Fitoterapia 105 (2015) 273–277

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Six new cassane diterpenes from the twigs and leaves of Tara (*Caesalpinia spinosa* Kuntze)

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ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 26 July 2015
Accepted 28 July 2015
Available online 2 August 2015

Keywords:
Tara
Caesalpinia spinosa
Leguminaceae
Cassane diterpenoid
Caespinosins A–E

ABSTRACT

Six new cassane diterpenes, isoneocaesalpin H (**1**), caespinosin A (**2**), caespinosin B (**3**), a cassane diterpene with unique 6/6/7 carbon rings, and caespinosins C–E (**4**–**6**) were isolated from the twigs and leaves of Tara (*Caesalpinia spinosa* Kuntze). The absolute configuration of isoneocaesalpin H (**1**) was determined by single-crystal X-ray crystallographic analysis. Compound **3** represents a class of rare natural cassane diterpene bearing unique 6/6/7 carbon rings. Their structures were identified by 1D and 2D NMR spectral data. Cassane diterpenes were firstly reported from Tara. Compounds **1**–**5** were evaluated for their cytotoxicity on HL-60, SMMC-7721, A-549, MCF-7 and SW-480 human cancer cell lines, but they were inactive.

1. Introduction

Caesalpinia spinosa Kuntze, commonly known as Tara, belongs to the family Caesalpiniaceae, which is a small tree or thorny shrub native to Peru. It has been introduced and largely cultivated in China's Yunnan and Sichuan provinces as a source of tannins rich in the fruit pods [1]. Tara infusions have been traditionally and extensively used by the Peruvian folk medicine to treat inflamed tonsils, fever, cold and stomachaches [2].

Studies have mainly been focused on the tannins of *C. spinosa* [3–7]. Few reports are available concerning the non-tanninic components of this species. Because of the various cassane-type diterpenoids, the plants of *Caesalpinia* have drawn wide attention. Cassane-type diterpenoids showed various activities [8], among which the cytotoxic activities [9,10] are interesting to us. As our continuous research on cassane diterpenoids of *Caesalpinia* plants in Yunnan Province, further investigation on Tara led to the isolation of six new cassane diterpenes, isoneocaesalpin H (**1**), and caespinosins A–E (**2**–**6**) (Fig. 1) from the ethanol extract of the brunches and leaves. Caespinosin B (**3**) represents a class of rare natural cassane diterpene bearing unique 6/6/7 carbon rings.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. IR Spectra were obtained on a Bruker Tensor 27 FT-IR polarimeter. NMR spectra were acquired on Bruker DRX-500 and Bruker Avance 600 MHz spectrometer. MS data were obtained using a VG AutoSpec-3000 and API QSTAR time-of-flight spectrometers. Fractions were monitored by TLC on silica gel plates (GF_{254}, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Column chromatography (CC) was performed on silica gel (100–200 mesh or 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare) and MCI gel (75–150 mm, Mitsubishi Chemical Corporation, Tokyo, Japan).

2.2. Plant material

The materials were collected by the corresponding author from Yimen County, Yunnan Province, P. R. China, in December 2012, and the voucher specimen was collected in August 2014 by the corresponding author and identified by Haiying Ma (Yunnan University). A voucher specimen (Ma H.Y. 2014290) was deposited at Herbarium of Yunnan University, Kunming, China.

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http://dx.doi.org/10.1016/j.fitote.2015.07.018
0367-326X/

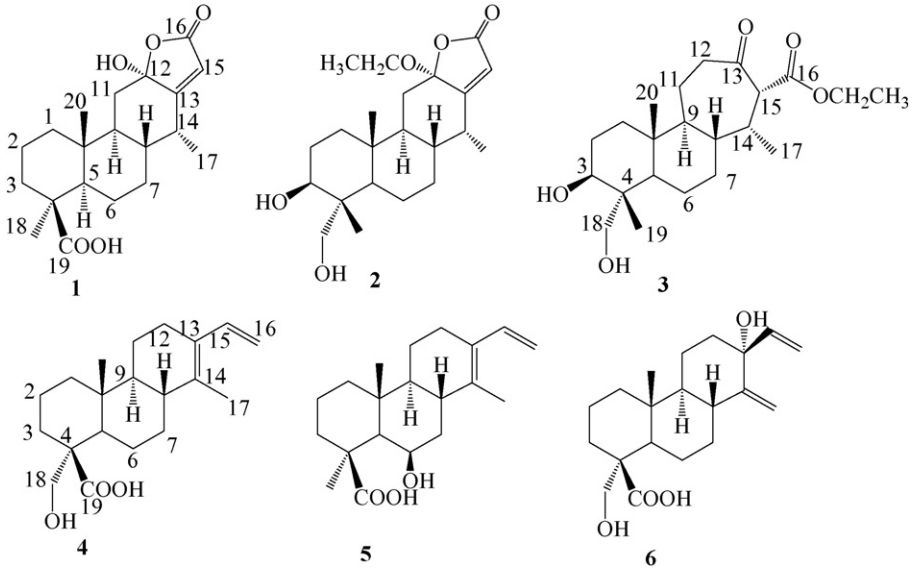

Fig. 1. The structures of compounds **1**–**6**.

2.3. Extraction and isolation

The powdered twigs and leaves (10 kg) of Tara were extracted with EtOH at room temperature, which afforded a dark residue after evaporation under reduced pressure. The residue was dissolved in H_2O and extracted by $CHCl_3$. The $CHCl_3$ extract (270 g) was subjected to CC (SiO_2, 100–200 mesh; petroleum ether/EtOAc 40:1, 20:1, 8:1, 5:1, 3:1, 1:1, 0:1) to gain sixteen fractions (Fr. T_1–T_{16}). Fr. T_{12} (44 g) was subjected to CC (SiO_2, $CHCl_3$/CH_3OH 100:1–1:1) to obtain nine subfractions (T_{12}a–T_{12}i). T_{12}e was resubjected to CC ($CHCl_3$/MeOH 100:1, petroleum ether/CH_3COCH_3 8:1; petroleum ether/EtOAc 4:1; Sephadex LH-20, $CHCl_3$/MeOH 1:1) to provide compound **5** (28.9 mg). T_{12}f was subjected to CC (petroleum ether/acetone 8:1, $CHCl_3$/MeOH 70:1, petroleum ether/EtOAc 3:1; Sephadex LH-20, $CHCl_3$/MeOH 1:1) to provide compounds **4** (123.0 mg), **6** (6.6 mg) and **1** (45.2 mg). T_{14} (21.5 g) was subjected to CC (MCI, MeOH/H_2O 4:6–1:0) to give seven subfractions (T_{14}a–T_{14}g). T_{14}c was subjected to CC (SiO_2, $CHCl_3$/EtOAc 15:1, petroleum ether/EtOAc 3:1; Sephadex LH-20, $CHCl_3$/MeOH 1:1; MCI, MeOH/H_2O 4:6–1:0), to provide compounds **3** (7.6 mg) and **2** (4.0 mg).

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**3** (**1** recorded at 500 MHz, **2** and **3** recorded at 600 MHz) (ppm, *J* in *Hz* in parentheses).

No.	1 δ_H	2 δ_H	3 δ_H
1	1.05 (overlap)	1.13 (overlap)	1.00 (overlap)
	1.86 (overlap)	1.79 (dt, 13.1, 3.5)	1.59 (m)
2	1.51 (overlap)	1.63 (overlap)	1.69 (m)
	1.86 (overlap)	1.63 (overlap)	1.71 (overlap)
3	1.05 (overlap)	3.70 (dd, 5.5, 10.0)	3.63 (dd, 5.5, 10.0)
	2.17 (br d, 13.5)		
5	1.18 (overlap)	1.36 (br s)	1.25 (br s)
6	1.86 (overlap)	1.46 (overlap)	1.44 (overlap)
	2.01 (br d, 13.5)	1.63 (overlap)	1.66 (overlap)
7	1.30 (m)	1.46 (overlap)	1.45 (overlap)
	2.43 (dd, 4.0, 13.0)	1.63 (overlap)	1.66 (overlap)
8	1.67 (overlap)	1.63 (overlap)	2.06 (m)
9	1.51 (overlap)	1.46 (m)	1.47 (overlap)
11	1.67 (overlap)	1.36 (overlap)	1.45 (overlap)
	1.39 (dd, 3.0, 13.0)	2.40 (dd, 3.2, 13.1)	2.17 (m)
12			2.73 (dd, 9.0, 16.5)
			2.16 (m)
14	2.99 (m)	3.01 (dq, 5.0, 7.4)	2.11 (m)
15	5.73 (s)	5.88 (s)	3.13 (m)
17	1.18 (3H, d, 7.5)	1.13 (3H, d, 7.4)	0.65 (3H, d, 7.5)
18	1.24 (3H, s)	3.32 (overlap)	3.29 (d, 11.0)
		3.63 (d, 11.0)	3.53 (d, 11.0)
19		0.78 (3H, s)	0.75 (3H, s)
20	0.77 (3H, s)	1.89 (3H, s)	1.04 (3H, s)
OCH_2CH_3		3.50 (dd, 5.9, 15.5),	4.12 (2H, dd, 7.0, 14.5)
		3.32 (overlap)	
OCH_2CH_3		1.16 (3H, t, 7.0)	1.25 (3H, t, 7.0, 14.5)

Isoneocaesalpin H (**1**), colorless needles (acetone/n-hexane); IR (KBr): ν_{max} = 3446, 2944, 1744, 1698, 1631, 1350, 934 cm^{-1}; $[\alpha]_D^{17.9}$ − 59.48 (*c* 0.320, CH_3OH); EIMS: *m/z* 348 $[M]^+$, 320, 319, 304; HREIMS: *m/z* 348.1933 $[M]^+$ (calcd. for $C_{20}H_{28}O_5$, 348.1937). ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3.

Caespinosin A (**2**), white amorphous powder; IR (KBr): ν_{max} = 3439, 2935, 1764, 1636, 1190, 1041 cm^{-1}; $[\alpha]_D^{19.6}$ − 66.54 (*c* 0.103, CH_3OH); positive ESIMS: *m/z* 379 $[M + H]^+$. Positive HRESIMS: *m/z* 379.2482 $[M + H]^+$ (calcd. for $C_{22}H_{35}O_5$, 379.2479). ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3.

Caespinosin B (**3**), colorless needles (acetone/n-hexane); IR (KBr): ν_{max} = 3435, 2933, 1734, 1711, 1630, 1447, 1038 cm^{-1}; $[\alpha]_D^{19.8}$ − 32.38 (*c* 0.140, CH_3OH); positive ESIMS: *m/z* 403 $[M + Na]^+$. Positive HRESIMS: *m/z* 403.2466 $[M]^+$ (calcd. for $C_{22}H_{36}O_5Na$, 403.2460); ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3.

Caespinosin C (**4**), white amorphous powder; IR (KBr): ν_{max} = 3446, 2919, 1698, 1631, 1350 cm^{-1}; $[\alpha]_D^{18.3}$ − 35.59 (*c* 0.230, CH_3OH); positive ESIMS: *m/z* 318 $[M]^+$; HREIMS: *m/z* 318.2194 $[M]^+$ (calcd. for $C_{20}H_{30}O_3$, 318.2195); ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3.

Caespinosin D (**5**), white amorphous powder; IR (KBr): ν_{max} = 3446, 2936, 1699, 1632, 1351 cm^{-1}; $[\alpha]_D^{17.6}$ − 33.92 (*c* 0.170, CH_3OH); EIMS: *m/z* 317 $[M - 1]^+$, 300 $[M - 18]^+$; HREIMS: *m/z* 318.2187 $[M]^+$ (calcd. for $C_{20}H_{30}O_3$, 318.2195); ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3.

Caespinosin E (**6**), white amorphous powder; IR (KBr): ν_{max} = 3470, 2942, 1706, 1631 cm, 1349 cm^{-1}; $[\alpha]_D^{17.7}$ + 23.96 (*c* 0.210, CH_3OH); positive ESIMS: *m/z* 334 $[M]^+$. HREIMS: *m/z* 334.2145 $[M]^+$ (calcd. for $C_{20}H_{30}O_4$, 334.2144); ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3.

2.4. X-ray crystallographic analysis

Single crystal X-ray diffraction analysis of **1**: X-ray data for **1** were collected on a Bruker APEX II DUO diffractometer using Cu Ka radiation: $C_{20}H_{28}O_5 \cdot 0.5(C_6H_{14}) \cdot H_2O$, *M* = 409.53, orthorhombic, *a* = 8.0451(2) Å, *b* = 13.6830(3) Å, *c* = 39.0617(10) Å, α = 90.00°, β = 90.00°, γ = 90.00°, *V* = 4299.96(18) $Å^3$, *T* = 100(2) K, space group *C*2221, *Z* = 8, μ(CuKα) = 0.729 mm^{-1}, 21215 reflections