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Characterization of Phenolic Compounds from *Rhododendron alutaceum*

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A new phenolic glycoside, 3'-keto rhododendrin (**1**) and a new sesquilignan, alutaceuol (**2**), together with twelve known phenolic compounds, were isolated from the leaves of *Rhododendron alutaceum*. Their structures were elucidated by extensive spectroscopic data analysis and comparison with literature values. In addition, the detailed analysis of 2D NMR data led us to conclude that the chemical shifts of dihydrobuddlenol B (**5**) need to be revised.

Key words: *Rhododendron alutaceum*, Ericaceae, 3'-Keto rhododendrin, Alutaceuol

INTRODUCTION

Many species of the genus *Rhododendron* which belongs to the family Ericaceae are famous ornamental flowers and have been used as Chinese traditional medicine with anti-inflammatory, antitussive and antipyretic activities (Flora of China Editorial Committee, 2005). *Rhododendron alutaceum* Balf. F., a shrub only growing in the high mountain forest of southwest China, is used as herbal medicine for relieving cough and eliminating phlegm (Jiangsu New Medical College, 1986). So far, there is no report on the phytochemical investigation of this plant. In a systematic study on chemical constituents of species in different genera belonging to the family Ericaceae (Wang et al., 2010a, 2010b; Wu et al., 2011), two new phenolic compounds, 3'-keto rhododendrin (**1**) and alutaceuol (**2**), along with three known 4-(4-hydroxyphenyl)-2-butanol derivatives, four known lignans and five known flavonoids, were isolated from the leaves of *R. alutaceum*. Herein, details of the isolation and structural elucidation of these two new compounds are presented. Moreover, through the extensive analyzing 2D NMR spectra, the chemical shifts of the previously reported dihydrobuddlenol B (**5**), which was elucidated on the basis of 1D NMR data, need to be revised for being consistent with the 2D NMR data.

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MATERIALS AND METHODS

General experimental procedures

Optical rotation was measured with a JascoDIP-370 digital polarimeter. 1D and 2D NMR spectra were recorded using Bruker AM-400 and DRX-500 instruments with tetramethylsilane (TMS) as an internal standard. FAB-MS was measured on a VG Auto Spec-3000 spectrometer, and HR-ESI-MS was taken on an API Qstar Pulsar instrument. Semipreparative HPLC was performed on an Agilent 1200 liquid chromatograph with a ZORBAX SB-C18 (5 μm, 9.4 × 250 mm) column. Column chromatography (CC) was carried out on silica gel (80-100 mesh, 100-200 mesh and 200-300 mesh, Qingdao Marine Chemical Factory), Lichroprep® RP-18 (43-63 μm, Merck), Sephadex LH-20 (Amersham Biosciences AB) and MCI (MCI-gel CHP-20P, 75-150 μm, Mitsubishi Chemical Corporation). Fractions were monitored by TLC plates (Si gel GF_{254}, Qingdao Marine Chemical Factory), and visualized by heating after having sprayed with 5% H_2SO_4-EtOH.

Plant material

The sample of *R. alutaceum* was collected from Cang Montain, Dali, Yunnan Province, P. R. China, in August 2008, and was identified by Dr. Yong-Peng Ma, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KMUST 20080001) was deposited at the Laboratory of Phytochemistry, Kunming Uni-

versity of Science and Technology.

Extraction and isolation

Air-dried leaves (4 kg) of *R. alutaceum* were powdered and extracted with 75% Me_2CO/H_2O (2×25 L, 2d, each) at room temperature and filtered. The filtrate was concentrated *in vacuo* and the resulting residue was extracted with petroleum ether and EtOAc, respectively.

The EtOAc extract (75 g) was chromatographed over Sephadex LH-20, eluting with 30%, 60%, 90% MeOH-H_2O to afford fractions I-IV. Fraction II (6 g) was subjected to MCI gel column (eluted with 30%, 60%, and 90% MeOH-H_2O) to give four fractions (fr. 2.1-fr. 2.4). Fraction 2.1 was further purified by repeated on silica gel column (CH_3Cl-MeOH 10:1) and yielded compounds **1** (10 mg) and **3** (15 mg). Fraction 2.2 was chromatographed over silica gel (CH_3Cl-MeOH 10:1), RP-18 (MeOH-H_2O 2:3) and HPLC (MeOH-H_2O 1:1) repeatedly, to obtain compounds **2** (7 mg), **5** (7 mg), **8** (6 mg) and **9** (5 mg). Compound **10** (15 mg) was purified from fraction 2.3 by RP-18 (eluted with MeOH-H_2O 1:4) and Sephadex LH-20 (eluted with 30%, 60%, 90% MeOH-H_2O). After repeated silica gel column chromatography (petroleum ether/Me_2CO 9:1 and petroleum ether/EtOAc 4:1, respectively), fraction 2.4 afforded **4** (11 mg) and **7** (10 mg). Fraction III (12 g) was subjected to MCI gel (30%, 60%, and 90% MeOH-H_2O), Sephadex LH-20 (30%, 60%, 90% MeOH-H_2O) and RP-18 (10%-60% MeOH eluate) to give compounds **11** (15 mg), **12** (20 mg), **13** (30 mg), **14** (13 mg) and **15** (15 mg).

3'-Keto rhododendrin (1)

Amorphous powder. $[\alpha]_D^{22}$ = –29.58° (*c* = 0.46, CH_3OH); FAB$^-$-MS: *m/z* 325 $[M-H]^-$; HR-ESI-MS ($[M-H]^-$, $C_{16}H_{21}O_7^-$, found 325.1285, calcd. 325.1287). IR (KBr) cm^{-1}: 3645, 1710, 1610, 1498, 1045. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.04 (2H, d, *J* = 8.4 Hz, H-2, 6), 6.67 (2H, d, *J* = 8.4 Hz, H-3, 5), 1.70 (1H, m, H-7a), 1.87 (1H, m, H-7b), 2.64 (2H, m, H-8), 3.93 (1H, m, H-9), 1.19 (3H, d, *J* = 6.4 Hz, H-10), 4.43 (1H, d, *J* = 7.8 Hz, H-1'), 4.11 (1H, d, *J* = 7.8 Hz, H-2'), 4.25 (1H, d, *J* = 1.6 Hz, H-4'), 3.30 (1H, m, H-5'), 3.93 (1H, dd, *J* = 2.0, 12.0 Hz, H-6'a), 3.83 (1H, dd, *J* = 4.4, 12.0 Hz, H-6'b).^{13}C-NMR (125 MHz, CD_3OD) δ: 134.5 (C-1), 130.1 (C-2, 6), 116.2 (C-3, 5), 156.3 (C-4), 31.8 (C-7), 40.5 (C-8), 75.7 (C-9), 20.0 (C-10), 103.8 (C-1'), 78.1 (C-2'), 207.3 (C-3'), 73.6 (C-4'), 78.3 (C-5'), 62.5 (C-6').

Acid hydrolysis of 1

A solution of **1** (7 mg) in 1 M HCl (1 mL) was heated at 90-100°C in a screw-capped vial for 5 hours. The mixture was partitioned between EtOAc (1 mL), and the EtOAc layer was further purified by Sephadex LH-20 (40-100% MeOH in H_2O) to yield (-)-rhododendrol (**4**) (2.4 mg, $[\alpha]_D^{22}$ = –16.7°, c = 0.3, CH_3OH), which was identified by ^{1}H-NMR data and negative optical rotation.

Alutaceuol (2)

Amorphous powder. $[\alpha]_D^{22}$ = –4.56° (*c* = 0.5, CH_3OH); CD (*c* = 0.29, MeOH) (mdeg): 205 (+9.68), 209 (-9.41). FAB$^-$-MS: *m/z* 571 $[M-H]^-$; HR-ESI-MS ($[M-H]^-$, $C_{30}H_{35}O_{11}^-$, found 571.2171, calcd. 571.2179). IR (KBr) cm^{-1}: 3435, 1610, 1508, 1040. ^{1}H- and ^{13}C-NMR spectral data: see Table I.

Rhododendrin (3)

Amorphous powder. $[\alpha]_D^{22}$ = –33.85° (*c* = 0.88, CH_3OH); ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 7.24 (1H, d, *J* = 8.4 Hz, H-2, 6), 7.16 (1H, d, *J* = 8.4 Hz, H-3, 5), 2.79 (2H, m, H-7), 1.75 (2H, m, H-8), 3.59 (1H, m, H-9), 1.24 (3H, d, *J* = 6.1 Hz, H-10), 4.90 (1H, d, *J* = 7.7 Hz, H-1'), 3.90-4.6 (5H, H-2'- H-5'). ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 133.4 (s, C-1), 130.2 (d, C-2, 6), 116.2 (d, C-3, 5), 157.0 (s, C-4), 31.3 (t, C-7), 40.2 (t, C-8), 73.6 (d, C-9), 20.2 (q, C-10), 102.5 (s, C-1'), 75.3 (d, C-2'), 78.7 (d, C-3'), 71.8 (d, C-4'), 78.5 (d, C-5'), 62.9 (t, C-6').

(–)-Rhododendrol (4)

Amorphous powder. $[\alpha]_D^{22}$ = –16.68° (*c* = 0.44, CH_3OH); ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 1.16 (3H, d, *J* = 6.1 Hz, H-10), 3.76 (1H, m, H-9), 1.64 (2H, m, H-8), 2.60 (2H, m, H-7), 7.02 (2H, d, *J* = 8.3 Hz, H-2, 6), 6.74 (2H, d, *J* = 8.3 Hz, H-3, 5); ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 133.9 (s, C-1), 129.9 (d, C-2, 6), 115.8 (d, C-3, 5), 156.0 (s, C-4) , 31.8 (t, C-7) , 42.2 (t, C-8), 67.0 (d, C-9), 23.9 (q, C-10).

Dihydrobuddlenol B (5)

Amorphous powder. FAB$^-$-MS: *m/z* 585 $[M-H]^-$. IR (KBr) cm^{-1}: 3435, 1610, 1509. ^{1}H- and ^{13}C-NMR spectral data: see Table I.

(–)-Rhodolatouchol (7)

Amorphous powder. $[\alpha]_D^{22}$ = –18.20° (*c* = 0.44, CH_3OH); ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 6.49 (1H, br s, H-2), 6.47 (1H, d, *J* = 7.8 Hz, H-5), 6.29 (1H, d, *J* = 7.8 Hz, H-6), 2.50 (2H, m, H-7), 1.64 (2H, m, H-8), 3.70 (1H, m, H-9), 1.11 (3H, d, *J* = 6.0 Hz, H-10); ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 135.0 (s, C-1), 116.2 (d, C-2), 143.7 (s, C-3), 145.7 (s, C-4), 115.9 (d, C-5), 120.2 (d, C-6), 32.1 (t, C-7) , 42.3 (t, C-8), 66.9 (d, C-9) , 23.9 (q, C-10).

Ficusequilignan A (8)

Amorphous powder. FAB$^-$-MS: *m/z* 585 $[M-H]^-$, ^{1}H-

NMR (CD_3OD, 400 MHz): δ 6.65 (s, H-2,6), 4.75 (d, *J* = 3.2 Hz, H-7), 3.13 (m, Hz, H-8), 3.89, 4.28 (m, H-9), 6.91 (d, *J* = 1.9 Hz, H-2'), 6.90 (d, *J* = 8.1 Hz, H-5'), 6.91 (dd, *J* = 8.0, 1.9 Hz, H-6'), 4.70 (d, *J* = 4.4 Hz, H-7'), 3.13 (m, H-8'), 3.89, 4.28 (m, H-9'), 6.96 (s, H-2"), 6.76 (d, *J* = 8.0 Hz, H-5"), 6.76 (dd, *J* = 8.0, 1.8 Hz, H-6"), 4.90 (t, *J* = 3.4 Hz, H-7"), 4.25 (1H, m, H-8"), 3.89 (1H, m, H-9"a), 3.59 (1H, dd, *J* = 2.7, 12.1 Hz, H-9"b). ^{13}C-NMR (CD_3OD, 100 MHz): δ 138.9 (s, C-1), 104.2 (d, C-2), 154.5 (s, C-3), 136.0 (s, C-4), 154.5 (s, C-5), 104.2 (d, C-6), 87.3 (d, C-7), 55.3 (d, C-8), 72.7 (t, C-9), 133.7 (s, C-1'), 110.9 (d, C-2'), 148.9 (s, C-3'), 147.3 (s, C-4'), 116.0 (d, C-5'), 120.1 (d, C-6'), 87.3 (d, C-7'), 55.7 (d, C-8'), 72.9 (t, C-9'), 133.7 (s, C-1"), 111.3 (d, C-2"), 148.5 (s, C-3"), 146.8 (s, C-4"), 115.6 (d, C-5"), 120.7 (d, C-6"), 74.0 (d, C-7"), 87.5 (d, C-8"), 61.6 (t, C-9").

Table I. ^{1}H- and ^{13}C-NMR Data of **2** and **5** (500 MHz for ^{1}H and 125 MHz for ^{13}C, CD_3OD)

No.	**2**		**5**	
	^{13}C	^{1}H	^{13}C	^{1}H
2	88.2	5.55 (dd, 2.1, 5.6)	88.0	5.56 (d, 6.5)
3	56.0	3.43 (m)	55.1	3.43 (m)
3a	65.2	3.86 (m) 3.75 (m)	64.7	3.81 (m) 3.75 (m)
4	116.7	6.59 (s)	117.5	6.82 (s)
4a	129.3	-	129.6	-
5	137.0	-	136.5	-
5a	32.7	2.56 (t, 8.0)	32.6	2.56 (t, 7.7)
5b	35. 8	1.78 (m)	35.9	1.77 (m)
5c	62.3	3.54 (m)	61.8	3.54 (m)
6	117.1	6.58 (s)	113.9	6.81 (s)
7	141.9	-	144.9	-
7a	146.8	-	147.1	-
1'	140.0	-	139.3	-
2'	103.8	6.75 (s)	104.0	6.73 (s)
3'	154.5	-	154.2	-
4'	136.1	-	135.9	-
5'	154.5	-	154.2	-
6'	103.8	6.75 (s)	104.0	6.73 (s)
1"	133.8	-	133.7	-
2"	111.3	6.96 (d, 2.0)	110.7	7.02 (d, 2.0)
3"	148.6	-	147.9	-
4"	146.4	-	146.4	-
5"	115.7	6.72 (d, 8.0)	115.2	6.75 (d, 8.0)
6"	120.6	6.77 (dd, 2.0, 8.0)	119.9	6.83 (dd, 2.0, 8.0)
7"	74.0	4.89 (d, 4.9)	73.3	4.97 (d, 4.9)
8"	87.4	4.23 (m)	87.8	4.19 (m)
9"	61.6	3.87 (m) 3.54 (m)	60.8	3.91 (m) 3.52 (m)
3'-OMe	56.6	3.79 (s)	56.5	3.82 (s)
5'-OMe	56.6	3.79 (s)	56.5	3.82 (s)
3"-OMe	56.3	3.82 (s)	56.1	3.84 (s)
7-OMe	-	-	56.4	3.81 (s)

Ehletianol C (9)

Amorphous powder. FAB$^-$-MS: *m/z* 555 [M-H]$^-$, ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 7.01 (d, *J* = 1.5 Hz, H-2), 6.74 (d, *J* = 8.0 Hz, H-5), 6.85 (dd, *J* = 8.0, 1.5 Hz, H-6), 4.79 (d, *J* = 6.2 Hz, H-7), 4.24 (dd, *J* = 11.7, 6.75 Hz, H-8), 3.71 (dd, *J* = 12.0, 4.0 Hz, H-9a), 3.46 (dd, *J* = 12.0, 5.2 Hz, H-9b), 6.87 (d, *J* = 1.5 Hz, H-2'), 6.97 (d, *J* = 8.0 Hz, H-5'), 6.71 (dd, *J* = 8.0, 1.5 Hz, H-6'), 2.95 (dd, *J* = 13.0, 5.0 Hz, H-7'), 2.72 (m, H-8'), 3.97 (1H, dd, *J* = 8.0, 10.2 Hz, H-9'a), 3.67 (1H, dd, *J* = 8.0, 10.2 Hz, H-9'b), 6.90 (d, *J* = 1.5 Hz, H-2"), 6.75 (d, *J* = 8.0 Hz, H-5"), 6.76 (dd, *J* = 8.0, 1.5 Hz, H-6"), 4.74 (dd, *J* = 6.8, 1.0 Hz, H-7"), 2.33 (1H, t, *J* = 8.5 Hz, H-8"), 3.82 (dd, *J* =11.0, 8.0 Hz, H-9"a), 3.63 (dd, *J* =11.0, 8.0 Hz, H-9"b). ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 134.3 (s, C-1), 111.3 (d, C-2), 147.9 (s, C-3), 146.6 (s, C-4), 113.9 (d, C-5), 120.5 (d, C-6), 73.0 (d, C-7), 86.9 (d, C-8), 61.8 (t, C-9), 136.5 (s, C-1'), 113.8 (d, C-2'), 151.8 (s, C-3'), 147.9 (s, C-4'), 119.2 (d, C-5'), 121.7 (d, C-6'), 33.5 (t, C-7'), 43.3 (d, C-8'), 73.7 (t, C-9'), 134.3 (s, C-1"), 110.6 (d, C-2"), 148.1 (s, C-3"), 147.1 (s, C-4"), 115.3 (d, C-5"), 119.7 (d, C-6"), 83.2 (d, C-7"), 53.9 (d, C-8"), 60.3 (t, C-9").

2,3-Dihydrobenzofuran-2-(4'-hydroxy-3'-methoxyphenyl)-3-α-L-rhamnopyranosyloxymethyl-7-methoxy-5-propanol (10)

Amorphous powder. ^{1}H-NMR (CD_3OD, 400 MHz): δ 1.18 (3H, d, *J* = 6.4 Hz, H-6"), 1.80 (2H, m, H-8'), 2.66 (2H, t, *J* = 7.4 Hz, H-9'), 4.75 (1H, d, *J* = 1.2 Hz, H-1"), 3.33-3.83 (4H, m, H-2"-5"), 3.65 (1H, m, H-8), 3.59 (2H, m, H-7'), 3.65 (1H, dd, *J* = 6.0, 12.0 Hz, H-9a), 3.84 (3H, s, -OCH_3), 3.85 (3H, s, -OCH_3), 4.00 (1H, dd, *J* = 6.0, 12.0 Hz, H-9b), 5.47 (1H, d, *J* = 6.9 Hz, H-7), 6.79 (1H, d, *J* = 2.0 Hz, H-2'), 6.72 (1H, d, *J* = 2.0 Hz, H-6'), 6.81 (1H, d, *J* = 2.0 Hz, H-2), 7.01 (1H, d, *J* = 8.0 Hz, H-5), 6.87 (1H, dd, *J* = 2.0, 8.0 Hz, H-6). ^{13}C-NMR (CD_3OD, 100 MHz): δ 134.1 (s, C-1), 119.4 (d, C-2), 147.1 (s, C-3), 144.8 (s, C-4), 117.2 (d, C-5), 115.6 (d, C-6), 88.6 (d, C-7), 52.2 (d, C-8), 69.5 (t, C-9), 129.0 (s, C-1'), 110.2 (d, C-2'), 147.1 (s, C-3'), 144.8 (s, C-4'), 136.3 (d, C-5'), 113.8 (d, C-6'), 35.6 (t, C-7'), 32.5 (t, C-8'), 61.6 (t, C-9'), 101.0 (s, C-1"), 71.8 (d, C-2"), 72.3 (s, C-3"), 73.5 (s, C-4"), 69.9 (d, C-5"), 18.1 (d, C-6").

Quercetin 3-*O*-β-D-galactopyranoside (11)

Amorphous powder. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.19 (1H, d, *J* = 1.9 Hz, H-6), 6.39 (1H, d, *J* = 1.9 Hz, H-8), 7.51 (1H, d, *J* = 2.1 Hz, H-2'), 6.80 (1H, d, *J* = 8.5 Hz, H-5'), 7.65 (1H, dd, *J* = 2.1, 8.3 Hz, H-6'), 5.36 (1H,

d, J = 7.7 Hz, H-1''), 3.15-3.63 (5H, m, H-2''~6''), 12.63 (1H, s, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 156.3 (s, C-2), 133.5 (s, C-3), 177.5 (s, C-4), 161.3 (s, C-5), 98.7 (d, C-6), 164.1 (s, C-7), 93.5 (d, C-8), 156.2 (s, C-9), 103.9 (s, C-10), 121.1 (s, C-1'), 115.2 (d, C-2'), 144.8 (s, C-3'), 148.5 (s, C-4'), 115.9 (d, C-5'), 122.0 (d, C-6'), 101.8 (d, C-1''), 71.2 (d, C-2''), 73.2 (d, C-3''), 67.9 (d, C-4''), 75.9 (d, C-5''), 60.1 (t, C-6'').

Quercetin 3-*O*-α-L-arabinofuranoside (12)
Amorphous powder. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.19 (1H, d, J = 1.8 Hz, H-6), 6.39 (1H, d, J = 1.8 Hz, H-8), 7.46 (1H, d, J = 2.2 Hz, H-2'), 6.84 (1H, d, J = 8.5 Hz, H-5'), 7.54 (1H, dd, J = 2.1, 8.5 Hz, H-6'), 5.57 (1H, s, H-Ara-1), 4.14-3.29 (6H, m, H-Ara-2~6), 12.64 (1H, s, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 156.9 (s, C-2), 133.4 (s, C-3), 177.7 (s, C-4), 161.2 (s, C-5), 98.7 (d, C-6), 164.2 (s, C-7), 93.6 (d, C-8), 156.3 (s, C-9), 103.9 (s, C-10), 120.9 (s, C-1'), 115.5 (d, C-2'), 144.8 (s, C-3'), 148.5 (s, C-4'), 115.5 (d, C-5'), 121.7 (d, C-6'), 107.8 (d, C-1''), 82.1 (d, C-2''), 76.9 (d, C-3''), 85.8 (d, C-4''), 60.6 (t, C-5'').

Quercetin (13)
Amorphous powder. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.17 (1H, d, J = 2.0 Hz, H-6), 6.41 (1H, d, J = 2.0 Hz, H-8), 7.66 (1H, d, J = 2.1 Hz, H-2'), 6.87 (1H, d, J = 8.4 Hz, H-5'), 7.54 (1H, dd, J = 2.1, 8.4 Hz, H-6'); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 146.8 (s, C-2), 135.8 (s, C-3), 175.9 (s, C-4), 156.2 (s, C-5), 98.2 (d, C-6), 163.9 (s, C-7), 93.4 (d, C-8), 160.8 (s, C-9), 103.0 (s, C-10), 122.0 (s, C-1'), 115.0 (d, C-2'), 145.1 (s, C-3'), 147.7 (s, C-4'), 115.6 (d, C-5'), 120.0 (d, C-6').

Catechin (14)
Amorphous powder. ^{1}H-NMR (CD_3COCD_3, 400 MHz): δ 4.53 (1H, d, J = 7.7 Hz, H-2), 4.42 (1H, m, H-3), 2.88 (1H, dd, J = 5.0, 16.7 Hz, H-4a), 2.56 (1H, dd, J = 8.0, 16.7 Hz, H-4b), 5.83 (1H, d, J = 2.3 Hz, H-6), 5.99 (1H, d, J = 2.3 Hz, H-8), 6.92 (1H, d, J = 1.9 Hz, H-2'), 6.73 (1H, d, J = 8.1 Hz, H-5'), 6.70 (1H, dd, J = 1.9, 8.1 Hz, H-6'). ^{13}C-NMR (CD_3COCD_3, 100 MHz): δ 82.8 (d, C-2), 68.8 (d, C-3), 28.5 (t, C-4), 157.8 (s, C-5), 96.3 (s, C-6), 156.9 (s, C-7), 95.5 (d, C-8), 157.6 (s, C-9), 99.4 (s, C-10), 131.8 (s, C-1'), 115.4 (d, C-2'), 145.4 (s, C-3'), 145.5 (s, C-4'), 115.5 (d, C-5'), 118.9 (d, C-6').

Myricetin (15)
Amorphous powder. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 4.49 (1H, d, J = 11.1 Hz, H-2), 4.91 (1H, d, J = 11.1 Hz, H-3), 5.94 (1H, d, J = 2.0 Hz, H-6), 5.91 (1H, d, J = 2.0 Hz, H-8), 6.56 (2H, br s, H-2', 6'). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 84.6 (d, C-2), 73.0 (d, C-3), 198.2 (s, C-4), 164.8 (s, C-5), 96.8 (d, C-6), 168.2 (s, C-7), 95.9 (d, C-8), 163.8 (s, C-9), 101.3 (s, C-10), 128.1 (s, C-1'), 107.9 (d, C-2'), 146.5 (s, C-3'), 134.2 (s, C-4'), 146.5 (s, C-5'), 107.9 (d, C-6').

RESULTS AND DISCUSSION

The EtOAc-soluble fraction of the 75% aq. acetone extract from the leaves of *R. alutaceum* was repeatedly chromatographed with various solvent systems on normal and reversed-phase columns, Sephadex LH-20 as well as C-18 semipreparative HPLC to afford a new phenolic glycoside, 3'-keto rhododendrin (**1**), and a new sesquilignan, alutaceuol (**2**), together with 12 known phenolic compounds.

Compound **1** was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{22}$ = –29.58°). A quasi-molecular ion peak at m/z 325.1285 ($[M-H]^-$) in the negative HR-ESI-MS indicated the molecular formula of $C_{16}H_{22}O_7$, and corresponding to six degrees of unsaturation. The IR spectrum showed the absorption bands of hydroxyl (3465 cm^{-1}), carbonyl (1710 cm^{-1}) and aromatic (1610, 1498 cm^{-1}) groups.

The ^{1}H- and ^{13}CNMR spectra of **1** exhibited the presence of a *p*-substituted benzene ring [δ_H 7.04 (2H, d, J = 8.4 Hz, H-2, 6; δ_C 130.1), 6.67 (2H, d, J = 8.4 Hz, H-3, 5; δ_C 116.2)], a methyl [δ_H 1.19 (3H, d, J = 6.4 Hz, H-10); δ_C 20.0], two methylenes [δ_H 2.64 (2H, m, H-8), 1.70, 1.87 (each, 1H, m, H-7); δ_C 40.5, 31.8], an oxygenated methine [δ_H 3.93 (1H, m, H-9); δ_C 75.7], and a ketohexopyranosyl moiety [δ_H 4.43 (1H, d, J = 7.8 Hz, H-1'), 4.11 (1H, d, J = 7.8 Hz, H-2'), 4.25 (1H, d, J = 1.6 Hz, H-4'), 3.30 (1H, m, H-5'), 3.93 (1H, dd, J = 2.0, 12.0 Hz, H-6'a), 3.83 (1H, dd, J = 4.4, 12.0 Hz, H-6'b); δ_C 103.8 (C-1'), 78.1 (C-2'), 207.3 (C-3'), 73.6 (C-4'), 78.3 (C-5'), 62.5 (C-6')].

Analysis of the ^{1}H-^{1}H COSY and HMBC spectra of **1** revealed that the aglycone moiety was 4-(3-hydroxybutyl) phenol. The proton and carbon signals of **1** were similar to those of rhododendrin (**3**) (Das et al., 1993), except for the signals of sugar moiety. The ketone group in the ketoside was located at C-3', as the evidence from the HMBC correlations of the protons H-1', H-2', and H-4' with C-3' (δ_C 207.3) (Fig. 2). Furthermore, the HMBC correlation from the anomeric proton (H-1') to C-9 of the aglycone indicated that the ketoside should be connected to C-9 with β-configuration due to large coupling constant ($J_{1',2'}$ = 7.8 Hz). Acidic hydrolysis of **1** with 1 M HCl afforded (–)-rhododendrol (**4**) (Das et al., 1993) with negative optical rotation ($[\alpha]_D^{22}$ = –16.7°), suggesting that the absolute configuration of 4-(4-hydroxyphenyl)-2-butanol was *R*-form. Thus, compound **1** was assigned as the 3'-keto derivative of rho-

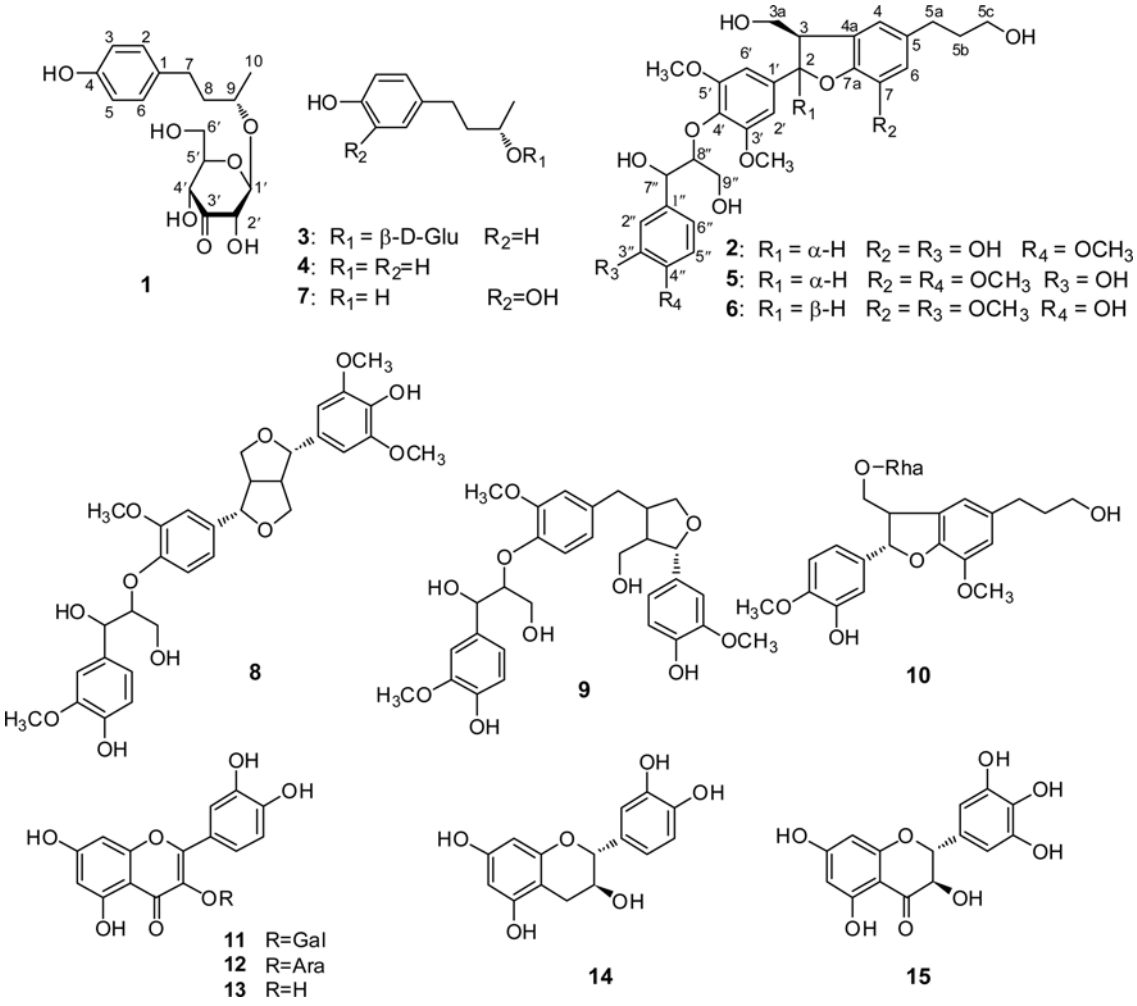

Fig. 1. Chemical structures of **1**-**15**.

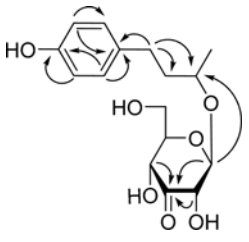

Fig. 2. Key HMBC Correlations of **1**.

dodendrin, and named 3'-keto rhododendrin.

Compound **2**, $[\alpha]_D^{22} = -4.56^o$ ($c = 0.51$, CH_3OH), was obtained as amorphous powder, and has the molecular formula of $C_{30}H_{36}O_{11}$ as determined by HR-ESI-MS. The IR spectrum of **2** showed absorption bands at 3435, 1610, and 1508 cm^{-1} ascribable to hydroxyl and aromatic functions.

The ^{13}C-NMR and DEPT spectra of **2** showed 30 carbon signals, including three methoxyl groups at δ_C 56.6, 56.6 and 56.3 ppm. The ^{1}H NMR spectrum of **2** exhibited three methoxyls [δ_H 3.79 (6H, s, 3', 5'-OCH_3), 3.82 (3H, s, 4''-OCH_3)], as well as seven aromatic protons including two symmetric cones of a tetra-substituted benzene ring [δ_H 6.75 (2H, s, H-2', 6')], two asymmetric cones [δ_H 6.59 (1H, s, H-4), 6.58 (1H, s, H-6)], and three ones of a 1,3,4-trisubstituted benzene ring as an ABX system [δ_H 6.96 (1H, d, J = 2.0 Hz, H-2''), 6.72 (1H, d, J = 8.0 Hz, H-5''), 6.77 (1H, dd, J = 2.0, 8.0 Hz, H-6'')]. Furthermore, the ^{1}H-^{1}H COSY spectrum revealed the presence of three C_3 units, Ar-$CH_2CH_2CH_2OH$ [δ_H: 2.56 (2H, t, J = 8.0 Hz, H-5a), 1.78 (2H, m, H-5b), 3.54 (2H, m, H-5c)], Ar-$CHCHCH_2OH$ [δ_H: 5.55 (1H, dd, J = 2.1, 5.6 Hz, H-2), 3.43 (1H, m, H-3), 3.75, 3.86 (each 1H, m, H-3a)], and Ar-$CHCHCH_2OH$ [δ_H: 4.89 (1H, d, J = 4.9 Hz, H-7''), 4.23 (1H, m, H-8''), 3.87, 3.54 (each 1H, m, H-9'')].

Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of dihydrobuddlenol B (**5**) (Yoshinari et al., 1990) showed that they were very similar, except for the signals of the methoxyl group and the chemical shifts of H-6, C-6 and C-7 (Table I). HMBC correlations (Fig.

3) from two methoxyls (δ_H 3.79, 6H, s) to C-3' and C-5' (δ_C 154.5), and from another methoxyl (δ_H 3.82, s) to C-4" (δ_C 146.4), indicated that above methoxyl groups were located at C-3', C-5' and C-4", respectively. No HMBC correlation observed between any methoxyl and C-7, indicated that the methoxyl at C-7 in **5** was replaced by a hydroxyl group in **2**. The C-6 signal of **2** (δ_C 117.1) was shifted downfield by 3.2 ppm in comparison with that of **5** (δ_C 113.9), while the signal of C-7 (δ_C 141.9) of **2** was at a higher field than that of **5** (δ_C 144.9), further confirmed that the hydroxyl should be assigned to C-7.

Comparison of the values of the ^{1}H NMR chemical shifts and the coupling constants of H-2, H-3, H-3a, H-7" and H-8" with those of related compounds (such as **5** and acernikol **6**) suggested that they have the same 2, 3-*cis* situation and the 7", 8"-*erythro* form (Yoshinari et al., 1990; Agrawal et al., 1983; Agrawal and Thakur, 1985; Lundgren et al., 1985). In addition, the absolute stereostructure of **2** was determined by circular dichroism (CD) spectroscopic analysis. The CD spectrum of **2** showed a positive Cotton effect at 205 nm and a negative Cotton effect at 209 nm, indicating the absolute configuration of the 2-position to be in the *R*-orientation (Morikawa et al., 2003). Thus, the whole stereochemistry of **2** was (2, 3-*trans*-7", 8"-*erythro*) 7-demethyldihydrobuddlenol B and named as alutaceuol.

Compound **5** was assigned the molecular formula of $C_{31}H_{38}O_{11}$ by FAB$^-$MS (*m/z* 585 [M-H]$^-$) and ^{13}C NMR spectral data. By interpretation of its ^{1}H-and ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC and HMBC spectra followed by comparison with published ^{1}H- and ^{13}C-NMR data, the structure was found to be dihydrobuddlenol B which was previously isolated from the bark of *Prunus jamasakura* (Yoshinari et al., 1990). The previously reported NMR assignments for C-4a, 5, 5a and 5b of **5** (Table I) required to be revised because of strong HMBC correlations (Fig. 4) from H-2 (δ_H 5.56, d, J = 6.5 Hz) to C-4a (δ_C 129.6), from H-4 (δ_H 6.82, s) to C-5 (δ_C 136.5), and from H-5a (δ_H 2.56, t, J = 7.7 Hz) to C-4 (δ_C 117.5), C-5 and C-6 (δ_C 113.9). The above assignments were further supported by one proton spin system from C-5a to C-5c based on ^{1}H-^{1}H COSY and HMQC correlations.

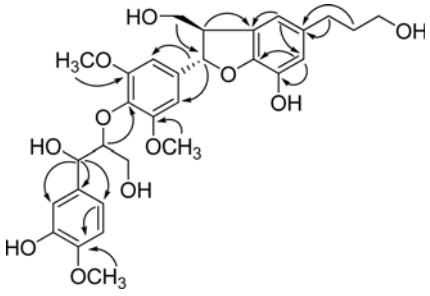

Fig. 3. Key HMBC Correlations of **2**.

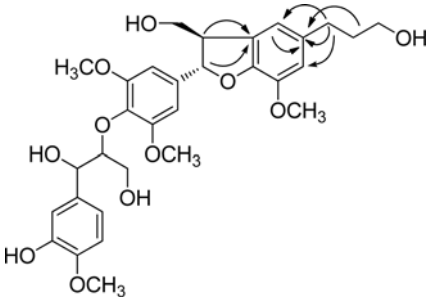

Fig. 4. Key HMBC Correlations of **5**.

By comparing physical and spectroscopic data with reported data, others known compounds were identified as rhododendrin (**3**) (Das et al., 1993), (–)-rhododendrol (**4**) (Das et al., 1993), (–)-rhodolatouchol (**7**) (Das et al., 1993), ficusequilignan A(**8**) (Cui et al., 2003), ehletianol C (**9**) (Yoshikawa et al., 1995), 2,3-dihydrobenzofuran-2-(4'-hydroxy-3'-methoxyphenyl)-3-α-L-rhamnopyranosyloxymethyl-7-methoxy-5-propanol (**10**) (Marinella and Alessandra, 2004), quercetin 3-*O*-β-D-galactopyranoside (**11**) (Zhang and Tan, 2007), quercetin 3-*O*-α-L-arabinofuranoside (**12**) (Li et al., 2005), quercetin (**13**) (Li et al., 2005), catechin (**14**) (Wang et al., 2010a), and myricetin (**15**) (Zhou et al., 2010), by comparison of their spectroscopic data with literatures.

Some species of the genus *Rhododendron* were used as Chinese traditional medicine for relieving cough and eliminating phlegm. But among of them maybe contain grayanane diterpenoids, which can be toxic to the heart and nervous systems of mammals. In this work, four 4-(4-hydroxyphenyl)-2-butanol derivatives, five lignans, and five flavonoids were isolated from *R. alutaceum*, while grayanane diterpenoids were undetectable. The above research results suggested that *R. alutaceum* is non-toxic plant, which could be used as an herbal medicine.

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