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Clerodane-type diterpenoids from *Nannoglottis ravida*

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Abstract

Chemical investigation of the roots of *Nannoglottis ravida* resulted in the characterization of two 5α,10α-*cis*-clerodane-type diterpenoides, ravidin A and B. Their structures and stereochemistry were established by spectroscopic methods, including X-ray crystallographic diffraction analysis of ravidin A. Their significance in terms of the chemotaxonomy of *Nannoglottis* is discussed.

Keywords: *Nannoglottis ravida*; Compositae; Structural determination; Stereochemistry; Clerodane-type diterpenoids; Ravidin A and B

1. Introduction

Nannoglottis Maxim. s.l., a small genus of the family Compositae, is endemic to the Qinghai–Tibet Plateau (Liu et al., 2000). It comprises about eight species, found in altitudes between 2400 and 4200 m. This genus has been classified into four different tribes of Asteraceae: Inuleae, Senecioneae, Liabeae and Astereae (Liu, 2000). Recently, researchers reported the geographically isolated occurrence of *Nannoglottis* in the tribe Astereae, and its ancient origin in the Southern Hemisphere around 23–32 million years ago (Liu, 2000, 2001; Liu et al., 2002). The altitude of the Qinghai–Tibet Plateau and climate fluctuation in the Pleistocene preserved the most ancestral species of *Nannoglottis*. The ancestor-like species, *N. ravida* (C. Winkl.) Y. L. Chen, a separate lineage in the genus, became narrowly distributed in the alpine shrub habitats of Chengduo and Qumalai County in Qinghai province, despite the fact that other lineages continued to produce new species and extend their distribution following the retreat and re-colonization of the coniferous forest in the plateau (Liu et al., 2002).

So far, no phytochemical research has been conducted on *Nannoglottis*. Elucidating the distribution patterns of the natural compounds and their biosynthetic pathways in the tribe Astereae can help to understand the systematic position of the genus. In the present study, two new clerodane-type diterpenoides, ravidin A (**1**) and B (**2**), were identified as the major components in EtOH extracts of the roots of *N. ravida*. The finding of these two new diterpenoide, which have a similar skeleton to those found in *Aster souliei* (Guo et al., 1997) and in *A. alpinus* (Bohlmann et al., 1985), suggested a classification of *Nannoglottis* in Astereae rather than in Senecioneae, Liabeae or Inuleae of Compositae. This is in accordance with the evidence obtained recently from floral microcharacter, pollen, chromosome and DNA sequences (Liu, 2000, 2001; Liu et al., 2000; Liu et al., 2002).

2. Results and discussion

The HREIMS spectrum of **1** exhibited its $[M]^+$ at *m/z* 344.16292, corresponding to the molecular formula $C_{20}H_{24}O_5$. Its IR spectrum (KBr) showed furyl (3141, 3132, 3114, 1508, 876 cm^{-1}), δ-lactone and ketone (1724, 1709 cm^{-1}) absorptions. In the EIMS, the

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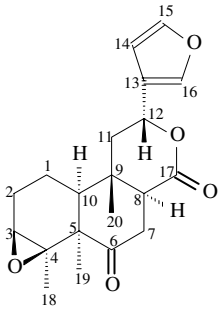
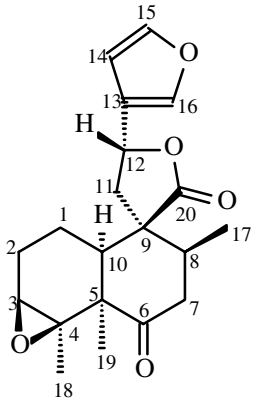
fragment at *m/z* 81 resulted from the cleavage of the C-11/C-12 bond. The ions at *m/z* 94 and 95 were due to the cleavage of the δ-lactone ring along the C-9/C-11 and C-12 bonds (ring C). These observations clearly indicated that the furan ring occupied the C-12 position, as in other furanoid diterpenoids (Atta-ur-Rahman and Ahmad, 1988; Atta-ur-Rahman et al., 1991; Bohlmann et al., 1985). The ^{13}C and DEPT NMR spectra contained 20 carbon resonances attributable to $6\times C$, $7\times CH$, $4\times CH_2$ and $3\times CH_3$ groups (Table 1). The presence of a lactone function and a ketone carbonyl were confirmed further by analysis of the ^{13}C NMR resonances at δ 72.2 (*d*, C-12), 171.1 (*s*, C-17), and 210.2 (*s*, C-6), and the presence of a β-substituted furan ring was also confirmed further by low-field signals at δ 124.8 (*s*, C-13), 108.3 (*d*, C-14), 139.6 (*d*, C-16), and 143.8 (*d*, C-15). In addition, the appearance of resonances at δ 57.1 (*d*, C-3) (δ 2.91 in ^{1} H NMR spectrum) and 59.1 (*s*, C-4) suggested the presence of an oxirane ring. The ^{1}H NMR spectrum showed a one-proton double doublet at δ 5.39 ($J_{12,11\alpha}=12.5$, $J_{12,11\beta}=4.0$ Hz) which was assigned to the C-12 proton, while the two double doublets at δ 1.77 ($J_{11\alpha,11\beta}=13.5$, $J_{11\alpha,12}=12.5$ Hz) and 2.24 ($J_{11\beta,11\alpha}=13.5$, $J_{11\beta,12}=4.0$ Hz) each integrating for one proton were assigned to the C-11α and C-11β protons, respectively. In the ^{1}H–^{1}H COSY spectrum, the signals at δ 5.39 (H-12), 1.77 (H-11$_\alpha$) and 2.24 (H-11$_\beta$) clearly correlated with one another. The three methyl groups at C-4, C-5 and C-9 were observed as 3H singlets at δ 1.40, 1.44 and 1.01, respectively. The chemical shift of the methyl group at C-9 was observed at high field due to the influence of the furan ring on the same side as the C-9 methyl group (Atta-ur-Rahman and Ahmad, 1988). The position of the three methyl groups was also confirmed by HMBC analysis. The C-7α, C-7β, and C-8 protons appeared as three one-proton double doublets at δ 3.02 ($J_{7\alpha,7\beta}=17.5$, $J_{7\alpha,8}=8.0$ Hz), 2.66 ($J_{7\beta,7\alpha}=17.5$, $J_{7\beta,8}=11.0$ Hz), and 3.08 ($J_{8,7\alpha}=8.0$, $J_{8,7\beta}=11.0$ Hz), respectively. Similarly in the ^{1}H–^{1}H COSY spectrum, the signals at δ 3.02, 2.66, and 3.08 showed mutual correlations, indicating connectivity between the C-7α, C-7β, and C-8 protons. The C-2β and C-3 protons were found to resonate at δ 2.12 (1H, *dd*, $J_{2\beta,2\alpha}=14.5$, $J_{2\beta,3}=3.0$ Hz) and 2.91 (1H, *d*, $J_{3,2\beta}=3.0$ Hz), respectively, while the C-1α, C-1β, C-2α, and C-10 protons appeared together as overlapping multiplets at δ 1.37–1.75. The furan protons at C-14, C-15, and C-16 were found to resonate at δ 6.42 (*br s*), 7.42 (*br s*), and 7.46 (*br s*) (Atta-ur-Rahman and Ahmad, 1988; Atta-ur-Rahman et al., 1991), respectively. With the aid of the ^{13}C and DEPT NMR spectral analyses, as well as detailed ^{1}H–^{1}H COSY, HMQC, and HMBC experiments, these signals could be assigned accordingly.

Table 1
Table **1** ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for **1** and **2** ($CDCl_3$, TMS)

	1		**2**	
	δ_H	δ_C (DEPT)	δ_H	δ_C (DEPT)
1a	~ 1.37 overlapped	17.6 *t*	~ 1.42 overlapped	24.6 *t*
1b	~ 1.63 overlapped		~ 1.42 overlapped	
2α	~ 1.75 overlapped	22.6 *t*	~ 1.77 overlapped	25.5 *t*
2β	2.12 *dd* (14.5, 3.0)		2.19 *dd* (14.0, 3.0)	
3	2.91 *d* (3.0)	57.1 *d*	2.96 *d* (3.0)	59.7 *d*
4		59.1 *s*		60.8 *s*
5		49.9 *s*		49.7 *s*
6		210.2 *s*		211.5 *s*
7α	3.02 *dd* (17.5, 8.0)	36.5 *t*	2.93 *dd* (16.0, 13.5)	43.8 *t*
7β	2.66 *dd* (17.5, 11.0)		2.33 *dd* (16.0, 3.5)	
8	3.08 *dd* (11.0, 8.0)	45.3 *d*	2.24 *m*	32.4 *d*
9		36.8 *s*		51.3 *s*
10	~ 1.63 overlapped	55.4 *d*	~ 1.80 overlapped	48.5 *d*
11α	1.77 *dd* (13.5, 12.5)	43.3 *t*	2.48 *dd* (13.0, 6.5)	45.3 *t*
11β	2.24 *dd* (13.5, 4.0)		2.39 *dd* (13.0, 10.0)	
12	5.39 *dd* (12.5, 4.0)	72.2 *d*	5.36 *dd* (10.0, 6.5)	70.8 *d*
13		124.8 *s*		123.6 *s*
14	6.42 *br s*	108.3 *d*	6.40 *br s*	108.1 *d*
15	7.42 *br s*	143.8 *d*	7.44 *br s*	144.1 *d*
16	7.46 *br s*	139.6 *d*	7.48 *br s*	139.9 *d*
17		171.1 *s*	1.11 *d* (6.5)	16.7 *q*
18	1.40 *s*	21.6 *q*	1.53 *s*	22.6 *q*
19	1.44 *s*	27.9 *q*	1.47 *s*	27.1 *q*
20	1.01 *s*	16.5 *q*		175.8 *s*



1



2

The IR, MS, ^{1}H, and ^{13}C NMR spectroscopic data are characteristic for a diterpenoid with a clerodane skeleton. The HMBC experiment was used to elucidate the relevant connectivities in **1**. The position of the ketone carbonyl at C-6 was determined by significant HMBC correlations of the protons at δ 1.44 (3H, *s*, CH_3-19), $\sim$1.63 (H-10), 2.66 (1H, *dd*, $J_{7\beta,7\alpha} = 17.5$, $J_{7\beta,8} = 11.0$ Hz, H-7β), and 3.02 (1H, *dd*, $J_{7\alpha,7\beta} = 17.5$, $J_{7\alpha,8} = 8.0$ Hz, H-7α) with the carbon at δ 210.2 (C-6). The epoxide moiety was supported by significant HMBC correlations of the proton at δ 2.91 (1H, *d*, $J_{3,2\beta} = 3.0$ Hz) with the carbons at δ 17.6 (C-1) and 22.6 (C-2), the protons at δ 1.40 (3H, *s*, CH_3-18) with the carbons at δ 57.1 (C-3) and 59.1 (C-4), and the protons at δ 1.44 (3H, *s*, CH_3-19) with the carbon at δ 59.1 (C-4). Thus, the structure of **1** was determined to be 3β, 4β: 15,16-diepoxy-6-keto-clerodane-13 (16), 14-dien-17,12-olide, and was designated ravidin A.

The relative configuration of **1** was established by NOESY experiments. Starting from a conventional α-orientation of CH_3-19 in clerodane, NOESY correlations of CH_3-19 with H-3, H-8, and H-10, and CH_3-20 with H-12 suggested that H-3, H-8, and H-10 had α-orientations, and CH_3-20, and H-12 had β-orientations. In addition, correlations of CH_3-19 with H-7_α, H-8, and H-10 suggested that the cyclohexanone ring had a boat conformation. Because the chemical shift of CH_3-18 was superimposed with that of H-1a, the stereochemistry of CH_3-18 was not definable. Therefore, the structure and relative stereochemistry of **1** was studied by single-crystal X-ray diffraction analysis, confirming the structure in Fig. 1. The X-ray crystallography affirmed the relative configuration of **1** and the result was consistent with the data from the NOSEY experiment. The CH_3-18, CH_3-19, H-3, H-8, and H-10, and the furan ring were found to be in the α-orientations, and the CH_3-20 and H-12 in β-orientations. Ring A and C are in semi-chair conformation, ring B approximately in a twisted boat conformation, and the furan ring in a plane conformation. A/B rings were found to be fused *cis* and the B/C rings fused *trans*.

Although in *cis*-clerodane type diterpenes, the absolute configuration of a 5β,10β-*cis*-clerodane has been determined by a structural correlation with a confirmed absolute structure (Anderson et al., 1974; McCrindle et al., 1976), and that of a 5α,10α-*cis*-clerodane has been established by X-ray structural studies of the heavy-atom derivative (Nishino et al., 1984), there are no references for the C-6 ketone derivatives of the *cis*-clerodane type diterpenes. The absolute stereochemistry of **1** was thus established as shown in Fig. 1 by the positive Cotton effect ($\Delta\varepsilon_{297}$ + 4.22) for the C-6 ketone in combination with the octant rule for ketones (Kirk et al., 1972) and X-ray analysis (see Section 3).

Compound **2** was obtained as a colorless, amorphous solid. Its HREIMS spectrum exhibited the $[M]^+$ at *m/z*

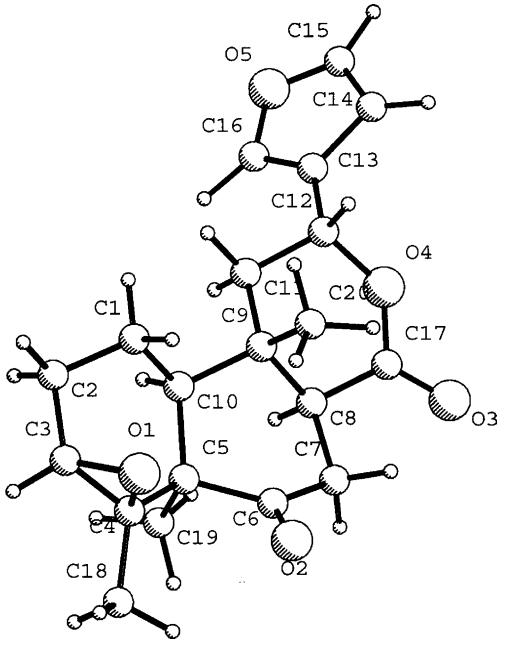


Fig. 1. ORTEP drawing of ravidin A (**1**).

344.16205, corresponding to the molecular formula $C_{20}H_{24}O_5$. The IR spectrum (KBr) showed furyl (3143, 3136, 3114, 1508, 876 cm^{-1}), γ-lactone and ketone (1755, 1707 cm^{-1}) absorptions. The ^1H, ^{13}C, and DEPT NMR spectral data of **2** closely resembled those of **1**with characteristic signals for a β-substituted furan ring, an oxirane ring, and a ketone carbonyl. The main difference was the absence of one tertiary methyl group and the appearance of a resonance of one secondary methyl group at δ 1.11 (3H, *d*, $J = 6.5$ Hz, CH_3-17) in the ^1H NMR spectrum and at δ 16.7 (*q*, C-17) in the ^{13}C NMR spectrum. HMQC and HMBC experiments permitted unambiguous definition of the structure of **2**, an isomer of compound **1**. Especially, HMBC correlations of the CH_3-17 at δ 1.11 with the carbons at δ 43.8 (C-7), 32.4 (C-8), and 51.3 (C-9), and those of the C-11 protons at δ 2.39 (1H, *dd*, $J_{11\beta,11\alpha} = 13.0$, $J_{11\beta,12} = 10.0$ Hz, H-11_β) and 2.48 (1H, *dd*, $J_{11\alpha,11\beta} = 13.0$, $J_{11\alpha,12} = 6.5$ Hz, H-11_α) with the carbons at δ 32.4 (C-8), 51.3 (C-9), 48.5 (C-10), 70.8 (C-12), 123.6 (C-13), and 175.8 (C-20) indicated the position of the secondary methyl group to be on C-8, the position of the β-substituted furan ring on C-12, and that of the γ-lactone group on C-12 and C-20. In addition, when inspecting the molecular model, NOESY correlations of CH_3-19 with CH_3-18 and H-10 indicated α-orientation of CH_3-18 and H-10. NOESY correlations of CH_3-19 with H-8 and H-10 suggested a chair conformation of the cyclohexanone ring, as well as β-orientation of CH_3-17. The H-3 proton was oriented equatorially, because it appeared as a doublet with $J = 3.0$ Hz, and in the NOESY spectrum, it showed correlation with CH_3-18. In particular, the α-orientation of the C-11 methylene relative to ring B followed from the NOESY correlations of H-11_α with H-10 and CH_3-19, and of H-11_β with H-8 and CH_3-17. Consequently, the structure of **2** was determined to be 3β, 4β: 15,16-diepoxy-6-keto–clerodane-13(16), 14-dien-20,12-olide with CH_3-17 in the unusual β-orientation, and was named ravidin B.

Finally, the CD curve of **2** showed a negative Cotton effect ($\Delta\varepsilon_{298} - 11.17$) for the C-6 ketone. By comparing with ravidin A, the absolute configuration of **2** was established as 5α,10α-*cis* clerodane possessing a chair conformation for ring B (Fayos et al., 1979; Martinez-Ripoll et al., 1981).

From a phytochemical point of view, it is of interest to note that clerodane diterpenoids, ravidin A (**1**) and B (**2**), were found in *Nannoglottis* species for the first time, although more than 1000 compounds possessing this carbon skeleton have been isolated from plants and liverworts in the last years (Bruno et al., 2000; Tazaki et al., 1998, 1999). This is additional evidence for the botanical classification of this genus in Astereae, and they might be important chemical markers of the genus.

3. Experimental

3.1. General experimental procedures

Melting point was measured on a XT_4-100× apparatus, and is uncorr. Optical rotations were measured on a Perkin–Elmer 241 automatic polarimeter in MeOH at 25 °C. IR spectra were recorded on a Nicolet Impac 400 FTIR (KBr) spectrophotometer. ^1H, ^{13}C and 2D NMR spectra were taken on a Varian Inova-500 NMR spectrometer using tetramethylsilane as internal standard. CD measurements were performed at room temp. on a Jasco J-715 spectropolarimeter. HREIMS and EIMS were measured on a England VG ZAB-HS mass spectrometer at 70 eV. ESIMS was measured on a Agilent 100 series LC/MSD Trap mass spectrometer. The single X-ray crystallography was determined by using MAC DIP-2030K. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Co., Qingdao, PR China) was used for CC.

3.2. Plant materials

The roots of *N. ravida* were both collected from Qumulai Country, Qinghai Province, PR China in August, 2002 (alt., 3800 m), and authenticated by Professor Liu Jian-quan of the Northwest Plateau Institute of Biology, Chinese Academy of Sciences. Two voucher specimens (Liu Jianquan 645; No. 337-03) were deposited both in NPIB, Chinese Academy of Sciences, and the Department of Natural Medicinal Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

3.3. Extraction and isolation

The air-dried and powdered roots (2 kg) of *N. ravida* were extracted repeatedly three times under reflux with H_2O (5:95) EtOH. The combined EtOH extracts were evaporated under reduced pressure to yield a viscous residue (131 g), the latter being dissolved in EtOH H_2O (4:1) (ca. 1000 mL) and the soln. was extracted with petroleum ether (60–90 °C) (3 × 800 mL). Evaporation of the aq. layer under reduced pressure yielded a brown residue which was again dissolved in water (ca. 1000 mL), and then extracted with EtOAc (3 × 800 mL). The combined EtOAc extracts were washed with aq. 5% $NaHCO_3$ (3 × 800 mL), then H_2O (2 × 800 mL) and dried (Na_2SO_4). After removal of the organic solvent under reduced pressure, 13 g of residue was obtained as a brown gummy solid. The residue was applied to a silica gel column (200–300 mesh, 210 g) eluted with a mixture of petroleum ether (60–90 °C)–EtOAc of increasing polarity to obtain 53 fractions (250 mL for each) (petroleum ether–EtOAc = 9:1 × 8

frs., 8:2 × 23 frs., 7:3 × 10 frs., 6:4 × 12 frs.) and finally with MeOH (1000 mL). After evaporation of each eluate, compound **1** (597 mg) was obtained from frs. 18 to 23 as colorless prisms, and compound **2** (33 mg) from frs. 25 to 26 as colorless amorphous solid.

3.4. Ravidin A (*1*)

Colorless crystals, m.p. 171–173 °C. $[\alpha]_D^{25}$: −22.45° ($CHCl_3$; *c* 0.530). IR ν_{max}^{KBr} cm^{-1} : 3141, 3132, 3114, 2987, 2941, 2877, 1724, 1709, 1508, 1225, 1184, 1147, 1020, 955, 876. CD nm (Δε): 325 (+0.62), 304 (+4.15), 297 (+4.22), 270 (+1.01), 259 (0) (MeOH; *c* 0.151). For ^1H and ^{13}C NMR spectral data, see Table 1. HREIMS *m/z* : 344.16292 $[M]^+$ (calc. for $C_{20}H_{24}O_5$, 344.16237). EIMS *m/z* rel. int.): 344 $[M]^+$ (4), 329 $[M\text{-}CH_3]^+$ (28), 311 (10), 219 (20), 201 (9), 191 (10), 173 (12), 147 (19), 125 (24), 107 (34), 95 (18), 94 (23), 81 (38), 43 (100).

3.5. Ravidin B (*2*)

White amorphous solid. $[\alpha]_D^{25}$: −77.50°($CHCl_3$; *c* 0.120). IR ν_{max}^{KBr} cm^{-1} : 3143, 3136, 3114, 2939, 1755, 1707, 1508, 1477, 1184, 1157, 1016, 876. CD nm (Δε): 325 (−2.58), 310 (−8.43), 298 (−11.17), 292 (−10.10), 253 (−0.39) (MeOH; *c* 0.071). For ^1H and ^{13}C NMR spectral data, see Table 1. HREIMS *m/z*: 344.16205 $[M]^+$ (calc. for $C_{20}H_{24}O_5$, 344.16237). EIMS *m/z* (rel. int.): 344 $[M]^+$ (4), 329 $[M\text{-}CH_3]^+$ (3), 311 (3), 220 (100), 202 (43), 187 (27), 178 (78), 161 (32), 147 (27), 121 (47), 107 (73), 95 (28), 94 (30), 81 (33), 43 (73). ESIMS *m/z*: 344 $[M]^+$, 367 $[M + Na]^+$.

3.6. X-ray crystal analysis of ravidin A (*1*)

Crystal data: $C_{20}H_{24}O_5$, M_r = 344.41, orthorhombic, space group $P2_1$, *a* = 6.405(1) Å, *b* = 10.456(1) Å, *c* = 13.506(1) Å, *V* = 888.84(5) $Å^3$, *Z* = 2, D_c = 1.287 g/cm^3, μ = 0.09 mm^{-1}. Intensity data were collected with MAC DIP-2030K Image Plate diffractometer with a graphite monochromator (ω–2θ scans, $2\theta_{max}$ = 50.0°), MoKα (λ = 0.71073 Å) radiation. A total of 1886 unique reflections were collected, of which 1502 were observed ($|F|^2 = 8\sigma|F|^2$). The structure was solved by direct methods using SHELX-86 and expanded using difference Fourier techniques, refined by the program and the NOMCSDP and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were $R_f = 0.059$, $R_w = 0.064 (w = 1/\sigma|F|^2)$. The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as Deposition No. CCDC-232785. Copies of the data can be obtained free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44 1223 336 033, or e-mail: deposit@ccdc.cam.ac.uk].

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