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Two Enantiomeric Pairs of Meroterpenoids from *Rhododendron capitatum*

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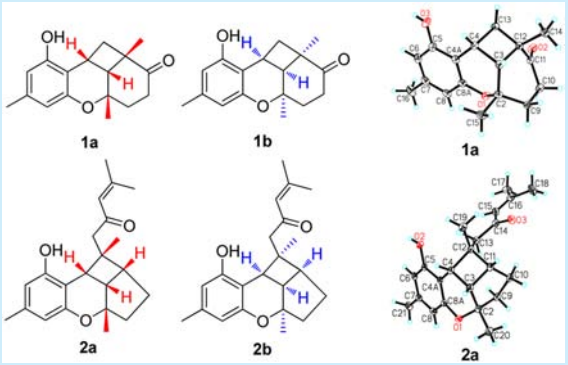
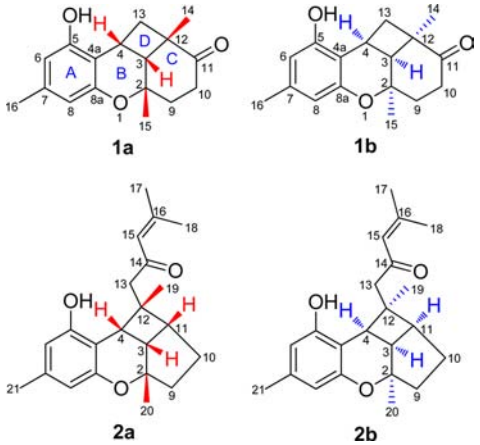
Supporting Information

ABSTRACT: Two enantiomeric pairs of meroterpenoids, (−)- and (+)-rhodonoids A (**1a** and **1b**) and B (**2a** and **2b**), were isolated unprecedentedly from partially racemic mixtures that naturally occurred in *Rhododendron capitatum*. Their structures were fully determined by spectroscopic data, X-ray crystallography, and electronic circular dichroism analysis. Compounds **1a** and **1b** are the first examples of meromonoterpenes featuring a unique 6/6/6/4 ring system. Compounds **2a** and **2b** showed PTP1B inhibitory activity.



The genus *Rhododendron* (Ericaceae) comprises about 1000 species mainly distributed in East and Southeast Asia, and China is considered to be the *Rhododendron* distribution center in the world with 571 species.[1] Many *Rhododendron* plants have been used as folk medicine for the treatment of bronchitis, cough, rheumatism, pain, diabetes, and skin ailments.[2] A variety of compounds with significant bioactivities, such as iridoids,[3] diterpenoids,[4] triterpenoids,[5] and chromane derivatives,[6] were discovered from this genus.



Rhododendron capitatum Maxim. is a small deciduous shrub with rich resources in the Qinghai province of China and has been used in Tibetan medicine against gastric cold, abdominal pain, pharyngalgia, cough, and inflammation.[7] Grayanane diterpenoids, flavonoids, and coumarins have been isolated from this plant previously.[8] In our continuing search for natural products with diverse structures and antimetabolic disease activities from Chinese medicinal plants,[4a,9] investigations on the chemical constituents of *R. capitatum* were carried out. Rhodonoids A and B, two unexpected partially racemic mixtures of meroterpenoids, were isolated from the aerial parts of *R. capitatum*. By chiral HPLC separation, two pairs of enantiomers were obtained. (−)-Rhodonoid and (+)-rhodonoid A (**1a** and **1b**) are the first examples of meromonoterpenes featuring a unique 6/6/6/4 ring system. (−)-Rhodonoid and (+)-rhodonoid B (**2a** and **2b**) are an enantiomeric pair of new merosesquiterpenes with a 6/6/5/4 ring system, which showed inhibition on protein tyrosine phosphatase 1B (PTP1B), a significant target for treating obesity and type 2 diabetes.[10] The structures of these enantiomeric pairs were assigned by spectroscopic data, single-crystal X-ray crystallography, and electronic circular dichroism (ECD) analysis. This is the first separation of the partially racemic meroterpenoids from the *Rhododendron* genus. We herein present the structural elucidation and biological evaluation of these compounds.

Rhodonoid A was obtained as colorless crystals with the specific rotation $[\alpha]_D^{25}$ −34.8. It possessed a molecular formula of $C_{17}H_{20}O_3$ with 8 degrees of unsaturation, as deduced from HRESIMS (*m*/*z* 567.2720 [2M + Na]$^+$; calcd 567.2723). The NMR data including DEPT and HSQC spectra revealed the presence of three methyls, three methylenes, four methines, and seven quaternary carbons (Table 1). The NMR spectra further displayed some diagnostic signals for a hydroxyl group (δ_H

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Rhodonoids A and B in $CDCl_3$

	rhodonoid A[a]		rhodonoid B[b]	
no.	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
2		73.6		83.9
3	2.59 (d, 9.5)	51.2	2.59 (dd, 9.0, 8.0)[c]	39.1
4	3.75 (td, 9.5, 7.0)	21.9	3.17 (d, 9.0)	36.6
4a		112.6		109.6
5		154.3		154.2
6	6.33 (br s)	112.3	6.25 (br s)	109.6
7		137.6		137.8
8	6.21 (br s)	109.3	6.33 (br s)	111.9
8a		152.6		154.6
9	2.03 (ddd, 14.0, 11.0, 7.0)	34.2	1.94 (m)	39.5
	2.33 (ddd, 14.0, 7.0, 3.5)[c]		1.58 (m)	
10	2.78 (ddd, 18.0, 11.0, 7.0)	33.7	1.72 (m)	26.9
	2.42 (ddd, 18.0, 7.0, 3.5)		1.63 (m)	
11		215.7	2.62 (m)[c]	47.0
12		43.9		41.7
13	2.18 (dd, 12.0, 7.0)	39.0	2.41 (d, 18.6)	45.6
	2.36 (br dd, 12.0, 9.5)[c]		2.49 (d, 18.6)	
14	1.44 (s)	25.0		201.6
15	1.15 (s)	25.6	5.89 (br s)	125.0
16	2.21 (s)	21.4		154.4
17			1.78 (br s)	27.7
18			2.04 (br s)	20.7
19			1.53 (s)	30.5
20			1.32 (s)	26.4
21			2.22 (s)	21.4
5-OH	4.78 (br s)		5.42 (br s)	

[a]Data were measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Data were measured at 600 MHz (^{1}H) and 150 MHz (^{13}C). [c]Signals overlapped within the same column.

4.78, OH-5), two tertiary methyls (δ_H 1.44, H_3-14; 1.15, H_3-15; δ_C 25.0, C-14; 25.6, C-15), an aromatic methyl (δ_H 2.21, H_3-16; δ_C 21.4, C-16), a 1,2,3,5-tetrasubstituted benzene (δ_H 6.33, H-6; 6.21, H-8; δ_C 112.6, C-4a; 154.3, C-5; 112.3, C-6; 137.6, C-7; 109.3, C-8; 152.6, C-8a), an oxygenated sp^3 quaternary carbon (δ_C 73.6, C-2), and a keto carbonyl group (δ_C 215.7, C-11). These data combined with the degrees of unsaturation suggested three additional rings in the structure of rhodonoid A besides the benzene ring.

By interpretation of HSQC and HMBC spectra, the planar structure of rhodonoid A was established. In the HMBC spectrum (Figure 1), the correlations of H-4/C-2, C-3, C-4a, C-

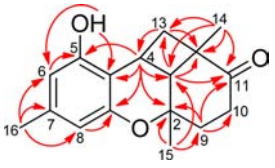

Figure 1. Key HMBC correlations for rhodonoid A.

5, C-8a; H_3-15/C-2, C-3; OH-5/C-4a, C-6; and H_3-16/C-6, C-7, C-8 indicated the presence of a benzopyran moiety (rings A and B). The HMBC cross-peaks of H_3-15/C-9; H_2-9/C-2, C-10, C-11; H-3/C-11, C-12, C-13; H_2-13/C-4, C-12; and H_3-14/C-11, C-12, C-13 defined the formation of a cyclohexanone (ring C) and a cyclobutane (ring D). The planar structure of rhodonoid A was thus constructed, which is the first example of meromonoterpene with a unique benzo[b]-2-oxatricyclo-[5.3.1.0^{5,11}]undecane ring system.

The single-crystal X-ray diffraction experiment showed that the crystal was a racemate, and chiral HPLC analysis of rhodonoid A indicated a ratio of about 5:1 (Supporting Information (SI) Figure S1). After chiral HPLC separation, compounds **1a** and **1b** were obtained. The MS and NMR data of **1a** and **1b** were identical with those of rhodonoid A (SI Table S1). The specific rotations ($[\alpha]_D^{20}$ −39.0 for **1a** and $[\alpha]_D^{20}$ +38.0 for **1b**) and CD curves of **1a** and **1b** were opposite (Figure 2).

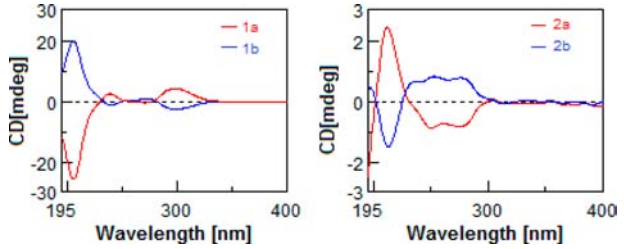

Figure 2. ECD spectra of **1a**, **1b**, **2a**, and **2b**.

The absolute configuration of **1a** was eventually assigned as 2*S*, 3*R*, 4*R*, and 12*S* [absolute structure parameter: −0.03(8)] by a single-crystal X-ray diffraction experiment performed with Cu Kα (λ = 1.54178 Å) radiation (Figure 3). Thus, the

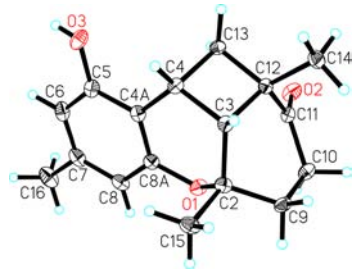

Figure 3. Single-crystal X-ray structure of **1a**.

structure of **1a** was elucidated and named (−)-rhodonoid A. Subsequently, the absolute configuration of **1b** was determined as 2*R*, 3*S*, 4*S*, and 12*R* and named (+)-rhodonoid A.

Rhodonoid B, an optically active substance ($[\alpha]_D^{25}$ −37.2), was assigned a molecular formula of $C_{22}H_{28}O_3$ by HRESIMS (m/z 703.3976 $[2M + Na]^+$; calcd 703.3975), corresponding to 9 degrees of unsaturation. The NMR spectra showed resonances for five methyls, three methylenes, six methines, and eight quaternary carbons (including one carbonyl, two oxygenated sp^2, and one oxygenated sp^3) (Table 1). In addition to signals of a hydroxyl group and a 1,2,3,5-tetrasubstituted benzene similar to those of rhodonoid A, the presence of a 4-methylpent-3-en-2-one moiety (δ_H 2.41, 2.49, H_2-13; 5.89, H-15; 1.78, H_3-17; 2.04, H_3-18; δ_C 45.6, C-13; 201.6, C-14; 125.0, C-15; 154.4, C-16; 27.7, C-17; 20.7, C-18) was differentiated. These data suggested a tetracyclic merosesquiterpene scaffold for rhodonoid B.

Chiral HPLC analysis showed that rhodonoid B contained two enantiomers with a ratio of about 2:1 (SI Figure S2). Chiral HPLC separation afforded compounds **2a** and **2b**. The MS and NMR data of **2a** and **2b** were exactly consistent with those of rhodonoid B (SI Table S2). The specific rotations ($[\alpha]_D^{20}$

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−39.0 for **2a** and $[\alpha]_D^{20}$ +42.0 for **2b**) and CD curves of **2a** and **2b** were opposite (Figure 2).

The planar structure of **2b** was elucidated by 2D NMR spectra, especially the HMBC spectrum (Figure 4). The

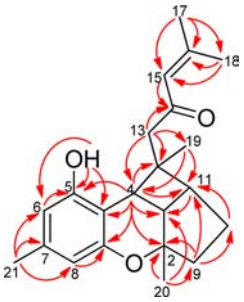

Figure 4. Key HMBC correlations for **2b**.

HMBC data confirmed that **2b** possessed the same A/B ring system as rhodonoid A and a C/D ring moiety formed by a cyclopentane (ring C) and a cyclobutane (ring D). The HMBC correlations of H_3-17/C-15, C-16, C-18; H_3-18/C-15, C-16; H-15/C-14; and H-13/C-4, C-12, C-14, C-19 verified the presence of the 4-methylpent-3-en-2-one side chain and its location at C-12. Compound **2b** was thus deduced to be a merosesquiterpene bearing a benzo[*b*]-2-oxatricyclo-[5.2.1.$0^{5,10}$]decane ring system. Its relative configuration was assigned by ROESY spectrum. Fortunately, the qualified crystals of **2a** were obtained from MeOH, which allowed us to assign its absolute configuration by X-ray diffraction using Cu Kα (λ = 1.54178 Å) radiation. The absolute configuration of **2a** was determined as 2*S*, 3*S*, 4*S*, 11*R*, and 12*R* [absolute structure parameter: 0.00(8)] (Figure 5). Thus, the structure of

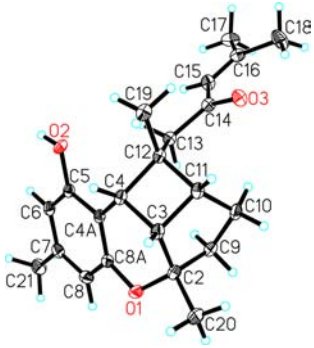

Figure 5. Single-crystal X-ray structure of **2a**.

2a was assigned and named (−)-rhodonoid B. As its enantiomer, the absolute configuration of **2b** was determined as 2*R*, 3*R*, 4*R*, 11*S*, and 12*S* and named (+)-rhodonoid B.

The biogenetic precursors of two enantiomeric pairs could be tracked back to the terpene−shikimate adducts. After enzyme-involved epoxidation, cyclization, and dehydroxylation, the benzopyran intermediates with both *R* and *S* configurations at C-2 would be produced. Subsequently, compounds **1a**, **1b**, **2a**, and **2b** could be obtained through a classic [2 + 2] cycloaddition in enantiomeric forms.

Compounds **1a**, **1b**, **2a**, and **2b** were assayed in vitro for the inhibitory effects on PTP1B. Compounds **2a** and **2b** showed inhibition with IC_{50} values of 43.56 ± 8.53 and 30.38 ± 13.41 μM, respectively. Compounds **1a** and **1b** were inactive. Oleanolic acid, an effective natural PTP1B inhibitor,[11] was used as positive control in this test (IC_{50} = 2.46 ± 0.18 μM).

In summary, this is the first report of partially racemic meroterpenoids that naturally occurred in the genus *Rhododendron*. By chiral separation, two enantiomeric pairs of optically pure meroterpenoids were obtained from this genus for the first time. The absolute configurations of the enantiomers were successfully resolved by single-crystal X-ray crystallography and ECD analysis. (−)-Rhodonoid and (+)-rhodonoid A (**1a** and **1b**) are the first examples of meromonoterpenes featuring a unique 6/6/6/4 ring system. The enantiomeric pair of merosesquiterpenes, (−)- and (+)-rhodonoid B (**2a** and **2b**), showed inhibitory effects on PTP1B in vitro. This finding shows that the chemical constituents of the genus *Rhododendron* is worthy of in-depth and meticulous research.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02515.

Experimental details and data for structure characterization (PDF)

X-ray crystallographic data for rhodonoid A (CIF)

X-ray crystallographic data for **1a** (CIF)

X-ray crystallographic data for **2a** (CIF)

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Notes

The authors declare no competing financial interest.

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