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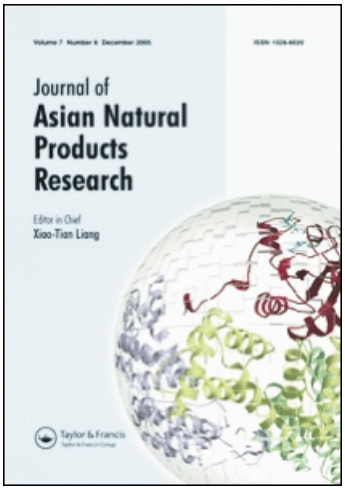

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Three new cassane diterpenes from the seeds of *Caesalpinia minax* Hance

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ORIGINAL ARTICLE

Three new cassane diterpenes from the seeds of *Caesalpinia minax* Hance

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Three new cassane-diterpene-lactones, methyl 1α,7β-diacetoxy-5α,12α-dihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate (**1**), methyl 7β-acetoxy-1α,5α,12α-trihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate (**2**), and 12α-ethoxyl-1α,6α,7β-triacetoxy-5α,14β-dihydroxy-cass-13(15)-en-16,12-olide (**3**), were isolated from the seeds of *Caesalpinia minax* Hance. Their structures were established on the basis of HR-ESI-MS, 1D and 2D NMR spectral analysis.

Keywords: *Caesalpinia minax* Hance; Fabaceae; cassane diterpenes

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1. Introduction

Plants belonging to the genus *Caesalpinia* (Fabaceae) are widely distributed throughout the tropical and subtropical regions, and many species are used in different systems of traditional medicine for the treatment of many diseases such as antiviral [1–4], anti-inflammatory, anti-analgesic [5], radical growth regulation [6], and anti-tumor [7] agents. In addition, a large number of chemical investigations on this genus have found that diterpene derivatives with a cassane skeleton are the main bioactive components, and much attention has been given to these compounds recently. The seeds of *Caesalpinia minax* Hance are called 'Ku-Shi-Lian' in China, as a folk medicine, especially in Guangxi Zhuang Autonomous Region and Sichuan Province, to treat influenza, fever, and dysentery [8]. For the purpose of finding new anti-flu viral agents from this plant, the chemical study on the seeds led to the isolation of three new cassane-diterpene-lactones. In this paper, we describe the separation and structural elucidation of these new compounds **1**–**3** (Figure 1).

2. Results and discussion

Compound **1** was obtained as colorless needles ($CHCl_3$). Its molecular formula was established as $C_{25}H_{34}O_{10}$ by the positive ion peak at *m/z* 495.2222 $[M + H]^+$ in HR-ESI-MS. The absorption bands at 3408 and 1744 cm^{-1} in the IR spectrum indicated the presence of the hydroxyl and carbonyl groups. The ^1H NMR spectrum (Table 1) exhibited signals due to six methyl singlets [δ_H 1.03 (6H, s),

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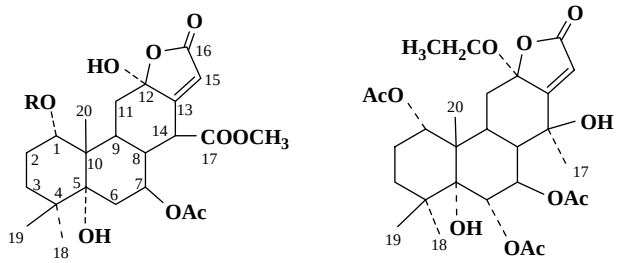

1 R = –$COCH_3$
2 R = H

3

Figure 1. Structures of compounds **1**–**3**.

1.09 (3H, s), 2.17 (3H, s), 2.00 (3H, s), 3.79 (3H, s)], two oxygen-substituted methines [δ_H 5.23 (1H, td, $J = 10.8$, 5.4 Hz), 4.90 (1H, br s)], one olefin proton at δ_H 5.69 (1H, d, $J = 1.8$ Hz), along with other alkyl signals. The ^{13}C NMR spectrum (Table 1) showed 25 carbon signals, including four carbons for two acetoxyl groups (δ_C 21.2, 169.9, 21.3, 169.7), three tertiary methyls (δ_C 17.5, 24.5, 27.9), one methoxy (δ_C 52.2), five methylenes (δ_C 22.6, 29.9, 32.1, 36.0, 36.1), five methines (δ_C 44.0, 48.4, 74.7, 74.8, 115.4), and seven quaternary carbons (δ_C 38.3, 43.4, 78.2, 104.1, 164.8, 169.3, 171.1). The ^1H and ^{13}C NMR spectral data (Table 1) for **1** suggested that it was a cassane derivative with the α,β-unsaturated γ-lactone moiety [9]. The existence of the α,β-unsaturated γ-lactone moiety could be verified by four carbon signals at δ_C 104.1, 115.4, 164.8, and 169.3. Moreover, the locations of two acetoxyl groups were determined to be at C-1 and C-7, respectively, on the basis of long-range correlations of H-1 (δ_H 4.90) with one carbonyl at δ_C 169.7 and H-7 (δ_H 5.23) with the other at δ_C 169.9. Compound **1** was considered to be a derivative of neocaesalpin N with two acetoxyl groups in the structure, which was confirmed by the analysis of HSQC and HMBC spectra completely (Figure 2) [9]. In addition, compound **1** persisted the same stereochemistry as that of neocaesalpin N, according to the NOESY correlations (Figure 2) of CH_3-20/H-1, H-6_{ax}, H-8; H-14/H-7, H-9 and the coupling constant for H-14 (δ_H 3.42, dd, $J = 10.5$, 1.8 Hz). Therefore, compound **1** was elucidated as methyl 1α,7β-diacetoxy-5α,12α-dihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate.

Compound **2** was obtained as white needles ($CHCl_3$) and its molecular formula was assigned as $C_{23}H_{32}O_9$ by the positive ion peak at m/z 475.1941 $[M + Na]^+$. The IR spectrum of **2** showed the absorption bands at 3366 (hydroxyl) and 1747 cm^{-1} (ester carbonyls). The ^1H NMR and ^{13}C NMR spectra exhibited almost the same signal patterns as those of **1** except for the lack of an acetoxyl group [δ_H 2.17 (3H, s) in the ^1H NMR spectrum and δ_C 21.3, 169.7 in the ^{13}C NMR spectrum of **1**]. Comparing with compound **1**, the chemical shift for C-1 in **2** was upfielded from δ_C 74.8 to 72.1, which indicated the presence of a hydroxyl at this position, and this hypothesis could be confirmed by its HMBC experiment. The long-range correlations of H-1 (δ_H 3.66) with C-3 (δ_C 29.7), C-5 (δ_C 80.1), and C-10 (δ_C 43.4) were observed. Thus, compound **2** was elucidated as methyl 7β-acetoxy-1α,5α,12α-trihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate.

Compound **3** was obtained as a white amorphous solid ($CHCl_3$). Its molecular formula was determined as $C_{28}H_{40}O_{11}$ by

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Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**3** ($CDCl_3$, *J* in Hz).

	1		**2**		**3**	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.90 (br s)	74.8	3.66 (br s)	72.1	4.87 (br s)	75.1
2	1.78 (m) α	22.6	1.74 (m) α	25.6	1.76 (m) α	22.3
	2.00 (m) β		1.99 (m) β		1.92 (m) β	
3	1.15 (m) α	29.9	1.13 (m) α	29.7	1.13 (m) α	32.2
	1.74 (m) β		1.95 (m) β		1.76 (m) β	
4	–	38.3	–	38.5	–	38.4
5	–	78.2	–	80.1	2.93 (s, 5-OH)	79.1
6	1.56 (dd, 13.2, 10.8) β	32.1	1.63 (dd, 13.2, 10.8) β	32.9	5.47 (d, 8.4)	74.7
	2.18 (dd, 13.2, 5.4) α		2.08 (dd, 13.2, 5.4) α			
7	5.23 (td, 10.8, 5.4)	74.7	5.11 (td, 10.8, 5.4)	75.1	5.65 (t, 9.0)	72.6
8	2.24 (m)	44.0	2.28 (m)	43.7	1.92 (m)	50.8
9	2.84 (td, 12.6, 2.4)	36.0	2.97 (td, 12.0, 3.0)	35.9	2.69 (t, 12.6)	33.5
10	–	43.4	–	43.4	–	44.4
11	1.44 (dd, 13.2, 12.6) β	36.1	1.55 (dd, 13.2, 12.6) β	36.1	1.33 (t, 12.6) β	37.6
	2.06 (dd, 13.2, 2.4) α		2.56 (dd, 13.2, 3.0) α		2.12 (d, 10.8) α	
12	–	104.1	–	104.1	–	106.3
13	–	164.8	–	164.2	–	172.5
14	3.42 (dd, 10.5, 1.8)	48.4	3.44 (dd, 10.2, 1.8)	48.2	2.31 (s, 14-OH)	74.9
15	5.69 (d, 1.8)	115.4	5.67 (d, 1.8)	115.4	6.06 (s)	116.4
16	–	169.3	–	169.3	–	168.6
17	–	171.1	–	171.2	1.57 (s)	20.0
18	1.03 (s)	27.9	0.99 (s)	27.7	1.14 (s)	30.4
19	1.03 (s)	24.5	1.03 (s)	24.6	1.13 (s)	24.4
20	1.09 (s)	17.5	1.02 (s)	17.5	1.15 (s)	16.6
1-$OCOCH_3$	2.17 (s)	169.7	–	–	2.14 (s)	168.8
–	–	21.3	–	–	–	21.1
6-$OCOCH_3$	–	–	–	–	2.08 (s)	170.2
–	–	–	–	–	–	21.5
7-$OCOCH_3$	2.00 (s)	169.9	2.01 (s)	170.3	2.01 (s)	170.7
–	–	21.2	–	21.2	–	21.3

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Table 1 – *continued*

No.	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
12-OCH_2CH_3	–	–	–	–	*CH_2* 3.19(m)/3.55(m)	58.8
–	–	–	–	–	*CH_3* 1.16 (t, 6.6)	14.8
17-OCH_3	3.79 (s)	52.2	3.79 (s)	52.1	–	–

the positive ion peak at *m/z* 553.2645 $[M + H]^+$. Its IR spectrum exhibited absorption bands at 3570 and 1745 cm^{-1}, indicating the existence of the hydroxyl and ester carbonyl groups. The presence of an α,β-unsaturated γ-lactone moiety was substantiated by the signals for the olefin proton and carbon signals at δ_H 6.06 and δ_C 116.4, respectively, the downfield olefin quaternary carbon at δ_C 172.5, the lactone carbonyl at δ_C 168.6, and the hemiketal carbon at δ_C 106.3. The ^1H NMR spectrum (Table 1) showed the presence of four tertiary methyl groups at δ_H 1.13 (3H, s), 1.14 (3H, s), 1.15 (3H, s), 1.57 (3H, s), three acetoxyl groups at δ_H 2.01 (3H, s), 2.14 (3H, s), 2.08 (3H, s), three low-field protons attached to carbon atoms bearing an oxygen function at δ_H 5.65 (1H, t, $J = 9.0$ Hz), 5.47 (1H, d, $J = 8.4$ Hz), 4.87 (1H, br s), and the signals at δ_H 2.93 (s, C_5-OH), 2.31 (s, C_{14}-OH) for two hydroxyl groups. Except the signals for the α,β-unsaturated γ-lactone moiety and three acetoxyl groups, the ^{13}C NMR spectrum (Table 1) also showed 18 carbon signals for four tertiary methyl groups, six carbon atoms bearing the oxygen atom (δ_C 79.1, 75.1, 74.9, 74.7, 72.6, 58.8), and other eight alkyl carbons. These ^1H and ^{13}C NMR spectral data were similar to those of neocaesalpin L [9], except for the presence of the ethyl group at δ_H 1.16 (3H, t, 6.6 Hz), 3.55 (1H, m)/3.19 (1H, m), and δ_C 14.8, 58.8. This was confirmed by the analysis of the HSQC and HMBC spectra (Figure 2). The location of the CH_3CH_2O- group was determined to be at C-12 on the basis of the long-range correlations of the protons at δ_H 3.55 and 3.19 with the hemiketal carbon at δ_C 106.3. Moreover, in the NOESY spectrum of compound **3** (Figure 2), the presence of mutual correlations of CH_3-20/H-6_{ax}, H-8, CH_3-19; CH_3-19/H-1; H-9/C_5-OH/C_1-OCO*CH_3*; H-9/CH_3-17, C_{12}-O*CH_2*CH_3, and H-7/CH_3-17 agreed with the relative structure of neocaesalpin L. Based on the above analysis, **3** was determined as

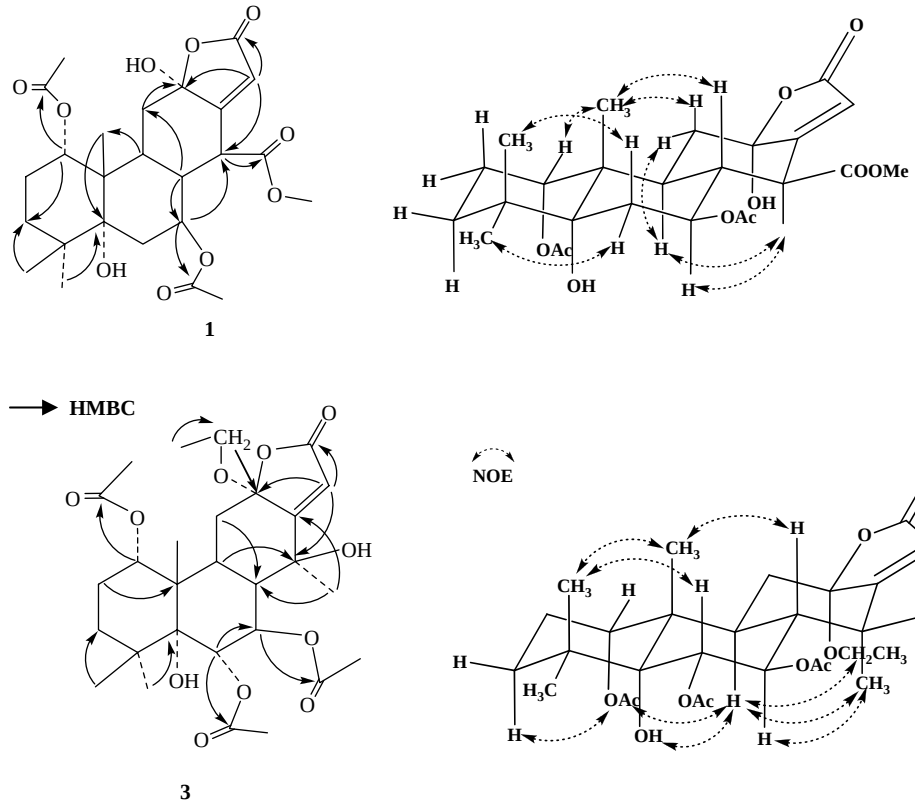

Figure 2. Key HMBC and NOESY correlations of **1** and **3**.

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12α-ethoxyl-1α,6α,7β-triacetoxy-5α,14β-dihydroxy-cass-13(15)-en-16,12-olide.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco P-1010 polarimeter. Melting points were determined on a Yanagimoto MP-500D. UV spectra were performed on a UV-1700 spectrophotometer. IR spectra were recorded on KBr discs with a Bruker IFS-55 spectrometer. 1D and 2D NMR spectral data were taken on a Bruker AV-600 spectrometer with TMS as the internal standard. HR-ESI-MS were recorded on a Bruker micro-TOFQ mass spectrometer. HPLC separation was carried out on a reversed phase Mightysil packed column using the gradient CH_3CN-H_2O and MeOH–H_2O solvent systems with detection at 210 nm. Silica gel for column chromatography (CC, 200–300 mesh) and TLC plates (GF_{254}) were purchased from Qingdao Marine Chemical Ltd (Qingdao, China), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. Sephadex LH-20 was purchased from Pharmacia Biotech (Pharmacia, Kalamazoo, MI, USA). All chemical agents used were of biochemical reagent grade.

3.2 Plant material

The seeds of *C. minax* Hance were purchased from Nanning City, Guangxi Zhuang Autonomous Region of China in March, 2008 and identified by Prof. Qi-shi Sun of the School of Traditional Chinese

Medicine, Shenyang Pharmaceutical University, in which a voucher specimen (No. ZB-2008-017) has been deposited.

3.3 Extraction and isolation

Powdered seeds (15 kg) were extracted with 95% ethanol under reflux for three times. The solution was evaporated in vacuum to give a brown viscous residue (1200 g), which was suspended in water and partitioned successively with chloroform, ethyl acetate, and *n*-butanol. The chloroform soluble fraction (175 g) was subjected to silica gel column chromatography, eluted with a chloroform–methanol (100:1–1:1) gradient system, to give 10 fractions (A–J). Fraction B (1.0 g) was subjected to silica gel column chromatography, eluted with a gradient of petroleum ether–acetone (10:1–1:1) to afford three subfractions. Subfraction 2 was then purified by Sephadex LH-20 column chromatography with chloroform–methanol (1:1) and RP-HPLC with MeOH–H_2O (50:50) to afford **3** (12.2 mg, 0.001%). Fraction D (3.6 g) was subjected to silica gel column chromatography, eluted with a gradient of petroleum ether–acetone (20:1–1:1) to afford five fractions. Subfraction 3 was purified by repeated silica gel column chromatography and preparative TLC with petroleum ether–acetone (3:1) to afford **1** (8.0 mg, 0.00067%) and **2** (3.5 mg, 0.00029%).

3.3.1 *Methyl 1α,7β-diacetoxy-5α,12α-dihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate (**1**)*

Colorless needles ($CDCl_3$), $[\alpha]_D^{20}$: −17.3 ($c = 0.22$, $CHCl_3$); mp 253–255°C; UV (MeOH) λ_{max} (log ε): 214 (3.59) nm; IR (KBr, cm^{-1}) ν_{max}: 3579, 3408, 2955, 1744, 1367, 1236, 1023, 757; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data: see Table 1; HR-ESI-MS: *m/z* 495.2222 $[M + H]^+$ (calcd for $C_{25}H_{35}O_{10}$, 495.2225).

3.3.2 *Methyl 7β-acetoxy-1α,5α,12α-trihydroxy-cass-13(15)-en-16,12-olide-17β-carboxylate (**2**)*

White needles ($CHCl_3$), $[\alpha]_D^{20}$: −29.1 ($c = 0.12$, $CHCl_3$); mp 277–279°C; UV (MeOH) λ_{max} (log ε): 214 (3.57) nm; IR (KBr, cm^{-1}) ν_{max}: 3366, 2974, 2925, 1747, 1383, 1217, 1048, 758; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data: see Table 1; HR-ESI-MS: *m/z* 475.1941 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_9Na$, 475.1939).

3.3.3 *12α-Ethoxyl-1α,6α,7β-triacetoxy-5α,14β-dihydroxy-cass-13(15)-en-16,12-olide (**3**)*

White amorphous solid, $[\alpha]_D^{20}$: −94.1 ($c = 0.09$, $CHCl_3$); mp 239–241°C; UV (MeOH) λ_{max} (log ε): 216 (3.29) nm; IR (KBr, cm^{-1}) ν_{max}: 3570, 2981, 2936, 1745, 1373, 1232, 1033, 756; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data: see Table 1; HR-ESI-MS: *m/z* 553.2645 $[M + H]^+$ (calcd for $C_{28}H_{41}O_{11}$, 553.2643).

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