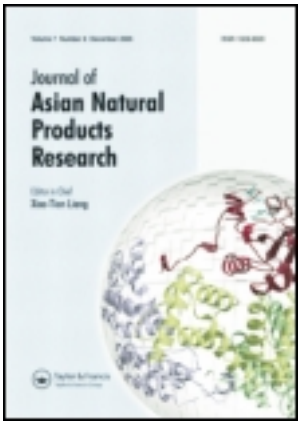
This article was downloaded by: [Malmo Hogskola]
On: 18 December 2011, At: 23:10
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

A new diterpene from the stems of *Manihot esculenta*

Shan-Shan Li [a c], Li-Fei Hu [a], You-Xing Zhao [a b], Wen-Jian Zuo [a b], Yan-Bo Zeng [a b], Xiao-Na Li [a b], Wen-Li Mei [a b] & Hao-Fu Dai [a b]

[a] Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, China

[b] Haikou Key Laboratory for Research and Development of Tropical Natural Products, Haikou, 571101, China

[c] College of Agriculture, Hainan University, Haikou, 570228, China

Available online: 05 Oct 2011

To cite this article: Shan-Shan Li, Li-Fei Hu, You-Xing Zhao, Wen-Jian Zuo, Yan-Bo Zeng, Xiao-Na Li, Wen-Li Mei & Hao-Fu Dai (2011): A new diterpene from the stems of *Manihot esculenta*, Journal of Asian Natural Products Research, 13:10, 961-964

To link to this article: http://dx.doi.org/10.1080/10286020.2011.600249

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research
Vol. 13, No. 10, October 2011, 961–964

Taylor & Francis
Taylor & Francis Group

A new diterpene from the stems of *Manihot esculenta*

Shan-Shan Li[ac], Li-Fei Hu[a], You-Xing Zhao[ab], Wen-Jian Zuo[ab], Yan-Bo Zeng[ab], Xiao-Na Li[ab], Wen-Li Mei[ab]* and Hao-Fu Dai[ab]*

[a]*Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China;* [b]*Haikou Key Laboratory for Research and Development of Tropical Natural Products, Haikou 571101, China;* [c]*College of Agriculture, Hainan University, Haikou 570228, China*

(*Received 20 April 2011; final version received 22 June 2011*)

A new diterpene, named yucalexin P-23 (**1**), together with three known compounds, yucalexin P-15 (**2**), protocatechuic acid (**3**), and catalpinic acid (**4**), was isolated from the stems of *Manihot esculenta*. Their structures were determined by means of physicochemical evidence and spectral analysis. Compounds **3** and **4** exhibited antimicrobial activity.

Keywords: *Manihot esculenta*; diterpene; yucalexin P-23

Downloaded by [Malmo Hogskola] at 23:10 18 December 2011

1. Introduction

Manihot esculenta Crantz, belonging to the family Euphorbiaceae and commonly known as tapioca or cassava, is extensively cultivated as an annual crop in tropical and subtropical regions. In many countries, cassava has been used as an ethanol biofuel feedstock, which was also used as a traditional Chinese medicine to cure furuncle and acariasis [1]. Cassava has long been known to contain the cyanogenic glycosides, linamarin, and lotaustralin, in both roots and aerial parts. In addition, hydroxycoumarins, flavanols, fatty acids, and esters were also identified from cassava [2], together with 22 diterpenic stress metabolites isolated and identified from the cassava root tissues damaged by cutting or fungal infection [3]. The oil extracted from the seeds of cassava was reported to show cytotoxicity and antimicrobial activities against five skin pathogenic microorganisms [4–6]. The current phytochemical study on this plant resulted in the isolation of a new diterpene, named yucalexin P-23 (**1**), along with three known compounds, yucalexin P-15 (**2**), protocatechuic acid (**3**), and catalpinic acid (**4**). The bioassay result showed that both compounds **3** and **4** possessed inhibitory effects on methicillin-resistant *Staphyloccus aureus* (MRSA), and compound **3** showed inhibitory effects on *S. aureus* (SA). In this paper, we report the isolation, structural elucidation, and antibacterial activity of these compounds.

2. Results and discussion

Compound **1** was obtained as yellow oil. The molecular formula of **1** was deduced as $C_{20}H_{28}O_4$ by HR-ESI-MS at *m/z* 333.2062 $[M + H]^+$ and ^{13}C NMR spectral data. IR spectrum revealed the presence of hydroxyls (3743 cm^{-1}), carbonyl (1715 cm^{-1}), CH_3 (2984 cm^{-1}), and conjugated double bond (1641, 1540 cm^{-1}) in **1**. The ^1H NMR spectrum (Table 1) showed four methyl singlets [δ 1.18 × 2 (s), 1.07 (s), 0.98 (s)].

*Corresponding authors. Email: meiwenli@yahoo.com.cn; hfdai2001@yahoo.com.cn

ISSN 1028-6020 print/ISSN 1477-2213 online
© 2011 Taylor & Francis
http://dx.doi.org/10.1080/10286020.2011.600249
http://www.tandfonline.com

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of compound 1 (in CD_3OD).

Position	δ_C	δ_H (*J* in Hz)	HMBC (H to C)
1	44.2 t	2.20 (1H, dd 12.4, 1.8) 1.47 (1H, m)	C-2
2	69.3 d	3.79 (1H, m)	
3	83.7 d	2.99 (1H, d 8.8)	C-2, C-18, C-19
4	39.7 s		
5	49.6 d	3.30 (1H,m)	
6	25.4 t	2.39 (2H, m)	C-8
7	135.7 d	5.70 (1H, m)	
8	134.3 s		
9	164.3 s		
10	40.8 s		
11	117.2 d	6.40 (1H, s)	C-8, C-10, C-13
12	205.3 s		
13	55.0 s		
14	80.5 d	4.15 (1H, s)	C-8, C-9, C-12, C-17
15	139.8 d	6.26 (1H, dd 11.2, 4.0)	
16	116.0 t	5.23 (2H, m)	C-13, C-15
17	21.2 q	1.18 (3H, s)	C-12, C-13, C-15
18	28.8 q	1.07 (3H, s)	C-3, C-4, C-5, C-19
19	17.5 q	0.98 (3H, s)	C-3, C-4, C-5, C-18
20	21.7 q	1.18 (3H, s)	C-1, C-9, C-10

The ^{13}C NMR spectrum of **1** in CD_3OD contained 20 carbon signals, including four methyls [δ 21.2 (q), 28.8 (q), 17.5 (q), and 21.7 (q)], one carbonyl group [δ 205.3 (s)], and six olefinic carbons [δ 117.2 (d), 134.3 (s), 164.3 (s), 135.7 (d), 139.8 (d), and 116.0 (t)], and three oxygenated methine carbons resonating at δ 69.3 (d), 83.7 (d), and 80.5 (d). These data suggested that **1** was an ent-pimarane diterpene [3]. And the NMR spectral data of **1** were very similar to those of yucalexin P-15 [3], except that an oxygenated methine carbon at δ 69.3 in **1** substituted a carbonyl group (C-2) in yucalexin P-15. A hydroxyl at C-2 position was assigned by $^{1}H-^{1}H$ COSY correlations from H-1 and H-3 to H-2, whereas C-1 and C-3 positions were assigned by long-range correlations from H-20 to C-1, as well as H-18 and H-19 to C-3 in the HMBC spectrum. In addition, a carbonyl group at C-12 was assigned by long-range correlations from H-14 and H-17 to C-12 in the HMBC spectrum (Figure 1). Considering that the known diterpene (ent-3β,14α-hydroxypimara-7,9(11),15-triene-12-one [7]) isolated from the same species was ent-pimarane, in which C-5 and C-10 were (*S*)- and (*R*)-configured, respectively; the configurations of C-5 and C-10 in **1** were presumed to be the same as in

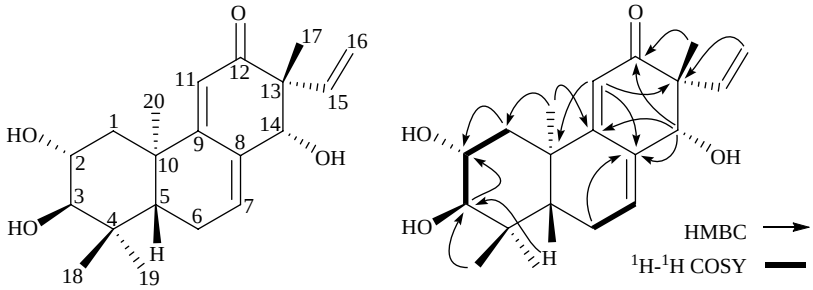


Figure 1. Key HMBC and $^{1}H-^{1}H$ COSY correlations of compound **1**.

Downloaded by [Malmo Hogskola] at 23:10 18 December 2011

Downloaded by [Malmo Hogskola] at 23:10 18 December 2011

it. The α configurations of 2-OH and 18-CH_3 were determined on the basis of correlations of H-2/H-5 and H-18/H-5, and the β orientation of 3-OH was deduced by the correlation of H-3/H-19 in the NOESY spectrum. Consequently, the structure of **1** was assigned as ent-2α,3β,14α-trihydroxypimara-7,9(11),15-triene-12-one, named yucalexin P-23.

Three known compounds, yucalexin P-15 (**2**) [3], protocatechuic acid (**3**) [8], and catalpinic acid (**4**) [9], were identified by comparing their spectral data (^{1}H and ^{13}C NMR) with those reported in the literature.

Antibacterial assay exhibited that compounds **3** and **4** had inhibitory effects on MRSA, and diameters of inhibition zones were 14 and 12 mm, respectively. Compound **3** showed inhibitory effects on SA, and the diameter of inhibition zone was 7.5 mm (Table 2). Compounds **1** and **2** showed no antibacterial activity to the test strains.

Table 2. Antibacterial activities of compounds **3** and **4** on MRSA and SA (D/mm).

Compounds	MRSA	SA
3	14	7.5
4	12	–
Kanamycin sulfate[a]	31	20

[a] Positive control.

3. Experimental

3.1 General experimental procedures

The IR spectra were obtained on a Nicolet 380 FT-IR instrument from KBr pellets. The UV spectra were measured on a Shimadzu UV-2550 spectrometer. Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-ESI-MS were measured with an API QSTAR Pulsar mass spectrometer. Column chromatography was carried out with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). TLC was carried out with silica gel GF_{254} (Marine Chemical Industry Factory).

3.2 Plant material

The stems of cassava were collected from Wenchang City, Hainan Province, China, in November 2008, and were identified by associate Professor Zheng-Fu Dai of Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science, where a voucher specimen (BL20081106) is deposited.

3.3 Extraction and isolation

The fresh, milled stems of cassava (39.7 kg) were extracted three times with EtOH (95%) at room temperature. After evaporation, the residue was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH successively. The EtOAc fraction (21.6 g) was separated into seven fractions (A1–A7) on a silica gel column using step gradient elution of $CHCl_3$–MeOH (50:1, 30:1, 15:1, 10:1, 7:1, 4:1, 2:1, 1:1, and 0:1, v/v). Fraction A6 (2.5 g) was subjected to chromatography on silica gel column with petroleum ether–acetone gradients (4:1–2:1, v/v) as eluent, and then subjected to silica gel chromatography eluted with $CHCl_3$–MeOH (30:1) and petroleum ether–EtOAc (1:1) yielding compounds **1** (4.3 mg) and **2** (3.8 mg). Fraction A7 (4.3 g) subjected to Sephadex LH–20 with $CHCl_3$–MeOH (1:1, v/v) and then subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (20:1) and petroleum ether–EtOAc (6:1) led to the isolation of compounds **3** (30.0 mg) and **4** (10.0 mg).

3.3.1 Yucalexin P-23 (*1*)

Yellow oil: $[\alpha]_D^{22} + 6.0$ (c 0.5, MeOH). UV (MeOH) λ_{max}: 284, 207, 202 nm. IR (KBr) ν_{max}: 3743 (OH), 1715 (C=O), 1641, 1540

(conjugated double bond), 2984 (CH_3) cm^{-1}. ^1H and ^{13}C NMR spectral data in CD_3OD see Table 1. HR-ESI-MS: *m/z* 333.2062 $[M]^+$ (calculated for $C_{20}H_{28}O_4$, 333.2065).

3.4 Antibacterial activity

These compounds were tested for *in vitro* antibacterial activity against SA and MRSA strains (obtained from Professor Kui Hong of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences) by the filter paper disk agar diffusion method [10]. The nutrient agar medium was used to culture the bacteria. The sterile agar medium was poured into Petri-plates to a uniform depth of 5 mm and was allowed to solidify. The microbial suspensions were streaked over the surface of the medium using a sterile cotton swab. Fifty microliters (10 mg/ml) of the compounds were impregnated on sterile filter paper disks of 6 mm size, respectively. These disks were then aseptically applied to the surface of the agar plates at well spaced intervals. Control disks impregnated with 50 μl of acetone and 50 μl of kanamycin sulfate (0.64 mg/ml) were also used alongside the test disks in the experiment. The plates were incubated at 36°C for 24 h. Experiments were done in triplicate, and the results are presented as the mean values of the three measurements.

Acknowledgement

This research was financially supported by a Major Project of Chinese National Programs for Fundamental Research and Development Grants (2010CB126606).

References

[1] L.R. Song, *Chin. Mater. Med.* **4**, 834 (1999).
[2] I.S. Blagbrough, S.A.L. Bayoumi, and M.G. Rowan, *Phytochemistry* **71**, 1940 (2010).
[3] S. Tsutomu and N. Yoshiko, *Phyto chemistry* **27**, 3769 (1988).
[4] T.O.S. Popola, O.D. Yangomodou, and A.K. Akintokun, *Res. J. Med. Plant* **1**, 60 (2007).
[5] T.O.S. Popola and O.D. Yangomodou, *Biotechnology* **5**, 38 (2006).
[6] L.F. Hu, W.L. Mei, J. Wu, W.Q. Wang, M. Peng, and H.F. Dai, *Chin. J. Trop. Crops* **31**, 126 (2010).
[7] H.Y. Liu, S.J. Li, and Y. Zhao, *Helv. Chim. Acta* **90**, 2017 (2007).
[8] H.Y. Ren, X.Y. Cai, and Z.R. Xu, *J. Chin. Med. Mater.* **32**, 862 (2007).
[9] L.Y. Wang, N.L. Wang, and X.S. Yao, *J. Chin. Med. Mater.* **30**, 792 (2007).
[10] S.Y. Xu, R.L. Bian, and X. Chen, *Methods of Pharmacology Experiment* (People's Sanitation Press, Beijing, 2003), p. 1651.

Downloaded by [Malmo Hogskola] at 23:10 18 December 2011