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

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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

\textit{Caesalpinia spinosa} Kuntze, commonly known as Tara, belongs to the family Caesalpiniiaceae, 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 \cite{1}. Tara infusions have been traditionally and extensively used by the Peruvian folk medicine to treat inflamed tonsils, fever, cold and stomachaches \cite{2}.

Studies have mainly been focused on the tannins of \textit{C. spinosa} \cite{3–7}. Few reports are available concerning the non-tanninic components of this species. Because of the various cassane-type diterpenoids, the plants of \textit{Caesalpinia} have drawn wide attention. Cassane-type diterpenoids showed various activities \cite{8}, among which the cytotoxic activities \cite{9,10} are interesting to us. As our continuous research on cassane diterpenoids of \textit{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 branches 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 (GF254, 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.
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 H2O and extracted by CHCl3. The CHCl3 extract (270 g) was subjected to CC (SiO2, 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. T1–T16). Fr. T2 (44 g) was subjected to CC (SiO2, CHCl3/CH3OH 100:1, petroleum ether/CH3OH 8:1; petroleum ether/EtOAc, 4:1; Sephadex LH-20, CHCl3/MeOH 1:1) to provide compound (27.4 mg).

T12f was subjected to CC (petroleum ether/acetone 8:1, CHCl3/MeOH 5:1, 3:1, 1:1, 0:1) to gain sixteen fractions (Fr. T12a–T12q). Fr. T12f (21.5 g) was subjected to CC (SiO2, CHCl3/CH3OH 15:1, petroleum ether/EtOAc 3:1; Sephadex LH-20, CHCl3/MeOH 1:1; PCI, MeOH/H2O 4:6–1:0) to give seven subfractions (T12a–T12g): T12f was subjected to CC (SiO2, CHCl3/CH3OH 15:1, petroleum ether/EtOAc 3:1; Sephadex LH-20, CHCl3/MeOH 1:1; PCI, MeOH/H2O 4:6–1:0) to provide compounds (7.6 mg) and (2.4 mg).

The structures of compounds were determined by 1H and 13C NMR spectroscopy, and the positive ESIMS of [M + Na]+ peaks at m/z 334.2144 and 334.2194 were consistent with the calculated values for C22H35O5 (333.2127) and C22H36O5Na (333.2460), respectively. The positive ESIMS gave the molecular ion peaks at m/z 3439.2466, 2944.2600, 1744.1794, 1631.0043, 1350.9517, 934.8287, and 598.7165, corresponding to C22H35O5 (3439.2460), C22H35O5Na (3439.2460), C22H36O5Na (3439.2460), C22H36O5Na (3439.2460), C22H36O5Na (3439.2460), C22H36O5Na (3439.2460), and C22H36O5Na (3439.2460), respectively.

NMR spectroscopy data of compounds 1–3 (1 recorded at 500 MHz, 2 and 3 recorded at 600 MHz) are listed in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
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<th>13C NMR (125 MHz)</th>
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<tbody>
<tr>
<td>1</td>
<td>1.05 (overlap)</td>
<td>1.13 (overlap)</td>
</tr>
<tr>
<td>2</td>
<td>1.13 (overlap)</td>
<td>1.20 (overlap)</td>
</tr>
<tr>
<td>3</td>
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Fig. 1. The structures of compounds 1–6.

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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 Kα radiation: C20H30O4, 334.2144); 1HN M Ra n d13C NMR data, see Tables 1 and 3.

IR (KBr): ν 3446, 2944, 1744, 1698, 1631, 1350, 934 cm−1; [M + H]+ (calcd. for C22H35O5, 3434.2144); 1HN M Ra n d13C NMR data, see Tables 1 and 3.

IR (KBr): ν 3446, 2944, 1744, 1698, 1631, 1350, 934 cm−1; [M + H]+ (calcd. for C22H35O5, 3434.2144); 1HN M Ra n d13C NMR data, see Tables 1 and 3.