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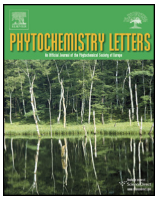

Four new cassane diterpenes from the seeds of *Caesalpinia minax*

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ABSTRACT

In our survey on the chemical composition of traditional Chinese medicines to further elucidate their chemical substances for the treatment of diseases, we investigated the chemical constituents of the seeds of plants *Caesalpinia minax*. The investigation led to the isolation and identification of four new cassane diterpenes, caesalpines A–D (**1**–**4**). Their structures were elucidated on the basis of extensive 1D and 2D NMR (COSY, HMQC, HMBC, and NOESY) and mass (ESIMS and HR-ESIMS) spectroscopic data analyses. The phytochemical results imply that cassane diterpenes are maybe regarded as the characteristic constituents of *C. minax*.

1. Introduction

Caesalpinia minax Hance, belonging to the family Caesalpinaceae, is a prickly shrub growing in the tropical and subtropical regions of Southeast Asia. The seeds of this plant, called "ku-shi-lian" in China, have been used as a traditional Chinese medicine for the treatment of common cold, fever and dysentery since ancient times (Jiangsu New Medical College, 1977). Previous phytochemical investigations on *C. minax* led to the isolation and identification of diterpenes, especially cassane-type diterpenes (Ma et al., 2012a,b,c; Wu et al., 2010; Li et al., 2006; Jiang et al., 2002, 2001), which showed the cytotoxic, antiproliferative, and antiviral activities (Ma et al., 2012a,b; Jiang et al., 2002, 2001). In our continuous survey on the chemical composition of traditional Chinese medicines to further elucidate their chemical substances for the treatment of diseases (Guo et al., 2011, 2012; Xu et al., 2012), we investigated the chemical constituents of the seeds of plants *C. minax*. As a result, four new cassane diterpenes, designated caesalpines A–D (**1**–**4**) (Fig. 1), have been isolated from the seeds of *C. minax*. Their structures were elucidated on the basis of extensive 1D and 2D NMR (COSY, HMQC, HMBC, and NOESY) and mass (ESIMS and HR-ESIMS) spectroscopic data analyses. Herein, we describe the isolation and structural elucidation of these new natural cassane diterpenes.

2. Results and discussion

The ethyl acetate-soluble part of the methanol extract of the seeds of *C. minax* was fractionated by column chromatography and purified by HPLC to obtain four new cassane diterpenes (**1**–**4**).

Compound **1** was obtained as an amorphous powder. Its HR-ESIMS provided the molecular formula, $C_{23}H_{32}O_7$, through the presence of a peak at *m*/*z* 443.2042 $[M+Na]^+$ (calcd. for $C_{23}H_{32}O_7Na$, 443.2046), which was compatible with the NMR data. The ^1H NMR spectrum for **1** exhibited four methyl groups [δ_H 1.01 (3H, s, H_3-18), 1.07 (3H, s, H_3-19), 1.13 (3H, s, H_3-20), and 2.00 (3H, s, $COCH_3$-7)], two olefinic protons [δ_H 6.12 (1H, s, H-15), and 7.24 (1H, s, H-16)], and two oxygenated methine protons [δ_H 5.13 (1H, ddd, $J = 10.8$, 10.8, 4.2 Hz, H-7), and 3.73 (1H, m, H-1)] (Table 1). In addition, a methoxy singlet at δ_H 3.74 (3H, s, OCH_3-17) was also displayed in the ^1H NMR spectrum of **1**. The ^{13}C NMR spectrum of **1** showed 23 carbon resonances. Based on the aforementioned proton signals and the following carbon resonances (δ_C 51.9, 170.5, and 21.1) in the ^{13}C NMR spectrum, one acetyl group and one methoxy group were deduced and defined. Apart from the above three signals for the acetyl and the methoxy group, there are additional 20 resonances exhibited for the parent skeleton in the ^{13}C NMR spectrum, which comprised three methyls (C-18, C-19, and C-20), four methylenes (C-2, C-3, C-6, and C-11), seven methines (C-1, C-7, C-8, C-9, C-14, C-15, and C-16), and six quaternary carbons (C-4, C-5, C-10, C-12, C-13, and C-17) based on the DEPT and HMQC spectra. According to the reported diterpenes from the genus *Caesalpinia*, a careful comparison of the chemical shifts of C-1–C-20 of compound **1** with those of cassane-type

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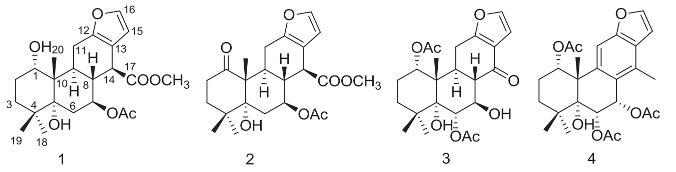

Fig. 1. Structures of compounds **1–4** from *C. minax*.

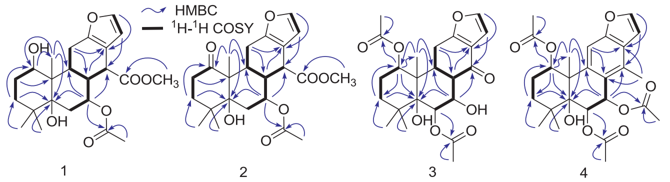

Fig. 2. Selected ^{1}H–^{1}H COSY and HMBC correlations of compounds **1–4**.

diterpenes, bonducellpin C and caesalpinin E (Peter et al., 1997; Linn et al., 2005), suggested compound **1** had the same cassane-type furanoditerpene skeleton. The following HMQC and HMBC interpretation confirmed the presence of the cassane-type diterpene skeleton. The oxygenated quaternary carbon signal at δ_C 79.7 was assigned to C-5 by the HMBC correlations of H$_3$-20/C-5, H$_3$-18/C-5, H$_3$-19/C-5, H$_2$-6/C-5, and H-7/C-5. Similarly, the remaining oxygenated carbons at δ_C 72.3 and 76.7 and the carbonyl carbon at δ_C 175.0 were attributed to C-1, C-7, and C-17, respectively, by the corresponding HMBC correlations. The long-range coupling of the proton at δ_H 5.13 (H-7) with the carbonyl carbon at δ_C 170.5 (CO of the acetoxy) demonstrated the presence of the acetoxy group at C-7. The methoxy group was verified at C-17 by the HMBC correlation of OCH$_3$/C-17. There were no additional acyloxy groups in **1** and no long-range correlations observed for H-1 (δ_H 3.73) with the carbonyl carbons, which indicated that the substituent at C-1 should be a hydroxy group, which was supported by the HR-RSIMS data. By further analyzing the HMQC, HMBC, and ^{1}H–^{1}H COSY spectra (Fig. 2), all the proton and carbon signals were assigned unambiguously, which resulted in the establishment of the planar structure for **1**.

The relative configuration of **1** was elucidated as follows. For the reported natural cassane-type diterpenes, the three six-membered rings (6/6/6) comprising C-1–C-14 are generally *trans*-fused with each other, H-8, and H$_3$-20 are β-oriented based on biosynthetic considerations, and H-9, and the hydroxy at C-5 are α-oriented (Pudhom et al., 2007; Linn et al., 2005; Peter et al., 1997). NOESY correlations (Fig. 3) observed for H-1/H$_3$-20, H-7/H-9, H-7/H-14, H-8/H-11β, H-11β/H$_3$-20, and H$_3$-20/H$_3$-19, but not for H-9/H$_3$-20, suggested that the C-1 hydroxy group was in an α-position with an axial orientation, and the C-7 acetoxy group and the C-14 methoxycarbonyl group were both in β-positions with an equatorial orientation. These assignments were consistent with the orientations of H-8, H-9, H$_3$-18, H$_3$-19, and H$_3$-20 of cassane-type diterpenes reported in the literature (Pudhom et al., 2007; Linn et al., 2005; Peter et al., 1997). The structure of compound **1** was therefore elucidated and named caesalpine A.

Compound **2**, a white powder, possessed a molecular formula of $C_{23}H_{30}O_7$ as determined from the HR-ESIMS (*m*/*z* 441.1886 [M+Na]$^+$, calcd. for $C_{23}H_{30}O_7Na$ 441.1889) and NMR analyses. From the ^{1}H NMR spectrum of **2**, four methyl and one methoxy group (Table 1) were exhibited. The ^{13}C NMR spectrum of **2** showed 23 carbon resonances. Based on the ^{1}H and ^{13}C NMR spectra, one acetoxy group and one methoxy group were deduced from the observation of the proton signals [δ_H 1.99 (3H, s, OCOCH$_3$-7), 3.72 (3H, s, OCH$_3$-17)] and the corresponding carbons (δ_C 170.5, 21.1,

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1–4** (in CDCl$_3$, δ in ppm, *J* in Hz).[a]

No.	1		2		3		4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	72.3	β 3.73 m	213.2		75.3	β 4.90 br. s	75.9	β 5.66 br. s
2	26.0	α 1.68 m	35.4	α 2.22 m	22.3	α 1.13 m	22.4	α 1.96 m
		β 2.02 m		β 2.88 m		β 1.75 m		β 2.25 m
3	29.7	α 1.44 m	37.6	α 1.68 m	32.2	α 1.17 m	32.6	α 1.17 m
		β 2.04 m		β 2.05 m		β 1.80 m		β 1.91 m
4	38.5		38.8		38.5		38.7	
5	79.7		82.9		79.0		79.6	
6	32.7	α 1.70 m	32.6	α 1.86 m	74.8	β 5.48 d (9.2)	76.9	β 5.94 d (5.0)
		β 2.10 m		β 2.08 m				
7	76.7	α 5.13 ddd (10.8, 10.8, 5.4)	75.6	α 5.03 ddd (11.0, 11.0, 4.9)	70.7	α 4.34 t (9.2)	75.3	6.48 d (5.0)
8	38.8	β 2.49 m	39.6	β 2.39 m	50.0	β 2.58 m	126.7	
9	36.6	α 2.92 m	37.6	α 2.80 m	37.9	α 3.11 m	139.8	
10	43.5		55.4		44.9		48.8	
11	21.4	β 2.51 m	23.7	β 2.41 m	23.0	β 2.56 m	104.2	7.04 s
		α 2.82 dd (15.8,5.4)		α 3.26 dd (15.7,4.2)		α 2.75 m		
12	150.6		151.3		166.3		154.6	
13	113.0		112.3		119.8		126.8	
14	46.1	α 3.39 d (8.6)	46.0	α 3.39 d (8.4)	196.8		130.9	
15	108.3	6.12 s	108.2	6.10 s	106.3	6.67 d (1.9)	105.3	6.76 d (2.2)
16	141.3	7.24 s	141.1	7.21 s	143.6	7.36 d (1.9)	145.1	7.57 d (2.2)
17	175.0		174.9				16.0	2.32 s
18	27.8	1.01 s	27.1	1.01 s	30.4	1.15 s	30.2	1.22 s
19	24.8	1.07 s	25.6	1.29 s	24.4	1.20 s	24.9	1.21 s
20	17.6	1.13 s	16.3	1.49 s	17.4	1.32 s	28.8	1.60 s
1-OAc					169.2		169.3	
					21.4	2.12 s	21.3	1.92 s
6-OAc					170.7		171.2	
					21.8	2.19 s	22.0	2.18 s
7-OAc	170.5		170.5				170.7	
	21.1	2.00 s	21.1	1.99 s			21.0	2.04 s
OMe	51.9	3.74 s	51.9	3.72 s				

[a] The assignments are based on DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY, and NOESY experiments.