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A new amide and a new monoterpene from the seeds of *Clausena lansium*

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A new amide (**2**) and a new monoterpene (**6**) were isolated from the seeds of *Clausena lansium* together with four known ones (**1**, **3–5**). The structures of the new compounds were elucidated by extensive spectroscopic analyses. The absolute configurations of **1**, **2** and **6** were determined by optical rotation and the modified Mosher's method.

Keywords: *Clausena lansium*; rutaceae; amide; monoterpene

1. Introduction

The plant *Clausena lansium*, a fruit tree, is widely distributed in the southern area of China (Huang, 1959). The leaves and roots of the plant are used as folk medicines for the treatment of coughs, asthma, dermatological disease, viral hepatitis and gastro-intestinal diseases (Li, James, & Farouk, 1991). The seeds are used to treat acute and chronic gastro-intestinal inflammation, ulcers, etc. (Lin, 1989). Previous phytochemical investigations on the plant had led to the isolation of some carbazole alkaloids and amides (Kumar, Vallipuram, Adebajo, & Reisch, 1995; Li et al., 1991; Lin, 1989; Yang, Chen, & Huang, 1988). In this article, we report a new amide (**2**) and a new monoterpene (**6**) from the seeds of *C. lansium* together with four known ones (**1**, **3–5**). The structures of the new compounds were elucidated by extensive spectroscopic analyses. The known compounds were identified by comparing their physical and spectroscopic data with those reported in the literatures. The absolute configurations of **1**, **2** and **6** were determined by optical rotation and the modified Mosher's method.

2. Results and discussion

The air-dried seeds of *C. lansium* were extracted with 95% EtOH. The extract was suspended in H_2O and successively partitioned with cyclohexane, ethyl acetate and *n*-butanol. The EtOAc extract was chromatographed over silica gel, Sephadex LH-20 and ODS columns to afford compounds **1–6** (Figure 1).

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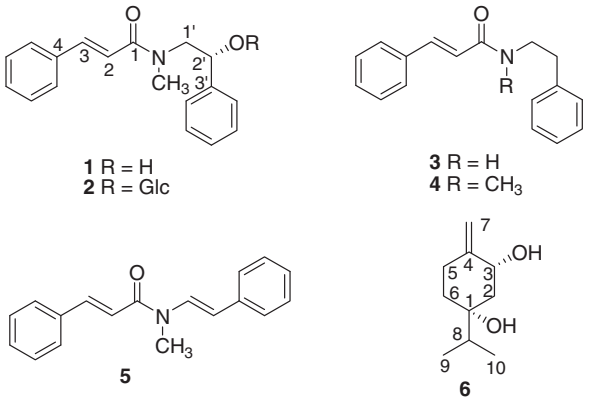

Figure 1. Chemical structures of **1**–**6**.

Compound **2** was isolated as yellow oil. The HR–ESI–MS of **2** showed a quasi-molecular ion at m/z 466.1842 $[M+Na]^+$ (calcd for $C_{24}H_{29}NO_7Na$, 466.1836), consistent with the molecular formula $C_{24}H_{29}NO_7$. The IR spectrum suggested the presence of hydroxyl group (3407 cm^{-1}) and carbonyl group (1646 cm^{-1}). The ^{1}H-NMR spectrum of **2** displayed two olefinic protons at δ_H 7.40 (1H, d, $J = 15.2$ Hz) and 6.98 (1H, d, $J = 15.2$ Hz), 10 aromatic protons at δ_H 7.27–7.58 (10H, m), one methyl at δ_H 3.02 (3H, s) and an anomeric proton of sugar at δ_H 4.13 (1H, d, $J = 6.3$ Hz). A total of 24 carbon signals were observed in the ^{13}C-NMR and DEPT spectra of **2**, including signals of a carbonyl (δ_C 166.1), a double bond (δ_C 139.6, 119.2), a methyl (δ_C 34.6), a glucose unit (δ_C 100.4, 73.5, 76.5, 70.1, 76.3, 61.1) and the signals of two benzene rings. The above data were very similar to those of **1** (Maneerat, Tha-in, Cheenpracha, Prawat, & Laphookhieo, 2011), except that **2** had an additional glucose unit signals. The HMBC spectrum of **2** showed the correlation between H-1″ (δ_H 4.13) and C-2′ (δ_C 76.5), indicating the glucose unit was connected to C-2′ position. Hydrolysis of **2** yielded **1** and D-glucose. The NMR data of **1** were consistent with those of Clausenalansamide B (Maneerat et al., 2011), but the configuration of C-2′ was not determined. The optical rotation of **1** was then measured, and it was negative as that of (*R*)-aegeline (Ahmed, Ahmad, Mahendra, & Shaheer, 2004), suggesting the configuration of C-2′ in **1** was *R*. Thus, **2** was elucidated as (2′*R*)-*N*-(2′phenylethyl)-*N*-methylcinnamamide 2′-*O*-*β*-D-glucopyranoside.

Compound **6** was obtained as white powder. The HR–ESI–MS gave a quasi-molecular ion at m/z 171.1375 $[M+H]^+$ (calcd for $C_{10}H_{19}O_2$, 171.1380), suggesting the molecular formula of **6** was $C_{10}H_{18}O_2$. The ^{1}H-NMR spectrum showed the signals of two olefinic protons at δ_H 5.00 (1H, d, $J = 0.5$ Hz) and 4.91 (1H, d, $J = 0.5$ Hz), one oxy-methine at δ_H 4.43 (1H, m), and an isopropyl group at δ_H 1.59 (1H, m), 0.91 (3H, d, $J = 6.9$ Hz) and 0.93 (3H, d, $J = 6.9$ Hz). The ^{13}C-NMR and DEPT spectra displayed 10 carbon signals including two olefinic carbons at δ_C 151.6 and 103.4, a quaternary carbon at δ_C 75.7, as well as an isopropyl group at δ_C 44.5, 16.9 and 16.9. All the above NMR data suggested that **6** was a menthane-type monoterpene (Ohloff & Uhde, 1965). The positions of the double bond at C-4 and C-7 and two hydroxyl groups at C-1 and C-3 were elucidated by the ^{1}H-^{1}H COSY and HMBC spectra. The relative configuration of **6** was elucidated by the ROESY correlations (Figure 2). The absolute configuration of **6** was confirmed by application of the modified Mosher's method (Ohtani, Kusumi, Kashman, & Kakisawa, 1991). Differences of proton chemical shift ($\Delta\delta$ values, δ_S–δ_R) between (*S*)-MTPA ester

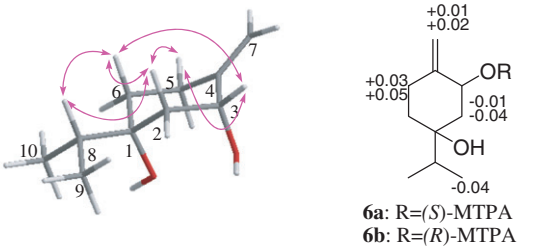

Figure 2. Key ROESY correlations of **6**, and $\Delta\delta$ values (δ_S–δ_R) of the MTPA esters **6a** and **6b**.

(**6a**) and (*R*)-MTPA ester (**6b**) (Figure 2) indicated the configuration of C-3 was *R*. Hence, **6** was identified as (1*R*), (3*R*)-4-methylene-1-(1-methylethyl)-(1,3)-cyclohexanediol.

The other three known compounds were identified as phenethyl cinnamide (**3**) (Que, Zhao, & Zhang, 2008), lansiumamide C (**4**) (Lin, 1989), and lansiumamide I (**5**) (Bayer & Maier, 2004), respectively, by a comparison of their physical and spectroscopic data with those of literatures.

3. Experimental

3.1. *General methods*

Melting points were determined on an XT-4 micro-melting-point apparatus. Optical rotations were measured on a JASCO-P-1020 polarimeter. UV spectra were recorded on a JASCO V-550 UV/VIS spectrophotometer; λ_{max} ($\log\varepsilon$) in nm. IR spectra (KBr) were measured by a JASCO FT/IR-480 plus spectrometer; in cm^{-1}. ^{1}H-, ^{13}C- and 2D-NMR spectra were recorded on a Bruker-AV-400 spectrometer; δ in ppm relative to $(CH_3)_4Si$, J in Hz. HR–ESI–MS spectra were obtained on an Agilent 6210 ESI/TOF mass spectrometer; in m/z. For column chromatography (CC), silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, P.R. China) and Sephadex LH-20 (Pharmacia) were used. Precoated silica gel GF_{254} plates (Qingdao Marine Chemical Factory, Qingdao, P.R. China) were used for TLC.

3.2. *Plant material*

The seeds of *C. lansium* were collected in Qingpin TCM Trade Center, Guangzhou, Guangdong province of China, in July 2008, and authenticated by Professor Zhou Guang-Xiong of Jinan University. A voucher specimen (no. 080723) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, P.R. China.

3.3. *Extraction and isolation*

The seeds of *C. lansium* (10.0 kg) were extracted with 95% EtOH. The extract was suspended in H_2O and successively partitioned with cyclohexane, ethyl acetate and *n*-butanol to afford 125, 135 and 147 g residues, respectively. The EtOAc extract was subjected to a silica gel column ($CHCl_3$–CH_3OH, $100:0 \rightarrow 0:100$) to give 10 major fractions. Fraction 3 was re-chromatographed on a silica gel column ($CHCl_3$–CH_3OH, $99:1 \rightarrow 70:30$) to afford **1** (10 mg), **3** (21 mg) and **4** (29 mg). Fraction 4 was re-chromatographed on a silica gel column ($CHCl_3$–CH_3OH, 98:2→70:30) to afford **2** (13 mg), **5** (32 mg) and **6** (15 mg).

3.3.1. *Compound* **2**

Yellow oil, $[\alpha]_D^{20} = -46^\circ$ (*c* 0.40, CH_3OH). UV (MeOH) λ_{max}: 209, 281 nm. IR (KBr) ν_{max}: 3407, 2924, 1646, 1591, 1404, 1078, 763, 703 cm^{-1}. HR–ESI–MS *m/z*: 466.1842 $[M + Na]^+$ (calcd for $C_{24}H_{29}NO_7Na$, 466.1836). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.98 (1H, d, $J = 15.2$ Hz, H-2), 7.40 (1H, d, $J = 15.2$ Hz, H-3), 3.72 (2H, m, H-1′), 5.10 (1H, d, $J = 5.8$ Hz, H-2′), 7.27–7.58 (10H, m, Ar), 3.02 (3H, s, Me), 4.13 (1H, d, $J = 6.3$ Hz, H-1″), 3.05–3.74 (6H, m, H-2″–H-6″). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 166.1 (C-1), 119.2 (C-2), 139.6 (C-3), 135.1 (C-4), 55.1 (C-1′), 76.7 (C-2′), 139.0 (C-3′), 126.6–128.3 (Ar), 34.6 (Me), 100.4 (C-1″), 73.5 (C-2″), 76.5 (C-3″), 70.1 (C-4″), 76.3 (C-5″), 61.1 (C-6″).

3.3.2. *Compound* **6**

White powder, m.p. 75–78°C, $[\alpha]_D^{20} = +27^\circ$ (*c* 0.50, CH_3OH). UV (MeOH) λ_{max}: 240 nm. IR ν_{max}: 3383, 2983, 2364, 1655, 1396, 1073 cm^{-1}. HR–ESI–MS *m/z*: 171.1375 $[M + H]^+$ (calcd for $C_{10}H_{19}O_2$, 171.1380). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.31 (1H, m, H-2a), 2.07 (1H, m, H-2b), 4.43 (1H, m, H-3), 2.28 (1H, m, H_a-5), 2.42 (1H, m, H_b-5), 1.43 (1H, m, H_a-6), 1.64 (1H, m, H_b-6), 5.00 (1H, d, $J = 0.5$ Hz, H_a-7), 4.91 (1H, d, $J = 0.5$ Hz, H_b-7), 0.91 (3H, d, $J = 6.9$ Hz, H-9), 0.93 (3H, d, $J = 6.9$ Hz, H-10). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 75.7 (C-1), 52.5 (C-2), 69.2 (C-3), 151.6 (C-4), 29.4 (C-5), 35.1 (C-6), 103.4 (C-7), 44.5 (C-8), 16.9 (C-9, C-10).

3.4. *Hydrolysis of 2 and determination of the absolute configuration of sugar*

Compound **2** (3.0 mg) was dissolved in 1N HCl (2.0 mL) and heated at 80°C for 3 h. The solution was evaporated with a stream of N_2. The reaction mixture was dissolved in H_2O and the aglycone was extracted with CH_2Cl_2, which was identified as **1**. The aqueous layer was evaporated to give a residue. Then, dry pyridine (1.0 mL) and L-cysteine methyl ester hydrochloride (3.0 mg) were added to the residue. The mixture was heated at 80°C for 2 h and then dried by N_2. *N*-(trimethylsilyl) imidazole (0.2 mL) was added, and the mixture was heated at 80°C for 1 h. Finally, H_2O (1.0 mL) was added to stop the reaction, and the aqueous layer was extracted by cyclohexane (1.0 mL). The organic layer was analysed by gas chromatography using trimethylsilylated derivatives of standard sugars as references (Ikeda, Tsumagari, & Nohara, 2000). As a result, D-glucose from the hydrolyzate of **2** was detected.

3.5. *Preparation of MTPA esters of 6*

Compound **6** (3.5 mg) was dissolved in 0.5 mL pyridine, and treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μL) for 24 h in an anhydrous circumstance at room temperature and then dried *in vacuum*. The reaction mixture was poured into water (5.0 mL) and extracted with EtOAc (5.0 mL). The EtOAc extract was purified by silica gel CC [*n*-hexane-EtOAc (75 : 25)] to yield (*S*)-MTPA ester (**6a**, 1.5 mg). (*R*)-MTPA ester (**6b**, 1.4 mg) was obtained using the same method by treatment of **6** (3.7 mg) with (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μL).

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