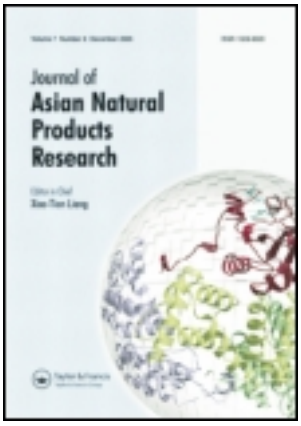

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Qing Zhao [a], Jing-Zhi Yang [a], Chuang-Jun Li [a], Nai-Hong Chen [a] & Dong-Ming Zhang [a]

[a] The Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education and Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100050, China

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A new megastigmane glucoside and a new amide alkaloid from the leaves of *Clausena lansium* (Lour.) Skeels

Qing Zhao, Jing-Zhi Yang, Chuang-Jun Li, Nai-Hong Chen and Dong-Ming Zhang*

The Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education and Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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A new megastigmane glucoside (6*S*,7*E*,9*S*)-6,9,10-trihydroxy-4,7-megastigmadien-3-one 9-*O*-β-D-glucopyranoside (**1**) and a new amide alkaloid (*E*)-*N*-(4-methoxyphenethyl)-2-methylbut-2-enamide (**2**), together with three known amide alkaloids (**3**–**5**), were isolated from the leaves of *Clausena lansium* (Lour.) Skeels. Their structures were elucidated by their physicochemical properties and analysis of their spectral data.

Keywords: *Clausena lansium*; amide alkaloid; megastigmane glucoside

1. Introduction

Clausena lansium is a plant of the genus *Clausena* of the Rutaceae family, widely distributed in southern China. The crude extract from its leaves has been used for the treatment of acute and chronic viral hepatitis, and its effect of lowering elevated serum glutamic pyruvic transaminase level was observed. Previous investigations revealed that the genus *Clausena* mainly contained coumarins, carbazole alkaloids, amide alkaloids, volatile oil, and terpenes [1,2]. Our effort to discover the bioactive constituents from the plant *C. lansium* has led to the isolation of a new megastigmane glucoside [(6*S*,7*E*,9*S*)-6,9,10-trihydroxy-4,7-megastigmadien-3-one 9-*O*-β-D-glucopyranoside (**1**)], a new amide alkaloid [(*E*)-*N*-(4-methoxyphenethyl)-2-methylbut-2-enamide (**2**)], and three known amide alkaloids [(2*S*,3*R*)-*N*-phenethyl-3-phenyloxirane-2-carboxamide (**3**), (2*S*,3*R*)-*N*-methyl-*N*-(*Z*)-styryl-3-phenyloxirane-2-carboxamide (**4**), and *N*-(4-methoxyphenethyl)benzamide (**5**)] (Figure 1). We report herein the isolation and structure elucidation of these compounds.

2. Results and discussion

Compound **1** was obtained as a colorless oil with $[\alpha]_D^{20}$ +19.7 ($c = 0.08$, MeOH). The UV spectrum showed absorption maxima at 237 nm. The IR spectrum indicated the presence of α,β-unsaturated carbonyl (1650 cm^{-1}) and hydroxyl (3375 cm^{-1}) groups. The negative ESI-MS of **1** gave a quasi-molecular ion peak at *m/z* 401.7 $[M - H]^-$, whereas the positive ESI-MS exhibited $[M + Na]^+$ at *m/z* 425.3. The HR-ESI-MS displayed the quasi-molecular ion peak at *m/z* 425.1788 $[M + Na]^+$, which was consistent with a molecular formula of $C_{19}H_{30}O_9$ with five degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1) showed signals of three methyls

*Corresponding author. Email: zhangdm@imm.ac.cn

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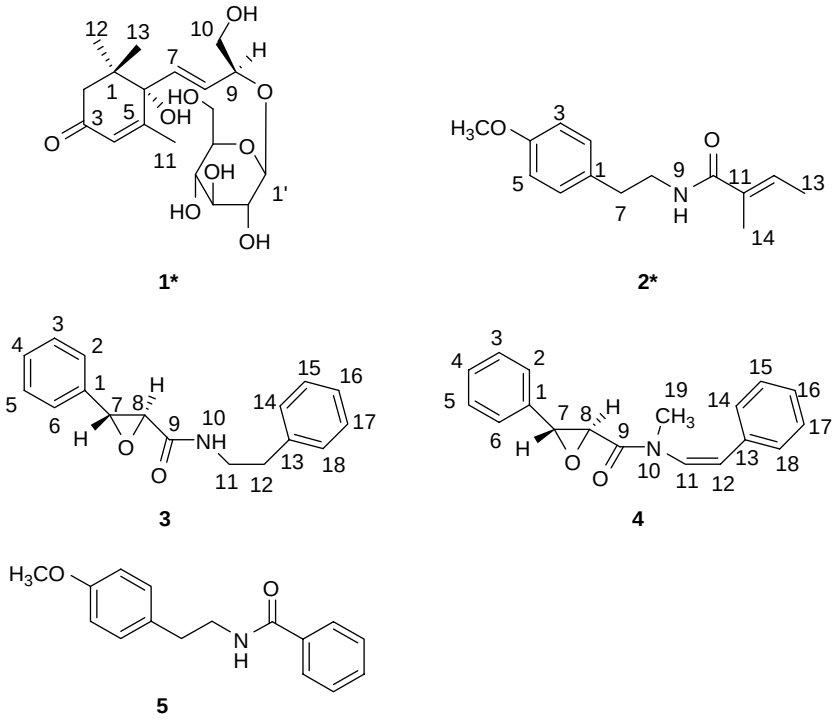


Figure 1. Structures of compounds **1**–**5**.

[δ_H 0.97 (3H, s), 0.98 (3H, s), 1.88 (3H, s)]; geminally coupled protons [δ_H 2.59 (1H, d, $J = 16.8$ Hz) and 2.14 (1H, d, $J = 16.8$ Hz)]; one oxymethine [δ_H 4.42 (1H, m)]; a vinyl proton [δ_H 5.80 (1H, s)]; two *trans*-olefinic protons [δ_H 6.08 (1H, d, $J = 16.0$ Hz) and 5.76 (1H, dd, $J = 16.0$, 6.8 Hz)]. The ^{13}C NMR spectrum of **1** (Table 1) exhibited a carbonyl carbon at δ_C 201.6; four olefinic carbons at δ_C 127.4, 129.1, 136.1, 167.3; an oxymethine carbon at δ_C 79.7; and three methyls at δ_C 19.8, 23.8, 25.0. The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of corchoionoside C [3] and cucumegastigmane II [4], except for some signals around the 9-position. Anomeric proton [δ_H 4.24 (1H, d, $J = 7.6$ Hz)] and other signals in the ^{1}H NMR spectrum at δ_H 3.09–3.80, together with their corresponding carbon signals in the ^{13}C NMR spectrum at δ_C 101.7, 75.2, 78.4, 71.8, 78.4, 62.9, were characteristic of a β-glucopyranosyl residue [3,4]. The D-glucose was determined by acid hydrolysis of **1** followed by the optical rotation analysis [$[\alpha]_D^{20}$ +34.5 ($c = 0.13$, H_2O)]. The β-D-glucose was localized at C-9, which could be confirmed by the HMBC spectrum (Figure 2). H-8 showed correlation with C-10; H-7 with C-9; H-1′ with C-9; and H-9 with C-1′. Because the CD spectrum of **1** showed a positive Cotton effect ($\Delta\varepsilon$ + 7.92) at 241 nm, the 6-position was determined to have an *S*-configuration in accordance with Ref. [5]. As shown in Ref. [5], the remarkable chemical shift difference between C-7 (δ_C 136.1) and C-8 (δ_C 129.1) of **1** indicated that the 9-position had an *S*-configuration. Therefore, the structure was assigned as (6*S*,7*E*,9*S*)-6,9,10-trihydroxy-4,7-megastigmadien-3-one 9-*O*-β-D-glucopyranoside.

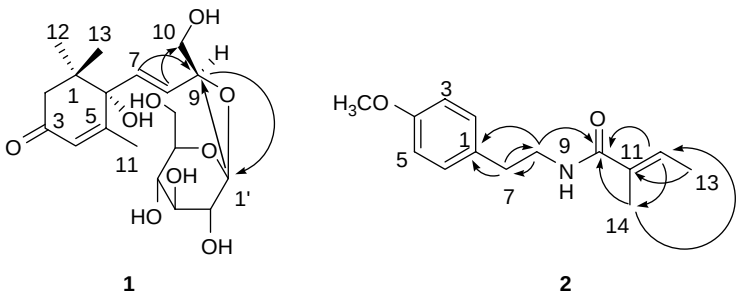
Compound **2** was obtained as a colorless oil. The positive Dragendorff reaction showed that **2** was an alkaloid. The UV

Table 1. ^{1}H and ^{13}C NMR spectral data for compound **1** in CD_3OD (400 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR).

Position	δ_H	δ_C
1		42.7
2	2.59 (1H, d, 16.8)	51.2
	2.14 (1H, d, 16.8)	
3		201.6
4	5.80 (1H, s)	127.4
5		167.3
6		80.5
7	6.08 (1H, d, 16.0)	136.1
8	5.76 (1H, dd, 16.0, 6.8)	129.1
9	4.42 (1H, m)	79.7
10	3.60 (1H, dd, 12.4, 4.0)	66.3
	3.54 (1H, dd, 12.4, 6.4)	
11	1.88 (3H, s)	19.8
12	0.98 (3H, s)	23.8
13	0.97 (3H, s)	25.0
Glc 1′	4.24 (1H, d, 7.6)	101.7
2′	3.09–3.27 (1H, m)	75.2
3′	3.09–3.27 (1H, m)	78.4
4′	3.09–3.27 (1H, m)	71.8
5′	3.09–3.27 (1H, m)	78.4
6′	3.80 (1H, dd, 12.0, 2.0)	62.9
	3.57 (1H, dd, 12.0, 6.4)	

spectrum showed absorption maxima at 278 and 241 nm. The IR spectrum presented the absorption bands of an amino group (3294 cm^{-1}), an amide group (1661 cm^{-1}), and a double bond (1613 cm^{-1}). The EI-MS of **2** gave a molecular ion peak at *m/z* 233 $[M]^+$. The HR-ESI-MS displayed a quasi-molecular ion peak at *m/z* 234.1493 $[M + H]^+$, which was consistent with a molecular formula of $C_{14}H_{19}NO_2$ with six degrees of unsaturation. The ^{1}H NMR spectrum (Table 2) of **2** showed aromatic protons [δ_H 7.12 (2H, d, $J = 8.5$ Hz), 6.86 (2H, d, $J = 8.5$ Hz)]; a vinyl proton [δ_H 6.38 (1H, q, $J = 7.0$ Hz)]; a methoxyl [δ_H 3.79 (3H, s)]; two methylenes [δ_H 3.55 (2H, m), 2.79 (2H, t, $J = 6.5$ Hz)]; two methyls [δ_H 1.78 (3H, s), 1.73 (3H, d, $J = 7.0$ Hz)]. The ^{13}C NMR spectrum of **2** (Table 2) exhibited a carbonyl carbon at δ_C 169.3 (C-10); aromatic carbons at δ_C 158.3, 130.6, 129.7 (×2), 114.0 (×2); two olefinic carbons at δ_C 131.7, 130.9; a methoxyl at δ_C 55.3; two methylene carbons at δ_C 40.9, 34.7; and two methyls at δ_C 13.9, 12.3. The ^{1}H and ^{13}C NMR spectral data of **2** suggested the presence of *p*-CH_3O-Ph—, —CH_2CH_2NHCO—, and —CH_3C=$CHCH_3$, respectively. The planar structure of **2** was subsequently established by the HMBC spectrum (Figure 2). H-12 showed correlations with C-10 and C-14; H-14 with C-10 and C-12; H-13 with C-11; H-7 with C-1 and C-8; H-8 with C-10, C-1, and C-7. According to Refs [6–10], the configuration of the olefinic bond was determined to be tigloyl, rather than angeloyl, due to the typical signals at δ_H 1.78 (3H, s, H-14), 1.73 (3H, d, $J = 7.0$ Hz, H-13), and δ_C 13.9 (C-13), 12.3 (C-14). Therefore, compound **2** was assigned as (*E*)-*N*-(4-methoxyphenethyl)-2-methylbut-2-enamide.

Three known amide alkaloids (2*S*,3*R*)-*N*-phenethyl-3-phenyloxirane-2-carboxamide (**3**) [11] (Table 3), (2*S*,3*R*)-*N*-methyl-*N*-(*Z*)-styryl-3-phenyloxirane-2-carboxamide (**4**) [12] (Table 3), and *N*-(4-



1 **2**

Figure 2. Key HMBC correlations of compounds **1** and **2**.

Table 2. ^{1}H and ^{13}C NMR spectral data for compound **2** in $CDCl_3$ (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR).

Position	δ_H	δ_C
1		130.6
2/6	7.12 (2H, d, 8.5)	129.7
3/5	6.86 (2H, d, 8.5)	114.0
4		158.3
7	2.79 (2H, t, 6.5)	34.7
8	3.55 (2H, m)	40.9
9	5.70 (1H, br s, NH)	
10		169.3
11		130.9
12	6.38 (1H, q, 7.0)	131.7
13	1.73 (3H, d, 7.0)	13.9
14	1.78 (3H, s)	12.3
OCH_3	3.79 (3H, s)	55.3

methoxyphenethyl)benzamide (**5**) [13] were identified by the comparison of their physical and spectral data (UV, ^{1}H, ^{13}C NMR, MS) with those values reported in the literature.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured by a JASCO P-2000 polarimeter. IR spectra were carried out on a Nicolet IMPACT 400 spectrophotometer with KBr disks. UV spectra were determined by a JASCO V-650 spectrophotometer. CD spectra were obtained using a JASCO J-815 spectrometer. ESI-MS were run on a VG Autospec-300. ESI-MS were performed on an Agilent 1100 LC/MSD Trap-SL mass spectrometer. HR-ESI-MS were performed on an Agilent 6520 Accurate-Mass LC/MS Q-TOF. ^{1}H NMR (400 and 500 MHz) spectra were recorded by a Mercury-400 spectrophotometer and an Inova-500 spectrometer, respectively. ^{13}C NMR (160–200 mesh; 125 MHz) and HMBC spectra were run on an Inova-500 with TMS as an internal standard. Silica gel (Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and RP-18 (40–60 μm; Merck, Darmstadt, Germany) were used for column chromatography and silica gel GF-254 (Qingdao Marine Chemical Factory, Qingdao, China) was used for TLC. Macroporous resin (PRP-512, Tianjin, China) was used for column chromatography. HPLC experiments were performed on a preparation YMC-Pack ODS-A column (20 mm × 250 mm, 5 μm) equipped with a Shimadzu SPD-20A UV spectrophotometric detector and a Shimadzu LC-6AD dual pumping system.

3.2 Plant material

The leaves of *C. lansium* (Lour.) Skeels were collected in Liuzhou, Guangxi, China, in December 2008, and identified

Table 3. ^{1}H and ^{13}C NMR spectral data for compounds **3** and **4** in $CDCl_3$ (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR).

	Compound **3**		Compound **4**	
Position	δ_H	δ_C	δ_H	δ_C
1		138.4		135.3
2–6/14–18	7.21–7.37 (10H, m)	125.7–129.0	6.99–7.29 (10H, m)	125.7–133.5
7	3.71 (1H, d, 1.5)	59.1	3.80 (1H, d, 2.0)	57.9
8	3.49 (1H, d, 1.5)	58.9	3.77 (1H, d, 2.0)	56.8
9		167.4		166.6
10	6.24 (1H, br s, NH)			
11	3.58 (2H, m)	39.8	6.35 (1H, d, 8.5)	126.9
12	2.87 (2H, m)	35.5	6.22 (1H, d, 8.5)	127.6
13		134.8		
19(NCH_3)			3.13 (3H, s)	34.5

by Engineer Guang-Ri Long, Liuzhou Forestry Bureau. A voucher specimen (ID-S-2320) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The air-dried leaves (9 kg) of *C. lansium* were extracted with 70% EtOH (3 × 27 liters, 2 h) under reflux condition. The 70% EtOH extract was concentrated under reduced pressure. Subsequently, it was partitioned with petroleum ether (60–90°C), EtOAc, and *n*-BuOH, respectively. The *n*-BuOH portion (50 g) was chromatographed over a silica gel column using EtOAc–MeOH as a gradient eluent (50:1–1:1, v/v) to provide 32 parts. Parts 14–19 (7.1 g) were combined and subjected to column chromatography on silica gel and eluted with $CHCl_3$–MeOH–H_2O (8:2.5:0.3, v/v) to yield eight sections. Section 3 (978 mg) was further separated by RP-18 column chromatography using a gradient of MeOH–H_2O (10, 20, 30, 40, 100%, v/v) to yield several fractions. Fractions 8 and 9 (130 mg) were combined and subjected to a Sephadex LH-20 column using 70% MeOH–H_2O and purified on HPLC [YMC-Pack ODS-A column (20 mm × 250 mm, 5 μm)] with 14% MeOH–H_2O to yield compound **1** (80 mg). The EtOAc portion (240 g) was subjected to column chromatography on silica gel and eluted with petroleum ether–acetone (100:0, 50:1, 20:1, 10:1, 5:1, 3:1, v/v) to yield 40 corresponding parts. Parts 14–18 (4 g) were combined and separated by PRP-512 macroporous resin and eluted with MeOH–H_2O (70, 100%, v/v) to yield 34 sections. Sections 9–12 (447 mg) were combined and subjected to a Sephadex LH-20 column using $CHCl_3$–MeOH (1:1) and prepared by HPLC with 65% MeOH–H_2O as the mobile phase to yield four fractions. Fraction 1 (312 mg) was purified on HPLC once again to yield compound **3** (54 mg) and compound **4** (205 mg) with 53% MeOH–H_2O. Parts 21–25 (19.6 g) were combined and separated by PRP-512 macroporous resin with MeOH–H_2O (50–90%, v/v) to yield 20 sections. Sections 7–12 (4.8 g) were combined and subjected to RP-18 column chromatography and eluted using a gradient of MeOH–H_2O (50–100%, v/v) to afford 19 fractions. Fraction 4 (180 mg) was prepared by HPLC with 34% CH_3CN–H_2O to yield compound **2** (7 mg). Fraction 6 (210 mg) was prepared by HPLC with 48% MeOH–H_2O to yield compound **5** (56 mg).

3.3.1 *(6S,7E,9S)-6,9,10-Trihydroxy-4,7-megastigmadien-3-one 9-O-β-D-glucopyranoside* ***(1)***

Colorless oil, $[\alpha]_D^{20}$ +19.7 ($c = 0.08$, MeOH). UV (MeOH) λ_{max} (log ε): 237 (3.09) nm. IR (KBr) ν_{max}: 3375, 2966, 2920, 2877, 1650, 1432, 1373, 1076, 641 cm^{-1}. CD (MeOH) $\Delta\varepsilon_{241\,nm}$ +7.92. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) spectral data, see Table 1. ESI-MS: *m/z* 425 $[M + Na]^+$. HR-ESI-MS: *m/z* 425.1788 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_9Na$, 425.1782).

3.3.2 *(E)-N-(4-Methoxyphenethyl)-2-methylbut-2-enamide* ***(2)***

Colorless oil, UV ($CHCl_3$) λ_{max} (log ε): 278 (2.26), 241 (2.52) nm. IR (KBr) ν_{max}: 3294, 1661, 1613, 1513, 1245, 1033, 817, 703 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectral data, see Table 2. EI-MS: *m/z* 233 $[M]^+$. HR-ESI-MS: *m/z* 234.1493 $[M + H]^+$ (calcd for $C_{14}H_{20}NO_2$, 234.1489).

3.4 Acid hydrolysis

A solution of **1** (5.03 mg) in 10% HCl (2 ml) was heated at 75°C for 3.5 h. After that, the solution was extracted with

EtOAc (3×2 ml). The H_2O fraction was concentrated under reduced pressure to yield glucose (2.68 mg). D-Glucose: $[\alpha]_D^{20}$ $+34.5$ ($c = 0.13$, H_2O).

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