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Five Pairs of Meroterpenoid Enantiomers from *Rhododendron capitatum*

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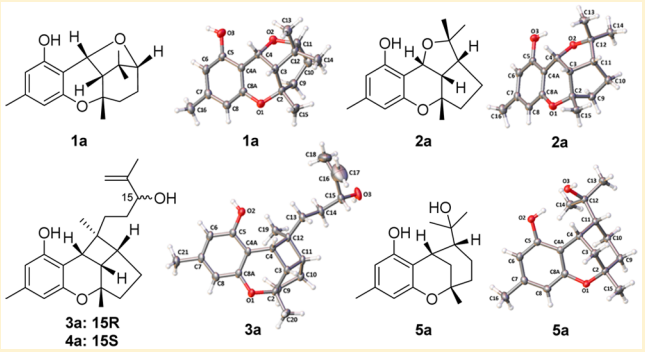
Supporting Information

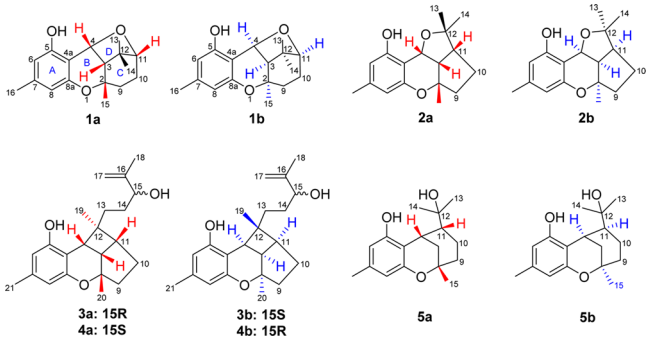
ABSTRACT: Chemical investigation on the aerial parts of *Rhododendron capitatum* resulted in the discovery of five enantiomeric pairs of new meroterpenoids, (+)-/(−)-rhodonoids C (**1a** and **1b**), E (**3a** and **3b**), F (**4a** and **4b**), and (−)-/(+)-rhodonoids D (**2a** and **2b**) and G (**5a** and **5b**). These enantiomeric pairs existed as partial racemates in a plant and were obtained by chiral HPLC separation. Their structures with absolute configurations were assigned by spectroscopic data, single-crystal X-ray diffraction, and ECD analysis. Compounds **1a** and **1b** are the first pair of meromonoterpenes incorporating an unprecedented 6/6/6/5 ring system, and **1a** showed antiviral activity against herpes simplex virus type 1 (HSV-1) in vitro. Compounds **2a** and **2b** are the first examples of meromonoterpenes featuring a unique 6/6/5/5 ring system.



INTRODUCTION

Meroterpenoids represent a special class of natural products that biogenetically originate from polyketide-terpenoids and non-polyketide-terpenoids.[1] Because of the hybrid nature, these natural products exhibit diverse structures and important bioactivities.[1,2] Although meroterpenoids are widely found in fungi and marine organisms, higher plants are also considered to be an important source of them. Plants of the genus *Rhododendron* (Ericaceae) have rich resources in China and important medicinal values. They are well-known to produce grayanane diterpenoids,[3] but meroterpenoids also have aroused attentions of chemists and pharmacologists. Some *Rhododendron* meroterpenoids with significant anti-HIV activity, inhibition of histamine release and PTP1B, and cytotoxicity have been discovered previously.[4] Total syntheses of a few *Rhododendron* meroterpenoids have been reported.[5]

Aiming to explore structurally interesting and bioactive meroterpenoids from the *Rhododendron* species, we examined the ethanol extract of *Rhododendron capitatum* Maxim., a plant being used as a Tibetan medicine in China.[6] Two enantiomeric pairs of meroterpenoids have been isolated recently.[4c] In this paper, we report five additional pairs of meroterpenoid enantiomers, (+)-/(−)-rhodonoids C (**1a** and **1b**), E (**3a** and **3b**), F (**4a** and **4b**), and (−)-/(+)-rhodonoids D (**2a** and **2b**) and G (**5a** and **5b**), which existed as partial racemates in a plant and were obtained by chiral HPLC separation. Their structures with absolute configurations were determined by spectroscopic data, X-ray crystallography, and electronic circular dichroism (ECD). Compounds **1a** and **1b** are the first pair of meromonoterpenes incorporating a novel 6/6/6/5 ring system, and **1a** showed antiviral activity against herpes simplex virus type 1 (HSV-1) in vitro. Compounds **2a** and **2b** are also the first examples of meromonoterpenes featuring a particular 6/6/5/5 ring system. Compounds **3a** and **3b**, an enantiomeric pair of new merosesquiterpenes bearing a 6/6/5/4 ring system, are C-15 epimers of **4a** and **4b**, respectively. Different from the former four enantiomeric pairs, compounds **5a** and **5b** are two new tricyclic meromonoterpenes with a 6/6/6 ring system. The isolation, structural elucidation, and biological evaluation of these meroterpenoids are described in this paper.



RESULTS AND DISCUSSION

Rhodonoid C was obtained as white amorphous powder with a specific rotation of $[\alpha]_D^{28}$ +43.1. It was assigned the molecular formula $C_{17}H_{22}O_3$ by (+) HR-ESI-MS at m/z 297.1464 [M +

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Table 1. ^{1}H and ^{13}C NMR Data of 1a−5a in $CDCl_3$

	1a[a]		2a[a]		3a[b]		4a[b]		5a[b]	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		77.7		83.0		83.5		83.6		74.5
3	1.84 (d, 4.2)	51.3	2.82 (t, 9.0)	51.7	2.56 (dd, 9.6, 7.8)	39.1	2.55 (dd, 9.6, 7.8)	39.2	1.79 (dd, 13.0, 2.8) 1.73 (dt, 13.0, 3.2)	38.4
4	5.04 (d, 4.2)	69.0	4.93 (d, 9.0)	68.9	3.09 (d, 9.6)	35.3	3.10 (d, 9.6)	35.2	3.46 (br s)	27.2
4a		108.0		107.5		108.6		108.7		108.4
5		155.7		156.2		154.1		154.1		154.4
6	6.30 (br s)	108.5	6.35 (br s)	109.4	6.16 (br s)	108.2	6.17 (br s)	108.3	6.24 (br s)	108.4
7		140.4		140.1		137.6		137.6		138.4
8	6.26 (br s)	110.0	6.30 (br s)	110.1	6.31 (br s)	111.5	6.31 (br s)	111.4	6.24 (br s)	108.5
8a		152.7		151.9		154.6		154.6		157.1
9	α 1.82 (dd, 13.7, 10.0) β 1.49 (br dd, 13.7, 5.9)	27.7	α 1.80 (m) β 1.42 (m)	35.0	α 1.98 (m) β 1.60 (m)[c]	38.7	α 1.98 (m) β 1.61 (m)[c]	38.8	α 2.01 (br d, 13.2) β 1.53 (td, 13.2, 5.0)	39.9
10	1.71 (2H, m)	27.1	α 1.74 (m) β 1.64 (m)	24.3	1.64 (m)[c]	25.6	1.65 (m)[c]	25.6	1.36 (m)[c]	22.4
11	3.85 (br s)	82.2	2.56 (m)	51.6	2.43 (td, 7.8, 3.0)	44.5	2.41 (td, 7.8, 3.0)	44.7	1.84 (dt, 12.0, 3.0)	51.7
12		42.8		83.3		42.3		42.4		74.2
13	1.29 (s)	28.1	1.33 (s)	28.2	1.65 (m)[c] 1.72 (m)[c]	41.9	1.58 (m)[c] 1.83 (m)	42.2	1.32 (s)	32.6
14	1.26 (s)	23.1	1.29 (s)	24.2	1.51 (m) 1.72 (m)[c]	29.4	1.56 (m)[c] 1.67 (m)[c]	29.5	0.88 (s)	24.3
15	1.63 (s)	30.3	1.48 (s)	27.6	4.10 (br t, 5.7)	76.6	4.10 (br t, 6.0)	76.7	1.35 (s)	28.8
16	2.23 (s)	21.7	2.23 (s)	21.6		147.7		147.6	2.22 (s)	21.4
17					4.87 (br s) 4.99 (br s)	111.3	4.88 (br s) 4.98 (br s)	111.5		
18					1.76 (br s)	17.8	1.76 (br s)	17.7		
19					0.73 (s)	15.3	0.73 (s)	15.3		
20					1.35 (s)	27.3	1.34 (s)	27.2		
21					2.22 (s)	21.4	2.22 (s)	21.4		
5-OH	5.53 (br s)		6.93 (br s)		4.69 (br s)		4.81 (br s)			

[a]Data were measured at 400 MHz (^{1}H) and 150 MHz (^{13}C). [b]Data were measured at 600 MHz (^{1}H) and 150 MHz (^{13}C). [c]Signals overlapped within the same column.

Na]$^+$ (calcd 297.1461). Analysis of the NMR data (Supporting Information (SI) Table S1) revealed a meromonoterpene scaffold for rhodonoid C. Chiral HPLC analysis showed the existence of two enantiomers with a ratio of about 10:1 (see the SI). After chiral separation, an enantiomeric pair (**1a** and **1b**) was provided.

Compound **1a**, obtained as colorless crystals, was assigned the same molecular formula as rhodonoid C by (+) HR-ESI-MS, corresponding to 7 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3429 cm^{-1}) and aromatic (1633, 1589, and 1451 cm^{-1}) moieties. The NMR data including DEPT and HSQC spectra showed resonances for four methyls, two methylenes, five methines, and six quaternary carbons (Table 1). Some characteristic signals were differentiated, including one hydroxyl group (δ_H 5.53, OH-5), three tertiary methyls (δ_H 1.29, H_3-13; 1.26, H_3-14; 1.63, H_3-15; δ_C 28.1, C-13; 23.1, C-14; 30.3, C-15), one aromatic methyl (δ_H 2.23, H_3-16; δ_C 21.7, C-16), two *meta*-coupled aromatic methines (δ_H 6.30, H-6; 6.26, H-8; δ_C 108.5, C-6; 110.0, C-8), two oxygenated methines (δ_H 5.04, H-4; 3.85, H-11; δ_C 69.0, C-4; 82.2, C-11), and three oxygenated quaternary carbons (δ_C 77.7, C-2; 155.7, C-5; 152.7, C-8a). These data in combination with the degrees of unsaturation indicated that **1a** possessed a tetracyclic system with a benzene ring.

The ^{1}H−^{1}H COSY and HSQC spectra established two structural fragments of C-3/C-4 and C-9/C-10/C-11 as shown in bold lines (Figure 1), which were connected with the benzene

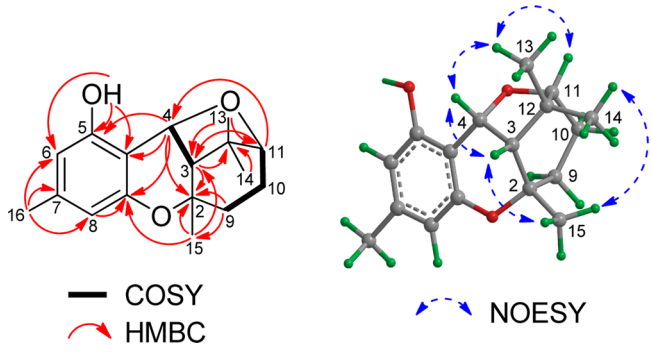

Figure 1. Key 2D NMR correlations for **1a**.

moiety and other isolated groups through quaternary carbons and heteroatoms by interpretation of the HMBC spectrum. The HMBC correlations of H-4/C-2, C-4a, C-5, C-8a; H_3-15/C-2, C-3, C-8a; OH-5/C-4a, C-5, C-6; and H_3-16/C-6, C-7, C-8 (Figure 1) indicated the presence of a benzopyran moiety (rings A and B). The HMBC cross-peaks of H-3/C-2, C-12; H_3-13, 14/C-3, C-11, C-12; H_2-9/C-2, C-15; and H-11/C-3, C-4 corroborated the formation of a cyclohexane (ring C) and a furan ring (ring D). The aforementioned analyses indicated that **1a** was the first example of meromonoterpene with a unique benzo[*d*]-2,6-dioxatricyclo[5.2.2.0^{3,8}]undecane ring system.

The relative configuration of **1a** was established by a NOESY experiment (Figure 1). The NOESY correlations of H-3/H_3-15

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and H_3-14/H_3-15 indicated their synperiplanar relationship, suggesting a β-orientation for H-3, H_3-14, and H_3-15. The NOESY cross-peaks of H-3/H-4, H-4/H_3-13, and H_3-13/H-11 revealed a β-orientation for H-4 and H-11. The absolute configuration of **1a** was determined as 2*S*, 3*S*, 4*R*, and 11*R* [absolute structure parameter: 0.01(6)] (Figure 2) by a single-crystal X-ray diffraction experiment with Cu Kα radiation.

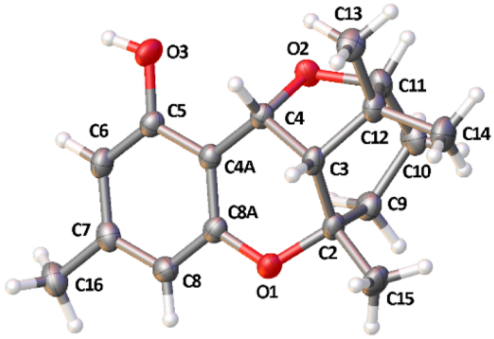

Figure 2. Single-crystal X-ray structure of **1a**. The thermal ellipsoid is scaled to the 30% probability level.

The NMR data of **1b** (SI Table S1) were exactly consistent with those of **1a**, but their specific rotations ($[\alpha]_D^{20}$ +53.0 for **1a** and $[\alpha]_D^{20}$ −51.7 for **1b**) and ECD data were opposite (Figure 3).

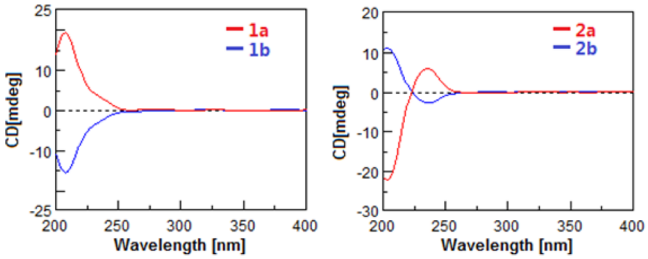

Figure 3. ECD spectra of **1a**, **1b**, **2a**, and **2b**.

It was confirmed that **1a** and **1b** should be a pair of enantiomers. Subsequently, the absolute configuration of **1b** was determined as 2*R*, 3*R*, 4*S*, and 11*S*. Thus, the structures of **1a** and **1b** were assigned and named (+)-rhodonoid C and (−)-rhodonoid C, respectively.

Rhodonoid D, an optically active substance ($[\alpha]_D^{25}$ −77.5), was assigned the same molecular formula $C_{17}H_{22}O_3$ as **1a** by (+) HR-ESI-MS. The NMR data (SI Table S2) revealed signals for one hydroxyl group, four methyls (including one aromatic and three tertiary ones), two methylenes, five methines (including one oxygenated and two *meta*-coupled aromatic ones), and six quaternary carbons (including four oxygenated ones). These observations accounted for 4 out of 7 degrees of unsaturation, indicating three additional rings besides the benzene ring.

The planar structure of rhodonoid D was constructed by 2D NMR data. The $^1H-^1H$ COSY experiment provided the structural unit as shown in bold lines (Figure 4), based on the correlations of H-4/H-3/H-11/H_2-10/H_2-9. The presence of an A/B ring system was deduced by the HMBC correlations similar to those of **1a** (Figure 4). The establishment of a cyclopentane (ring C) and a furan ring (ring D) was implied by the HMBC cross-peaks of H-3/C-2, C-9, C-12; H-4/C-2, C-12; H_3-15/C-2, C-3, C-9; and H_3-13,14/C-11, C-12. Therefore, rhodonoid D

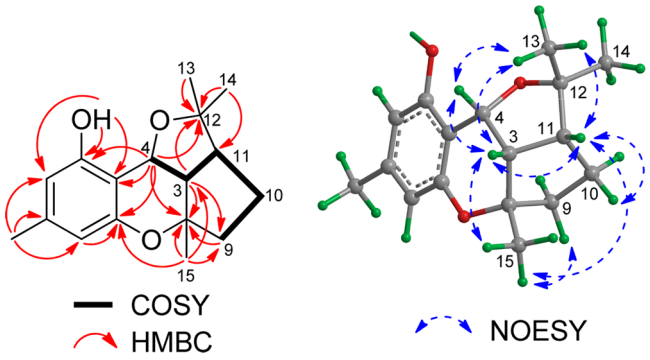

Figure 4. Key 2D NMR correlations for rhodonoid D.

was elucidated as a meromonoterpene bearing a benzo[*c*]-2,6-dioxatricyclo[6.2.1.$0^{5,11}$]undecane ring system.

In the NOESY spectrum, the correlations of H-3/H_3-15, H_3-15/H-9β, H-3/H-11, H-11/H_3-15, and H-11/H-10β demonstrated their *cis*-relationship, assigning a β-configuration for H-3, H-11, and H_3-15. The NOESY cross-peaks of H-3/H-4, H-4/H_3-13, H-3/H_3-13, and H-11/H_3-13 indicated that H-4 and H_3-13 were β-oriented (Figure 4).

Chiral HPLC analysis of rhodonoid D indicated the presence of a partial racemate with a ratio of about 4:1 (see the SI) and provided two enantiomers (**2a** and **2b**). Their specific rotations ($[\alpha]_D^{28}$ −84.3 for **2a** and $[\alpha]_D^{28}$ +85.8 for **2b**) and ECD data were opposite (Figure 3), NMR data being identical with those of rhodonoid D (Table 1 and SI Table S2). The absolute configuration of **2a** was established as 2*S*, 3*S*, 4*R*, and 11*R* [absolute structure parameter: 0.12(14)] (Figure 5) by a single-

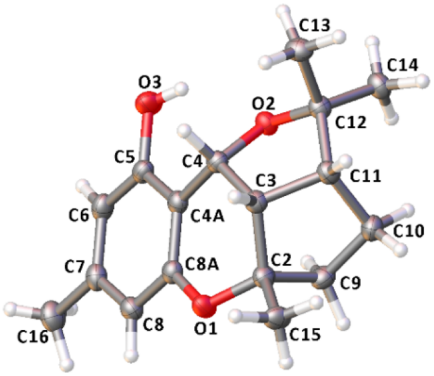

Figure 5. Single-crystal X-ray structure of **2a**. The thermal ellipsoid is scaled to the 30% probability level.

crystal X-ray diffraction with Cu Kα radiation. Subsequently, the absolute configuration of **2b** was determined as 2*R*, 3*R*, 4*S*, and 11*S*. Thus, the structures of **2a** and **2b** were assigned and named (−)-rhodonoid D and (+)-rhodonoid D, respectively.

Rhodonoid E, an optically active substance ($[\alpha]_D^{25}$ + 12.2), was isolated as a partially racemic mixuture with a ratio of about 5:1 (see the SI). By chiral HPLC separation, a pair of enantiomers (**3a** and **3b**) was obtained. Both compounds showed the identical molecular formula $C_{22}H_{30}O_3$ and NMR data (Table 1 and SI Table S3) with those of rhodonoid E.

Interpretation of HMBC spectrum of **3a** (Figure 6) corroborated the same benzo[*c*]-2-oxatricyclo[5.2.1.$0^{5,10}$]decane ring system as that of rhodonoid B.[4c] Different from rhodonoid B, it contained a 3-hydroxy-4-methylpent-4-en moiety (δ_H 1.65, 1.72, H_2-13; 1.51, 1.72, H_2-14; 4.10, H-15; 4.87, 4.99, H_2-17;

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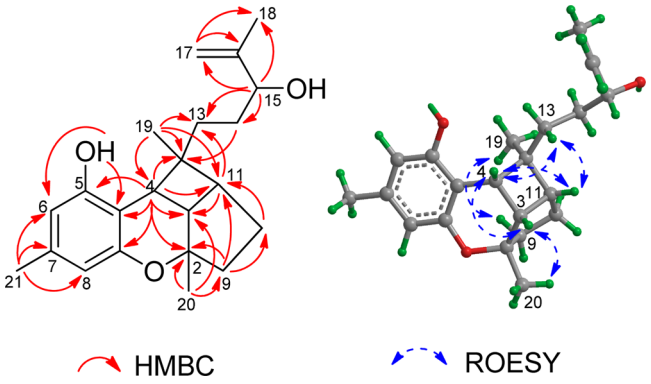

Figure 6. Key 2D NMR correlations for **3a**.

1.76, H_3-18; δ_C 41.9, C-13; 29.4, C-14; 76.6, C-15; 147.7, C-16; 111.3, C-17; 17.8, C-18) rather than 4-methylpent-3-en-2-one group in rhodonoid B. The HMBC correlations of H-15/C-13, C-14, C-17, C-18; H_3-17/C-16, C-18; H-11/C-13; and H-14/C-12 confirmed the presence of the 3-hydroxy-4-methylpent-4-en moiety and its location at C-12.

In the ROESY spectrum (Figure 6), the correlations of H-3/H-4, H-3/H_3-20, H-4/H-13, H-4/H-11, H-11/H-13, and H-9α/H_3-19 assigned a β-orientation for H-3, H-4, H-11, H_2-13, and Me-20 and an α-orientation for Me-19. Finally, a single-crystal X-ray diffraction experiment performed with Cu Kα radiation assigned the absolute configuration of **3a** as 2*S*, 3*S*, 4*S*, 11*R*, 12*S*, and 15*R* [absolute structure parameter: −0.05(12)] (Figure 7).

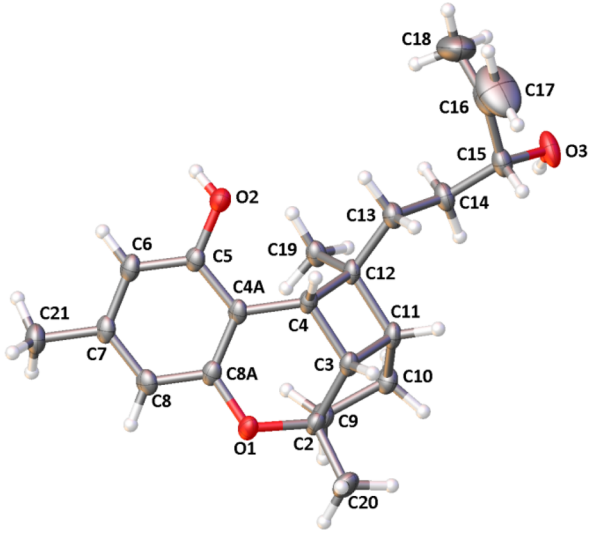

Figure 7. Single-crystal X-ray structure of **3a**. The thermal ellipsoid is scaled to the 30% probability level.

For compound **3b**, its specific rotation ($[\alpha]_D^{20}$ +23.1 for **3a** and $[\alpha]_D^{20}$ −21.7 for **3b**) and ECD curve were opposite to those of **3a** (Figure 8). As its enantiomer, the absolute configuration of **3b** was determined as 2*R*, 3*R*, 4*R*, 11*S*, 12*R*, and 15*S*. Thus, the structures of **3a** and **3b** were assigned and named (+)-rhodonoid E and (−)-rhodonoid E, respectively.

(+)-Rhodonoid F and (−)-rhodonoid F (**4a** and **4b**) with a ratio of about 2.5:1 was isolated by chiral HPLC (see the SI). Both compounds were assigned the same molecular formula $C_{22}H_{30}O_3$ as **3a** and **3b** by (+) HR-ESI-MS. Furthermore, they showed identical NMR data (Table 1 and SI Table S4) and

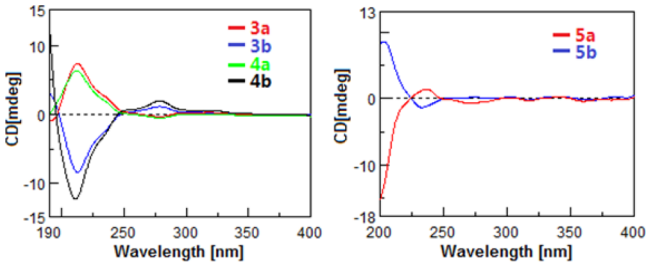

Figure 8. ECD spectra of **3a**, **3b**−**5a**, **5b**.

opposite specific rotations ($[\alpha]_D^{20}$ +19.5 for **4a** and $[\alpha]_D^{20}$ −18.3 for **4b**) and ECD curves (Figure 8). It was inferred that **4a** and **4b** should be a pair of enantiomers. The NMR data of **4a** including HSQC and HMBC spectra exhibited that it possessed the same planar structure as **3a**. The ROESY correlations of H-3/H-4, H-3/H_3-20, H-4/H-11, H-4/H-13, and H-9α/H_3-19 and the ECD data with Cotton effects around 212 (+) and 281 (−) nm of **4a** defined its relative and absolute configurations at C-2, C-3, C-4, C-11, and C-12, which were identical with those of **3a**. However, the chemical shifts of H_2-13, H_2-14, and C-13 in **3a** and **4a** were slightly different. This evidence verified that **4a** was an epimer of **3a** at C-15. The absolute configuration of **4a** was finally assigned as 2*S*, 3*S*, 4*S*, 11*R*, 12*S*, and 15*S*. Subsequently, the absolute configuration of **4b** was determined as 2*R*, 3*R*, 4*R*, 11*S*, 12*R*, and 15*R*. Thus, the structures of **4a** and **4b** were elucidated and named (+)-rhodonoid F and (−)-rhodonoid F, respectively.

Rhodonoid G, an optically active compound ($[\alpha]_D^{25}$ −15.4), was assigned the molecular formula $C_{17}H_{24}O_3$ by (+) HR-ESI-MS at *m*/*z* 277.1811 $[M + H]^+$ (calcd 277.1804). The NMR spectra revealed signals of 1,2,3,5-tetrasubstituted benzene moiety and additional three methyls, three methylenes, two methines, and two quaternary carbons (SI Table S5). These observations suggested a tricyclic meromonoterpene skeleton for rhodonoid G, which was constructed by 2D NMR spectra. The HMBC correlations (Figure 9) confirmed that rhodonoid G had

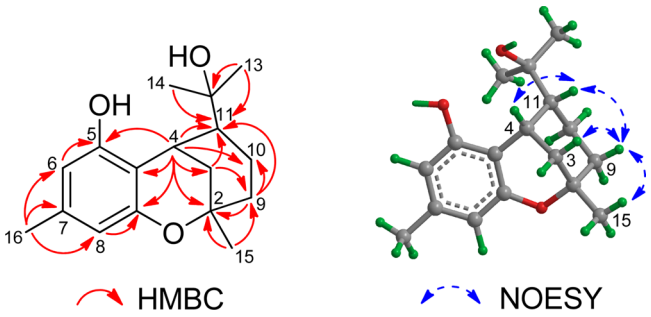

Figure 9. Key 2D NMR correlations for rhodonoid G.

a benzopyran moiety (A/B ring system) similar to rhodonoids C−F. The HMBC cross-peaks of H-3/C-9, C-11; H-4/C-10, C-11; H_2-9/C-2, C-10, C-11; H_3-15/C-2, C-9; and H_3-13,14/C-11, C-12 defined a cyclohexane moiety (ring C) with 2-hydroxypropanyl at C-11. The planar structure of rhodonoid G was thus established to be a meromonoterpene with a benzo[*c*]-2-oxadicyclo[3.3.1]nonane ring system. The relative stereochemistry was established by a NOESY experiment (Figure 9). The NOESY correlations of H-4/H-11, H-11/H-9β, and H-9β/H_3-15 indicated their synperiplanar relationship, shown as β-orientation.

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Chiral HPLC analysis showed that rhodonoid G was a partial racemate with a ratio of about 37:1 (see the SI). A pair of enantiomers, compounds **5a** and **5b** with identical NMR data (Table 1 and SI Table S5) and opposite specific rotations ($[\alpha]_D^{25}$ −19.0 for **5a** and $[\alpha]_D^{25}$ +18.2 for **5b**) and ECD curves (Figure 8), were obtained by chiral HPLC separation. The absolute configuration of **5a** was assigned as 2*S*, 4*S*, and 11*R* [absolute structure parameter: 0.05(9)] by a single-crystal X-ray diffraction using Cu Kα radiation (Figure 10). Accordingly, the absolute configuration of **5b** was determined as 2*R*, 4*R*, and 11*S*. Thus, the structures of **5a** and **5b** were elucidated and named (−)-rhodonoid G and (+)-rhodonoid G, respectively.

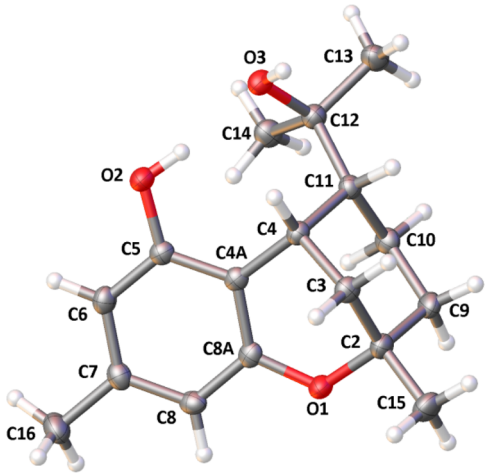

Figure 10. Single-crystal X-ray structure of **5a**. The thermal ellipsoid is scaled to the 30% probability level.

The antiviral activity of **1a** and **2a** against HSV-1 was evaluated in vitro with acyclovir as a positive control using the cytopathic effect (CPE) assay.[7] Compound **1a** showed inhibitory activity with an IC_{50} value of 80.6 ± 4.7 μM and selective index (SI = CC_{50}/IC_{50}) of 2.7 ± 0.2, while compound **2a** was inactive. Compounds **1a**−**5a** were assayed in vitro for the inhibitory effects on protein tyrosine phosphatase 1B (PTP1B), but none of them showed inhibition. In combination with the previous results on PTP1B,[4c] a preliminary summary of structure−activity relationship for the *Rhododendron* meroterpenoids could be suggested: the presence of 6/6/5/4 ring system is important for the PTP1B inhibition. Meanwhile, the *cis*-relationship of Me-19 with H-3, H-4, H-11, and Me-20 is essential for the activity. (−)- and (+)-Rhodonoid B showed inhibitory activity,[4c] while (+)-rhodonoids E (**3a**) and F (**4a**) had no activity.

CONCLUSION

In summary, five enantiomeric pairs of new meroterpenoids occurring as partial racemates in a plant were isolated from *R. capitatum*. The absolute configurations of the enantiomers were assigned by X-ray crystallography and ECD analysis. These *Rhododendron* meroterpenoids showed structural diversity. Meroterpenoids with 6/6/5/4, 6/6/6/4, 6/6/6/6, 6/5/6, and 6/6/6 polycyclic ring systems have been reported previously.[4,8] However, meroterpenoids incorporating 6/6/6/5 and 6/6/5/5 ring systems, such as (+)-/(−)-rhodonoid C (**1a** and **1b**) and (−)-/(+)-rhodonoid D (**2a** and **2b**), have been found for the first time. Compound **1a** showed an antiviral effect on HSV-1 in vitro. The findings enrich the meroterpenoids of the genus *Rhododendron*.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on a melting point apparatus. Optical rotations were recorded on a polarimeter. UV spectra were obtained on a spectrophotometer. ECD spectra were recorded on a spectropolarimeter. IR spectra were recorded on an IR spectrometer. NMR spectra were acquired on 400, 500, and 600 MHz spectrometers using $CDCl_3$ as solvent. Chemical shifts were reported with respect to $CDCl_3$ (δ_H 7.26 and δ_C 77.16). ESI-MS data were obtained on an ion trap mass spectrometer or a quadrupole linear ion trap mass spectrometer. HR-ESI-MS analyses were obtained using a Q-TOF mass spectrometer. X-ray crystallographic analyses were performed on an X-ray diffractometer employing graphite-monochromated Cu Kα radiation (λ = 1.54178 Å). D101 macroporous resin, silica gel (200−300 mesh), C_{18} reversed-phase (RP-18) silica gel (150−200 mesh), and MCI gel CHP-20P (75−150 μm) were used for column chromatography (CC), and precoated silica gel GF254 plates were used for TLC. Semipreparative HPLC was performed with a UV detector and an ODS column (250 × 10 mm, 5 μm). Chiral column (250 × 20 mm, 5 μm) was used for chiral separation on HPLC chromatographs.

Plant Material. The aerial parts of *Rhododendron capitatum* were collected in Qinghai province, People's Republic of China, in August 2012. The plant material was identified by Prof. Yanduo Tao, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, and a voucher specimen (TCM 12-09-1 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

Extraction and Isolation. The aerial parts of *R. capitatum* (10 kg) were powdered and percolated with EtOH−H_2O (95:5) at room temperature. The filtrate was evaporated under reduced pressure to be concentrated. The obtained extract (2 kg) was partitioned with water and EtOAc. The EtOAc fraction (1.5 kg) was applied to D101 macroporous resin CC using gradient eluent (EtOH−H_2O, from 1:9 to 9:1) to yield five fractions A−E. Fraction D (250 g) was purified by MCI gel CC (from MeOH−H_2O 4:6 to MeOH) to afford fractions D1−D8. Fraction D4 (25 g) was passed over a silica gel column (petroleum ether−Me_2CO, from 10:1 to 1:1) to give fractions D4a−D4d. Fraction D4b (2 g) was separated by RP-18 CC (MeOH−H_2O, from 3:7 to 8:2) to yield fractions D4b.1−D4b.4. Fraction D4b.3 (25 mg) was further purified by semipreparative HPLC (CH_3CN−H_2O, 55:45) to afford rhodonoid E (6.0 mg) and fraction D4b.3.2 (10 mg). After chiral HPLC separation (*n*-hexane−isopropanol, 92:8), compounds **3a** (3.5 mg) and **3b** (1.0 mg) from rhodonoid E and **4a** (2.0 mg) and **4b** (0.6 mg) from fraction D4b.3.2 were obtained, respectively. Fraction D4c (150 mg) was applied to RP-18 CC with gradient eluent from MeOH−H_2O 3:7 to MeOH, followed by semipreparative HPLC (MeOH−H_2O, 65:35) to afford rhodonoid G (9.08 mg). By chiral HPLC separation (*n*-hexane−isopropanol, 75:25), compounds **5a** (6.5 mg) and **5b** (1.0 mg) were obtained from it. Fraction D6 (35 g) was chromatographed on a silica gel column (petroleum ether−Me_2CO, from 10:1 to 1:1) to yield fractions D6a−D6h. Fraction D6c (150 mg) was further purified by semipreparative HPLC (MeOH−H_2O, 7:3) to afford rhodonoid C (28 mg). Fraction D6d (1.2 g) was separated by RP-18 CC (MeOH−H_2O, from 3:7 to 8:2) to yield fractions D6d.1−D6d.3. Fraction D6d.2 (200 mg) was purified by semipreparative HPLC (CH_3CN−H_2O, 55:45) to afford rhodonoid D (15 mg). Rhodonoids C and D were further separated by chiral HPLC (*n*-hexane−isopropanol, 90:10) to give compounds **1a** (22 mg), **1b** (0.8 mg), **2a** (8.5 mg), and **2b** (2.0 mg), respectively.

Characterization Data. *(+)-Rhodonoid C (**1a**).* Colorless crystals (MeOH); mp 171−172 °C; $[\alpha]_D^{20}$ = +53.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.53), 232 (4.00), 285 (3.18) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 208 (+12.39), 233 (+2.43), 256 (+0.11, observed as valley); IR (KBr) ν_{max} 3429, 2961, 2934, 2870, 1633, 1589, 1451, 1381, 1327, 1144, 1066, 1011, 997, 920, 873, 824 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 1; (+) ESI-MS *m*/*z* 275 $[M + H]^+$, 571 $[2 M + Na]^+$; (+) HR-ESI-MS *m*/*z* 275.1652 $[M + H]^+$ (calcd for $C_{17}H_{23}O_3$, 275.1647).

*(−)-Rhodonoid C (**1b**).* White amorphous powder; $[\alpha]_D^{20}$ = −51.7 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.52), 232 (4.00), 285

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(3.16) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 208 (−11.64), 233 (−2.34), 256 (−0.26, observed as peak); for ^{1}H NMR data, see SI Table S1; (+) HR-ESI-MS m/z 297.1460 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_3Na$, 297.1461).

*(−)-Rhodonoid D (**2a**)*. Colorless crystals (MeOH); mp 143−145 °C; $[\alpha]_D^{28}$ = −84.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.74), 232 (4.04), 285 (3.41) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 206 (−13.49), 236 (+3.78); for ^{1}H NMR and ^{13}C NMR data, see Table 1; (+) ESI-MS m/z 275 $[M + H]^+$, 297 $[M + Na]^+$; (+) HR-ESI-MS m/z 297.1461 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_3Na$, 297.1461).

*(+)-Rhodonoid D (**2b**)*. White amorphous powder; $[\alpha]_D^{28}$ = +85.8 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.72), 232 (4.02), 285 (3.40); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 206 (+12.77), 236 (−3.16); for ^{1}H NMR and ^{13}C NMR data, see SI Table S2; (+) HR-ESI-MS m/z 297.1462 $[M + Na]^+$ (calcd for $C_{17}H_{22}O_3Na$, 297.1461).

*(+)-Rhodonoid E (**3a**)*. Colorless crystals (MeOH); mp 172−173 °C; $[\alpha]_D^{20}$ = +23.1 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.57) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 212 (+10.44), 281 (−0.44); IR (KBr) ν_{max} 3440, 2923, 2853, 1626, 1058 cm^{-1}; for ^{1}H NMR and ^{13}C NMR data, see Table 1; (+) ESI-MS m/z 343 $[M + H]^+$; (−) ESI-MS m/z 341 $[M - H]^-$; (+) HR-ESI-MS m/z 343.2265 $[M + H]^+$ (calcd for $C_{22}H_{31}O_3$, 343.2273).

*(−)-Rhodonoid E (**3b**)*. White amorphous powder; $[\alpha]_D^{20}$ = −21.7 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.57) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 212 (−10.67), 279 (+1.41); for ^{1}H NMR and ^{13}C NMR data, see SI Table S3; (+) HR-ESI-MS m/z 343.2275 $[M + H]^+$ (calcd for $C_{22}H_{31}O_3$, 343.2273).

*(+)-Rhodonoid F (**4a**)*. White amorphous powder; $[\alpha]_D^{20}$ = +19.5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.55) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 212 (+10.11), 281 (−0.82); IR (KBr) ν_{max} 3439, 2972, 2924, 2852, 1625, 1050 cm^{-1}; for ^{1}H NMR and ^{13}C NMR data, see Table 1; (+) ESI-MS m/z 343 $[M + H]^+$; (−) ESI-MS m/z 341 $[M - H]^-$; (+) HR-ESI-MS m/z 343.2263 $[M + H]^+$ (calcd for $C_{22}H_{31}O_3$, 343.2273).

*(−)-Rhodonoid F (**4b**)*. White amorphous powder; $[\alpha]_D^{20}$ = −18.3 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.55) nm; ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 211 (−10.45), 279 (+1.63); for ^{1}H NMR data, see SI Table S4; (+) HR-ESI-MS m/z 343.2265 $[M + H]^+$ (calcd for $C_{22}H_{31}O_3$, 343.2273).

*(−)-Rhodonoid G (**5a**)*. Colorless crystals (MeOH); mp 173−175 °C; $[\alpha]_D^{25}$ = −19.0 (*c* 0.10 MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.80); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 203 (−9.20), 237 (+1.01), 271 (−0.50); for ^{1}H and ^{13}C NMR data, see Table 1; (+) HR-ESI-MS m/z 299.1620 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_3Na$, 299.1618).

*(+)-Rhodonoid G (**5b**)*. White amorphous powder; $[\alpha]_D^{25}$ = +18.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.50); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 203 (+9.01), 233 (−1.38), 274 (+0.27); for ^{1}H and ^{13}C NMR data, see SI Table S5; (+) HR-ESI-MS m/z 277.1801 $[M + H]^+$ (calcd for $C_{17}H_{25}O_3$, 277.1798).

Biological Evaluation. The antiviral activity of the tested compounds against herpes simplex virus type 1 (HSV-1) was measured by the cytopathic effect (CPE) assay as reported previously.[7] Acyclovir was used as the positive control (IC_{50} = 4.2 ± 0.3 μM, SI > 100). A colorimetric assay to measure inhibition on PTP1B was performed as reported previously.[9] Oleanolic acid was used as the positive control (IC_{50} = 2.5 ± 0.2 μM).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02800.

Data for structure characterization and chiral HPLC chromatograms (PDF)

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

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

X-ray crystallographic data for **3a** (CIF)

X-ray crystallographic data for **5a** (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Geris, R.; Simpson, T. J. *Nat. Prod. Rep.* **2009**, *26*, 1063−1094.

(2) Itoh, T.; Tokunaga, K.; Matsuda, Y.; Fujii, I.; Abe, I.; Ebizuka, Y.; Kushiro, T. *Nat. Chem.* **2010**, *2*, 858−864.

(3) (a) Liu, C. C.; Lei, C.; Zhong, Y.; Gao, L. X.; Li, J. Y.; Yu, M. H.; Li, J.; Hou, A. J. *Tetrahedron* **2014**, *70*, 4317−4322. (b) Li, Y.; Liu, Y. B.; Yu, S. S. *Phytochem. Rev.* **2013**, *12*, 305−325. (c) Wang, S.; Lin, S.; Zhu, C.; Yang, Y.; Li, S.; Zhang, J.; Chen, X.; Shi, J. *Org. Lett.* **2010**, *12*, 1560−1563. (d) Li, Y.; Liu, Y. B.; Liu, Y. L.; Wang, C.; Wu, L. Q.; Li, L.; Ma, S. G.; Qu, J.; Yu, S. S. *Org. Lett.* **2014**, *16*, 4320−4323.

(4) (a) Kashiwada, Y.; Yamazak, K.; Ikeshiro, Y.; Yamagishi, T.; Fujioka, T.; Mihashi, K.; Mizuik, K.; Cosentino, L. M.; Fowke, K.; Morris-Natschke, S. L.; Lee, K. H. *Tetrahedron* **2001**, *57*, 1559−1563. (b) Iwata, N.; Kitanaka, S. *J. Nat. Prod.* **2010**, *73*, 1203−1206. (c) Liao, H. B.; Lei, C.; Gao, L. X.; Li, J. Y.; Li, J.; Hou, A. J. *Org. Lett.* **2015**, *17*, 5040−5043. (d) Seephonkai, P.; Popescu, R.; Zehl, M.; Krupitza, G.; Urban, E.; Kopp, B. *J. Nat. Prod.* **2011**, *74*, 712−717.

(5) (a) Luo, G. Y.; Wu, H.; Tang, Y.; Li, H.; Yeom, H. S.; Yang, K.; Hsung, R. P. *Synthesis* **2015**, *47*, 2713−2720. (b) Kurdyumov, A. V.; Hsung, R. P.; Ihlen, K.; Wang, J. *Org. Lett.* **2003**, *5*, 3935−3938.

(6) Jia, M. R.; Li, X. W. *Zhongguo Minzu Yaozhi Yao*; China Medical Science Press: Beijing, 2005; pp 516−517.

(7) Zhang, L. B.; Liao, H. B.; Zhu, H. Y.; Yu, M. H.; Lei, C.; Hou, A. J. *Tetrahedron* **2016**, *72*, 8036−8041.

(8) (a) Kane, V. K.; Martin, A. R.; Peters, J. A. *J. Org. Chem.* **1984**, *49*, 1793−1796. (b) Turner, C. E.; Mole, M. L.; Hanus, L.; Elsohly, H. N. *J. Nat. Prod.* **1981**, *44*, 27−33.

(9) (a) Shi, L.; Yu, H. P.; Zhou, Y. Y.; Du, J. Q.; Shen, Q.; Li, J. Y.; Li, J. *Acta Pharmacol. Sin.* **2008**, *29*, 278−284. (b) Wang, M.; Yu, B. W.; Yu, M. H.; Gao, L. X.; Li, J. Y.; Wang, H. Y.; Li, J.; Hou, A. J. *Chem. Biodiversity* **2015**, *12*, 937−945.

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