文章编号:1001-6880(2016)8-1171-05

苦楝果实中具有细胞毒活性的苯丙素类成分

曾发古[1,2],苏　倩[1],邸迎彤[2*],郝小江[1,2*]

[1]昆明医科大学药学院暨云南省天然药物药理重点实验室,昆明 650031;

[2]中国科学院昆明植物研究所 植物化学与西部植物资源国家重点实验室,昆明 650201

摘　要:为了研究苦楝(*Melia azedarach*)中的化学成分,我们采用柱层析的方法从苦楝的果实中分离得到6个化合物 mesendannin A(**1**)、(+)-Pinoresinol(**2**)、(-)-Eudesmin(**3**)、(-)-Drodehyrodiconiferyl alcohol(**4**)、(-)-Jatrointelignan D(**5**)、(-)-Dihydrodehyrodiconiferyl alcohol(**6**)。其中化合物**1**为一个新的苯丙素类二聚体化合物。所有化合物的结构主要通过各种光谱方法,特别是二维核磁谱的方法进行鉴定。化合物**1**对**5**种人体肿瘤细胞表现出中等强度的细胞毒活性。

关键词:苦楝;楝科;苯丙素;细胞毒活性

中图分类号:Q946.91;R284.1　　**文献标识码**:A　　DOI:10.16333/j.1001-6880.2016.8.001

Penylpropanoids with Cytotoxic Activity from the Fruits of *Melia azedarach*

ZENG Fa-gu[1,2], SU Qian[1], DI Ying-tong[2*], HAO Xiao-jiang[1,2*]

[1]*Yunnan Key Laboratory of Pharmacology for Natural Products Research, Kunming Medical University, Kunming 650031, China*; [2]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

Abstract: In this study, a new penylpropanoid dimer, mesendannin A (**1**), along with **5** known ones (+)-Pinoresinol (**2**), (-)-Eudesmin (**3**), (-)-Drodehyrodiconiferylalcohol (**4**), (-)-Jatrointelignan D (**5**) and (-)-Dihydrodehyrodiconiferyl alcohol (**6**) were isolated from the fruits of *Melia azedarach*. Their structures was elucidated on the basis of spectroscopic methods, especially **2**D NMR techniques. Compound **1** showed medium cytotoxic against five human tumor cell lines.

Key words: *Melia azedarach*; Meliaceae; penylpropanoid; cytotoxic activity

Introduction

The genus Melia (Meliaceae) comprises three species in the world and is widely distributed in Asian and the south of tropical Africa[1]. As a traditional Chinese medicine, the fruit and bark of this plant have long been used as insect antifeedant and anthelmintic [2]. The chemical components of different parts of this plant have been well studied previously, leading to isolation of diverse bioactive compounds including limonoids, penylpropanoids and steroids[3-5]. As a part of our continuing search for bioactive compounds from Meliaceae family, six penylpropanoids (**1-6**) were obtained, including a new one. In addition, the cytotoxicity of the isolated compounds against five human tumor cell lines (Hela, MCF-7, A549, MGC-803 and COLO-205) was evaluated by an MTT assay. Herein, we report the isolation, structural elucidation, and cytotoxicity of these compounds.

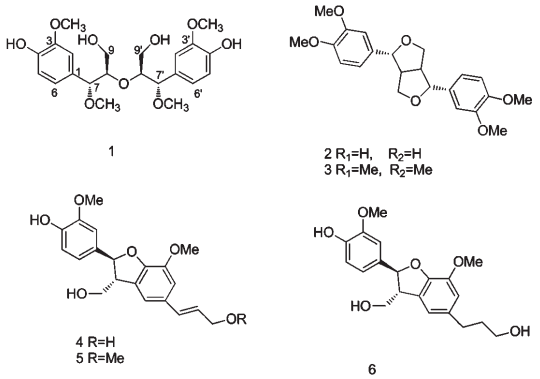

Fig. 1　Chemical structures of compounds 1-6

Received: April 22, 2016　Accepted: June 15, 2016

Foundation item: This work was supported financially by the Science Foundation of Yunnan (2014A050); Technological Leading Talent Project of Yunnan (2015HA020); The Xibuzhiguang Project (grant to Y.-T. Di), Central Asian Drug Discovery and Development Center of Chinese Academy of Sciences (to Xiao jiang Hao)

* Corresponding author Tel: 86-871-5223070; E-mail: diyt@mail.kib.ac.cn; haoxj@mail.kib.ac.cn

Materials and Methods

General experimental procedures

NMR spectra were performed on Bruker AM-400 instruments with TMS as the internal standard. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets, whereas UV date were measured using a UV-2410A spectrophotometer. Bruker HCT/E Squire and Waters Autospec Premier P776 mass spectrometers were used to measure ESI-MS and HR-ESI-MS, respectively. Semi-preparative HPLC was performed on a waters X-select (5 μm;25 cm × 9.4 mm i. d.), Rp-C18 (40-63 μm, Merck, Darmstadt, Germany). Column chromatography was performed on silica gel (60-80, 200-300 and 300-400 mesh, Qingdao Marine Chemical Inc., China), Sephadex LH-20 (40-70 μm, Amersham Pharmacia Biotech AB), MCI gel 20P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC (GF_{254}, Qingdao Marine Chemical Co. Ltd., Qingdao, China), and by heating silica gel plates sprayed with 5% H_2SO_4 in ethanol.

Plant material

The dried fruits of *M. azedarach* were collected in Yunnan province of China in October 2013 and was identified by Dr. Jia-Hui Zhang. A voucher specimen (KIB-HXJ20130021) was deposited at the Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried powdered fruits (40 kg) were extracted 3 times (4, 3 and 3 h) with MeOH. The combined MeOH extracts were concentrated in vacuo at 50 ℃ to give the crude residue (3 kg), which was re-suspended in water and then partitioned with EtOAc. The EtOAc fraction was processed with a silica gel column (0.2 m × 0.1 m, 100 to 200 mesh), and eluted with a gradient of petroleum ether-acetone (from 10∶1 to 0∶1) to yield 5 fractions (1-5). Fr. 3 (5 g) was then separated over a RP-C18 column (MeOH-H_2O 4∶6-10∶0) to obtain Fractions (3A-3C). Fr. 3A (300 mg) was chromatographed on a silica gel column (300-400 mesh), eluted with petroleum ether/acetone (20∶1), further purified by semi-preparative HPLC (MeOH/H_2O 60∶40, v/v, t_R = 15 min) to yield compound **1** (10 mg). Fr. 3B (2 g) was then purified on a silica gel column (300-400 mesh) eluted with petroleum ether/actone (10∶0-1∶1) to yield **2** (200 mg) and **3** (400 mg). Fr. C (1.5 g) was separated by Sephadex LH-20 eluted with MeOH and then applied to a silica gel column (300-400 mesh) eluted with petroleum ether/acetone (30∶1, 20∶1 and 10∶1) to yield compounds **4** (50 mg), **5** (18 mg), **6** (170 mg). The purity of compounds **1-6** were 95% as determined by TLC and HPLC.

Cytotoxicity assays

Cytotoxicity evaluations were performed on five human cell lines (Hela, MCF-7, A549, MGc-803 and COLO-205) using the MTT method described in literature elsewhere[6]. Cytotoxicity evaluations were performed according to a previously described protocol[7]. Doxorubicin was used as a positive control substance. The IC_{50} values were calculated by the Reed and Muench method[8].

Results and Discussion

Structural identification

Mesendannin A (**1**): white amorphous powder; ^1H NMR and ^{13}C NMR data: see Table 1. $[\alpha]_D^{22}$ = -0.66 (c = 0.6, MeOH). IR ν_{max} (KBr): v_m = 3420 (OH), 2935, 1517, 1431, 1277, 1121, 1154, 1115, 1087, 1035 cm^{-1}. ESI-MS: m/z = 439 $[M + H]^+$. HR-ESI-MS: m/z = 439.1876 $[M + H]^+$ (cald. for 439.1884). Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{22}H_{30}O_9$ by HR-ESI-MS from the ion at m/z 439.1876 $[M + H]^+$ (cald. for 439.1884). However, ^{13}C NMR resonances were observed for only 11 carbon atoms, indicating that **1** must be a symmetric dimer. The eight degrees of unsaturation implied by the molecular formula were accounted for two benzyl groups. The ^1H and ^{13}C NMR in combination with HSQC data (Table 1) revealed that each monomer of compound **1** possessed one 1, 3, 4-trisubstituted aromatic moiety (δ_H 6.90, 6.84, and 6.80), two methoxyls at δ_C 56.6 and 55.9, two sp^3 methines at δ_C 75.7 and 84.3, and one methylene at δ_C 62.5. The ^1H-^1H COSY and HSQC spectra of

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) data of 1 in $CDCl_3$ (δ in ppm, J in Hz)

Position	δ_H [a]	δ_C [a]
1,1′	–	129.5
2,2′	6.90 (2 × 1 H, d, J = 1.4 Hz)	109.4
3,3′	–	146.9
4,4′	–	145.8
5,5′	6.84 (2 × 1 H, d, J = 8.0 Hz)	114.4
6,6′	6.80 (2 × 1 H, dd, J = 8.0 Hz, 1.4 Hz)	120.9
7,7′	4.12 (2 × 1 H, d, J = 8.2 Hz)	84.3
8,8′	3.72 (2 × 1 H, m)	75.7
9a,9′a	3.54 (2 × 1 H, dd, J = 12.0 Hz, 3.6 Hz)	62.5
9b,9′b	3.36 (2 × 1 H, dd, J = 11.8 Hz, 5.8 Hz)	–
3,3′-OCH_3	3.82 (2 × 3 H, s)	55.9
7,7′-OCH_3	3.26 (2 × 3 H, s)	56.6

[a] Assignments were based on the HMBC, HSQC, COSY and DEPT experiments.

1 revealed the existence of two structural fragment of C-7 (C-7′) to C-9 (C-9′), and C-5 (C-5′) to C-6 (C-6′), drawn with bold bonds, as shown in Fig. 2. The HMBC correlations of MeO/C-3 and MeO/C-7 located the two MeO at C-3 and C-7, respectively. The connectivity of C-7 and C-1 was established by the HMBC correlations from H-7 to C-1, C-2, and C-6. The remaining methine C-8 was implied to join two monomers together via oxygen atom. Thus, compound **1** with a dimeric structure was unambiguously established as shown in Fig. 1.

Due to the structural flexibility of **1**, ROESY correlation of **1** could not provide direct evidence about the relative configuration of C-7(7′)/C-8(8′). However, the large coupling constant between H-7(7′) and H-8(8′) (J = 8.2 Hz) was observed. As shown in previous report, compounds with a guaiacylglycerol unit, have the $J_{7,8}$ value 7-9 in the threo-form, and have the $J_{7,8}$ value 3-6 in the erythro-form. [13-16] Thus, the C-7/C-8 system was determined as threo-configuration and compound **1** was elucidated as Mesendannin A. NMR data and detailed experimental data of **1** is available free of charge via the Internet at http://www.trcw.ac.cn.

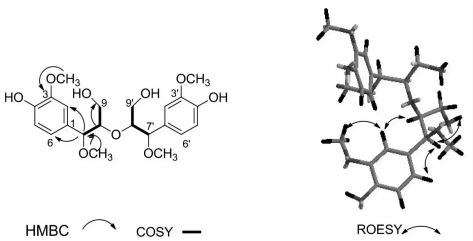

Fig. 1 ^{1}H-^{1}H COSY (Bold), Key HMBC and ROESY correlations of 1

(+)-Pinoresinol[9] (**2**): white amorphous powder; ESI-MS m/z 381 [M + Na]$^+$; molecular formula $C_{20}H_{22}O_6$; ^{1}H NMR (400 MHz, $CDCl_3$): 6.93 (2H, d, J = 1.4 Hz, H-2′,2″), 6.85 (2H, d, J = 8.2 Hz, H-5′,5″), 6.78 (2H, dd, J = 8.0, 1.6 Hz, H-6′,6″), 4.73 (2H, d, J = 4.5 Hz, H-2,6), 4.20 (2H, dd, J = 9.0, 7.0 Hz, H-4,8), 3.86 (6H, s, 3′,3″-OCH_3), 3.83 (2H, dd, J = 9.3, 3.62 Hz, H-4,8), 3.08 (2H, m, H-1,5); ^{13}C NMR (100 MHz, $CDCl_3$): 148.7 (C-3′,3″), 145.6 (C-4′,4″), 132.3 (C-1′,1″), 117.2 (C-5′,5″), 114.3 (C-6′,6″), 108.7 (C-2′,2″), 85.4 (C-2,6), 71.2 (C-4,8), 55.6 (3′,3″-OCH_3), 54.3 (C-1,5).

(-)-Eudesmin[10] (**3**): white amorphous powder; ESI-MS m/z 409 [M + Na]$^+$; molecular formula $C_{22}H_{22}O_6$; ^{1}H NMR (400 MHz, $CDCl_3$): 6.98 (2H, d, J = 1.4 Hz, H-2′,2″), 6.80 (2H, d, J = 7.8 Hz, H-5′,5″), 6.81 (2H, dd, J = 8.0, 1.4 Hz, H-6′,6″), 4.74 (2H, d, J = 4.5 Hz, H-2,6), 4.24 (2H, dd, J = 9.3, 6.9 Hz, H-4,8), 3.89 (6H, s, 3′,3″-OCH_3), 3.85 (6H, s, 3′,3″-OCH_3), 3.88 (2H, dd, J = 9.3, 3.6 Hz, H-4,8), 3.11 (2H, m, H-1,5); ^{13}C NMR (100 MHz, $CDCl_3$): 146.7 (C-3′,3″), 145.2 (C-4′,4″), 132.7 (C-1′,1″), 118.8 (C-5′,5″), 114.3

(C-6′,6″),108.7 (C-2′,2″),85.8 (C-2,6),71.5 (C-4,8),55.8 (3′,3″-OCH_3),55.4 (4′,4″-OCH_3),54.0 (C-1,5).

(-)-Drodehyrodiconiferyl alcohol[11] (**4**): white amorphous powder; ESI-MS *m/z* 381 $[M+Na]^+$ ($C_{20}H_{22}O_6$); ^{1}H NMR (400 MHz, $CDCl_3$): 6.89 (1H,s,H-6), 6.86 (2H,s,H-2,2′), 6.82 (1H,dd,*J* = 8.0,2.0 Hz,H-6′), 6.56 (1H,d,*J* = 8.0 Hz,H-5′), 6.51 (1H,d,*J* = 15.2 Hz,H-7), 6.13 (1H,m,H-8), 5.50 (1H,d,*J* = 6.0 Hz,H-7′),4.18 (2H,d,*J* = 6.2 Hz,H-9),3.90 (3H,s,3-OCH_3),3.80 (2H,m,H-9′),3.79 (3H,s,3′-OCH_3),3.57 (1H,dd,*J* = 12.2,5.8 Hz,H-8′); ^{13}C NMR (100 MHz, $CDCl_3$): 148.1 (C-4),146.6 (C-3′),145.6 (C-4′),144.2 (C-3),132.8 (C-1′),130.8 (C-7),130.1 (C-1),128.2(C-5),124.2 (C-8),119.9 (C-6′),116.7 (C-6),116.2 (C-5′),111.9 (C-2),108.5 (C-2′),88.2 (C-7′),73.8 (C-9),63.8(C-9′),56.8 (3-OCH_3),56.5 (3′-OCH_3),53.1 (C-8′).

(-)-Jatrointelignan D[11] (**5**): white amorphous powder; ESI-MS *m/z* 395 $[M+Na]^+$ ($C_{21}H_{24}O_6$); ^{1}H NMR (400 MHz, $CDCl_3$) δ:6.91 (1H,s,H-6),6.90 (2H,s,H-2,2′),6.87 (1H,dd,*J* = 8.1,1.8 Hz,H-6′),6.57 (1H,d,*J* = 8.1 Hz,H-5′),6.55(1H,d,*J* = 15.8 Hz,H-7),6.19 (1H,m,H-8),5.52 (1H,d,*J* = 6.2 Hz,H-7′),4.29 (2H,d,*J* = 6.2 Hz,H-9),3.90 (3H,s,3-OCH_3),3.80 (2H,m,H-9′),3.80 (3H,s,3′-OCH_3),3.75 (3H,s,9-OCH_3),3.50 (1H,dd,*J* = 12.4,6.2 Hz,H-8′); ^{13}C NMR (100 MHz, $CDCl_3$): 149.4 (C-4),149.1 (C-3′),147.5 (C-4′),145.5 (C-3),134.5 (C-1′),134.4 (C-7),132.2 (C-1),130.3 (C-5),124.2 (C-8),119.9 (C-6′),116.7 (C-6),116.2 (C-5′),111.9 (C-2),110.5 (C-2′),89.5 (C-7′),74.3 (C-9),64.8 (C-9′),56.8 (3-OCH_3),56.5 (3′-OCH_3),55.2 (9-OCH_3),55.1 (C-8′).

(-)-Dihydrodehyrodiconiferyl alcohol[12] (**6**): white amorphous powder; ESI-MS *m/z* 383 $[M+Na]^+$ ($C_{20}H_{24}O_6$); ^{1}H NMR (400 MHz, $CDCl_3$): 6.89 (1H,s,H-6), 6.86 (2H,s,H-2,2′), 6.82 (1H,dd,*J* = 8.0,2.0 Hz,H-6′), 6.56 (1H,d,*J* = 8.0 Hz,H-5′),5.50 (1H,d,*J* = 6.0 Hz,H-7′),4.18 (2H,d,*J* = 6.2 Hz,H-9),3.90 (3H,s,3-OCH_3),3.80 (2H,m,H-9′),3.79(3H,s,3′-OCH_3),3.57 (1H,dd,*J* = 12.2,5.8 Hz,H-8′),2.65 (2H,m,H-7),2.13 (2H,m,H-8); ^{13}C NMR (100 MHz, $CDCl_3$): 148.1 (C-4), 146.6 (C-3′), 145.6 (C-4′), 144.2 (C-3), 132.8 (C-1′),130.1 (C-1),128.2 (C-5),119.9 (C-6′), 116.7 (C-6), 116.2 (C-5′), 111.9 (C-2), 108.5 (C-2′), 88.2 (C-7′), 73.8 (C-9), 63.8 (C-9′), 56.8 (3-OCH_3), 56.5 (3′-OCH_3), 53.1 (C-8′), 34.3 (C-7),32.5 (C-8).

Cytotoxicity assays

All the isolated compounds were evaluated for their cytotoxicities against five human tumor cell lines, Hela, MCF-7, A-549, MGC-803 and COLO-205, by the MTT methods. Doxorubicin was used as positive control with IC_{50} of 0.77, 1.56, 1.92, 1.05 and 2.22 μM. The results showed that compound **1** showed medium cytotoxic against Hela, MCF, A549, MGC-803 and COLO-205 cell lines with IC_{50} of 3.92, 5.63, 9.33, 5.95 and 6.26 μM, respectively.

References

1 Peng H, Mabberley DJ. Flora of China. Beijing: Science Press, 2008.11, 116-117.

2 Zhao L, Huo CH, Shen LR, *et al*. Chemical constituents of plants from the Genus Melia. *Chem Biodivers*, 2010, 7: 839-859.

3 Oelrichs PB, Hill MW, Vallely PJ, *et al*. Toxic tetranortriterpenes of the fruit of *Melia azedarach*. *Phytochemistry*, 1983, 22: 531-534.

4 Nakatani M, Takao H, Miura I, *et al*. Azedarachol, a steroid ester antifeedant from *Melia azedarach* var. japonica. *Phytochemistry*, 1985, 24: 1945-1948.

5 Carpinella MC, Giorda LM, Ferrayoli GG, *et al*. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. *J Agric Food Chem*, 2003, 51: 2506-2511.

6 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*, 1983, 65: 55-63.

7 Guo LL, He HP, Di YT, *et al*. Indole alkaloids from *Ervatamia chinensis*. *Phytochemistry*, 2012, 74: 140-145.

8 Zhu F, Di YT, Li XY, *et al*. Neoclerodane diterpenoids from *Scutellaria barbata*. *Planta Med*, 2011, 77: 1536-1541.

9 Cao L, Huang DL, Chen H. Chemical constituents from *Calli-*

carpa macrophylla. *Zhongguo Xiandai Zhongyao*, 2014, 16: 733-739.

10 Cai XF, Lee IS, Kim YH, *et al*. Inhibitory lignans against NFAT transcription factor from *Acanthopanax koreanum*. *Arch Pharmacal Res*, 2004, 27: 738-741.

11 Yuen MSM, Xue F, Mark TCM, *et al*. On the absolute structure of optically active neolignans containing a dihydrobenzo [b] furan skeleton. *Tetrahedron*, 1998, 54: 12429-12444.

12 Yang YP, Cheng MJ, Teng CM, *et al*. Chemical and antiplatelet constituents from Formosan Zanthoxylum simulans. *Phytochemistry*, 2002, 61: 567-572.

13 Li LY, Seeram NP. Further investigation into maple syrup yields 3 newlignans, a new phenylpropanoid, and 26 other phytochemicals. *J Agric Food Chem*, 2011, 59: 7708-7716.

14 Matsumori N, Kaneno D, Tachibana K, *et al*. Stereochemical determination of acyclic structures based on carbon-proton spin-coupling constants. Amethod of configuration analysis for natural products. *J Org Chem*, 1999, 64: 866-876.

15 Deyama T, Ikawa T, Kitagawa S, *et al*. The constituents of *Eucommia ulmoides* Oliv. V: isolation of dihydrox-ydehydroconiferyl alcohol isomers and phenolic compounds. *Chem Pharm Bull*, 1987, 35: 1785-1789.

16 Wang H, Geng CA, Xu HB, *et al*. Lignans from the fruits of *Melia toosendan* and their agonistic activities on melatonin receptor MT1. *Planta Med*, 2015, 81: 847-854.