

割舌树中的两个新四降三萜 walsurobustones F 和 G

侯 丽^{1, 2, 3}, 曹明明¹, 赵 庆², 何红平^{2*}, 郝小江^{1*}

(1. 中国科学院昆明植物研究所, 植物化学与西部植物资源持续利用国家重点实验室, 云南 昆明 650201;

2. 云南中医药大学, 云南 昆明 650500; 3. 湖南湘江新区管委会商务和市场监管局, 湖南 长沙 410205)

摘要: 四降三萜是众多芸香科及楝科中药材中的细胞毒性成分, 其化学结构上碳骨架多变、高度氧化。本文报道从割舌树叶片中分离得到了两个新的四降三萜类化合物, 被命名为 walsurobustones F(**1**)和 G(**2**)。它们的结构通过一维与二维核磁, 及质谱数据进行了解析。从结构上看, **1** 和 **2** 是一对 C-22 位的差向异构体。细胞毒测试显示 **1** 和 **2** 对人肿瘤细胞株 HL-60、SMMC-7721、A-549、MCF-7 和 SW480 表现出了显著的细胞毒性。

关键词: 割舌树; 柠檬苦素; 四降三萜; 细胞毒

中图分类号: R284.1; R285.5 文献标志码: A 文章编号: 1000-2723(2023)03-0095-05

DOI: 10.19288/j.cnki.issn.1000-2723.2023.03.017

Walsurobustones F and G: Two New Tetranortriterpenoids from *Walsura robusta*

HOU Li^{1, 2, 3}, CAO Mingming¹, ZHAO Qing², HE Hongping², HAO Xiaojiang¹

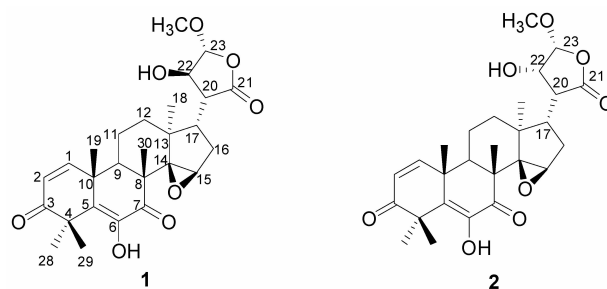
(1. State Key Laboratory of Photochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; 2. Yunnan University of Chinese Medicine, Kunming 650500, China;

3. Bureau of Commerce and Market Supervision of Management Committee of Hunan Xiangjiang New Area, Changsha 410205, China)

ABSTRACT: Tetranortriterpenoids are the major secondary metabolites in traditional Chinese medicine from family Meliaceae and Rutaceae, which structurally featured by diverse carbon skeletons and highly oxidized degree. Two new tetranortriterpenoids, walsurobustones F (**1**) and G (**2**), were isolated from the leaves of *Walsura robusta*. Their structures were elucidated by 1D NMR, 2D NMR, and MS spectra. Structurally, **1** and **2** are two geometric isomers that differ at position C-22. Both

1 and **2** showed obvious antitumor activity against the cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480. These findings suggest that walsurobustones F (**1**) and G (**2**) have the potential to be used as anti-cancer agents.

KEY WORDS: *Walsura robusta*; limonoids; tetranortriterpenoids; cytotoxicity



1 Introduction

Limonoids are a famous family of plant secondary metabolites that are characterized by complex chemical structures and obvious bioactivities. They are usu-

ally isolated from the families of Meliaceae and Rutaceae^[1-2]. The genus *Walsura* belongs to the family Meliaceae and is distributed in subtropical regions in Asia^[3]. It is commonly associated with producing limonoid

基金项目: 云南特色植物筛选与研发服务 CXO 平台建设(2022YKZY001)

作者简介: 侯丽(1987-),女,硕士,药物化学专业, E-mail: houli19870817@163.com

* 通信作者: 郝小江(1951-),男,研究员,博士生导师,研究方向:植物化学, E-mail: haoxj@mail.kib.ac.cn;

何红平(1972-),男,研究员,博士生导师,研究方向:中药物质基础及活性研究, E-mail: 95431111@qq.com

chemical structures^[4-11]. The structural diversity of limonoids is mainly due to their carbon skeleton and oxidations, or to the oxidation, degradation, and stereochemistry on the furan ring moiety^[12-16]. Previously, walsurobustones A–E were isolated from *Walsura robusta*^[17]. Herein, we report the isolation of walsurobustones F and G, with a focus on fixing their stereochemistry on the furan ring. Both walsurobustones F and G showed better antitumor activities than positive control cisplatin.

2 Results and discussion

Walsurobustone F (**1**), a white amorphous powder, showed the molecular formula of $C_{27}H_{34}O_8$ as established by the HRESIMS at m/z 509.2158 $[M + Na]^+$ (calcd. 509.2151). The 1D and 2D NMR spectra analysis of **1** revealed that **1** shared the same A, B, C and D rings in the limonoid core with walsurobustones (Table 1), while the different substitution on the furan ring was observed. Compared to walsurobustone C^[17], the conjugated $\Delta^{20(22)}$ double bond was substitute with the hydroxyl group at C-22 on structure of **1**, which were supported by the HMBC correlation from H-20 (δ_H 2.64) to C-22 (δ_C 73.6). H-15 (δ_H 3.74, s) and H-16 β (δ_H 2.85, dd, 14.0 and 6.4 Hz) (Figure 1) indicates $J_{H16-17} = 6.4$ Hz which requires the *cis* relation with H-17, accordingly δ_H 1.77 was assigned to H-16 α . The ROESY correlations of H-20/H-16 α , H-20/H₃-18, H-22/H₃-18 revealed that H-20 and H-22 were α -oriented. No ROESY cross-peaks can be observed between H-20 and H-23 which inferred H-23 was β -oriented (Figure 2). According to the above spectral data, the planar structure of **1** was established as hy-

drate products of walsurobustone C.

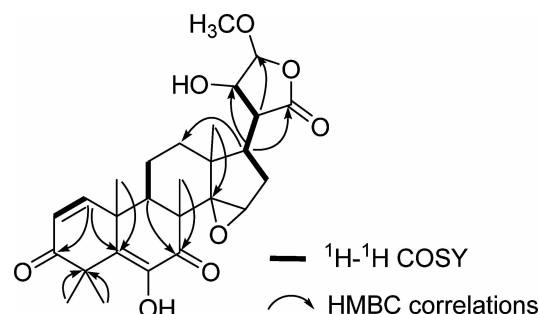


Figure 1. Key ¹H-¹H COSY, HMBC correlations of **1** and **2**

Walsurobustone G (**2**) was assigned the molecular formula of $C_{27}H_{34}O_8$ by the HRESIMS at m/z 509.2137 $[M + Na]^+$ (calcd. 509.2151). By comparison of the ¹H and ¹³C NMR data of **2** and **1** (Table 1), they showed high similarity. The 2D NMR spectra analysis of **2** revealed that **2** differed from **1** on the stereochemistry of furan ring. Regarding to the relative configuration of chiral centers of compound **2**, H-22 was assigned as β -oriented based on the ROESY correlation of H-22/H-17 (Figure 2). The configuration of H-20 was assigned as α -oriented due to the significant NOE cross-peaks between H-20 and H₃-18. H-23 was assigned as β -oriented since the absence of ROESY correlation between H-20 and H-23. Thus the planar structure of compound **2** was determined as shown.

The cytotoxicity of **1** and **2** against the cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) were evaluated by using the MTT method with taxol and cisplatin as positive controls. Both **1** and **2** showed obvious cytotoxicity, especially against SMMC-7721, A-549 and MCF-7 (Table 2).

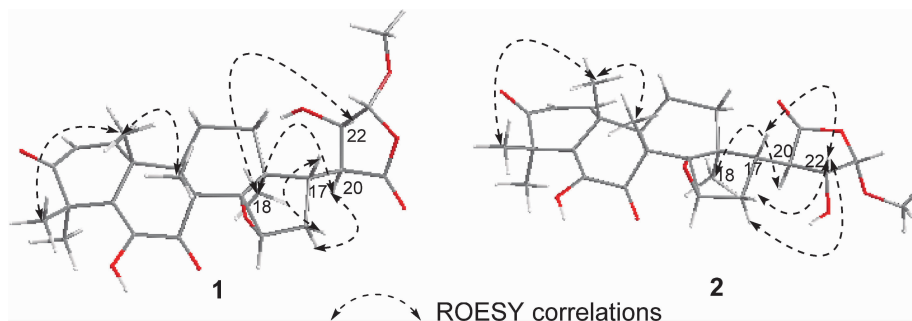


Figure 2. Key ROESY correlations of **1** and **2**

Tab.1 NMR spectroscopic data of **1** and **2** recorded in CDCl₃

position	1 ^a		2 ^b	
	¹³ C NMR	¹ H NMR	¹³ C NMR	¹ H NMR
1	152.5	6.11, d (9.8)	152.9	6.95, d (10.0)
2	127.4	6.11, d (9.8)	127.3	6.11, d (10.0)
3	203.9		204.1	
4	48.7		48.7	
5	134.0		134.3	
6	141.3		141.3	
7	197.9		198.2	
8	46.8		47.0	
9	43.3	2.64, m	43.3	2.71, d (12.0)
10	40.4		40.4	
11	19.6	1.79–1.82, m	20.0	1.83, dd (11.8, 7.4); 1.74, m
12	34.9	2.19, dd (14.1, 5.5); 1.55, m	37.1	2.04, m; 2.10, m
13	42.1		41.2	
14	69.3		70.7	
15	55.8	3.74, s	54.9	3.66, s
16	31.5	2.85, dd (14.0, 6.4); 1.77, m	33.3	2.32, dd (13.4, 6.5); 1.58, m
17	39.2	2.11, m	40.7	1.98, m
18	22.4	0.96, s	22.4	1.02, s
19	24.0	1.27, s	24.1	1.27, s
20	43.5	2.64, m	43.8	2.65, dd (11.9, 4.6)
21	176.0		175.4	
22	73.6	4.14, d (4.0)	73.7	4.19, d (4.5)
23	107.2	5.13, s	107.6	5.15, s
28	21.3	1.48, s	21.3	1.48, s
29	26.9	1.58, s	26.9	1.57, s
30	20.4	1.08 s	20.3	1.07 s
23–OCH ₃	56.8	3.48, s	56.8	3.48, s

a. ¹³C NMR and ¹H NMR were recorded at 100 MHz and 400 MHz, respectively;

b. ¹³C NMR and ¹H NMR were recorded at 125 MHz and 600 MHz, respectively.

3 Experimental section

3.1 General experimental procedure Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were detected on a Shimadzu

Tab.2 Cytotoxicity data for compounds **1** and **2** against the cancer cell lines

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
walsurobustone F (1)	7.07	3.64	5.71	8.36	9.10
walsurobustone G (2)	7.21	3.46	2.67	8.72	14.05
Cisplatin	1.81	8.86	11.68	15.92	16.65
taxol	<0.008	<0.008	<0.008	<0.008	<0.008

a. Results are expressed as IC₅₀ values in μM at the HL-60 leukemia cancer, SMMC-7721 liver cancer, A-549 lung cancer, MCF-7 breast cancer, and SW480 colon cancer cell lines.

UV-2401 spectrophotometer. IR spectra were determined on a Tenor 27 spectrophotometer with KBr pellets, whereas CD spectra were recorded with an Applied Photophysics Chirascan spectrometer. ESIMS and HRESIMS were measured on a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. 1D and 2D NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 with TMS as internal standard. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatographed with a Zorbax SB-C18 (9.4 mm 25 cm) column. Column chromatography was performed with silica gel (300–400 mesh, Qingdao Marine Chemical, Inc., Qingdao, People’s Republic of China), and MCI gel (75–150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH.

3.2 Plant material The leaves of *W. robusta* collected in Hainan Province, People’s Republic of China in December 2010. The plant was authenticated by Dr. Guangwan Hu, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. H20101202) was deposited in the State Key Laboratory of Photochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation The dried and pow-

dered leaves (12 kg) of *W. robusta* were extracted with MeOH three times under reflux, and the solvent was evaporated in vacuo. The residue was partitioned in H₂O and extracted successively with petroleum ether and EtOAc. The EtOAc fraction (200 g) was separated by silica gel column chromatography (CC) eluted with a gradient of petroleum ether/Me₂CO (50:1 to 1:1) and CHCl₃/MeOH in a gradient (15:1 to 3:1). Eight fractions (Fr. A–H) were obtained according to TLC monitor. Fr. C (17 g) was subjected to MCI–gel column (MeOH/H₂O, 6:4 to 9:1) to give sixteen sub-fractions (C1–C16). C7 (4.1 g) was then chromatographed on a column of reversed-phase C18 silica gel eluted with MeOH/H₂O (5:5 to 9:1) to get eight parts (C7a–C7h). Fr. C7h was purified by CC over silica gel and then applied to a Sephadex LH–20 column using solvent system acetone to provide **1** (11 mg) and **2** (15 mg).

3.3.1 Walsurobustone F (1) a white amorphous powder; $[\alpha]_D^{25} -102.8571$ (c 0.14, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 281 (3.35) nm; ¹H NMR (CDCl₃) and ¹³C NMR (CDCl₃), see Table 1. IR ν_{\max} cm⁻¹: 3448, 2925, 2852, 1781, 1696, 1674, 1356, 1123, 1034, 926 cm⁻¹; HRESIMS at *m/z* 509.2158 [M + Na]⁺ (calcd. 509.2151).

3.3.2 Walsurobustone G (2) a white amorphous powder; $[\alpha]_D^{25} -38.0606$ (c 0.11, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 283 (3.08) nm; ¹H NMR (CDCl₃) and ¹³C NMR (CDCl₃), see Table 1. IR ν_{\max} cm⁻¹: 3439, 1630 cm⁻¹; HRESIMS at *m/z* 509.2137 [M + Na]⁺ (calcd. 509.2151).

References:

- [1] LUO J, SUN Y P, LI Q R, et al. Research progress of meliaceous limonoids from 2011 to 2021[J]. Nat Prod Rep, 2022, 39: 1325–1365.
- [2] TAN Q G, LUO X D. Meliaceous limonoids: chemistry and biological activities[J]. Chem Rev, 2011, 111: 7437–7522.
- [3] PENG H, MABBERLEY D J. Flora of China[M]. Beijing: Science Press, 2008, 11: 119–120.
- [4] LUO X D, WU S H, MA Y B, et al. Tetranortriterpenoids from *Walsura yunnanensis* [J]. J Nat Prod, 2000, 63: 947–951.
- [5] Yin S, Wang X N, FAN C Q, et al. The first limonoid peroxide in the meliaceae family: Walsuronoid A from *Walsura robusta* [J]. Org Lett, 2007, 9: 2353–2356.
- [6] ZHOU Z W, YIN S, ZHANG H Y, et al. Walsucochins A and B with an unprecedented skeleton isolated from *Walsura cochinchinensis* [J]. Org Lett, 2008, 10: 465–468.
- [7] BALAKRISHNA K, SUKUMAR E, CONNOLLY J D. Piscidenone and piscidinol G, two new protolimonoids from *Walsura piscidia* [J]. Nat Prod Sci, 2003, 9: 192–194.
- [8] LUO X D, WU S H, MA Y B, et al. Chemical constituents from *Walsura yunnanensis* [J]. Acta Botanica Yunnanica, 2001, 23: 515–520.
- [9] YANG W, KONG L M, LI S F, et al. Five new mexicanolide type limonoids from *Heynea trijuga* [J]. Nat Prod Bioprospect, 2012, 2: 145–149.
- [10] WANG G C, YU J H, SHEN Y, et al. Limonoids and triterpenoids as 11 β -HSD1 inhibitors from *Walsura robusta* [J]. J Nat Prod, 2016, 79: 899–906.
- [11] WANG X Y, YUAN C M, TANG G H, et al. Studies on chemical constituents of the leaves and twigs of *Aphanamixis grandifolia* [J]. J Yunnan University of Trad Chin Med, 2012, 35(8): 34–36, 43.
- [12] AN F L, LUO J, LI R J, et al. Spirotrichilins A and B: two rearranged spirocyclic limonoids from *Trichilia connaroides* [J]. Org Lett, 2016, 18: 1924–1927.
- [13] LIN C P, XU J B, HAN Y S, et al. Trichiconins A–C, limonoids with new carbon skeletons from *Trichilia connaroides* [J]. Org Lett, 2014, 16: 5478–5481.
- [14] MURPHY B T, BRODIE P, SLEBODNICK C, et al. Antiproliferative limonoids of a *Malleastrum* sp. from the Madagascar rainforest [J]. J Nat Prod, 2008, 71: 325–329.
- [15] ZHANG Y, AN F L, HUANG S S, et al. Diverse triterpenoids from the fruits of *Walsura robusta* and their reversal of multidrug resistance phenotype in human breast cancer cells [J]. Phytochemistry, 2017, 136: 108–118.
- [16] CAI J Y, ZHANG Y, LLO S H, et al. Aphanamixoid A, a potent defensive limonoid, with a new carbon skeleton from *Aphanamixis polystachya* [J]. Org Lett, 2012, 14(10): 2524–2527.
- [17] HOU L, MEI C X, YUAN C M, et al. Five new limonoids isolated from *Walsura robusta* [J]. Nat Prod Bioprospect, 2023, 13: 7.

(收稿日期: 2023–03–22)