Phytochemistry 72 (2011) 1804–1813

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Triterpenes and neolignans from the roots of *Nannoglottis carpesioides*

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ARTICLE INFO

Article history:
Received 2 August 2010
Received in revised form 28 January 2011
Available online 12 June 2011

Keywords:
Nannoglottis carpesioides
Asteraceae
Triterpenes
Neolignans
Cytotoxic
Antioxidant

ABSTRACT

Seven oleanane-type triterpenes and two 8-*O*-4′-neolignans, along with five known compounds (three 28-noroleanane-type triterpenes, one sarratane triterpene, and one neolignan), were isolated from roots of *Nannoglottis carpesioides*. Their structures were elucidated by spectroscopic methods, including 1D and 2D NMR, HRMS, and CD. The absolute configurations of two triterpenes were determined by experimental and calculated circular dichroism (CD) and optical rotation values. Ten compounds were evaluated for their cytotoxicity against human promyelocytic leukaemia (HL-60) and human hepatoma (Hep-G2) cells using the MTT assay. The antioxidant activities of these compounds were assessed by ABTS radical-scavenging assays. Among the tested compounds, three compounds exhibited moderate radical-scavenging activity against $ABTS^{\cdot+}$, with IC_{50} values of 22.4, 17.4, and 23.2 μM, respectively.

1. Introduction

The genus *Nannoglottis* (Asteraceae) is an endemic Chinese genus that contains nine species and occurs mainly in the Himalayas between altitudes of 2400 and 4200 m and in the contiguous zone in the People's Republic of China (Gao and Chen, 2005). From this genus, only *N. ravida* had been previously investigated, with two clerodane-type diterpenoids and a dicaffeoyl quinic acid isolated (Qin and Li, 2004; Jiang et al., 2010). The systematic position of *Nannoglottis* has been a controversial subject for a long time, and it has been placed in different tribes of Asteraceae: Inuleae, Senecioneae, Liabeae, and Astereae (Gao and Chen, 2005; Liu et al., 2000). From morphology, cytobiology, and molecular biology, Gao and Liu et al. suggested a close affinity between the genus *Nannoglottis* and the Astereae tribe (Liu et al., 2000; Liu, 2001; Gao and Chen, 2005). From a chemtaxonomy, perspective Qin and Li also supported that *Nannoglottis* should be placed in the tribe of Asteraceae based on clerodane-type diterpenoids isolated from *N. ravida* (Qin and Li, 2004). These clerodane-type diterpenoids were considered as characteristic of the Astereae tribe (Faini et al., 1999; Simirgiotis et al., 2000; Tene et al., 2005).

As part of our ongoing effort to discover bioactive natural products in the Asteraceae and to supply useful evidence for chemtaxonomy on *Nannoglottis*, we report herein the first phytochemical and bioactive investigation on *Nannoglottis carpesioides* (Maxim.). This paper deals with the isolation and structural elucidation of nine compounds (**1**–**9**) from the roots of this plant, including three derivatives of 11α,12α-epoxyolean-28,13β-olide (**1**–**3**), four 28-noroleanane-type triterpenes (**4**–**7**), and two 8-*O*-4′-neolignans (**8**, **9**) (Fig. 1), along with five known compounds, **10**–**14** (one neolignan, three 28-noroleanane-type triterpenes, and one sarratane triterpene). The structures of the compounds were elucidated by spectroscopic methods, including 1D and 2D NMR, HRMS and CD. All compounds were obtained for the first time from this genus. To identify bioactive natural products from this plant, seven triterpenes (**1**, **3**, **5**, **6**, **11**–**13**) and three neolignans (**8**–**10**) were evaluated for their cytotoxic activities against two human cell lines, HL-60 and Hep-G2. The antioxidant activities of these compounds were assessed by ABTS radical-scavenging assays. The presence of these oleanane-type triterpenes further supports the chemosystematic position of *Nannoglottis* in Astereae.

2. Results and discussion

The molecular formula of compound **1** was determined to be $C_{30}H_{46}O_5$ based on HRESIMS data at *m*/*z* 504.3689 $[M+NH_4]^+$ (calcd. for $C_{30}H_{50}O_5N$, 504.3684). The IR spectrum showed absorption bands corresponding to OH (3469 and 3372 cm^{-1}), γ-lactone (1752 cm^{-1}), and epoxide groups (936 and 874 cm^{-1}). The ^{13}C NMR and DEPT spectra displayed 30 carbon signals, including seven methyls, eight methylenes, seven methines, and eight quaternary carbons (Table 1). Furthermore, the ^1H and ^{13}C NMR spectra showed seven oleanane-type methyl singlets (δ_C 15.1, 17.2, 18.4, 19.6, 24.2, 27.8, and 33.0; δ_H 0.80, 0.97, 0.99, 0.99, 1.01, 1.02, and 1.44), the characteristic resonances of an

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doi:10.1016/j.phytochem.2011.05.008

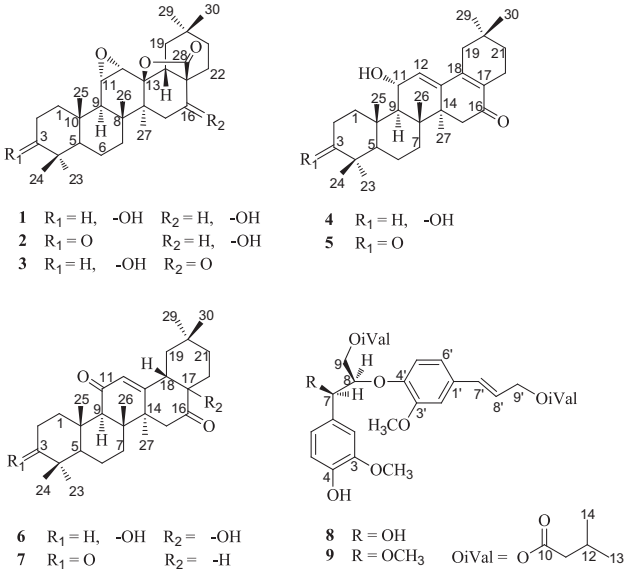

Fig. 1. Structures of new compounds **1–9** from the roots of *Nannoglottis carpesioides*.

11α,12α-epoxide moiety (δ_H 3.04, 3.07, δ_C 52.5, and 56.9), and the characteristic signals of a 28,13β-lactone unit (δ_C 178.2, C-28 and 88.5, C-13) (Ikuta et al., 1995; Wang et al., 2006). The NMR spectroscopic data of **1** (Tables 1 and 2) were similar to the published values of 3β-hydroxy-11α,12α-epoxyolean-28,13β-olide (Ikuta et al., 1995) and suggested that **1** was the 16-OH derivative of the reported triterpene. The most obvious distinction between two compounds was the chemical shift value of C-16, which appeared as an oxygen-bearing methine carbon at δ_C 72.9 for **1** instead of a methylene at δ_C 21.6 for the reported compound. In accordance, the ^{1}H NMR spectrum also showed an oxygen-bearing methine proton (δ_H 4.14 br s, H-16) for **1**. The structure of **1** was confirmed by ^{1}H–^{1}H COSY and HMBC correlations, as shown in Fig. 2. NOE experiments established the relative stereochemistry at C-3, C-5, C-16, C-23, and C-26. In the NOE experiments, irradiation of: H-3 led to an enhancement of H-5 (+3.09%) and H_3-23 (+2.04%) resonances; of H_3-27 led to an enhancement of H-3 (+1.75%), and H-5 (+2.07%) resonances, which confirmed that the 3-OH group was in a β-orientation; H-12 led to an enhancement of H-18 (+2.70%) resonance, which indicated that H-12 was in a β-orientation; and H-16 led to an enhancement of H_3-26 (+27.40%) resonance, which indicated that the 16-OH group was also in an α-orientation.

Table 1
^{13}C (100 Mz) NMR spectroscopic data for compounds **1–7** in $CDCl_3$.[a]

Position	1	2	3	4	5	6	7
1	38.1	38.6	38.1	40.1	40.3	39.1	39.6
2	27.0	33.8	26.7	27.3	34.1	27.2	34.0
3	78.8	216.3	78.6	78.6	217.7	78.7	217.0
4	38.8	47.6	38.9	39.1	47.6	39.0	47.6
5	54.6	54.6	55.1	55.3	55.2	55.0	55.2
6	17.6	18.7	17.5	18.2	19.1	17.8	18.8
7	31.1	30.5	31.1	34.2	33.6	33.0	32.6
8	39.9	40.0	41.6	41.4	41.2	45.5	45.6
9	49.8	49.1	49.9	54.1	52.3	61.0	60.5
10	36.4	36.2	36.5	38.1	37.6	36.3	37.0
11	52.5	52.3	52.6	67.8	68.4	199.5	199.0
12	56.9	56.8	56.1	128.3	128.6	129.1	123.3
13	88.5	88.3	87.3	140.8	140.6	165.0	166.5
14	41.2	41.0	46.4	44.6	45.0	49.3	47.6
15	36.8	36.7	46.4	43.5	43.6	42.8	43.5
16	72.9	72.9	202.7	199.8	199.5	211.5	212.2
17	47.6	47.6	59.9	131.6	131.5	76.5	48.0
18	49.9	49.9	54.6	145.7	145.8	52.4	38.1
19	38.7	38.6	39.8	40.5	41.1	45.8	41.9
20	31.3	31.3	31.4	29.1	29.1	30.7	30.6
21	35.9	35.9	34.8	34.0	34.2	36.3	37.9
22	26.8	27.0	21.3	20.7	20.7	30.4	23.5
23	27.8	26.0	27.8	28.2	27.1	28.1	26.6
24	15.1	21.1	15.1	15.6	21.2	15.6	21.2
25	17.2	16.4	17.1	16.7	16.3	16.0	15.9
26	18.4	18.3	19.9	19.2	19.1	19.6	18.3
27	19.6	19.3	20.6	23.5	23.2	24.7	22.7
28	178.2	178.0	173.6	–	–	–	–
29	33.0	33.0	32.9	28.3	28.7	32.1	32.8
30	24.2	24.2	23.2	28.3	28.0	23.5	24.5

[a] Assignments were made using ^{1}H–^{1}H COSY, HSQC, HMBC, and NOE experiments.

The molecular formula of compound **2** was determined to be $C_{30}H_{44}O_5$ based on the HRESIMS data at *m/z* 502.3521 $[M + NH_4]^+$ (calcd. for $C_{30}H_{48}O_5N$, 502.3527). The ^{13}C and ^{1}H NMR spectra of **2** were very similar to those of **1** (Tables 1 and 2). The main difference in the ^{13}C NMR spectra was the appearance of a signal at δ_C 216.3 in **2**, which replaced the signal for the 3-OH group at δ_C 78.8 in **1**. This observation suggested the presence of a 3-ketone group, which was confirmed by HMBC correlations from methyl protons H_3-23 at δ_H 1.11 and H_3-24 at δ_H 1.07 to C-3 at δ_C 216.3. Therefore, compound **2** was elucidated as 16α-hydroxy-3-oxo-11α, 12α-epoxyolean-28,13β-olide.

The molecular formula of compound **3** was determined to be $C_{30}H_{44}O_5$ based on the HRESIMS data at *m/z* 485.3265 $[M + H]^+$ (calcd. for $C_{30}H_{45}O_5$, 485.3262). The ^{13}C and ^{1}H NMR spectra of **3** (Tables 1 and 2) were consistent with those of **1** except for presence of a resonance at δ_C 202.7 in **3**, which replaced the resonance at δ_C 72.9 for the 16-OH group in **1**. This observation suggested the presence of a 16-ketone group in **3**, which was confirmed by HMBC correlations from H-15a and H-15b (δ_H 2.60, 1.99) to C-16 (δ_C 202.7). Because of the space effect, the carbonyl carbon signal at C-28 was shifted to a relatively upfield position (δ_C 173.6) compared to that observed in **1** (δ_C 178.1). Accordingly, compound **3** was elucidated as 3β-hydroxy-16-oxo-11α,12α-epoxyolean-28,13β-olide.

The molecular formula of compound **4** was determined to be $C_{29}H_{44}O_3$ based on HRESIMS data at *m/z* 441.3353 $[M + H]^+$ (calcd. for $C_{29}H_{45}O_3$, 441.3363). The IR spectrum showed absorption bands corresponding to OH (3417 cm^{-1}) and carbonyl (1655 cm^{-1}) group, that indicated of the presence of an α,β-unsaturated ketone group. The ^{13}C NMR and DEPT spectra showed 29 carbon signals (Table 1), including seven methyls, eight methylenes, five methines, and nine quaternary carbons. This information suggested that **4** was a noroleanane triterpene compared with that observed of **1**. Furthermore, the ^{1}H NMR and ^{13}C NMR spectroscopic data showed the presence of one olefinic proton at C-12 (δ_H 5.97, d, *J* = 3.6 Hz), four olefinic carbons at δ_C 128.3 (d), 131.6 (s), 140.8 (s) and 145.7 (s), and one carbonyl group at δ_C 199.8, all of which were attributed to a 12,17-dien-16-one (Itokawa et al., 1981). The UV absorption spectrum (289 nm) confirmed the presence of a heteroannular diene system. Two oxygenated signals (δ_C 78.6, 67.8; δ_H 3.24, 4.42) indicated the presence of two OH groups, which were assigned at C-3 and C-11, respectively. The assignment of a 3-OH group was based on the observed HMBC correlations from the methyl protons H_3-23 and H_3-24 (δ_H 1.01, 0.81) to C-3 (δ_C 78.6). The ^{1}H–^{1}H COSY correlations from H-11 (δ_H 4.42) to H-9 and H-12 (δ_H 1.56, 5.97) and the HMBC correlations from H-12 to C-9, C-14, and C-18 (δ_C 54.1, 44.6, 145.7) and from H-15 (δ_H 2.16) to C-16 (δ_C 199.8) confirmed the presence of 11-hydroxy-12,17-dien-16-one. The ^{13}C and ^{1}H NMR spectra of **4** (Tables 1 and 3) were similar to the published spectra of 3β-hydroxy-28-norolean-12,17-dien-16-one (Itokawa et al., 1981), except for the presence of the 11-OH in **4**.