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GRAYANANE DITERPENOIDS FROM THE BARKS OF *CRAIBIODENDRON HENRYI*

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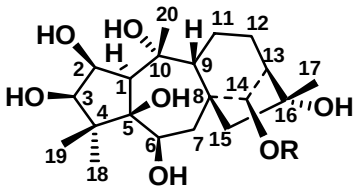
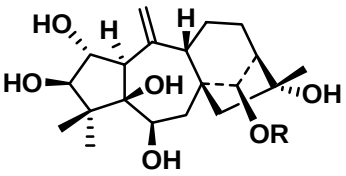
Abstract – Three new grayanane diterpenoids, 14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein XVIII (**1**), 14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein I (**2**) and 14*β*-*O*-(2*S*,3*R*-nilyl) rhodojaponin III (**3**) have been isolated from the barks of *Craibiodendron henryi*. Their structures were determined on the basis of chemical and spectral methods. Vasodilator effects of these compounds were assessed.

INTRODUCTION

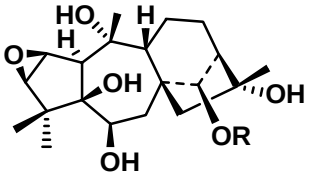
Grayanane diterpenoids possess a 5/7/6/5 (trans or *cis*/*cis*/*cis*) ring system, formed probably by rearrangement of the kaurane skeleton.[1] They have been found mainly in Ericaceae plants, some of which revealed potent vasodilator activities and significant antifeedant and insecticidal activities.[2,3] In order to search for bioactive natural products, we have initiated chemical studies on grayanane diterpenoids from *Craibiodendron henryi*, a well-known toxic Ericaceae plant. The *n*-BuOH soluble fraction of an ethanolic extract from the barks of the plant was found to show antioxidant activity in a microsomal lipid peroxidation induced by ferrous-cysteine model and moderate vasodilator activity in a phenylepherine-induced vasoconstriction assay using rat aortic rings, which encouraged us to undertake a chemical investigation of the plant. In a previous paper,[4] two grayanane diterpenoids, 14*β*-*O*-(2*S*,3*S* -nilyl)rhodojaponin VI and 14*β*-*O*-(2*S*,3*S*-nilyl)rhodomollein I have been reported from the roots of the plant. Our continuing studies on the barks of the plant has led to the isolation of three new grayanane diterpenoids: 14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein XVIII (**1**), 14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein I (**2**) and 14*β*-*O*-(2*S*,3*R*-nilyl)rhodojaponin III (**3**). In this paper we present the isolation and structural elucidation of the new compounds.



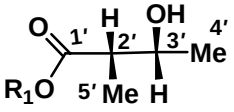
1 R = nilyl
1a R = H



2 R = nilyl
2a R = H



3 R = nilyl
3a R = H

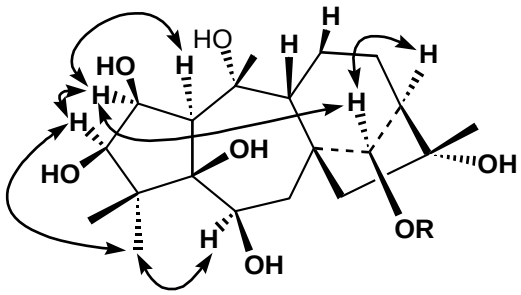


4 R_1 = H
5 R_1 = Me

RESULTS AND DISCUSSION

Compound (**1**) was isolated as an amorphous powder. The molecular formula was determined to be $C_{25}H_{42}O_9$ by HRFABMS. Its IR spectrum showed the presence of hydroxy groups (3389 cm^{-1}) and an ester carbonyl group (1722 cm^{-1}). The ^1H NMR spectrum (Table 1) contained signals for four methyl singlets (δ 1.46, 1.62, 1.84, 2.06), two methyl doublets (δ 1.18, 1.31), and five oxygenated methines (δ 4.14, 4.20, 4.45, 5.10, 6.63). The ^{13}C NMR (DEPT) spectrum revealed 25 carbon signals, including six methyls, four methylenes, nine methines (five oxygenated), and six quaternary carbons (one ester carbonyl and three oxygenated). The ^1H-^1H COSY spectrum indicated the existence of the following fragments: C_1-C_2-C_3, C_6-C_7, and C_9-C_{11}-C_{12}-C_{13}. These structural features suggested that **1** was a grayanane-type diterpenoid with seven sites of oxygenation. In addition to the signals for a grayanane-type diterpenoid skeleton, there were the resonances due to an extra nilyl group. The assignment of the nilyl group was determined on the basis of the fragment of successive connectivities, $C_{5'}$-$C_{2'}$-$C_{3'}$-$C_{4'}$, in the ^1H-^1H COSY spectrum. This assumption was confirmed by protons of C-5′ correlated with C-1′, C-2′ and C-3′, but protons of C-4′ only correlated with C-2′ and C-3′ in the HMBC spectrum of **1**. Alkaline hydrolysis of **1** yielded **1a**.[4] The ^1H and ^{13}C NMR, and optical rotation data of **1a** were in agreement with those of rhodomollein XVIII.[5] In addition, the presence of correlations between H-1/H-2, H-2/H-3, H-1/H-14, H-13/H-14, H-6/H-18, and H-3/H-18 in the NOESY spectra (Figure 1) revealed that the relative configuration of **1** was in accordance with those of rhodomollein XVIII. Thus, the structure of **1a** was established as 2*β*,3*β*,5*β*,6*β*,14*β*,16*α*-hexahydroxygrayanane. The downfield shifts of H-14 (δ 5.12 in rhodomollein XVIII (**1a**) to δ 6.63 in **1**) and C-14 (δ 79.7 in rhodomollein XVIII (**1a**) to δ 81.4 in **1**) indicated that nilyl group of **1** was located at C-14. This deduction was confirmed by the HMBC correlations among H-14/C-15, H-14/C-16 and the ester carbonyl carbon. The absolute stereochemistry of the nilate moiety was assigned by comparison of the specific rotations of methyl nilate (**5**) with reported values for the stereoisomers of this compound in the literature.[6-9] The specific rotation values ($[\alpha]_D^{23}$ +13.8 (*c* 5.00, $CHCl_3$)) of methyl nilate obtained by methylation of 3-hydroxy-2-methyl

butyric acid (**4**) derived from alkaline hydrolysis of **1** were as follows: (λ (nm)) 589, +13.8; 578, +14.2; 546, +15.9; 436, +23.8. These results indicated the nilate moiety present in **1** had the (2*S*,3*R*) configuration. Therefore the structure of **1** was determined as 14*β*-*O*-(2*S*,3*R*-nilyl)-2*β*,3*β*,5*β*,6*β*,16*α*-pentahydroxygrayanane, named 14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein XVIII.



1

Figure 1. Selected NOESY correlations of **1**

Compound (**2**), amorphous powder, was assigned a molecular formula of $C_{25}H_{40}O_8$, as deduced from its positive HRFABMS. The IR spectrum showed characteristic absorptions for hydroxy (3432 cm^{-1}), ester carbonyl (1710 cm^{-1}), and double-bond (1639 cm^{-1}) groups. The ^1H NMR spectrum contained three methyl singlets (δ 1.50, 1.54, 1.64), two methyl doublets (δ 1.24, 1.28), five oxygenated methines (δ 4.17, 4.21, 4.35, 4.96, 5.79), and two olefinic protons (δ 5.32, 5.48). The ^{13}C NMR (DEPT) spectrum revealed 25 carbon signals, including five methyls, five methylenes (one olefinic), nine methines (five oxygenated), and six quaternary carbons (one ester carbonyl, one olefinic and two oxygenated). The similarity of ^1H and ^{13}C NMR spectral data of **2** (Table 1) to those of 14*β*-*O*-(2*S*,3*S*-nilyl)rhodomollein I [4] indicated that they had the same skeleton structure except for the NMR data assignable to the nilyl unit (δ 2.70 (1H, *m*, H-2′), 4.17 (1H, *m*, H-3′), 1.28 (3H, *d*, *J* = 7.5 Hz, H-4′), 1.24 (3H, *d*, *J* = 6.5 Hz, H-5′) in the ^1H NMR spectrum; δ 175.2 (C-1′), 49.2 (C-2′), 70.1 (C-3′), 21.8 (C-4′), 14.3 (C-5′) in the ^{13}C NMR spectrum), which also implied that **2** was an optical isomer of 14*β*-*O*-(2*S*,3*S*-nilyl)rhodomollein I. The linkage position of the nilyl group was determined to be at the C-14 position on the basis of the HMBC correlations between the proton (δ 5.79) of C-14 and the ester carbonyl function (δ 175.2). Additionally, **2** was hydrolyzed with alkaline to afford **2a** and 3-hydroxy-2-methylbutyric acid (**4**). The ^1H and ^{13}C NMR data of **2a** were in accordance with those of rhodomollein I,[10] which was established as 2*α*,3*β*,5*β*,6*β*,14*β*,16*α*-hexahydroxygrayan-10(20)-ene. The absolute configuration of the nilate moiety in **2** was assumed to be the same as those of **1**. Thus, the structure of **2** was determined to be 14*β*-*O*-(2*S*,3*R*-nilyl)-2*β*,3*β*,5*β*,6*β*,16*α*-pentahydroxygrayan-10(20)-ene, named 14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein I.

Compound (**3)** was isolated as a colorless crystal. The molecular formula was determined to be $C_{25}H_{40}O_8$ by HRFABMS. Its IR spectrum showed the presence of hydroxy groups (3438 cm^{-1}) and an ester carbonyl group (1711 cm^{-1}). The ^1H NMR spectrum contained signals for four methyl singlets (δ 1.32,

1.40, 1.52, 1.83), two methyl doublets (δ 1.20, 1.28), and five oxygenated methines (δ 3.19, 4.05, 4.12, 4.16, 6.04). The ^{13}C NMR (DEPT) spectrum revealed 25 carbon signals, including six methyls, four methylenes, nine methines (five oxygenated at 61.2, 64.5, 70.1, 74.4, 82.1), and six quaternary carbons (one ester carbonyl at 175.7, and three oxygenated at 77.6, 78.8, 80.1). The ^{1}H-^{1}H COSY spectrum indicated the existence of the following fragments: C_1-C_2-C_3, C_6-C_7, and C_9-C_{11}-C_{12}-C_{13}. These structural features suggested that **3** was a grayanane-type diterpenoid with an extra nilyl group. The ^{1}H and ^{13}C NMR spectral data for **3** (Table 1) were found to be identical with those of craiobiotoxin III [1] except for

Table 1. ^{1}H and ^{13}C NMR spectral data of **1**, **2**, and **3**.[a]

position	**1**		**2**		**3**	
	proton	carbon	proton	carbon	proton	carbon
1	3.08 *d* (9.5)	54.4	3.22 *d* (9.0)	51.8	2.92 *s*	54.5
2	5.10 *dd* (10.0, 3.5)	78.2	4.96 *dd* (9.0, 4.0)	82.2	4.16 *d* (3.0)	61.2
3	4.14 *d* (4.5)	84.8	4.21 *d* (4.0)	88.4	3.19 *d* (3.0)	64.5
4		48.7		48.0		49.3
5		82.8		82.7		80.1
6	4.45 *dd* (10.0, 4.5)	74.9	4.35 *dd* (8.0, 4.0)	70.5	4.05 *d* (11.5)	74.4
7*α*	2.74 *dd* (13.5, 4.5)	39.6	2.56 *dd* (13.5, 4.0)	40.5	2.47 *dd* (13.0, 10.0)	44.4
7*β*	2.66 *dd* (13.5, 9.5)		2.67 *dd* (13.5, 8.0)		2.55 *d* (13.0)	
8		52.0		48.4		50.9
9	2.20 *d* (7.5)	57.3	2.12 *d* (7.0)	52.2	1.97 *d* (6.5)	55.9
10		78.3		149.7		77.6
11*α*	2.16 *m*	21.6	1.98 *m*	23.7	1.92 *m*	22.6
11*β*	1.64 *m*		1.65 *m*		1.59 *m*	
12*α*	2.69 *m*	26.8	2.04 *m*	24.2	2.63 *m*	27.5
12*β*	1.80 *m*		1.89 *m*		1.56 *m*	
13	2.61 *m*	57.3	2.98 *br. s*	55.7	1.96 *br. s*	55.6
14	6.63 *s*	81.4	5.79 *s*	82.8	6.04 *s*	82.1
15*α*	2.50 *d* (14.5)	57.6	2.48 *d* (13.5)	62.8	2.24 *d* (14.5)	60.5
15*β*	2.25 *d* (14.5)		2.14 *d* (13.5)		2.08 *d* (14.5)	
16		81.2		79.1		78.8
17	1.46 *s*	24.2	1.50 *s*	27.4	1.40 *s*	24.3
18	1.84 *s*	26.0	1.54 *s*	26.7	1.32 *s*	22.0
19	1.62 *s*	20.7	1.64 *s*	20.1	1.52 *s*	20.9
20	2.06 *s*	30.0	5.32, 5.48, each *s*	113.4	1.83 *s*	31.0
nilate						
1′		175.1		175.2		175.7
2′	2.68 m	48.7	2.70 *m*	49.2	2.67 *m*	48.2
3′	4.20 m	69.6	4.17 *m*	70.1	4.12 *m*	70.1
4′	1.31 d (7.0)	22.3	1.28 *d* (7.5)	21.8	1.28 *d* (7.0)	21.6
5′	1.18 d (6.0)	14.1	1.24 *d* (6.5)	14.3	1.20 *d* (6.0)	14.5

[a] ^{1}H NMR spectral data measured at 500 MHz; ^{13}C NMR spectral data measured at 125 MHz; Proton coupling constants (*J*) in Hz given in parentheses. pyridine-d_5 as solvent.

The chemical shifts of the nilyl group, indicating that **3** was a diastereoisomer of craiobiotoxin III. Alkaline hydrolysis of **3** yielded **3a** and 3-hydroxy-2-methylbutyric acid (**4**). The ^{1}H and ^{13}C NMR, and optical rotation data of **3a** were in agreement with those of rhodojaponin III.[11] Thus, the structure of **3a** was established as 2,3-epoxy-5*β*,6*β*,10*α*,14*β*,16*α*-pentahydroxygrayanane. The absolute configuration of the nilate moiety in craiobiotoxin III was confirmed as the (2*S*,3*S*) or (2*R*,3*R*) configuration by X-ray diffraction analysis,[1] while that of the nilate moiety in **3** was assumed to be the (2*S*,3*R*) configuration, the same as those of **1**. Therefore the structure of **3** was determined as 2,3-epoxy-5*β*,6*β*,10*α*,16*α*-tetrahydroxy-14*β*-*O*-(2*S*,3*R*-nilyl)grayanane, named 14*β*-*O*-(2*S*,3*R*-nilyl)rhodojaponin III.

EXPERIMENTAL SECTIONG

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR Spectrophotometer. 1D- and 2D-NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on an Inova 500 FT-NMR spectrometer using TMS as an internal referece. ESI-MS were measured on Agilent 1100 Series LC/MSD Trap mass spectrometer. HR-FAB-MS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (200-300 mesh), RP-18 (40-70 μm) and Sephadex LH-20. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. The barks of *Craibiodendron henryi* were collected from Mengzi city of Yunnan province in China, in September of 2006. A voucher specimen of the plant (No. 211) was identified by associate Prof. Lin Ma and deposited at the herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation. The barks of *Craibiodendron henryi* (6.5 kg) were air-dried, ground and extracted with EtOH under reflux. The ethanolic extract was evaporated to almost dryness *in vacuo* and the resulting mixture was partitioned successively between H_2O and EtOAc, H_2O and *n*-BuOH. The *n*-BuOH phase was concentrated to give a black mass (106 g), which was subjected to a silica gel column eluting with a gradient increasing MeOH in $CHCl_3$ to yield Frs. A_1-A_{11}. Fr. A_5 (2.6 g) was applied to silica gel column eluting with $CHCl_3$-MeOH (20:1~9:1) to give Frs. B_1-B_4. Fr. B_2 (760 mg) was chromatographed on Sephadex LH-20 column with $CHCl_3$-MeOH (2:1) and RP-18 column with MeOH-H_2O (48:52) to provide **1** (31 mg) and **2** (24 mg). From Fr. A_4 (2.1 g), repeated silica gel and Sephadex LH-20 column chromatography led to the isolations of **3** (117 mg).

14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein XVIII (1): Amorphous powder; mp 121- 123 °C; $[\alpha]_D^{23}$ -24.3 (*c* 0.31,

EtOH); UV (MeOH) λ_{max} (logε) 275 (0.83) nm; IR (KBr) ν_{max} 3389, 2962, 1722, 1455, 1377, 1266, 1041, 797 cm^{-1}; ^1H and ^{13}C NMR spectra, Table 1; ESIMS (positive-ion mode) *m/z* 509 $[M + Na]^+$; HRFABMS *m/z* 509.2755 $[M + Na]^+$ (calcd for $C_{25}H_{42}O_9Na$, 509.2727).

14*β*-*O*-(2*S*,3*R*-nilyl)rhodomollein I (2): Amorphous powder; mp 153-155 °C; $[\alpha]_D^{23}$ -31.1 (*c* 0.29, EtOH); UV (MeOH) λ_{max} (logε) 214 (1.51) nm; IR (KBr) ν_{max} 3432, 2968, 1710, 1639, 1451, 1380, 1284, 1037, 887 cm^{-1}; ^1H and ^{13}C NMR spectra, Table 1; ESIMS (positive-ion mode) *m/z* 491 $[M + Na]^+$; HRFABMS *m/z* 491.2689 $[M + Na]^+$ (calcd for $C_{25}H_{40}O_8Na$, 491.2621).

14*β*-*O*-(2*S*,3*R*-nilyl)rhodojaponin III (3): Colorless needles; mp 135-136 °C; $[\alpha]_D^{23}$ -20.6 (*c* 0.31, EtOH); UV (MeOH) λ_{max} (logε) 213 (1.47) nm; IR (KBr) ν_{max}: 3438, 2943, 1711, 1452, 1288, 1203, 1082 cm^{-1}; ^1H and ^{13}C NMR spectra, Table 1; ESIMS (positive-ion mode) *m/z* 491 $[M + Na]^+$; HRFABMS *m/z* 491.2647 $[M + Na]^+$ (calcd for $C_{25}H_{40}O_8Na$, 491.2621).

Methylation of 4. According to reference,[4] alkaline hydrolysis of compound (**1**), (**2**) and (**3**) yielded **1a**, **2a**, **3a** and 3-hydroxy-2-methylbutyric acid (**4**), respectively. **4** (8 mg) was methylated to give **5** (6 mg) which was identified as methyl (2*S*,3*R*)-nilate.

Methyl (2*S*,3*R*)-Nilate (5): colorless oil; $[\alpha]_D^{23}$ +13.8 (*c* 5.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 4.03 (1H, *m*, H-3′), 3.70 (3H, *s*, OCH_3), 2.45 (1H, *m*, H-2′), 1.17 (3H, *d*, *J* = 7.0 Hz, H-4′), 1.13 (3H, *d*, *J* = 6.0 Hz, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.3 (C, C-1′), 68.1 (CH, C-3′), 51.7 (CH_3, OCH_3), 45.7 (CH, C-2′), 19.9 (CH_3, C-4′), 11.7 (CH_3, C-5′); ESIMS *m/z* 155 $[M + Na]^+$.

Preliminary Vasodilator Assays. Vasodilator effects of three grayanane diterpenoids (1.0×10^{-5} M) on phenylepherine-induced vasoconstriction of rat aortic rings in the presences of indomethacin (Indo) and N^{ω}-L-nitroarginine (L-NA) [12] were (27 ± 1.4), (33 ± 1.6), and (41 ± 2.0) %, respectively, compared to (103 ± 4.6)% for sodium nitroprusside as a positive control. All those compounds exhibited weak vasodilator activities.

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