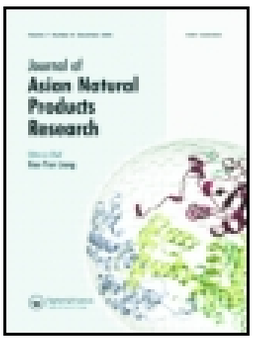

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A new ascorbic acid derivative and two new terpenoids from the leaves and twigs of *Rhododendron decorum*

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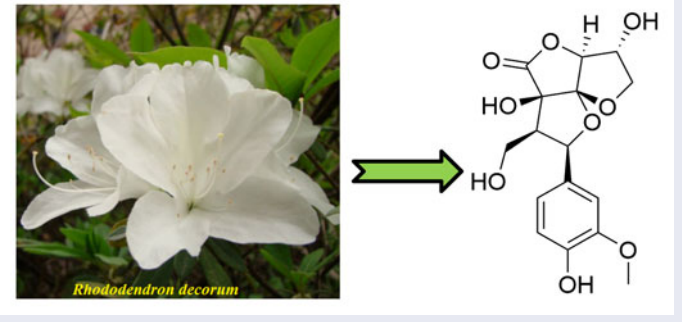
ABSTRACT

The phytochemical study of the ethanol extract of the leaves and twigs of *Rhododendron decorum* afforded a new ascorbic acid derivative (**1**), a new ionone analogue (**2**), a new ursane-type triterpenoid glucoside (**3**), and four known compounds (**4**–**7**). The structures were elucidated by spectroscopic analyses, including HRESIMS, 1D, and 2D NMR. The anti-neuroinflammatory activities of the compounds were evaluated by measuring inhibitory effects of LPS-induced NO production in BV2 cells.



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Rhododendron decorum; ascorbic acid derivative; terpenoid

1. Introduction

Rhododendron decorum is an evergreen shrub that distributes mainly in southwestern China and northeastern Burma. It is used as folk medicine in China to treat rheumatism and pain [1]. The family of Ericaceae is rich in terpenoids, lignans, and flavonoids, some of which show valuable anti-inflammatory, antinociceptive, antiviral, sodium channel antagonistic, and cytotoxic activities [2–3]. To explore the chemical

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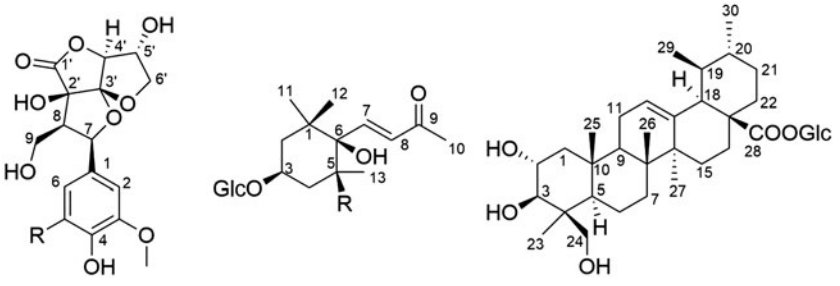

Figure 1. Structures of compounds **1**−**5**.

diversity and bioactivity, leaves and twigs of *R. decorum* were collected from Yunnan Province. In the present investigation, three new compounds along with five known compounds were isolated (Figure 1). Herein, the isolation and structural elucidation as well as the anti-neuroinflammatory activities of these compounds are described.

2. Results and discussion

Compound **1** was obtained as white solid. Its molecular formula was determined to be $C_{16}H_{18}O_9$ based on HRESIMS (*m/z* 377.0844 $[M+Na]^+$, calcd 377.0843), corresponding to 8 degrees of unsaturation. The IR spectrum showed typical signals of hydroxy (3378 cm^{-1}) and ester carbonyl (1782 cm^{-1}) functionalities. The ^1H NMR data (Table 1) showed resonances of an oxygenated methyl group (δ_H 3.67), two oxygenated methylenes (δ_H 4.33, 4.58, 4.48, and 4.28), and three oxygenated methines (δ_H 4.69, 5.50, and 5.90). The ^{13}C NMR (Table 2) coupled with DEPT spectra exhibited 16 carbon resonances, including a methyl group, two methylenes, seven methines (four sp^3 and three sp^2), and six quaternary carbons (two sp^3 and four sp^2). The ^1H-^1H COSY (Figure 2) and HSQC spectra established three fragments: CH_2-CH(OH)-CH, CH_2(OH)-CH-CH, and CH-CH.

The ^1H and ^{13}C NMR data of **1** were similar to those of amarusine A (**4**) [4], which possesses a skeleton composed of a spirocyclic di-tetrahydrofuran fused to a butyrolactone ring and an aromatic ring. NMR data of **1** indicated the existence of a 3,4-dioxygenated aromatic ring, which is different from amarusine A (**4**). This was confirmed by the HMBC correlations of H-5 (δ_H 7.24) to C-1 (δ_C 131.0) and C-4 (δ_C 149.0). In the NOESY spectrum (Figure 3), the correlations of H-8/H-4′ and H-7/H-8 indicated H-4′, H-7, and H-8 are α-oriented. H-7/H-8 and H-4′/H-5′ were deduced to be in the *erythro* and *threo* configuration, respectively, based on the coupling constants of $J_{7,8} = 10.4$ Hz and $J_{4',5'} = 2.0$ Hz. In addition, the CD spectrum of **1** was consistent with amarusine A (**4**). Thus, the structure of **1** was elucidated as depicted and named rhododeonin A.

Compound **2** has a molecular formula of $C_{20}H_{34}O_9$ based on HRESIMS. The ^1H NMR data showed resonances of five methyls (δ_H 1.17, 1.33, 1.40, 2.27, and 3.30), six oxygenated methines (δ_H 4.01, 4.10, 4.30, 4.32, 4.50, and 5.07), and two olefinic protons

Table 1. ^{1}H NMR spectroscopic data of compounds 1 – 3 in pyridine-d_5 (δ in ppm, J in Hz).

No	**1**[a]	No	**2**[a]	No	**3**[b]
1	–	1	–	1	2.28 (dd, 12.5, 4.2)
	–		–		1.27–1.33, (m)
2	7.35 (d, 1.7)	2	2.21 (dd, 13.8, 4.3)	2	4.30–4.35, (m)
	–		2.00 (dd, 13.7, 7.8)		–
3	–	3	4.48–4.52, (m)	3	3.58 (d, 8.7)
4	–	4	2.36 (dd, 14.3, 3.4)	4	–
	–		2.31 (d, 7.3)		–
5	7.23–7.25, (m)	5	–	5	1.13–1.16, (m)
6	7.26 (d, 1.9)	6	–	6	1.68 (d, 12.1)
	–		–		1.43–1.45, (m)
7	5.90 (d, 10.4)	7	6.88 (d, 16.0)	7	1.51–1.58, (m)
	–		–		1.40 (d, 11.8)
8	3.24–3.27, (m)	8	7.73 (d, 16.0)	8	–
9	4.58 (dd, 11.6, 6.3)	9	–	9	1.72–1.76, (m)
	4.33 (dd, 11.6, 4.2)		–		–
1′	–	10	2.27, (s)	10	–
2′	–	11	1.17, (s)	11	1.99–2.06, (m)
	–		–		1.69–1.75, (m)
3′	–	12	1.33, (s)	12	5.45, (brs)
4′	5.50 (d, 2.0)	13	1.40, (s)	13	–
5′	4.69–4.70, (m)	1′	5.07 (d, 7.7)	14	–
			–		
6′	4.48 (dd, 9.5, 4.5)	2′	4.10 (t, 8.1)	15	2.43–2.50, (m)
	4.28 (dd, 9.5, 5.3)		–		1.17–1.20, (m)
–OCH_3	3.67, (s)	3′	4.28–4.33, (m)	16	2.06–2.13, (m)
					1.96–2.01, (m)
		4′	4.28–4.33, (m)	17	–
		5′	4.00–4.03, (m)	18	2.54 (d, 11.3)
		6′	4.59, (dd, 11.6, 1.8)	19	1.36–1.51, (m)
			4.43 (dd, 11.6, 5.2)		
		–OCH_3	3.30, (s)	20	0.87–0.94, (m)
					–
				21	1.36–1.39, (m)
					1.25–1.30, (m)
				22	1.92 (brd, 13.1)
					1.72–1.78, (m)
				23	1.60, (s)
				24	4.47 (d, 11.0)
					3.72–3.76, (m)
				25	1.04, (s)
				26	1.16, (s)
				27	1.18, (s)
				28	–
				29	0.95 (d, 6.4)
				30	0.91 (brs)
				1′	6.29 (d, 8.1)
				2′	4.20–4.25, (m)
				3′	4.29–4.33, (m)
				4′	4.36–4.40, (m)
				5′	4.02–4.08, (m)
				6′	4.47–4.49, (m)
					4.40–4.42, (m)

[a]Recorded at 600 MHz.
[b]Recorded at 500 MHz.

(δ_H 6.88 and 7.73). The ^{13}C NMR and DEPT spectra exhibited 20 carbon resonances, including five methyls, three methylenes, eight methines, and four quaternary carbons. The NMR data of **2** were consistent with an ionone-type glycoside and comparable to those of pisumionoside (**5**) [5]. The HMBC correlation from H_3-14 (-OCH_3, δ_H 3.30) to C-5 (δ_C 81.2) indicated that 5-OH is methylated. In the NOESY spectrum, the

Table 2. ^{13}C NMR spectroscopic data of compounds 1 − 3 in pyridine-d_5 (δ in ppm).

No	**1**[a]	No	**2**[a]	No	**3**[b]
1	131.0	1	39.6	1	48.5
2	112.1	2	44.3	2	69.2
3	149.2	3	74.3	3	86.2
4	149.0	4	37.7	4	44.5
5	117.0	5	81.2	5	57.0
6	121.1	6	82.0	6	19.8
7	86.4	7	130.7	7	34.4
8	58.1	8	152.8	8	40.7
9	58.4	9	198.5	9	48.7
1′	176.1	10	28.0	10	38.7
2′	82.2	11	26.9	11	24.5
3′	118.3	12	29.4	12	126.4
4′	91.1	13	21.0	13	138.9
5′	75.5	1′	104.4	14	42.9
6′	75.3	2′	76.1	15	29.1
-OCH_3	56.3	3′	79.5	16	25.1
		4′	72.5	17	48.8
		5′	79.1	18	53.8
		6′	63.6	19	39.8
		-OCH_3	50.0	20	39.6
				21	31.3
				22	37.3
				23	24.7
				24	66.2
				25	18.0
				26	18.1
				27	24.3
				28	176.7
				29	17.9
				30	21.8
				1′	96.2
				2′	74.6
				3′	79.4
				4′	71.7
				5′	79.7
				6′	62.8

[a]Recorded at 150 MHz.
[b]Recorded at 125 MHz.

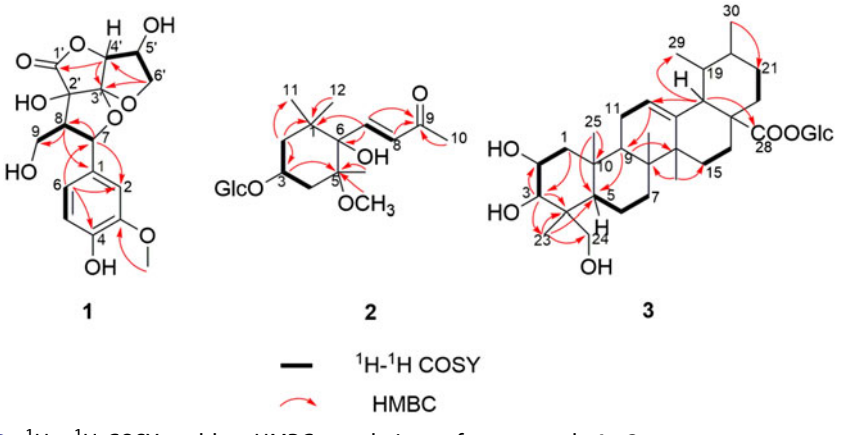

Figure 2. ^{1}H - ^{1}H COSY and key HMBC correlations of compounds **1**−**3**.

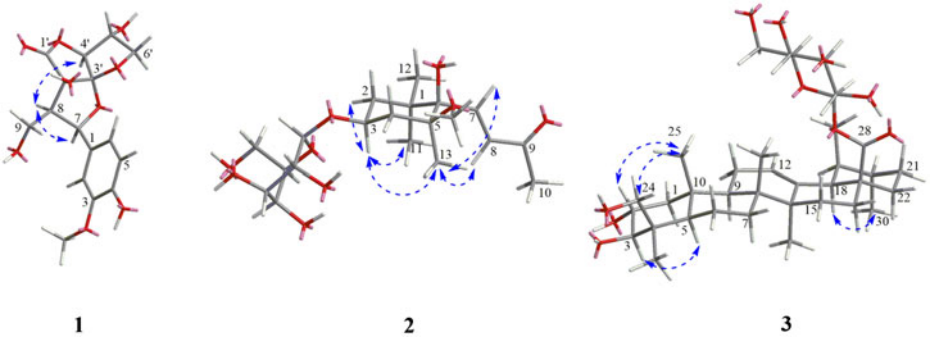

Figure 3. Conformations and key NOESY correlations of compounds **1**−**3**.

correlations of H_3-11/H-3, H_3-13/H-3, H-2α/H_3-11, H-2α/H-3, H-8/H_3-13, and H-7/H_3-13 indicated that H-3 and H_3-13 are α-oriented and HO-6 is β-oriented. In addition, the anomeric proton H-1′ (δ_H 5.07) was observed with a large coupling constant (7.7 Hz), which indicated the β-configuration of the glucose. Acid hydrolysis and GC analysis of the sugar moiety of **2** confirmed the sugar is $_D$-glucose. The absolute configuration of **2** was deduced to be the same as pisumionoside (**5**) due to the approximate optical value (–4.4 for **2** in our test and –3.4 for pisumionoside in the literature). Thus, the structure of **2** was elucidated as depicted and named rhododeoside A.

Compound **3** has a molecular formula of $C_{36}H_{58}O_{10}$ based on the HRESIMS. The ^{1}H and ^{13}C NMR data of **3** resembled those of 2α,3α,24-trihydroxyurs-12-en-28-oic acid-28-O-β-$_D$-glucopyranosyl ester [6], with the exception of the relative configuration of C-3. Compound **3** contains a β-OH at C-3 instead of an α-OH at C-3 in the known compound, which was supported by the downfield chemical shifts of C-3 and the NOESY correlations of H-3/H-5 and H-3/H_3-23. The NOESY correlations of H_3-25/H_2-24, H-2/H_3-25, H_3-30/H-18 indicated that H-2 is β-oriented and CH_3-23 and CH_3-30 are α-oriented. In addition, the anomeric proton at H-1′ (δ_H 6.29) was observed with a large coupling constant (8.1 Hz), which indicated the β-configuration of the glucose. Acid hydrolysis and GC analysis of the sugar moiety of **3** confirmed the sugar is $_D$-glucose. Thus, the structure of **3** was elucidated as depicted and named rhododeoside B.

Other known compounds isolated from the plant are amarusine A (**4**) [4], pisumionoside (**5**) [5], linarionoside A (**6**) [7], and grasshopper ketone (**7**) [8]. These compounds were identified by comparing their NMR data with published literature.

Compounds were assayed for their anti-neuroinflammatory activities by measuring inhibitory effects of LPS-induced NO production in BV2 cells. The inhibition rates of compounds **5**−**7** (10 μM) are 18.08%, 16.67%, and 17.37%, respectively.

3. Experimental

3.1. General experimental procedures

IR data were measured on a Nicolet 5700 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA). Optical rotations were acquired with a Rudolph automatic polarimeter (DKSH, Zurich, Switzerland). HRESIMS were carried out on an Agilent

6520 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent, Polo Alto, CA). NMR spectra were obtained on INOVA-500 (Varian, Polo Alto, CA) and Bruker AV600-III (Bruker Corporation, Karlsruhe, Germany) spectrometers. A Shimadazu LC-6AD instrument (SPD-20A and RID-10A detectors) was used for preparative HPLC separations. D101 type macroporous resin, Baoen Corporation (Cangzhou, China); Sephadex LH-20, GE Chemical Corporation (Waupaca); silica gel and GF254 TLC plates, Jiangyou Corporation (Yantai, China); and ODS (50 μm), Merck (Darmstadt, Germany) were used for column chromatography.

3.2. Plant material

Twigs and leaves of *Rhododendron decorum* were collected from Chuxiong, Yunnan Province in April 2014. The plant was authenticated by associate Prof. Lin Ma of Peking Union Medical College. A voucher specimen (ID-s-2603) was deposited in the herbarium of our institute.

3.3. Extraction and isolation

Twigs and leaves of *R. decorum* (100 kg) were extracted twice with EtOH at reflux. The crude extract was then loaded on a Soxhlet extractor and extracted successively with petroleum ether, CH_2Cl_2, EtOAc, and MeOH. The EtOAc extract (1.4 kg) was separated on a macroporous resin column eluted sequentially with 70:30, 40:60, and 5:95 (v/v) H_2O-EtOH solutions. Then, a silica gel CC was used to separate the 30% EtOH fraction (376 g). The column was eluted with a step gradient of CH_2Cl_2/MeOH (20:1, 10:1, 5:1, and 1:1, v/v) and yielded fractions $E_{30}G_1$-$E_{30}G_6$. Fraction $E_{30}G_3$ (20.1 g) was further separated via Sephadex LH-20 column eluted with MeOH – H_2O (60:40, v/v) and yielded six fractions ($E_{30}G_3L_1$–$E_{30}G_3L_6$). Fraction $E_{30}G_3L_1$ (12.1 g) was separated via ODS column and eluted with a step gradient of MeOH/H_2O (10:90, 20:80, 30:70, 50:50, 30:70, and 100:0, v/v) to yield 7 fractions, $E_{30}G_3L_1M_1$ to $E_{30}G_3L_1M_7$. Fraction $E_{30}G_3L_1M_4$ (2.3 g) was then separated via preparative HPLC (MeOH-H_2O, 24:76, v/v, 5 ml/min) and yielded five fractions, $E_{30}G_3L_1M_4$-1 to $E_{30}G_3L_1M_4$-5. Fraction M_4-2 (103.2 mg) was purified via semi-preparative HPLC (MeCN-H_2O, 9:91, v/v, 3.5 ml/min) to afford **1** (2.2 mg, $t_R = 20.4$ min) and **4** (29.6mg, $t_R = 24.2$ min). Fraction $E_{30}G_3L_1M_6$ (1.2 g) was then separated via preparative HPLC (MeOH-H_2O, 24:76, v/v, 5 ml/min) and yielded eight fractions, $E_{30}G_3L_1M_6$-1 to $E_{30}G_3L_1M_6$-8. Fraction M_6-3 (35.2 mg) was purified via semi-preparative HPLC (MeCN-H_2O, 14:86, v/v, 3.5 ml/min) to afford **7** (4.5 mg, $t_R = 24.8$ min). Fraction $E_{30}G_4$ (37.3 g) was separated via Sephadex LH-20 column and yielded two fractions ($E_{30}G_4L_1$ and $E_{30}G_4L_2$). Fraction $E_{30}G_4L_1$ (24.1 g) was separated via ODS column to yield 6 fractions, $E_{30}G_4L_1M_1$ to $E_{30}G_4L_1M_6$. Compound **2** (11.6 mg, $t_R = 22.4$ min), **5** (7.8mg, $t_R = 20.7$ min) and **6** (1.9 mg, $t_R = 32.0$ min) were isolated from $E_{30}G_4L_1M_3$ using HPLC with MeCN – H_2O (14:86, v/v, 3.5 ml/min). Compound **3** (7.4 mg, $t_R = 37.3$ min) was isolated from the 60% EtOH fraction of the macroporous resin column using HPLC with MeCN – H_2O (40:60, v/v, 3.5 ml/min).

3.3.1. Rhododeonin A

White powder; $[\alpha]^{20}_{D}$–28.8 (*c* 0.03, MeOH); IR ν_{max} 3378, 2958, 1782, 1679, 1610, 1521, 1462, 1390, 1279, 1201, 1131, 1076, 1040, 971, 821, 724 cm^{-1}; ^{1}H NMR (600 MHz, Pyridine-d_5) and ^{13}C NMR (150 MHz, Pyridine-d_5) spectral data, see Tables 1 and 2; HRESIMS *m/z*: 377.0844 $[M+Na]^+$ (calcd for $C_{16}H_{18}O_9Na$, 377.0843)

3.3.2. Rhododeoside A

White powder; $[\alpha]^{20}_{D}$–4.4 (*c* 0.12, MeOH); IR ν_{max} 3399, 2935, 1671, 1644, 1621, 1514, 1460, 1424, 1365, 1274, 1078, 1035, 900, 832 cm^{-1}; ^{1}H NMR (600 MHz, Pyridine-d_5) and ^{13}C NMR (150 MHz, Pyridine-d_5) spectral data, see Tables 1 and 2; HRESIMS *m/z*: 441.2115 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_9Na$, 441.2095).

3.3.3. Rhododeoside B

White powder; $[\alpha]^{20}_{D}$ +58.3 (*c* 0.14, MeOH); IR ν_{max} 3383, 2923, 2867, 1730, 1645, 1453, 1378, 1306, 1268, 1225, 1071, 1049, 1030, 964, 809 cm^{-1}; ^{1}H NMR (500 MHz, Pyridine-d_5) and ^{13}C NMR (125 MHz, Pyridine-d_5) spectral data, see Tables 1 and 2; HRESIMS *m/z*: 673.395 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922)

3.4. Acid hydrolysis and GC analysis of the sugar moieties of compounds 2 and 3

Compounds **2** and **3** (1.0 mg) were added to 2 N HCl (2 ml). The solution was heated at 90 °C for 12 h. The reaction mixture was evaporated and partitioned with EtOAc and H_2O. The aqueous layer was concentrated to furnish the sugar mixture, which was dissolved in dry pyridine and reacted with $_{L}$-cysteine methyl ester hydrochloride (2 mg) at 80 °C for 1 h. After removal of the solvent, *N*-trimethylsilylimidazole (1 ml) was added, and the mixture was heated at 80 °C for 0.5 h. The mixture was evaporated to dryness, and the residue partitioned into *n*-hexane and H_2O. The sample was analyzed on a GC system equipped with an FID (detector temperature, 300 °C). Chromatography conditions: injection temperature, 280 °C; column, HP-5 (60 m × 0.32 mm × 0.25 μm); initial column temperature, 200 °C; column temperature increased to 280 °C (10 °C/min) and kept at 280 °C for 40 min under N_2 carrier gas (1.8 ml/min).

In the GC chromatogram, the derivatives of the acid hydrolysate of **2**, **3** and the $_{D}$-glucose (standard) showed approximate retention times of 29.61, 29.61, and 29.62 min, respectively.

3.5. Anti-neuroinflammatory activity assays

Compounds (**5**–**7**) were tested for their anti-neuroinflammatory activity by measuring inhibitory effects of LPS-induced NO production in BV2 cells. Curcumin was used as the positive control. The BV2 macrophage cell line was obtained from the Cell Culture Center at the Institute of Basic Medical Sciences, Peking Union Medical College. LPS was bought from Sigma-Aldrich company. After preincubation for 24 h

in a 96-well plate, the cells were treated with the test compounds (10^{-5} mol/L), then stimulated with LPS for 24 h. The production of NO was determined via measuring the concentration of nitrite in the culture supernatant. $NaNO_2$ was utilized to generate a standard curve. The absorbances at 550 nm were measured.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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