# Isolation and Characterization of Secondary Metabolites from *Rhododendron microphyton*

by **Ya-Zhu Zhu**, **Yan-Hong Li**, **Dan Liu**, **Ling-Huan Gao**, **Hong-Mei Li**, and **Rong-Tao Li***

Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, P. R. China

(phone: +86-871-65920569; fax: +86-871-65920570; e-mail: rongtaolikm@163.com)

Four new iridoids, incarvoids D–F (**1**–**3**, resp.) and incarvoid B 9-*O*-*β*-D-glucopyranoside (**4**), and one new monoterpenoid, argutoid B (**5**), along with 14 known compounds, were isolated from *Rhododendron microphyton*. Their structures were established by comprehensive 1D- and 2D-NMR spectroscopic analysis.

**Introduction.** – Plants of the genus *Rhododendron* (Ericaceae), evergreen shrubs or trees, are well-known poisonous plants widely distributed in hilly and valley regions of southern China [1]. They have long been used for the treatment of various diseases, such as injury, pain, bronchitis, cough, and osteomyelitis in Chinese folk medicine [2][3].

*Rhododendron microphyton* FRANCH. was widely used as Chinese folk medicine for the treatment of convulsion and nephritides by people of the minority areas of Yunnan province [4]. Since 2010, the systematical chemical investigation of the family Ericaceae conducted by our group led to the discovery of a series of new highly acylated diterpenoids [5–8]. As a continuation of our search for more new natural compounds with biological activities, four new iridoids, **1**–**4**, and one new monoterpenoid, **5**, along with 14 known compounds, including two iridoids, (+)-incarvoid B and (−)-incarvoid A, three sesquiterpenes, diincarvilones A and B and lemnacarnol, two triterpenes, oleanolic acid and tormentic acid, and seven phenolic derivatives, *erythro*-guaiacylglycerol *β*-*O*-4′-coniferyl ether, *threo*-guaiacylglycerol *β*-*O*-4′-coniferyl ether, clerodeoside A, verbascoside, isoverbascoside, caffeic acid methyl ester, and 4-hydroxy-3-methoxy benzoic acid, were isolated from stems of *R. microphyton.* This is the first phytochemical report of *R. microphyton.*

**Results and Discussion.** – Compound **1** was obtained as colorless needles. The molecular formula $C_{24}H_{34}O_{11}$ was established based on HR-ESI-MS (*m*/*z* 521.1996 ($[M+Na]^+$)) in combination with $^1$H- and $^{13}$C-NMR data, indicating eight degrees of unsaturation. Analysis of the $^1$H-NMR spectrum (*Table 1*) indicated the typical signals of one MeO group at $\delta$(H) 3.79 (*s*), one Me group at 1.00 (*d*, *J* = 7.2, Me(10)), a $CH_2O$ group at 3.54 (*dd*, *J* = 12.0, 2.4, $H_a$–C(3)) and 3.74 (*dd*, *J* = 12.0, 4.8, $H_b$–C(3)), one $HOCH_2$ group at 4.48 (*s*, $CH_2$(7″)), and a set of an *ABX* spin system due to an 1,3,4-trisubstituted aromatic ring (6.95 (*d*, *J* = 1.8, H–C(3″)), 6.80 (*dd*, *J* = 8.2, 1.8, H–C(5″)), and 6.98 (*d*, *J* = 8.2, H–C(6″))). Additionally, a series of typical signals of a sugar

residue were recognized, including the anomeric H-atom at $\delta$(H) 4.81 (*d*, $J = 7.4$, H–C(1′)), revealing a $\beta$-configuration present in the sugar residue on the basis of the coupling constant [9][10]. Acid hydrolysis of **1** gave D-glucose (Glc) as sugar residue, which was confirmed by GC analysis of its corresponding trimethylsilylated L-cysteine derivative. The $^{13}$C-NMR and DEPT spectra (*Table 1*) showed resonances for 24 C-atoms: one COO group ($\delta$(C) 173.5 (*s*, C(11))), three aromatic $C_q$-atoms including two O-bearing ones (146.8 (*s*, C(1″)) and 151.1 (*s*, C(2″))), 13 CH groups (of which three were aromatic and six O-bearing), five $CH_2$ groups including three O-bearing ones (61.7 (*t*, C(3)), 64.5 (*t*, C(6′)), and 64.9 (*t*, C(7″))), one Me (17.4 (*q*, C(10))), as well as one MeO group (56.6 (*q*)). The $HOCH_2$ group was unambiguously located at C(4″) ($\delta$(C) 138.0 (*s*)) of the 1,3,4-trisubstituted aromatic ring, according to the HMBCs (*Fig. 1*) of $CH_2$(7″) with C(3″) ($\delta$(C) 112.7 (*d*)), C(4″), and C(5″) (120.4 (*d*)), of H–C(3″) with C(1″), C(5″), and C(7″), as well as of H–C(5″) with C(1″) and C(7″). The HMBC of the MeO H-atoms at $\delta$(H) 3.79 with C(2″) revealed that the MeO group

Table 1. *$^1$H- and $^{13}$C-NMR Data* (600 and 150 MHz, resp.; in $CD_3OD$) *of* **1** *and* **2**. $\delta$ in ppm, $J$ in Hz.

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| 1 | 4.40 (*d*, $J = 9.0$) | 95.1 | 4.28 (*d*, $J = 7.2$) | 104.2 |
| 3 | 3.54 (*dd*, $J = 12.0, 2.4$), 3.74 (*dd*, $J = 12.0, 4.8$) | 61.7 | 4.34 (*d*, $J = 15.6$), 4.21 (*d*, $J = 15.6$) | 67.3 |
| 4 | 2.78–2.82 (*m*) | 43.1 | | 128.9 |
| 5 | 2.51–2.56 (*m*) | 41.4 | | 137.1 |
| 6 | 1.48–1.54 (*m*), 1.35–1.42 (*m*) | 23.7 | 2.35[a]) | 26.0 |
| 7 | 1.69–1.75 (*m*), 1.17–1.22 (*m*) | 32.0 | 1.74–1.80 (*m*), 1.46–1.50 (*m*) | 32.5 |
| 8 | 1.94–2.00 (*m*) | 38.4 | 2.39–2.45 (*m*) | 34.9 |
| 9 | 1.60–1.63 (*m*) | 50.2 | 2.35[a]) | 49.6 |
| 10 | 1.00 (*d*, $J = 7.2$) | 17.4 | 0.75 (*d*, $J = 6.8$) | 14.7 |
| 11 | | 173.5 | 4.04 (*d*, $J = 12.0$), 4.06 (*d*, $J = 12.0$) | 60.2 |
| 1′ | 4.81 (*d*, $J = 7.4$) | 102.4 | | |
| 2′ | 3.36–3.39 (*m*) | 74.9 | | |
| 3′ | 3.39–3.42 (*m*) | 77.7 | | |
| 4′ | 3.24–3.27 (*m*) | 71.8 | | |
| 5′ | 3.47–3.50 (*m*) | 75.4 | | |
| 6′ | 4.07 (*dd*, $J = 12.0, 7.2$), 4.38 (*dd*, $J = 12.0, 2.4$) | 64.5 | | |
| 1″ | | 146.8 | | |
| 2″ | | 151.1 | | |
| 3″ | 6.95 (*d*, $J = 1.8$) | 112.7 | | |
| 4″ | | 138.0 | | |
| 5″ | 6.80 (*dd*, $J = 8.2, 1.8$) | 120.4 | | |
| 6″ | 6.98 (*d*, $J = 8.2$) | 118.2 | | |
| 7″ | 4.48 (*s*) | 64.9 | | |
| 1-MeO | | | 3.47 (*s*) | 56.3 |
| 2″-MeO | 3.79 (*s*) | 56.6 | | |

[a]) Overlapped signals assigned by HMBC and HSQC.

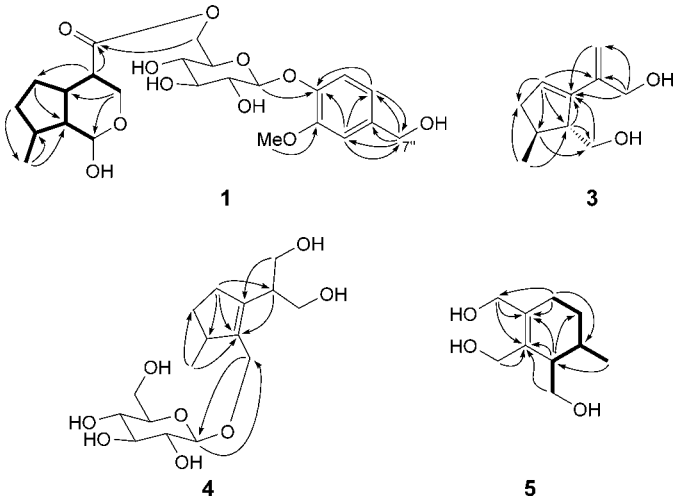


Fig. 1. *Key* $^1H,^1H$*-COSY* (—) *correlations for* **1** *and* **5***, and HMBCs* (H → C) *for* **1** *and* **3**–**5**

was located at C(2″). Moreover, the HMBC of H–C(1′) of the Glc moiety with C(1″) exhibited that the Glc moiety was linked to C(1″) of the 1,3,4-trisubstituted aromatic ring. Except for the signals belonging to the 1,3,4-trisubstituted aromatic ring and the glucosyl group, the remaining signals were extremely similar to those of incarvillic acid [11], a known iridoid, which was supported by the linkages of $CH_2$(3)/H–C(4)/H–C(5)/$CH_2$(6)/$CH_2$(7)/H–C(8)/Me(10), H–C(1)/H–C(9)/H–C(5), and H–C(8)/H–C(9) fragments in the $^1H,^1H$-COSY spectrum (*Fig. 1*). The HMBCs of $CH_2$(6′) ($\delta$(H) 4.07 (*dd*, $J = 12.0$, 7.2, $H_a$–C(6′)) and 4.38 (*dd*, $J = 12.0$, 2.4, $H_b$–C(6′))) with C(11) implied the connection of C(11) and the glucosyl group.

From biogenetic considerations, H–C(5) and H–C(9) of the iridoid skeleton must be $\beta$-oriented [11][12]. The ROESY spectrum (*Fig. 2*) displayed cross-peaks of H–C(8) with H–C(9) and H–C(5), which revealed that H–C(8) was $\beta$-oriented. The configuration of H–C(4) was determined as $\beta$ on the basis of the NOE correlations of H–C(4) with H–C(5) and H–C(9). Based on the above findings, the structure of **1** was finally determined, and the compound was named incarvoid D.

Compound **2** was isolated as colorless oil. Analysis of the HR-ESI-MS (*m*/*z* 221.1151 ($[M + Na]^+$)) established the molecular formula of $C_{11}H_{18}O_3$, requiring three degrees of unsaturation. The $^1H$- and $^{13}C$-NMR spectra (*Table 1*) revealed the presence of one MeO ($\delta$(H) 3.47 (*s*), $\delta$(C) 56.3 (*q*)), one Me ($\delta$(H) 0.75 (*d*, $J = 6.8$, Me(10)), $\delta$(C) 14.7 (*q*)), a $CH_2O$ ($\delta$(H) 4.34 (*d*, $J = 15.6$, $H_a$–C(3)) and 4.21 (*d*, $J = 15.6$, $H_b$–C(3)), $\delta$(C) 67.3 (*t*)), and a CH–O group ($\delta$(H) 4.28 (*d*, $J = 7.2$, H–C(1)), $\delta$(C) 104.2 (*d*)), and a pair of $HOCH_2$ H-atoms ($\delta$(H) 4.04 (*d*, $J = 12.0$, $H_a$–C(11)), 4.06 (*d*, $J = 12.0$, $H_b$–C(11)), $\delta$(C) 60.2 (*t*)). In comparison to incarvoid C [13], the only difference between these two compounds is an EtO group at C(1) of incarvoid C being replaced by a MeO group in **2**. Accordingly, the structure of **2** was assigned as shown in *Fig. 3*, and the compound was named incarvoid E.

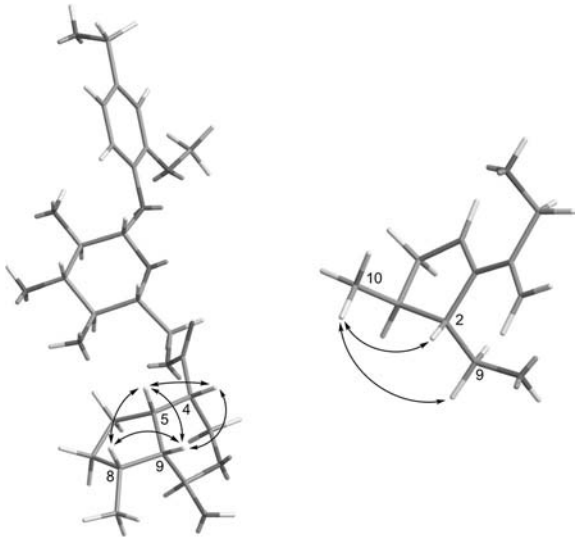


Fig. 2. *Key ROESY correlations represented by* **1** *and* **3**

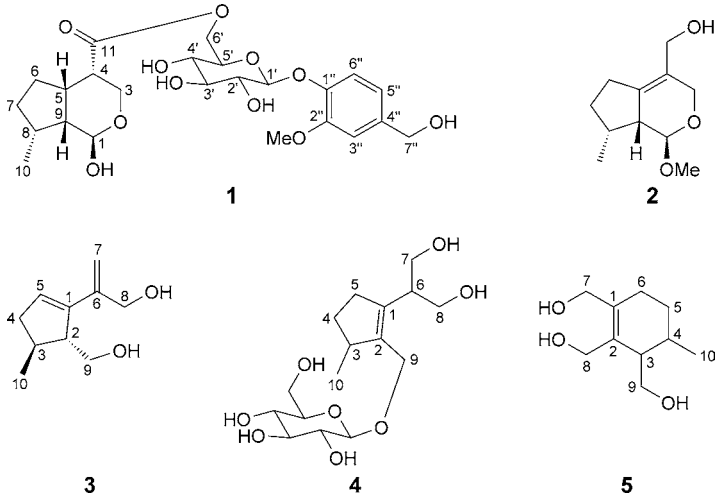


Fig. 3. *Compounds* **1**–**5***, isolated from* Rhododendron microphyton

Compound **3**, isolated as colorless oil, was shown to have a molecular formula of $C_{10}H_{16}O_2$ based on the HR-ESI-MS (*m*/*z* 191.1042 ($[M+Na]^+$)), which was suggestive of three degrees of unsaturation. The UV spectrum showed absorptions at 204 and 234 nm, which indicated the presence of a conjugated system. The $^1$H-NMR spectrum (*Table 2*) showed signals of one Me group at $\delta$(H) 1.25 (*d*, $J = 7.0$, Me(10)), four $CH_2$ groups including two O-bearing ones at 4.27 (*s*, $CH_2$(8)), 3.67 (*dd*, $J = 11.4$, 3.0,

Table 2. *$^1$H-NMR Data of* **3**–**5** (in $CD_3OD$). $\delta$ in ppm, $J$ in Hz.

| Position | **3** (600 MHz) | **4** (400 MHz) | **5** (600 MHz) |
|---|---|---|---|
| 2 | 2.84–2.87 (*m*) | | |
| 3 | 2.45–2.52 (*m*) | 2.74–2.79 (*m*) | 2.72–2.76 (*m*) |
| 4 | 2.15–2.20 (*m*), 2.39–2.43 (*m*) | 1.21–1.30 (*m*), 1.92–2.01 (*m*) | 1.96–2.02 (*m*) |
| 5 | 5.91 (*t*, *J* = 2.7) | 2.14–2.27 (*m*) | 1.34–1.42 (*m*), 1.71–1.76 (*m*) |
| 6 | | 2.81–2.88 (*m*) | 2.31–2.37 (*m*), 2.39–2.43 (*m*) |
| 7 | 5.15 (*s*), 5.27 (*s*) | 3.37 (*dd*, *J* = 10.4, 7.6), 3.44 (*dd*, *J* = 10.4, 7.6) | 4.04 (*d*, *J* = 11.7), 4.06 (*d*, *J* = 11.7) |
| 8 | 4.27 (*s*) | 3.53[a]) | 4.13 (*d*, *J* = 11.7), 4.16 (*d*, *J* = 11.7) |
| 9 | 3.67 (*dd*, *J* = 11.4, 3.0), 3.72 (*dd*, *J* = 11.4, 5.4) | 4.16 (*d*, *J* = 12.0), 4.39 (*d*, *J* = 12.0) | 3.30 (*dd*, *J* = 10.8, 7.6), 3.59 (*dd*, *J* = 10.8, 5.4) |
| 10 | 1.25 (*d*, *J* = 7.0) | 0.97 (*d*, *J* = 6.8) | 1.01 (*d*, *J* = 7.0) |
| 1′ | | 4.12 (*d*, *J* = 7.2) | |
| 2′ | | 3.04–3.09 (*m*) | |
| 3′ | | 3.16[a]) | |
| 4′ | | 3.16[a]) | |
| 5′ | | 3.23–3.25 (*m*) | |
| 6′ | | 3.53[a]), 3.81 (*d*, *J* = 12.0) | |

[a]) Overlapped signals assigned by HMBC and HSQC.

$H_a$–C(9)), and 3.72 (*dd*, *J* = 11.4, 5.4, $H_b$–C(9)), and a terminal C=C bond at 5.15 (*s*, $H_a$–C(7)) and 5.27 (*s*, $H_b$–C(7)). The $^{13}$C-NMR and DEPT spectra (*Table 3*) showed ten C-atoms, attributed to two $sp^2$ $C_q$-atoms ($\delta$(C) 143.3 (*s*, C(1)) and 144.0 (*s*, C(6))), three CH groups including an unsaturated one (129.6 (*d*, C(5))), four $CH_2$ groups including two O-bearing ones (64.1 (*t*, C(8)) and 60.9 (*t*, C(9))) and an olefinic one

Table 3. *$^{13}$C-NMR Data* (in $CD_3OD$) *of* **3**–**5**. $\delta$ in ppm.

| Position | **3** (150 MHz) | **4** (100 MHz) | **5** (150 MHz) |
|---|---|---|---|
| 1 | 143.3 | 142.4 | 132.6 |
| 2 | 51.2 | 140.4 | 147.9 |
| 3 | 38.2 | 41.5 | 49.3 |
| 4 | 41.3 | 32.2 | 38.6 |
| 5 | 129.6 | 31.5 | 32.4 |
| 6 | 144.0 | 45.4 | 29.3 |
| 7 | 111.7 | 62.9 | 61.3 |
| 8 | 64.1 | 63.2 | 61.2 |
| 9 | 60.9 | 62.5 | 61.6 |
| 10 | 15.6 | 19.5 | 15.6 |
| 1′ | | 101.2 | |
| 2′ | | 75.0 | |
| 3′ | | 77.7 | |
| 4′ | | 71.9 | |
| 5′ | | 78.1 | |
| 6′ | | 63.2 | |

(111.7 (*t*, C(7))), and one Me group (15.6 (*q*, C(10))). All the above mentioned NMR data suggested that **3** was similar to 2-[(1*S*,2*R*,3*S*)-2-(hydroxymethyl)-3-methylcyclopentyl]prop-2-en-1-ol [14]. However, compared with this known iridoid, **3** had one more degree of unsaturation, which was induced by the additional C=C bond between C(1) and C(5), based on the HMBCs (*Fig. 1*) of H–C(5) ($\delta$(H) 5.91 (*t*, $J = 2.7$)) with C(2) ($\delta$(C) 51.2 (*d*)), C(3) (38.2 (*d*)), C(4) (41.3 (*d*)), and C(6), as well as of H–C(2) ($\delta$(H) 2.84–2.87 (*m*)), $CH_2$(4) (2.15–2.20 (*m*, $H_a$–C(4)) and 2.39–2.43 (*m*, $H_b$–C(4))), $CH_2$(7), $CH_2$(8), and $CH_2$(9) with C(1) ($\delta$(C) 143.3 (*s*)). In addition, the position of the C=C bond between C(6) and C(7) was evidenced from the HMBCs of $CH_2$(8) with C(6) and C(7), and of $CH_2$(7) with C(1).

In general, the relative configuration of H–C(2) in the iridoid skeleton is $\beta$ [12][13]. In the ROESY spectrum of **3** (*Fig. 2*), the cross-peak observed of H–C(2) with Me(10) revealed that Me(10) was $\beta$-oriented. Consequently, the structure of **3** was fully established, and the compound was named incarvoid F.

The molecular formula of **4** was deduced as $C_{16}H_{28}O_8$ by the HR-ESI-MS at *m*/*z* 371.1683 ($[M + Na]^+$) in combination with the $^{13}$C-NMR and DEPT spectra, requiring three degrees of unsaturation. In the $^1$H-NMR spectrum (*Table 2*), the anomeric H-atom at $\delta$(H) 4.12 (*d*, $J = 7.2$, H–C(1′)) revealed the presence of a sugar residue with $\beta$-configuration [9][10]. GC Analysis of the corresponding trimethylsilylated L-cysteine derivative of the sugar residue obtained by acid hydrolysis of **4** proved the existence of Glc. In addition, one Me ($\delta$(H) 0.97 (*d*, $J = 6.8$, Me(10))) and three $CH_2O$ groups (3.37 (*dd*, $J = 10.4$, 7.6, $H_a$–C(7)) and 3.44 (*dd*, $J = 10.4$, 7.6, $H_b$–C(7)), 3.53 (overlapped, $CH_2$(8)), and 4.16 (*d*, $J = 12.0$, $H_a$–C(9)) and 4.39 (*d*, $J = 12.0$, $H_b$–C(9))) were also observed in the $^1$H-NMR spectrum. Except for the signals belonging to the glucosyl group, the $^{13}$C-NMR and DEPT spectra (*Table 3*) of **4** exhibited resonances for ten C-atoms: one Me group ($\delta$(C) 19.5 (*q*, C(10))), five $CH_2$ groups including three O-bearing ones (62.9 (*t*, C(7)), 63.2 (*t*, C(8)), and 62.5 (*t*, C(9))), two CH groups (41.5 (*d*, C(3)) and 45.4 (*d*, C(6))), and two olefinic $C_q$-atoms (142.4 (*s*, C(1)) and 140.4 (*s*, C(2))), which were assigned to the iridoid skeleton. The above mentioned $^1$H- and $^{13}$C-NMR data for the aglycone of **4** were extremely similar to those of (+)-incarvoid B [13], except for the signals of C(1), C(2), and C(9), suggesting that the Glc moiety formed the glycoside with HO–C(9) of (+)-incarvoid B, which was supported by the HMBCs (*Fig. 1*) of $CH_2$(9) with C(1′) ($\delta$(C) 101.2 (*d*)) and of H–C(1′) with C(9). Thus, the structure of **4** was assigned as shown in *Fig. 3* and the compound was named incarvoid B 9-*O*-$\beta$-D-glucopyranoside.

Compound **5** was isolated as colorless oil. Analysis of the HR-ESI-MS data at *m*/*z* 209.1156 ($[M + Na]^+$) established the molecular formula of $C_{10}H_{18}O_3$, requiring two degrees of unsaturation. A series of signals of one Me group at $\delta$(H) 1.01 (*d*, $J = 7.0$, Me(10)), and three $CH_2O$ groups (4.04 (*d*, $J = 11.7$, $H_a$–C(7)) and 4.06 (*d*, $J = 11.7$, $H_b$–C(7)), 4.13 (*d*, $J = 11.7$, $H_a$–C(8)) and 4.16 (*d*, $J = 11.7$, $H_b$–C(8)), and 3.30 (*dd*, $J =$ 10.8, 7.6, $H_a$–C(9)) and 3.59 (*dd*, $J = 10.8$, 5.4, $H_b$–C(9))) were obviously observed in the $^1$H-NMR spectrum (*Table 2*). Meanwhile, the $^{13}$C-NMR and DEPT spectra (*Table 3*) showed ten C-atoms attributive to one Me ($\delta$(C) 15.6 (*q*, C(10))), five $CH_2$ (three O-bearing: 61.3 (*t*, C(7)), 61.2 (*t*, C(8)), and 61.6 (*t*, C(9))), and two CH groups (49.3 (*d*, C(3)) and 38.6 (*d*, C(4))), and two olefinic $C_q$-atoms (132.6 (*s*, C(1)) and 147.9 (*s*, C(2))), which were assigned to the monoterpenoid skeleton. The 1D-NMR data of **5**

were similar to those of (+)-argutoid A [13]. The exocyclic C=C bond in (+)-argutoid A was replaced by a $HOCH_2$ group in **5**, which was confirmed by the $^1H,^1H$-COSY correlations (*Fig. 1*) of $CH_2(9)$/H–C(3)/H–C(4)/$CH_2(5)$/$CH_2(6)$, and the featured chemical shift of C(9) changed from $\delta$(C) 109.1 to 61.6 in **5**. Furthermore, in combination with the molecular formula and the degrees of unsaturation of **5**, HO–C(7) and HO–C(8) existed as free OH groups rather than forming a ring by dehydration. Based on the above evidence, the structure of **5** was established as shown in *Fig. 3*, and the compound was named argutoid B.

Additionally, our study afforded 14 known compounds, which were identified as (+)-incarvoid B, (−)-incarvoid A [13], diincarvilone A, diincarvilone B [15], lemnacarnol [16], oleanolic acid [17], tormentic acid [18], *erythro*-guaiacylglycerol *β-O*-4′-coniferyl ether, *threo*-guaiacylglycerol *β-O*-4′-coniferyl ether [19], clerodeoside A [20], verbascoside, isoverbascoside [21], caffeic acid methyl ester [22], and 4-hydroxy-3-methoxy benzoic acid [23]. Their structures were established by comparison of their spectroscopic data with those reported in the literature.

In addition, all new compounds were tested for their cytotoxic activities against Hela cells with paclitaxel as positive control. However, all compounds were inactive. Meanwhile, none of the tested compounds exhibited inhibitory effects on lipopolysaccharide-induced NO production in RAW264.7 cells.

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## Experimental Part

*General.* Thin layer chromatography (TLC) and column chromatography (CC): silica gel ($SiO_2$; 100–200 mesh, 200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), *Sephadex LH-20* (40–70 μm; *Amersham Pharmacia Biotech AB*, Uppsala, Sweden), and *YMC-GEL ODS-A* (50 μm; *YMC*, Milford, MA). HPLC: *Agilent 1200* liquid chromatography system; *Zorbax SB-C18* (5 μm, 9.4 × 250 mm); flow rate 3 ml $min^{-1}$. UV Spectra: *UV-210A* spectrophotometer; $\lambda_{max}$ (log $\varepsilon$) in nm. $^1H$- and $^{13}C$-NMR spectra: *Bruker AM-400*, *DRX-500*, or *Avance III-600* spectrometer (*Bruker BioSpin Group*, Germany); in $CD_3OD$; $\delta$ in ppm rel. to $Me_4Si$ as internal standard, *J* in Hz. ESI-MS: *VG-Autospec-3000* spectrometer; in *m*/*z*. HR-ESI-MS: *API-QSTAR-Pulsar* instrument; in *m*/*z*.

*Plant Material.* Dried stems of *R. microphyton* were collected from Lanping County, Yunnan Province, P. R. China, in July 2011, and identified by Prof. *Shiming Guo*, Traditional Chinese Medicine Research Institute of Yunnan Province. A voucher specimen (KM 20110701) has been deposited with the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science and Technology.

*Extraction and Isolation.* Air-dried and powdered stems of *R. microphyton* (4.5 kg) were extracted three times with 70% acetone (each 25 l, 24 h) at r.t. to give an extract, which was suspended in $H_2O$ and partitioned sequentially with AcOEt and BuOH (each 4 × 8 l). The AcOEt fraction (60 g) was subjected to CC ($SiO_2$; $CHCl_3$/MeOH 1:0 to 0:1) to give six fractions, *Frs. 1–6*.

*Fr. 2* (5 g) was subjected to CC (*ODS*; MeOH/$H_2O$ 10:90, 30:70, 50:50, 70:30, 90:10, and 100:0) to yield four fractions, *Frs. 2.1–2.4*. *Fr. 2.1* was further subjected to CC ($SiO_2$; petroleum ether (PE)/AcOEt 5:1) to give 4-hydroxy-3-methoxy benzoic acid (30 mg). *Fr. 2.2* was purified by repeated CC ($SiO_2$; PE/AcOEt 5:1 and $CHCl_3$/acetone 5:1) and HPLC (flow rate 3 ml $min^{-1}$) to afford **3** (35% MeOH/$H_2O$; $t_R$ 17.5 min; 3 mg) and **7** (35% MeOH/$H_2O$; $t_R$ 18.5 min; 5 mg). *Fr. 2.3* was subjected to CC (*Sephadex LH-20*; PE/$CHCl_3$/MeOH 5:5:1), followed by prep. TLC ($CHCl_3$/MeOH 20:1) to give oleanolic acid (40 mg), tormentic acid (25 mg), and caffeic acid methyl ester (17 mg). *Fr. 3* (5.2 g) was subjected to CC

($SiO_2$; $CHCl_3$/acetone 10:1 to 1:1) to yield three subfractions, *Frs. 3.1–3.3*. *Fr. 3.1* was further purified by repeated CC ($SiO_2$, PE/acetone 2:1 and $CHCl_3$/acetone 5:1; then *Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to give **2** (1 mg), diincarvilone A (17 mg), and diincarvilone B (19 mg). *Fr. 3.2* was separated by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) and by repeated CC ($SiO_2$; $CHCl_3$/MeOH 20:1) to give **5** (9 mg) and a mixture (49 mg). Then, lemnacarnol (40% MeCN/$H_2O$; $t_R$ 10.4 min; 3 mg), *erythro*-guaiacylglycerol *β-O-4′*-coniferyl ether (20% MeCN/$H_2O$; $t_R$ 11.5 min; 28 mg), *threo*-guaiacylglycerol *β-O-4′*-coniferyl ether (20% MeCN/$H_2O$; $t_R$ 10.5 min; 6 mg), and clerodeoside A (40% MeCN/$H_2O$; $t_R$ 8.5 min; 11 mg) were isolated from the mixture by HPLC (flow rate 3 ml $min^{-1}$). *Fr. 4* (5 g) was subjected to CC (*ODS*; MeOH/$H_2O$ 10:90–100:0) to give three fractions, *Frs. 4.1–4.3*. *Fr. 4.1* was further purified by repeated CC ($SiO_2$, $CHCl_3$/MeOH 10:1 and $CHCl_3$/acetone 5:1; then *Sephadex LH-20*, PE/$CHCl_3$/MeOH 5:5:1) to give (+)-incarvoid B (4 mg), **1** (6 mg), and **4** (87 mg). Verbascoside (20% MeCN/$H_2O$; $t_R$ 19.1 min; 10 mg) and isoverbascoside (20% MeCN/$H_2O$; $t_R$ 26.8 min; 10 mg) were isolated from *Fr. 4.2* by CC ($SiO_2$; $CHCl_3$/MeOH 10:1) and HPLC (flow rate 3 ml $min^{-1}$).

*Acid Hydrolysis and GC Analysis* [24][25]. Compounds **1** and **4** (1 mg each) were hydrolyzed with 9% HCl at 90–100° for 5 h. The mixture was evaporated to dryness, dissolved in anh. pyridine (100 µl) containing 0.1M L-cysteine methyl ester hydrochloride (200 µl) and reacted at 60° for 1 h. Then, $(Me_3Si)_2NH$ (HMDS)/$Me_3SiCl$/pyridine 2:1:10 (*Acros Organics*, Belgium) was added, and the mixture was stirred at 60° for 30 min. The final mixture was directly analyzed by GC (*30QC2/AC-5* quartz capillary column (30 m × 0.32 mm); column temperature, 150°/280°; programmed increase, 5° $min^{-1}$; carrier gas, $N_2$ (1 ml $min^{-1}$); injection temp., 230°; detector temp., 250°; split ratio, 1:10). The standards were prepared following the same procedure. Under these conditions, the retention time of the D-glucoside derivative was 22.03 min. During co-injection studies, identical retention times were observed between the hydrolysates and authentic standard.

*Incarvoid D* (*=4-(Hydroxymethyl)-2-methoxyphenyl 6-*O*-{[(1*R*,4*S*,4a*S*,7*R*,7a*R*)-Octahydro-1-hydroxy-7-methylcyclopenta[*c*]pyran-4-yl]carbonyl}-β-*D*-glucopyranoside*; **1**). Colorless needles. M.p. 254°. $[\alpha]_D^{20.6} = -43.9$ (*c* = 0.10, MeOH). UV (MeOH): 201 (4.33), 224 (3.82), 276 (3.22). $^1$H- and $^{13}$C-NMR: *Table 1*. ESI-MS (pos.): 521 ($[M+Na]^+$). HR-ESI-MS (pos.): 521.1996 ($[M+Na]^+$, $C_{24}H_{34}NaO_{11}^+$; calc. 521.1993).

*Incarvoid E* (*=[(1*R*,7*R*,7a*R*)-1,3,5,6,7,7a-Hexahydro-1-methoxy-7-methylcyclopenta[*c*]pyran-4-yl]methanol*; **2**). Colorless oil. $[\alpha]_D^{20.8} = -85.1$ (*c* = 0.10, MeOH). $^1$H- and $^{13}$C-NMR: *Table 1*. ESI-MS (pos.): 221 ($[M+Na]^+$). HR-ESI-MS (pos.): 221.1151 ($[M+Na]^+$, $C_{11}H_{18}NaO_3^+$; calc. 221.1148).

*Incarvoid F* (*=2-[(4*S*,5*R*)-5-(Hydroxymethyl)-4-methylcyclopent-1-en-1-yl]prop-2-en-1-ol*; **3**). Colorless oil. $[\alpha]_D^{20.8} = -31.5$ (*c* = 0.10, MeOH). UV (MeOH): 204 (3.61), 234 (3.63). $^1$H- and $^{13}$C-NMR: *Tables 2* and *3*. ESI-MS (pos.): 191 ($[M+Na]^+$). HR-ESI-MS (pos.): 191.1042 ($[M+Na]^+$, $C_{10}H_{16}NaO_2^+$; calc. 191.1043).

*Incarvoid B 9-*O*-β-*D*-Glucopyranoside* (*=[2-(1,3-Dihydroxypropan-2-yl)-5-methylcyclopent-1-en-1-yl]methyl β-*D*-glucopyranoside*; **4**). White powder. $[\alpha]_D^{20.4} = -47.6$ (*c* = 0.22, MeOH). $^1$H- and $^{13}$C-NMR: *Tables 2* and *3*. ESI-MS (pos.): 371 ($[M+Na]^+$). HR-ESI-MS (pos.): 371.1683 ($[M+Na]^+$, $C_{16}H_{28}NaO_8^+$; calc. 371.1676).

*Argutoid B* (*=(4-Methylcyclohex-1-ene-1,2,3-triyl)trimethanol*; **5**). Colorless oil. $[\alpha]_D^{20.5} = +21.5$ (*c* = 0.18, MeOH). $^1$H- and $^{13}$C-NMR: *Tables 2* and *3*. ESI-MS (pos.): 209 ($[M+Na]^+$). HR-ESI-MS (pos.): 209.1156 ($[M+Na]^+$, $C_{10}H_{18}NaO_3^+$; calc. 209.1148).

*Cytotoxicity Assays.* The cytotoxic activity was determined against HeLa cells, obtained from Kunming Institute of Zoology. Cells were seeded in 96-well plates at a cell density of 6000 per well and were treated 48 h later with various concentrations of **1**–**5**. After 48 h of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) was added to all wells. Plates were incubated for another 4 h, and cell viability was measured by observing absorbance at 490 nm on a *SpectraMax 190* microplate reader (*Molecular Devices*, USA) [26]. Paclitaxel was used as positive control.

*Inhibition of NO Production Assay.* RAW264.7 cells were purchased from Kunming Institute of Zoology. The assay was performed as described previously [27]. Wells with DMSO were used as negative control, and L-$N^G$-monomethyl arginine (L-NMMA) was used as positive control. Cytotoxic activities were determined by the MTT assay as described [28].

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