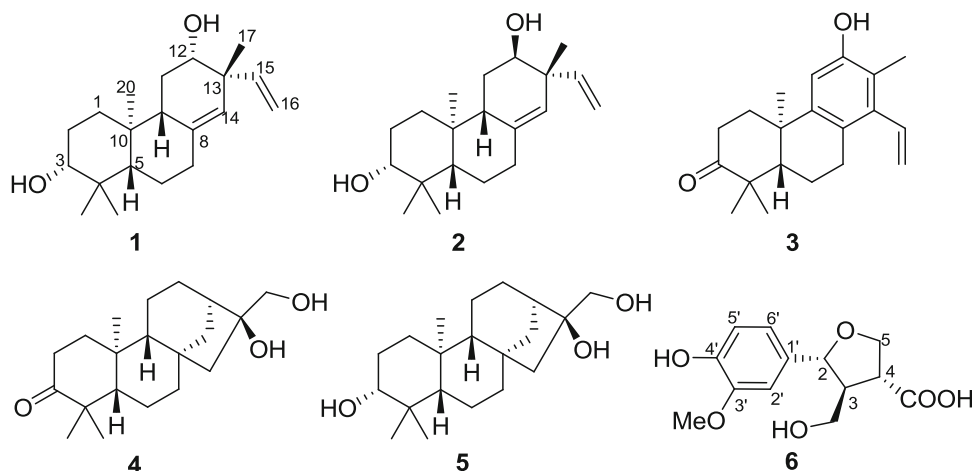
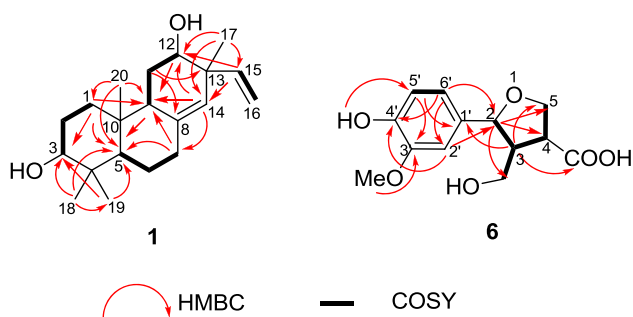
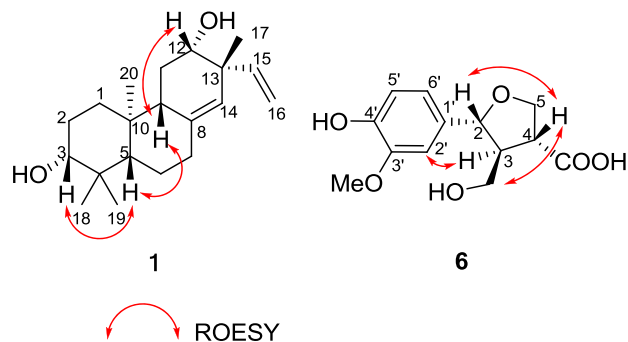


Table 1 NMR Data of **1** and **6** in CDCl₃ (δ in ppm and *J* in Hz)

No.	1		No.	6	
	δ_H	δ_C		δ_H	δ_C
1	1.65 (m)	36.7	2	4.62 (d, 7.0)	86.1
	1.16 (td, 13.4, 3.6)				
2	1.64 (m)	27.4	3	3.13 (m)	48.2
	1.54 (m)				
3	3.26 (dd, 11.4, 4.1)	78.8	4	3.46 (td, 9.0, 3.9)	46.0
4		38.9	5	4.37 (d, 9.0)	69.8
				4.19 (dd, 9.0, 3.9)	
5	1.09 (m)	54.1	1'		130.5
6	1.67 (m)	22.2	2'	6.89 (br s)	108.4
	1.43 (dd, 13.0, 4.1)				
7	2.37 (dd, 13.9, 5.0)	35.4	3'		146.9
	2.10 (td, 13.9, 5.0)				
8		138.1	4'		145.8
9	1.83 (t, 8.3)	46.3	5'	6.91 (d, 8.1)	114.4
10		38.2	6'	6.81 (dd, 8.1, 1.9)	119.1
11	1.68 (m)	25.9	CH ₂ OH	4.51 (dd, 9.8, 7.0)	69.9
	1.62 (m)		4.33 (dd, 9.8, 1.9)		
12	3.63 (s)	72.7	COOH		178.6
13		43.7	OCH ₃	3.92 (s)	55.9
14	5.07 (s)	125.7			
15	5.74 (dd, 17.6, 10.0)	146.1			
16	5.01 (d, 17.6)	114.2			
	5.00 (d, 10.0)				
17	1.07 (s)	23.5			
18	1.02 (s)	28.4			
19	0.82 (s)	15.6			
20	0.74 (s)	14.7			

**Fig. 2** Key ¹H-¹H COSY and HMBC correlations of **1** and **6****Fig. 3** Key ROESY correlations of **1** and **6**

including one 1,3,4-trisubstituted aromatic ring (δ_C 146.9, 145.8, 130.5, 19.1, 114.4, 108.4), one furan ring (δ_C 86.1, 46.0, 48.2, 69.9), one hydroxymethyl (δ_C 69.8), and one methoxy group (δ_C 55.9). The ¹H NMR spectrum also revealed characteristic signals of 1, 3, 4-trisubstituted aromatic ring [δ_H 6.89 (br s, H-2), 6.91 (d, *J* = 8.1 Hz, H-5), 6.81 (br d, *J* = 8.1 Hz, H-6)]. The ¹H NMR and ¹³C NMR

spectra of **6** displayed similarity to the known lariciresinol, a furan methanol lignan isolated from *Araucaria angustifolia* [16]. The major difference is one carbonyl group instead of 4'-hydroxy-3'-methoxyphenmethyl group at C-4 supported by the HMBC correlation (Fig. 2) from H-4 (δ_H 3.46) to COOH (δ_C 178.6). The hydroxymethyl group was

positioned at C-3 by means of the ^1H - ^1H COSY correlation of H-3 (δ_{H} 3.13) with the two protons of CH_2OH (δ_{H} 4.33 and 4.51), and the HMBC (Fig. 2) correlation from H-2 (δ_{H} 4.62) to CH_2OH (δ_{C} 69.9). Addition, in the HMBC spectrum (Fig. 2), the correlations from OH (δ_{H} 5.63) to C-5' (δ_{C} 114.4) and OMe (δ_{C} 55.9) to C-3' (δ_{C} 146.9) indicated that OH and OMe were connected to C-4' and C-3, respectively. On the basis of the above information, the planar structure of **6** was further confirmed in Fig. 1.

The ROESY correlation (Fig. 3) between H-2 and H-4 manifested that H-2 and H-4 were in the same side (assigned as β -orientation). The relative configuration of H-3 was α -orientation determined by coupling constants ($J = 7.0$ Hz), which was similar to the literature [17]. In addition, the ROESY correlations (Fig. 3) of $\text{CH}_2\text{OH}/\text{H-4}$ and H-3/H-2' further confirmed the relative configuration of H-3. Consequently, compound **6** was determined to be tetrahydro-2a-(4'-hydroxy-3'-methoxyphenyl)-4a-carboxyl-3b-hydroxymethyl furan (**6**), and named as maniesculentin B.

Maniesculentins A (**1**) and B (**6**) were assayed for antibacterial activity against four bacteria lines (*Staphylococcus aureus*, *Streptococcus*, *Escherichia.coli*, *Pseudomonas aeruginosa*) by double-dilution [18, 19]. The results of antibacterial activity showed that the two new compounds were inactive against four tested bacteria lines.

3 Experimental Part

3.1 General Experimental Procedures

Optical rotations were detected with a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were scanned with Bruker Tensor-27 infrared spectrometer with a KBr disk. 1D and 2D NMR spectra were measured on Bruker AM-400, DRX-600 spectrometer using TMS as internal standard. MS and HREIMS spectra were carried out on Bruker HCT/E squire and Waters Autospec Premier P776 spectrum. HPLC analysis was performed on an Agilent 1100 liquid chromatograph equipped with a Waters X-Bridge C18 column (4.6 \times 250 mm, 5 μm) with a flow rate of 3.0 mL/min, detected by a DAD detector. Column chromatography was carried out on silica gel (200–300 and 300–400 mesh; Qingdao Marine Chemical, Inc., Qingdao, P. R. China) and Sephadex LH-20 (40–70 μm , Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC spots were visualized under UV light and by dipping into 8 % H_2SO_4 in EtOH followed by heating.

3.2 Plant Material

The stems of *M. esculenta* Crantz were collected from Xishuangbanna, Yunnan Province, People's Republic of

China, in June 2012. The plant was authenticated by Mr. Yu Chen (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (No. H201206162) was deposited at the Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried and powdered stems of *M. esculenta* (13.0 kg) were extracted with 95 % aqueous EtOH (3 \times 20 L) under reflux for three times (4, 3, and 3 h, respectively). The combined EtOH extracts were concentrated under vacuum to give a crude extract (750.0 g), which was suspended in water and then partitioned with EtOAc. The EtOAc extract (140.0 g) was subjected to a silica gel column, eluted with petroleum ether-ethyl acetate (from 9:1 to 5:5) and then eluted with chloroform-methanol (from 9:1 to 8:2) to yield seven fractions (A–J). Fr. C (30.0 g) was separated over an MCI-gel column (MeOH/ H_2O from 4:6 to 10:0) to obtain four fractions (Fr. C1–C4). Fr. C3 (800.0 mg) was chromatographed on Sephadex LH-20 (MeOH) to obtain Fr. C3A (400.0 mg), which was further purified by a silica gel column (petroleum ether-acetone, 9:1) to obtain Fr. C3A2 (200.0 mg).

Fr. C3A2 was further purified by Semi-preparative HPLC using a Waters X-bridge C18 (4.6 \times 250 mm, 5 μm) column with 40 % MeOH/ H_2O to obtain compound **1** (10.0 mg) and **2** (16.0 mg). Fr.C2 (600.0 mg) was purified using Sephadex LH-20 (CHCl_3 -MeOH, 1:1) and then by the Waters X-bridge C18 (4.6 \times 250 mm, 5 μm) column with 60 % MeOH/ H_2O to afford compounds **3** (8.0 mg), **4** (5.0 mg), **5** (20.0 mg). Fr.C1 (102.0 mg) was chromatographed on Sephadex LH-20 (MeOH) to obtain Fr. C1A (56.0 mg), and further purified by a silica gel column (CHCl_3) to obtain compound **6** (9.0 mg).

3.4 Maniesculentins (1)

Amorphous powder. $[\alpha]_D^{23} = -51.7$ ($c = 0.40$, CHCl_3), UV (MeOH) λ_{max} (log ϵ) 204 (2.08); IR (KBr) ν_{max} 3443, 1633, 1456, 1385, 1179, 1038, 596 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see (Table 1); positive ESIMS m/z 305 $[\text{M} + \text{H}]^+$; HREIMS m/z 304.2408 $[\text{M}]^+$ (calcd for $\text{C}_{13}\text{H}_{16}\text{O}_6$, 304.2402).

3.5 Maniesculentins B (6)

Colorless oil. $[\alpha]_D^{23} = +14.7$ ($c = 0.60$, CHCl_3), UV (CHCl_3) λ_{max} (log ϵ) 280 (2.39), 240 (2.33); IR (KBr) ν_{max} 3442, 1764, 1631, 1517, 1384, 1277, 1038, 575 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see (Table 1); negative ESIMS m/z 267 $[\text{M}-\text{H}]^-$; HREIMS m/z 268.0943 $[\text{M}]^+$ (calcd for $\text{C}_{13}\text{H}_{16}\text{O}_6$, 268.0943).

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Conflict of interest The authors declare no conflict of interest.

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