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Micranthanone A, a New Diterpene with an Unprecedented Carbon Skeleton from *Rhododendron micranthum*

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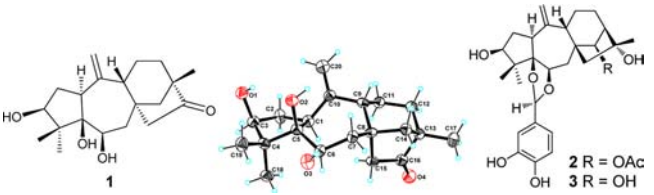
ABSTRACT



A new diterpene with an unprecedented carbon skeleton, micranthanone A (1), two new grayanane diterpenoids bearing an unusual 5,6-(3,4-dihydroxylbenzylidene acetal) motif, rhodomicranols A (2) and B (3), and three known grayanane diterpenoids (4−6) were isolated from *Rhododendron micranthum*. Their structures were elucidated by spectroscopic analyses, calculated ECD, and single-crystal X-ray diffraction. The in vitro immunomodulatory activities of 1−6 were evaluated, and a plausible biogenetic pathway for 1 is proposed.

Rhododendron micranthum Turcz. (Ericaceae) is a semi-evergreen shrub widely distributed in Northeast China, North China, Shandong, Henan, Hubei, Hunan, and Sichuan provinces of China, as well as North Korea.[1] Its branches and leaves have been used as a folk medicine in China to treat chronic tracheitis, puerperal arthrodynia, menoxenia, dysmenorrhea, fracture, and hypertension.[1] "Zhaoshanbai Jingao Pian", a Chinese patent medicine, is a tablet made from the extract of branches and leaves of *R. micranthum*. However, studies on the chemical constituents of *R. micranthum* are limited. Heretofore, only seven flavonoids were isolated.[2] In our search for new bioactive compounds from Chinese medicinal plants, a new diterpene with an unprecedented carbon skeleton, micranthanone A (**1**), two new grayanane diterpenoids bearing an unusual 5,6-(3,4-dihydroxylbenzylidene acetal) motif, rhodomicranols A (**2**) and B (**3**), and three known grayanane diterpenoids (**4**−**6**) were isolated from the leaves of *R. micranthum* (Figure 1). Herein, we report the isolation, structure elucidation, and in vitro immunomodulatory activities of **1**−**6** as well as the plausible biosynthetic pathway for **1**.

The $CHCl_3$ partition fraction of the 95% EtOH extract of *R. micranthum* was subjected to repeated column chromatography on silica gel, reversed-phase (RP) C_{18} silica gel, and Sephadex LH-20, followed by RP HPLC to afford compounds **1**−**6**. Compounds **4**−**6** were identified to be grayanotoxins IV, II, and XVIII,[3] respectively, based on spectroscopic analyses and comparison with literature data.

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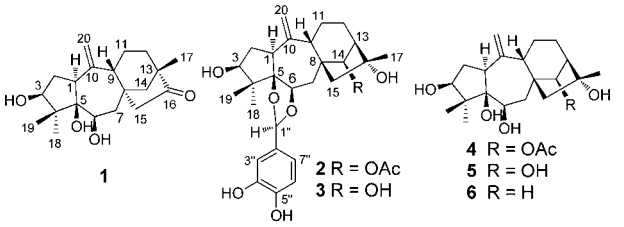

Figure 1. Structure of compounds **1**–**6**.

Micranthanone A (**1**) was obtained as colorless block crystals. The molecular formula of **1** is $C_{20}H_{30}O_4$ based on a quasimolecular ion peak at m/z 357.2032 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2036) in HRESIMS, requiring six degrees of unsaturation. The IR spectrum displayed the characteristic absorptions attributable to a carbonyl group (1735 cm^{-1}) and a hydroxyl group (3400 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** displayed resonances for an exocyclic double bond at δ_H 5.28 (s, H-20) and 5.20 (s, H-20), two oxygenated methines at δ_H 3.71 (dd, J = 11.6, 3.6 Hz, H-6) and 3.59 (dd, J = 6.5, 0.9 Hz, H-3), and three quaternary methyls at δ_H 1.26 (s, H_3-19), 1.01 (s, H_3-17), and 1.00 (s, H_3-18). The ^{13}C NMR and DEPT spectra (Table 1) revealed 20 carbon signals assignable to a ketone carbonyl, an exocyclic double bond, four quaternary carbons (an oxygenated), four methines (two oxygenated), six methylenes, and three methyls. The ketone carbonyl and double bond account for two degrees of unsaturation, and the remaining four degrees of unsaturation required the presence of four rings in **1**. The above data for **1** are consistent with a tetracyclic diterpene.

The planar structure of **1** was deduced from the detailed analyses of HSQC, $^1H-^1H$ COSY, and HMBC spectra. The $^1H-^1H$ COSY and HSQC spectra revealed the presence of three partial structures (a) C-1–C-3, (b) C-6/C-7, and (c) C-9–C-12, as shown in Figure 2. In the HMBC spectrum, the correlations from H_3-18 and H_3-19 to C-3, C-4, and C-5 and correlations from H-3 to C-4 and C-5 suggested that C-3, C-5, C-18, and C-19 are each connected to the quaternary carbon C-4. HMBC correlations from H-1 to C-5 and C-6, from H-6 to C-1 and C-5, and from H_2-7 to C-5 established the connection of C-1 and C-6 through C-5. The connection of C-1 to C-9 through C-10 was deduced based on HMBC correlations from H_2-20 to C-1, C-9, and C-10, from H-1 to C-9 and C-10, and from H-9 to C-1 and C-10. The connection of C-7, C-9, C-14, and C-15 to quaternary carbon C-8 was supported by HMBC correlations from H_2-7 to C-8/C-9/C-14/C-15, from H-9 to C-7/C-8/C-14/C-15, from H_2-14 to C-7/C-8/C-9/C-15, and from H_2-15 to C-7/C-8/C-9/C-14. The HMBC correlations of singlet H_3-17 to C-12/C-13/C-14/C-16 revealed that C-12, C-14, C-16, and C-17 are each connected to quaternary carbon C-13. Finally, the connection of C-15 to C-16 was established on the basis of the HMBC correlations from H_2-15 to C-16 and C-13.

Table 1. ^1H (400 MHz) and ^{13}C NMR (100 MHz) Data for **1** in $CDCl_3$

no.	δ_H (J in Hz)	δ_C	no.	δ_H (J in Hz)	δ_C
1	3.01 dd (11.0, 7.1)	43.3	12α	1.51 m	36.4
2α	2.34 ddd (15.5, 11.0, 6.5)	37.2	12β	1.45 m	
2β	1.81 ddd (15.5, 7.1, 0.9)		13		49.5
3	3.59 dd (6.5, 0.9)	82.6	14a	1.65 d (16.8)	55.1
4		51.0	14b	1.66 d (16.8)	
5		83.2	15α	2.50 d (18.3)	47.6
6	3.71 dd (11.6, 3.6)	71.8	15β	2.24 d (18.3)	
7α	1.51 dd (14.2, 3.6)	44.6	16		219.6
7β	1.74 dd (14.2, 11.6)		17	1.01 s	19.8
8		38.7	18	1.00 s	23.6
9	2.48 m	53.6	19	1.26 s	19.5
10		151.1	20	5.28 s; 5.20 s	116.6
11α	1.44 m	30.9			
11β	1.61 m				

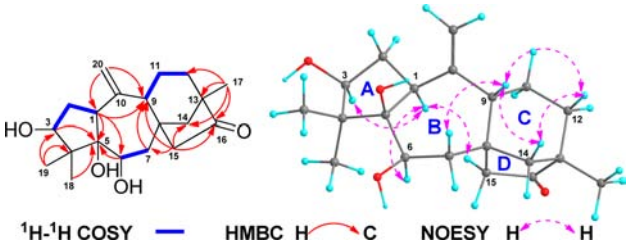

Figure 2. $^1H-^1H$ COSY, key HMBC, and NOESY correlations of **1**.

The relative configuration of **1** was assigned based on NOESY correlations (Figure 2). H-1 was randomly assigned as α-oriented. The NOESY cross peaks between H-1α and H_3-18 and between H_3-18 and H-3 require the α-orientations of H-3 and H_3-18. The NOESY correlation between H-1α and H-6 suggested that H-6 is also α-oriented, and the junction of rings A and B in **1** is *trans*. Accordingly, 5-OH must be in the β-orientation. The NOESY correlation between H-7 and H-9 and lack of the NOESY correlation between H-1α and H-9 are evidence that H-9 is β-oriented. The junction of rings C and D was established as *cis* on the basis of NOESY correlations from H-1α to H-15 and from H-9β to H-12 and H-14. The NOESY correlations from H_3-17 to H-12β and H-14β require β-orientation of H_3-17.

Finally, the planar structure and the relative configuration of **1** was further confirmed by single-crystal X-ray diffraction (Figure 3).

In order to determine the absolute configuration of **1**, the ECD spectra for (1*S*,3*S*,5*R*,6*R*,8*S*,9*R*,13*S*)-**1** and its enantiomer were calculated by the time-dependent density functional theory (TD-DFT) calculations (see the Supporting Information). The measured CD spectrum of **1** fits well with the calculated ECD of the 1*S*,3*S*,5*R*,6*R*,8*S*,9*R*,13*S*-**1** and is opposite to that of its enantiomer (Figure 4).

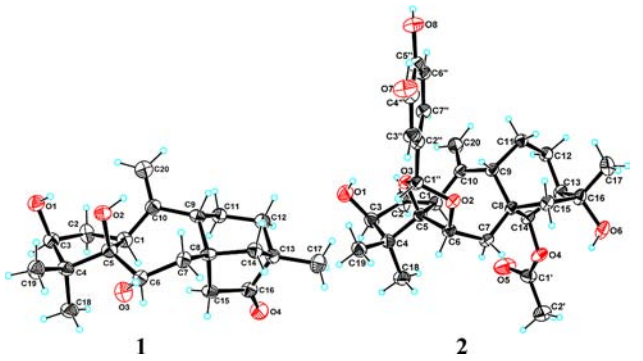

Figure 3. X-ray crystal structures of **1** and **2**.

Therefore, the absolute configuration of **1** was established as 1*S*,3*S*,5*R*,6*R*,8*S*,9*R*,13*S*.

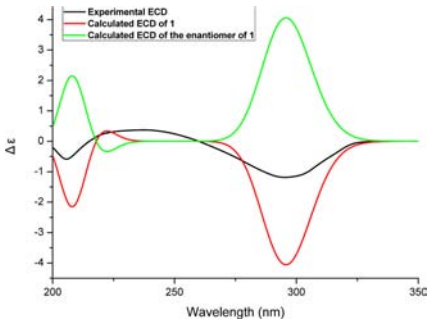

Figure 4. Experimental ECD spectrum of **1** and the calculated ECD spectra for (1*S*,3*S*,5*R*,6*R*,8*S*,9*R*,13*S*)-**1** and its enantiomer.

Rhodomicranol A (**2**) was obtained as colorless block crystals, and its molecular formula, $C_{29}H_{38}O_8$, was determined by its HRESIMS peak at *m*/*z* 537.2428 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_8Na$, 537.2459). The ^1H and ^{13}C NMR spectra (Table 2) of **2** resembled those of grayanotoxin IV (**4**), with major differences that included additional signals for a 3,4-dihydroxylbenzylidene in **2**. The differences were accompanied by a downfield shift of C-5 (δ_C 94.1) and C-6 (δ_C 76.9) and an upfield shift of C-9 (δ_C 45.2) in **2** compared with **4**. Furthermore, HMBC correlations from H-1″ to C-5 and C-6 were observed, and together these suggested that **2** is the 5,6-(3,4-dihydroxylbenzylidene acetal) derivative of **4**. The structure of **2** was confirmed by extensive 2D NMR analyses and single-crystal X-ray diffraction with Cu Kα radiation (Figure 3), and its absolute configuration was determined as 1*S*,3*S*,5*R*,6*R*,8*S*,9*S*,13*R*,14*R*,16*R*,1″*R* by the Flack parameter −0.04 (18)[4] for the given coordinate.

The molecular formula of rhodomicranol B (**3**) was deduced as $C_{27}H_{36}O_7$ by a HRESIMS peak at *m*/*z* 495.2339 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_7Na$, 495.2353). The ^1H and ^{13}C NMR data (Table 2) of **3** were very similar to those of **2**, except for the absence of an acetyl group in **3**, suggesting that **3** is the 14-deacetyl derivative of **2**. This was confirmed by detailed 2D NMR analyses of **3**, and its absolute configuration was assigned the same as that of **2** based on the similarity of their CD spectra.

Table 2. ^1H (400 MHz) and ^{13}C NMR (100 MHz) Data for **2** and **3** in CD_3OD

	2		**3**	
no.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	2.93 dd (11.4, 8.8)	46.4	2.91 dd (11.4, 8.8)	46.6
2α	2.79 ddd (13.4, 8.8, 7.6)	40.1	2.77 ddd (13.4, 8.8, 7.7)	40.0
2*β*	1.77 overlap		1.77 ddd (13.4, 11.4, 4.5)	
3	3.73 dd (7.6, 4.5)	79.8	3.73 dd (7.7, 4.5)	79.9
4		50.9		51.0
5		94.1		94.1
6	4.41 d (6.0)	76.9	4.45 d (6.0)	77.4
7α	1.85 d (15.9)	37.6	2.14 d (16.2)	38.4
7*β*	2.34 dd (15.9, 6.0)		2.33 dd (16.2, 6.0)	
8		50.4		51.6
9	3.49 d (6.4)	45.2	3.42 d (6.5)	44.7
10		152.0		152.4
11α	1.70 m	22.0	1.67 m	22.0
11*β*	1.43 m		1.39 m	
12α	1.78 m	25.7	1.69 m	25.3
12*β*	1.57 m		1.54 m	
13	2.04 s	54.1	1.93 s	55.4
14	4.85 s	87.3	3.61 s	84.3
15α	1.98 d (15.2)	60.0	1.91 d (15.1)	59.7
15*β*	2.21 d (15.2)		2.17 d (15.1)	
16		82.1		83.6
17	1.28 s	24.0	1.26 s	23.2
18	0.87 s	25.7	0.92 s	25.7
19	1.12 s	18.5	1.13 s	18.5
20	5.05 s; 5.03 s	112.3	4.973 s; 4.969 s	111.6
1″	5.75 s	103.9	5.74 s	104.0
2″		130.2		130.3
3″	6.88 d (1.2)	114.8	6.88 d (1.8)	114.9
4″		146.2		146.2
5″		147.1		147.1
6″	6.74 d (8.2)	116.0	6.73 d (8.2)	116.0
7″	6.78 dd (8.2, 1.2)	119.3	6.77 dd (8.2, 1.8)	119.4
$COCH_3$		173.2		
$COCH_3$	2.05 s	21.5		

To date, diterpenoids from the plants of family Ericaceae belong to one of nine carbon skeletons, grayanane,[5] 1,5-secograyanane,[6] 3,4-secograyanane,[7] 9,10-secograyanane,[8] leucothane,[9] kalmane,[10] *ent*-kaurane,[11] 4,5-seco-*ent*-kaurane,[12] and podocarpane,[13] and their biogenetic relationships are summarized in Scheme 1. Among them, grayanane diterpenoids are specific in Ericaceae.[11]

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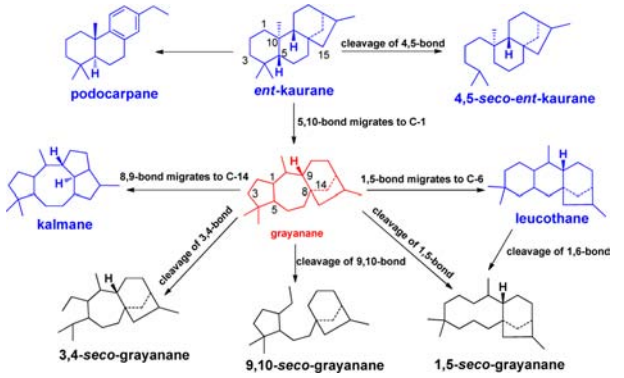
Micranthanone A (**1**) possesses a modified grayanane skeleton in which CH_3-17 connects to the quaternary carbon C-13, instead of C-16 in the grayanane skeleton. Interestingly, the conjunction of rings B and C in **1** is *trans*; however, it is *cis* in the grayanane diterpenoids.

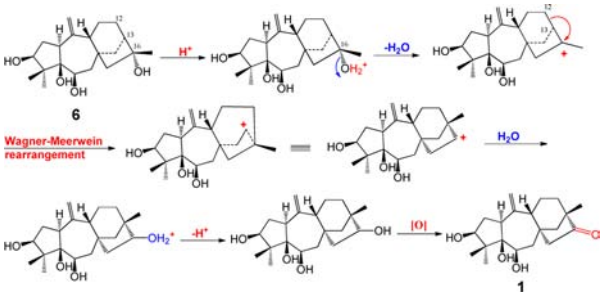
Scheme 1. Diterpenoid Skeletons from Ericaceae and Their Proposed Biogenetic Relationships



The grayanane diterpenoid **6** is proposed as the biosynthetic precursor of **1**, and a plausible biogenetic pathway of **1** is proposed in Scheme 2. We suggest the protonation of 16-OH in **6** and then the loss of H_2O to form a carbocation center at C-16. Subsequently, an enzymatic Wagner–Meerwein rearrangement[14] could lead to the migration of the 12,13-bond to C-16. The carbocation center created at C-13 would then be neutralized by H_2O with subsequent loss of a proton. Finally, the hydroxyl intermediate is oxidized to afford **1**.

In terms of reaction mechanism, the biogenetic relationship between **1** and the grayanane derivative **6** is similar to that between *ent*-beyerene and *ent*-kaurane.[15] It had been proven that *ent*-kaurane is the biosynthetic precursor of grayanane;[16] thus, grayanane must be the biosynthetic precursor of **1**.

Scheme 2. Plausible Biogenetic Pathway for **1**



Micranthanone A (**1**) represents a new tetracyclic diterpene carbon skeleton, and the name "micranthane" is suggested for this new skeleton type. Notable but of less significance, rhodomicranols A (**2**) and B (**3**) are the first examples of grayanane diterpenoids bearing a 5,6-(3,4-dihydroxylbenzylidene acetal) motif.

Compounds **1**–**6** were evaluated for their in vitro immunomodulatory activities against murine lymphocytes (see Supporting Information), however, none of them showed significant activities. Interestingly, **6** significantly enhanced the proliferation of murine lymphocytes, and **1**–**6** are nontoxic to murine lymphocytes.

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Supporting Information Available. Detailed experimental procedures, 1D and 2D NMR, HRESIMS, UV, IR, CD spectra of compounds **1**–**3**, and X-ray crystal data of **1** and **2** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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