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Nematicidal amide alkaloids from the seeds of *Clausena lansium*

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ABSTRACT

Five new amide alkaloids (**1–3**, **5–6**) were isolated from the seeds of *Clausena lansium* together with one new natural product (**4**) and four known analogues (**7–10**). The structures of the new amide alkaloids were elucidated based on a comprehensive spectroscopic data analysis including 1D and 2D NMR as well as HRESIMS, and by comparison with the literature. The bioactivity results showed that compound **8** expressed potent nematicidal activity against *Panagrellus redivevus*, with IC_{50} value of 0.12 *m*M, while compounds **3** and **5** presented moderate nematicidal activity with IC_{50} values of 2.75 and 3.93 *m*M, respectively (abamectin as the positive control with IC_{50} value of 1.05 *m*M).

1. Introduction

Clausena lansium, also known as “wampee”, is one of the Rutaceae family members widely distributed in Hainan, Fujian, Taiwan and other southern areas of China [1]. The leaves and roots of the plant have been used as a folk medicine for the treatment of coughs, asthma, dermatological disease, viral hepatitis and gastro-intestinal diseases, while the fruits are used for digestive disorders [2]. So far, phytochemical investigations on the plant have led to a number of carbazole alkaloids and coumarins, which are considered as the main secondary metabolites in *C. lansium*, exhibiting cytotoxic [3,4], hepatoprotective [5,6], and antimicrobial activities [7–9]. Previous study of our group on the peels of *C. lansium* also identified a serials of new carbazole alkaloids and coumarins with α-glucosidase inhibitory activity [10,11]. Although the constituents have been reported to be similar with other parts of the plant [12,13], the seeds of *C. lansium* are often discarded as waster, and only small amount of reports can be found. Therefore, in order to further study the chemical constituents from the seeds of *C. lansium*, especially the characteristic components of amide alkaloids, phytochemical studies on the seeds were carried out. Herein, the structures of five new amide alkaloids (**1–3**, **5–6**) and a new natural product (**4**) were described from the seeds, as well as their nematicidal activity evaluated for the first time, which not only enrich the amide alkaloid structures, but also provide the mind to exploit the utilization of this garbage and the development of innovative pesticides.

2. Experimental

2.1. General

NMR spectra were recorded on a Bruker AV-500 NMR spectrometer, using TMS as the internal standard. HRESIMS data were collected on API QSTAR Pulsar mass spectrometer (Bruker). Optical rotations were measured by a Rudolph Autopol III polarimeter. UV spectra were recorded on a Shimadzu UV-2550 spectrometer. IR spectra were acquired from a Thermo Nicolet 380 FT-IR instrument (Thermo) using KBr pellets. Circular dichroism data were obtained from a JASCO J-715 spectrophotometer. Silica gel (60–80, 200–300 mesh, Qingdao Marine Chemical Co. Ltd), Sephadex LH-20 (Merck), and ODS gel (150–200 mesh, Fuji Silysia Chemical Co. Ltd) were used for column chromatography. Semipreparative high-performance liquid chromatography (HPLC) was performed on a Dionex P680 with a reversed-phased column (YMC-packed C_{18}, 10 × 250 mm, 5 μm, 4 mL/min).

2.2. Plant material

The fruits of *Clausena lansium* were collected from Danzhou, Hainan province in May 2011, and identified by Dr. Jun Wang (Institute of

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Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences). The voucher specimen (CL20110501) was deposited at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science. The seeds were manually separated from the fruits, dried and finally powdered.

2.3. Extraction and isolation

The dried seeds of *C. lansium* (8.0 kg) were powdered and extracted with 95% EtOH (3 × 40 L) at room temperature to give 305.8 g extract. The crude extract was suspended in water and successively partitioned by petroleum ether (PE), ethyl acetate (EtOAc) and *n*-butanol. The EtOAc extract (84.6 g) was chromatographed on a MCI gel column eluting with a stepwise gradient MeOH-H_2O (30%–100%) to afford thirteen fractions (Fr.1–Fr.13). Fr.6 (7.2 g) was subjected to an ODS gel column employing a gradient of MeOH-H_2O (30%–100%) to obtain Fr.6.1–Fr.6.7. Followed by the same procedure, Fr.2 (7.6 g) provided Fr.2.1–Fr.2.4.

Fr.6.4 was then divided into four fractions (Fr.6.4.1-Fr.6.4.4) through Sephadex LH-20 column eluted with $CHCl_3$/MeOH (v/v, 1:1). Fr.6.4.1 (1.2 g) was further eluted by PE/EtOAc (1:0–0:1) through silica gel column to obtain nine fractions (Fr.6.4.1.1–Fr.6.4.1.9). Fr.6.4.1.7 (116.8 mg) was further purified by semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 45:55; flow rate 4.0 mL/min; UV detection at 220 nm) to afford compound **6** (t_R 11.0 min, 2.7 mg). Fr.6.4.1.6 (61.3 mg) was applied to semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 40:60; flow rate 4.0 mL/min; UV detection at 220 nm) to obtain compounds **1** (t_R 17.4 min, 1.4 mg) and **2** (t_R 14.0 min, 1.3 mg). Fr.6.6 was divided into five fractions (Fr.6.6.1–Fr.6.6.5) through silica gel column (PE/EtOAc, 1:0–0:1). Fr.6.6.2 was separated on semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 50:50; flow rate 4.0 mL/min; UV detection at 220 nm) to obtain compound **5** (t_R 17.2 min, 5.5 mg).

Fr.6.5 was chromatographed by Sephadex LH-20 column (MeOH), and then followed by silica gel column (PE/EtOAc, 1:0–0:1) to give Fr.6.5.2 (330.8 mg), which chromatographed on Sephadex LH-20 column (PE/CH_3Cl/MeOH = 2:1:1) to afford Fr.6.5.2.1–Fr.6.5.2.7. Fr.6.5.2.3 (89.8 mg) was chromatographed over the semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 50:50; flow rate 4.0 mL/min; UV detection at 220 nm) to afford compounds **4** (t_R 25.8 min, 2.0 mg) and **3** (t_R 13.3 min, 2.4 mg). Fr.6.5.2.6 (5.6 mg) was passed through the semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 40:60; flow rate 4.0 mL/min; UV detection at 220 nm) to afford compound **10** (t_R13.0 min, 1.8 mg). Fr.6.5.2.4 (120.7 mg) was purified to get compounds **8** (t_R 14.6 min, 60.0 mg) and **9** (t_R 34.4 min, 35.8 mg) by semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 50:50; flow rate 4.0 mL/min; UV detection at 220 nm).

Fr.2.4 was then divided into Fr.2.4.1–Fr.2.4.10 by silica gel column (PE/EtOAc, 1:0–0:1) and then Fr.2.4.10 (28.5 mg) was chromatographed over the semi-preparative HPLC (C_{18} column; MeCN/H_2O, v/v 20:80; flow rate 4.0 mL/min; UV detection at 220 nm) to afford compound **7** (t_R 16.6 min, 15.0 mg).

1′-Methoxyl-clausenalansamide B (**1**): colorless oil, $[\alpha]_D^{25}$ + 0.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 202 (4.43), 278 (4.04) nm; IR (KBr) ν_{max} 3466, 1741, 1649, 1608, 1496, 1451, 1398, 1373, 1234, 1026, 979, 764, 701 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 334.1417 $[M + Na]^+$ (calcd for $C_{19}H_{21}NO_3Na$, 334.1414).

3-Dehydroxy-3-methoxyl-secoclausenamide (**2**): colorless powder, $[\alpha]_D^{25}$ + 2.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε): 202 (4.27) nm; IR (KBr) ν_{max} 3673, 3031, 2825, 1665, 1543, 1099, 1030, 748, 701 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 322.1419 $[M + Na]^+$ (calcd for $C_{18}H_{21}NO_3Na$, 322.1414).

2′-Dehydroxy-2′-acetoxyl-clausenalansamide B (**3**): colorless oil, $[\alpha]_D^{25}$ − 8.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.44), 280 (4.35) nm; IR (KBr) ν_{max} 1741, 1651, 1606, 1496, 1398, 1234, 1024, 764, 701 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 346.1409 $[M + Na]^+$ (calcd for $C_{20}H_{21}NO_3Na$, 346.1414).

Anhydroelausenamide (**4**): colorless oil, $[\alpha]_D^{25}$ + 6.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 202 (3.95) nm; IR (KBr) ν_{max} 2919, 1698, 1494, 1447, 1387, 786, 766, 696 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 280.1316 $[M + H]^+$ (calcd for $C_{18}H_{18}NO_2$, 280.1332).

Neoclausenamide-A (**5**): colorless crystal, $[\alpha]_D^{25}$ − 2.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 202 (3.52) nm; IR (KBr) ν_{max} 1737, 1692, 1494, 1369, 1236, 1022, 764, 698 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 346.1422 $[M + Na]^+$ (calcd for $C_{20}H_{21}NO_3Na$, 346.1414).

Neoclausenamide-B (**6**): yellowish crystal, $[\alpha]_D^{25}$ + 27.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.64), 280 (4.34) nm; IR (KBr) ν_{max} 2927, 1702, 1635, 1494, 1449, 1250, 1161, 1069, 766, 701 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 450.1666 $[M + Na]^+$ (calcd for $C_{27}H_{25}NO_4Na$, 450.1676).

2.4. Nematicidal activity assay

Nematicidal activity against *Panagrellus redivevus* was carried out according to the previous method [14], with abamectin as the positive control and DMSO as a negative control (2.5 mg/mL), each treatment was repeated for 3 times. Nematicidal activity (NA) was assessed by counting the dead nematodes ($n > 100$) under a microscope after incubation at 28 °C for 24 h. The NA value was calculated using the formula NA = DN/SN × 100% (DN is the number of dead nematodes; SN is the sum of all counted nematodes, SN > 100). The RDR was calculated using the formula RDR = $NA_n - NA_0$ (NA_n is the nematicidal activity of compounds, and NA_0 is the nematicidal activity of the blank control). If the RDR of the test compound was beyond 40%, the IC_{50} of the compound was then assayed and calculated.

3. Results and discussion

Compound **1** was isolated as colorless oil. The HRESIMS showed a pseudomolecular ion peak at *m/z* 334.1417 $[M + Na]^+$ (calcd for $C_{19}H_{21}NO_3Na$, 334.1414), which was consistent with the molecular formula $C_{19}H_{21}NO_3$. The IR spectrum suggested the presence of hydroxyl group (3466 cm^{-1}) and amide carbonyl group (1649 cm^{-1}). The ^{13}C DEPT Q and ^1H NMR spectra (Table 1) exhibited two typical monosubstituted aromatic rings [δ_H 7.27–7.57 (10H, m); δ_C 128.3–142.9], two oxygenated methines [δ_H 5.77 (d, J = 7.7 Hz)/δ_C 88.9; δ_H 4.63 (d, J = 7.7 Hz)/δ_C 73.8], two olefinic protons [δ_H 7.64 (d, J = 15.5 Hz)/δ_C 144.7; δ_H 7.14 (d, J = 15.5 Hz)/δ_C 119.1], one methoxyl [δ_H 3.15 (s)/δ_C 56.4], one *N*-methyl [δ_H 3.11 (s)/δ_C 28.9], and one carbonyl group (δ_C 171.0), as supported by HSQC spectrum. In the HMBC spectrum (Fig. 2), the correlations observed from H-2 to C-1 and C-4, from H-3 to C-1, C-4, C-5 and C-9, as well as from N-CH_3 to C-1, suggested the existence of the fragment of Ph-CH=CH-C(O)-N-CH_3. Besides, the correlations from H-1′/H-2′ to C-3′, from N-CH_3 to C-1′, and from H-4′/H-8′ to C-2′, along with the ^1H-^1H COSY correlations of H-1′/H-2′, indicated the fragment of Ph-CH-CH-N-CH_3. The attachment of the methoxyl group to C-1′ was deduced by the HMBC correlation of H_3-9′ to C-1′. According to the above, as well as the molecular fomular, the structure was in agreement with that of the known clausenalansamide B [15], except for the presence of an additional methoxyl group located at C-1′, which accounted for the molecular weight difference of 30 amu observed between both compounds. Thus, the planar structure of compound **1** was assigned as shown in Fig. 1. The large coupling constant of $^3J_{H\text{-}2,\ H\text{-}3}$ = 15.5 Hz suggested the geometry of the double bond to be *E*. The relative configuration of H-1′/H-2′ was proposed to be *anti* according to the large coupling constant of $^3J_{H\text{-}1',\ H\text{-}2'}$ = 7.7 Hz, however, no significant signals can be observed in ROESY to determine the configuration of 2′-OCH_3 and 3′-OH [16]. The $[\alpha]_D^{25}$ value of **1** was close to zero indicating that **1** present as racemic mixture. Unfortunately, we failed to separate them on our available chiral columns. Finally, compounds **1** was reported as racemates, and named as 1′-