Terpenoids from the Roots and Rhizomes of *Nardostachys chinensis*

Yi Zhang,[†] Yang Lu,[‡] Li Zhang,[‡] Qi-Tai Zheng,[‡] Li-Zhen Xu,*[,†] and Shi-Lin Yang[†]

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, People's Republic of China, and Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

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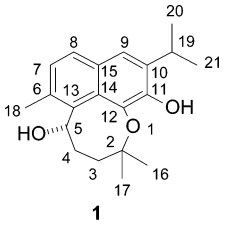
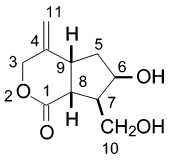
A new diterpene, 10-isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtha[1,8-*bc*]oxocine-5,11-diol (**1**), and a new monoterpene, 6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one, together with four known sesquiterpenes, $\Delta^{1(10)}$-aristolene-9β-ol, debilon, nardosinone, and kanshone A, were isolated from the roots of *Nardostachys chinensis*. The structures of the new compounds were established on the basis of their spectroscopic data, and the structure of **1** was confirmed by X-ray crystallographic analysis.

The roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae) have been used in Chinese traditional medicine to elicit stomachic and sedative effects.[1] The plant is known to be rich in sesquiterpenoids,[2] which have been found to exhibit antimalarial, antinociceptive,[3] and cytotoxic activities,[4] as well as to enhance nerve growth factor.[5] From the ethanol extracts of the roots and rhizomes of *N. chinensis*, we have isolated a new diterpene (**1**) based on a new carbon skeleton, a new monoterpene (**2**), and four known sesquiterpenes, $\Delta^{1(10)}$-aristolene-9β-ol,[6] debilon,[4] nardosinone,[7] and kanshone A.[7] In this report, we describe the structure elucidation of compounds **1** and **2**.



1



2

Compound **1** was obtained as colorless crystals. A molecular formula of $C_{20}H_{26}O_3$ was determined on the basis of HREIMS, which showed a molecular ion peak at *m/z* 314.1879 (calcd *m/z* 314.1882). The IR spectrum of **1** exhibited typical absorptions at 3359 (OH) and 1622 cm^{-1} (aromatic), while its UV spectrum showed intense absorptions at 239, 305, and 338 nm, suggesting the presence of a naphthalene moiety in the molecule.[8] The ^{13}C NMR spectrum of **1** displayed 20 carbon signals, of which 10 were attributable to the olefinic carbons from the naphthalene unit, and the rest resulted from five methyls, two methylenes, one methine, one oxymethine, and one oxygen-bearing quaternary carbon, respectively. In addition, when comparing NMR data of **1** with those reported for de-*O*-ethyl salvonitin,[8] signals in the downfield regions of their ^{1}H and ^{13}C NMR spectra were almost identical. Such observations indicated that de-*O*-ethyl salvonitin and **1** have the same positions of substitution in the naphthalene moiety. In addition, like that of de-*O*-ethyl salvonitin, the ^{1}H NMR spectrum of **1** also gave signals attributable to an isopropyl group (δ 1.31, 3H, d, J = 6.6 Hz, H-20; δ 1.34, 3H, d, J = 6.6 Hz, H-21; δ 3.33, 1H, m, H-19) and a methyl group (δ 2.49, 3H, s, H-18), which could be located at C-10 and C-6, respectively, according to the HMBC spectrum of **1** (Figure 1). The establishment of the rest of the structure was performed using the ^{1}H−^{1}H COSY and HMBC spectra of **1**, which suggested a partial connectivity between C-2 and C-5, with a partial structure being −CH(OH)−CH$_2$−CH$_2$−C(CH$_3$)$_2$−. In addition, the correlations between H-4b (δ 2.41) and C-13 (δ 131.6), H-5 and C-14 (δ 128.1), and H-7 (δ 7.08) and C-5 (δ 69.8) in the HMBC spectrum (Figure 1) indicated that C-5 is connected to C-13, while a correlation between the methyl proton signal (δ 1.72, H-16) and C-12 (δ 131.6) suggested that C-12 is connected to C-2 (δ 85.1) through an oxygen bridge. Through the above considerations, an eight-membered oxygen-containing ring could be proposed. The structure of **1** was confirmed using X-ray crystallographic analysis (Figure 2). As a result, **1** was determined as 10-isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtha[1,8-*bc*]oxocine-5,11-diol, which is representative of a new carbon skeleton.

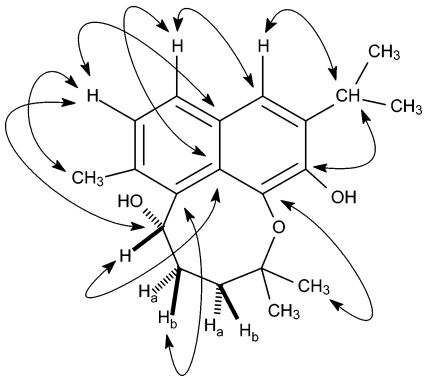


Figure 1. Key correlations in the HMBC spectrum of **1**.

Compound **2** was obtained as colorless needles, and a molecular formula of $C_{10}H_{14}O_4$ was established on the basis of its HRFABMS ([M + 1]$^+$ *m/z* 199.0989, calcd 199.0970). The IR spectrum showed absorptions at 3400 (OH), 2920 (C=CH$_2$), 1760, and 1630 (ester carbonyl group) cm^{-1}. The ^{13}C NMR spectrum of **2** showed 10 signals attributable to one carbonyl (δ 177.3, C-1), two olefinic (δ 113.7, C-11; δ 144.1, C-4), two oxymethylene (δ 61.9, C-10; δ 72.5, C-3), one oxymethine (δ 73.5, C-6), one methylene (δ 41.4, C-5), and three methine carbons (δ 40.9, C-9; δ 44.9, C-8; δ 52.0, C-7), respectively. After analyzing its ^{1}H and ^{13}C NMR, HMBC, and ^{1}H−^{1}H COSY spectroscopic data, **2** was

* To whom correspondence should be addressed. Tel: (86) 10-62899705. Fax: (86) 10-62895086. E-mail: xulizhen2002@hotmail.com.
[†] Institute of Medicinal Plant Development.
[‡] Institute of Materia Medica.

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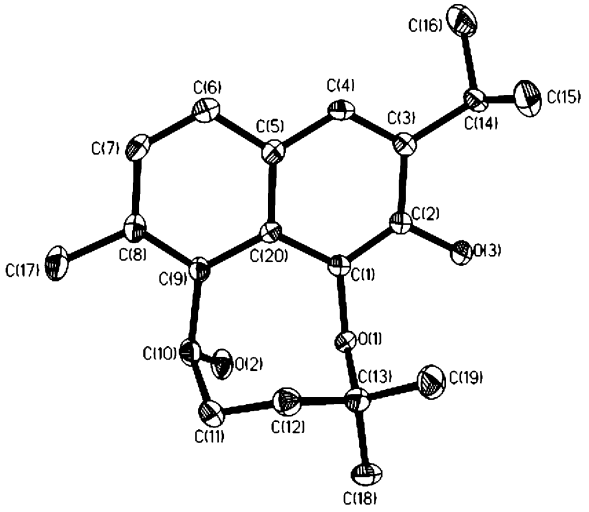

Figure 2. ORTEP diagram of **1**.

Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Spectroscopic Data of **1** (CD_3OD)[a]

position	δ_C	δ_H (*J* Hz)	position	δ_C	δ_H (*J* Hz)
2	85.1		11	148.9	
3	34.9	1.42, m	12	131.6	
		1.60, m	13	131.6	
4	30.6	2.03, m	14	128.1	
		2.41, m	15	129	
5	69.8	5.46, dd (1.2,6.6)	16	25.6	1.72, s
6	132.8		17	26.1	1.21, s
7	127.1	7.08, d (8.4)	18	21.1	2.49, s
8	128.4	7.56, d (8.4)	19	28.1	3.33, m
9	123.2	7.45, s	20	22.5	1.31, d (6.6)
10	136.4		21	22.5	1.34, d (6.6)

[a] Chemical shifts (δ) given in ppm.

Table 2. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Data of **2** (CD_3OD)[a]

position	δ_C	δ_H (*J* Hz)	HMBC (^{1}H to ^{13}C)
1	177.3		
3	72.5	4.73, m	1,4,9,11
4	144.1		
5	41.4	Hα, 1.65, m	4,6,9
		Hβ, 2.15, m	6,7,8,9,10
6	73.5	4.37, m	7,8,9
7	52.0	2.41, m	1,6,8,10
8	44.9	3.01, dd (9.6,11.4)	1,4,5,7,9,10
9	40.9	3.46, m	1,4,5,8,11
10	61.9	3.80, m	6,7,8
11	113.7	Ha, 5.06, s	3,4,9
		Hb, 5.17, s	3,4,9

[a] Chemical shifts (δ) given in ppm.

determined to be a monoterpene alcohol with a six/five-membered fused ring system, with NMR data (Table 2) being similar to those of the monoterpene unit of nardostachysin.[9] In addition, the relative configuration of **2** was established on the basis of the coupling constants in the ^{1}H NMR spectrum and correlations in the NOESY (Figure 3) spectrum. In particular, a coupling constant of 11.4 ($J_{8,9}$) Hz, identical to those of nardostachysin[9] and gardenone,[10] indicated that both H-8 and H-9 were β oriented. As a result, compound **2** was elucidated as shown.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded using a Shimadzu UV-3000 spectrophotometer and IR spectra using KBr pellets

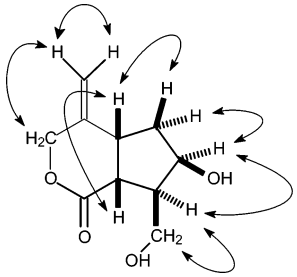

Figure 3. Key correlations in the NOESY spectrum of **2**.

on a Nicolet impact-400 Fourier transform infrared spectrometer. One- and two-dimensional NMR spectra were recorded on a Varian Unity Inova-600 spectrometer. The EIMS were measured in a Micromass ZabSpec spectrometer. Thin-layer chromatography employed precoated silica gel plates (Qingdao Haiyang). For column chromatography, silica gel (Qingdao Haiyang) and Sephadex LH-20 (Pharmacia) were used. The X-ray crystallographic data were collected with Mo Kα radiation (λ = 0.71073 Å) on a MAC DIP-2030K diffractometer, with a graphite monochromator.

Plant Material. The roots and rhizomes of *Nardostachys chinensis* Batalin were collected in August 2002, in Sichuan Province of the People's Republic of China, and identified by Professor Shou-Quan Lin (Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College). A voucher specimen (No. 2002417) has been deposited in the Herbarium of IMPLAD.

Extraction and Isolation. The dried roots and rhizomes (23 kg) of *N. chinensis* were extracted three times with 95% EtOH at room temperature, with the solvent removed under reduced pressure to give a 95% EtOH extract (1 kg). This extract was subsequently chromatographed over silica gel and eluted in a gradient manner with petroleum ether−EtOAc (0:10 → 10:0) followed by EtOAc−MeOH (0:10 → 10:0), to give 10 fractions. Fraction 3 (petroleum ether−EtOAc, 9:1) was submitted to repeated column chromatography over silica gel with petroleum ether−acetone to give $\Delta^{1(10)}$-aristolene-9β-ol (155 mg) and debilon (300 mg). Fraction 4 (petroleum ether−EtOAc, 8:2) was submitted to repeated column chromatography over silica gel with petroleum ether−acetone and purified further by passage with Sephadex LH-20, using $CHCl_3$−MeOH (1:1), to give compound **1** (25 mg). Fraction 5 (petroleum ether−EtOAc, 5:5) was submitted to repeated column chromatography over silica gel, with petroleum ether−acetone, to give nardosinone (160 mg) and kanshone A (200 mg). Fraction 7 (EtOH−MeOH, 9:1) was subjected to repeated column chromatography over silica gel with $CHCl_3$−MeOH and purified on Sephadex LH-20 with $CHCl_3$−MeOH (1:1) to give compound **2** (15 mg).

10-Isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtha-[1,8-*bc*]oxocine-5,11-diol (1): colorless crystals, mp 202−204 °C; $[\alpha]^{20}_D$ −36° (*c* 0.019, $CHCl_3$−CH_3OH, 1:1); UV (MeOH) λ_{max} (log ϵ) 239 (4.53), 305 (4.21), 338 (4.19) nm; IR (KBr) ν_{max} 3359, 2956, 2922, 1622, 1452, 1363 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 314 $[M]^+$ (27), 296 (23), 239 (43), 228 (64), 226 (100), 127 (10), 114 (9), 91 (5); HREIMS *m/z* 314.1879 (calcd for $C_{20}H_{26}O_3$, 314.1882).

X-ray Crystallographic Analysis of 1. Compound **1** (15 mg) was dissolved in 2 mL of $CHCl_3$−MeOH (1:1). The solution was left for a week to give suitable crystals (13 mg). Crystal data: $C_{20}H_{26}O_3$; M_r = 314.42; dimensions 0.20 × 0.20 × 0.40 mm; orthorhombic, space group $P2_12_12_1$, a = 9.095(1) Å, b = 10.533(1) Å, c = 17.957(1) Å, $\alpha = \beta = \gamma = 90°$, V = 1720.24(13) Å^3, Z = 4, D_{calc} = 1.328 g/cm^3, λ = 0.71073Å, μ(Mo Kα) = 0.080 mm^{-1}, $F(000)$ = 680, T = 295(2) K. Of the 2131 reflections collected, all were unique. The structure was solved by direct methods with SHELXS 97 and refined by full matrix least-squares on F^2. Final refinement: data/restraints/parameters = 2131/0/208; R_1 = 0.0741 (all data), wR_2 = 0.1079 (all data), and GOF = 1.231. The H coordinates were determined by the difference Fourier method and by the calculated

geometry. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.314 and −0.260 $e^-/Å^3$, respectively. These data have been deposited (CCDC 265458) at the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK, fax: +44 1223 336033, and can be obtained free of charge at www.ccdc.cam.ac.uk.

6-Hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one (2): colorless needles, mp 114−116 °C; $[\alpha]^{20}_D$ −13° (*c* 0 0.004, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.28), 225 (4.28), 288 (4.06), 323 (3.36) nm; IR (KBr) ν_{max} 3400, 2920, 1760, 1630 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; FABMS *m/z* 199 $[M + 1]^+$; HRFABMS *m/z* 199.0989 $[M + 1]^+$ (calcd for $C_{10}H_{15}O_4$, 199.0970).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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