# Iridoids from *Viburnum cylindricum*

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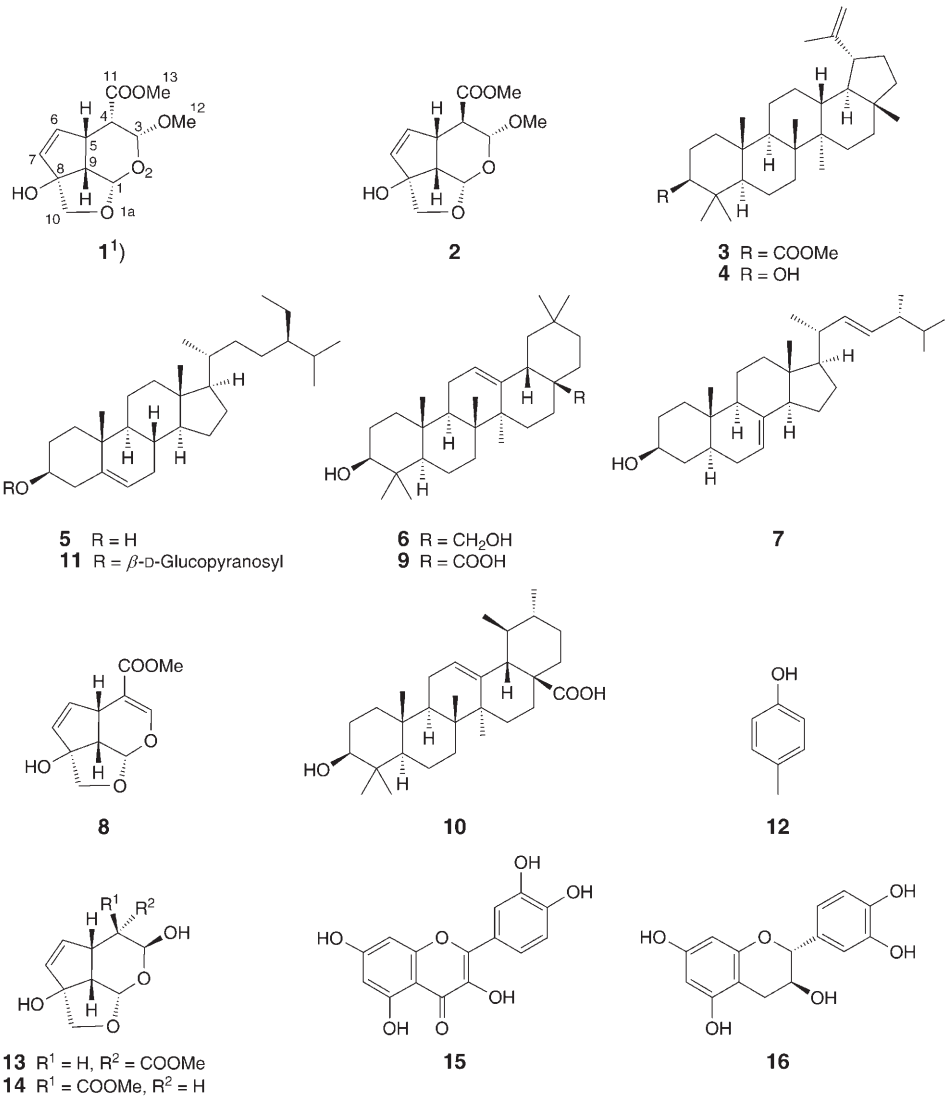
Two new iridoids, methyl (+)-*rel*-(1*R*,3*S*,4*R*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate (**1**) and methyl (+)-*rel*-(1*R*,3*S*,4*S*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate (**2**), were isolated from *Viburnum cylindricum* along with 14 known compounds. Their structures were determined by spectroscopic analyses. This type of iridoids bearing a MeO group at C(3) was discovered for the first time.

**Introduction.** – The genus *Viburnum* comprises over 200 species distributed from South America to Southeast Asia [1], 80 of which are distributed in China [2]. Previous chemical investigations showed that *Viburnum* species characteristically contained iridoids, terpenoids, glycosides, and flavones [3–8]. *Viburnum cylindricum* is found mainly in tropical Asia, which has been used as folk medicine to treat different diseases such as cough, diarrhea, rheumatoid arthritis, and tumefaction [9]. As part of ongoing phytochemical and pharmacological investigations on the genus *Viburnum*, the stems of *Viburnum cylindricum* were collected in Yanbian County, Sichuan Province. Two new iridoids, methyl (+)-*rel*-(1*R*,3*S*,4*R*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate (**1**) and methyl (+)-*rel*-(1*R*,3*S*,4*S*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate (**2**) were isolated along with 14 known compounds, lupenyl acetate (**3**), lupeol (**4**), $\beta$-sitosterol (**5**), erythrodiol (**6**), ergosta-7,22-dien-3$\beta$-ol (**7**), garjasmine (**8**), oleanolic acid (**9**), ursolic acid (**10**), $\beta$-daucosterol (**11**), 4-methylphenol (**12**), $\alpha$-gardiol (**13**) [10], $\beta$-gardiol (**14**), quercetin (**15**), and (+)-catechin (**16**) from the AcOEt extract. Their structures were elucidated on the basis of spectroscopic evidence. Compounds **1** and **2** are the first examples for iridoids with a MeO group at C(3).

**Results and Discussion.** – Compound **1** was obtained as a yellow gum. Its molecular formula $C_{12}H_{16}O_6$ was provided by the pseudomolecular-ion peak at *m*/*z* 279.0829 ($[M+Na]^+$) in the HR-ESI-MS, indicating five degrees of unsaturation. The IR spectrum of **1** exhibited strong absorption bands at 3435 (OH), 1738 (C=O), and 1631 $cm^{-1}$ (C=C). The $^1$H- and $^{13}$C-NMR (DEPT) spectroscopic data of **1** (*Table*) indicated twelve C-atoms, including two Me, one $CH_2$, and seven CH groups, along with two




quaternary C-atoms, and 15 H-atoms, suggesting that compound **1** is an iridoid with a similar skeleton to that of α-gardiol (**13**). The presence of a MeO group at C(3)[1]) was concluded on the basis of HMBC correlations from δ(H) 3.43 (H–C(12)) to δ(C) 97.8 (C(3)) and from δ(H) 4.94 (H–C(3)) to δ(C) 55.8 (C(12)) (*Fig. 1*). The signal at δ 97.8 (C(3)) in compound **1** instead at 90.9 (C(3)) in α-gardiol (**13**) confirmed this conclusion. The $^{13}$C-NMR signal for the quaternary C-atom at δ(C) 172.0 (C(11)), the $^{1}$H-NMR signal at δ(H) 3.77 (*s*, Me(13)) and the HMBC correlation between C(11) and

[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part.*

Me(13) demonstrated the presence of a COOMe group. The NOESY correlations $\delta$(H) 2.71 (H–C(4))/5.53 (H–C(1)), 4.94 (H–C(3)), and 3.51–3.53 (*m*, H–C(5)), and 2.79(H–C(9)/5.53 (H–C(1)) and 3.51–3.53 (H–C(5)) suggested that H–C(1), H–C(3), H–C(4), H–C(5), and H–C(9) are located on the same face of the molecule. Thus, the structure of **1** was finally determined as methyl (+)-*rel*-(1*R*,3*S*,4*R*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate[1]).

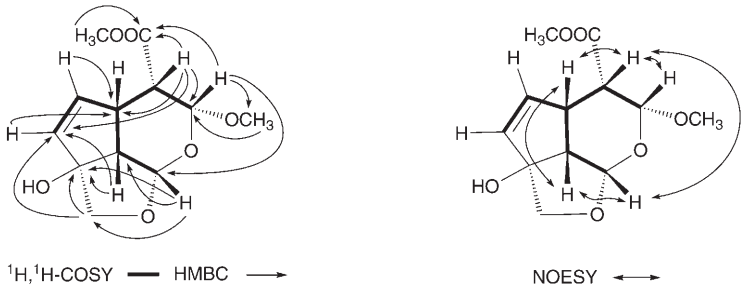



Fig. 1. *Key $^1$H,$^1$H-COSY, HMBC, and NOESY correlations of* **1**

Table. *$^1$H- and $^{13}$C-NMR Data of Compounds* **1** *and* **2** *in $CDCl_3$ and* **13** *in $CD_3OD$*[1]). $^1$H- and $^{13}$C-NMR at 600, 150 MHz, resp.; $\delta$ in ppm, *J* in Hz.

| Position | **1** | | **2** | | **13** | |
|---|---|---|---|---|---|---|
| | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) |
| 1 | 99.4 | 5.53 (*d*, *J* = 5.3) | 100.3 | 5.62 (*d*, *J* = 6.2) | 101.8 | 5.54 (*d*, *J* = 5.7) |
| 3 | 97.8 | 4.94 (*d*, *J* = 8.3) | 98.5 | 4.89 (*d*, *J* = 6.2) | 90.9 | 5.18 (*d*, *J* = 8.7) |
| 4 | 45.7 | 2.71 (*dd*, *J* = 8.1, 5.2) | 47.7 | 2.74 (overlap. with H–C(9)) | 48.7 | 2.71 (*dd*, *J* = 8.7, 5.3) |
| 5 | 42.1 | 3.51–3.53 (*m*) | 41.4 | 3.35–3.38 (*m*) | 40.6 | 3.54–3.57 (*m*) |
| 6 | 135.9 | 5.91 (*dd*, *J* = 5.6, 2.6) | 137.4 | 5.77 (*dd*, *J* = 5.6, 2.2) | 137.1 | 5.78 (*dd*, *J* = 5.5, 2.0) |
| 7 | 135.1 | 5.86 (*dd*, *J* = 5.6, 1.3) | 133.1 | 5.83 (*dd*, *J* = 5.5, 2.0) | 135.2 | 5.95 (*dd*, *J* = 5.5, 2.1) |
| 8 | 93.6 | – | 94.3 | – | 93.6 | – |
| 9 | 53.7 | 2.79 (*dd*, *J* = 8.0, 5.2) | 50.5 | 2.74 (overlap. with H–C(4)) | 52.5 | 2.63 (*dd*, *J* = 8.5, 5.7) |
| 10 | 76.8 | 4.14 (*d*, *J* = 9.5, $H_a$), 4.05 (*d*, *J* = 9.5, $H_b$) | 75.5 | 3.96 (*d*, *J* = 9.7, $H_a$), 3.79 (*d*, *J* = 9.7, $H_b$) | 76.6 | 3.78 (*d*, *J* = 9.2, $H_a$), 3.93 (*d*, *J* = 9.2, $H_b$) |
| 11 | 172.0 | – | 172.6 | – | 173.9 | – |
| 12 | 55.8 | 3.43 (*s*) | 55.9 | 3.75 (*s*) | – | – |
| 13 | 52.3 | 3.77 (*s*) | 52.3 | 3.43 (*s*) | 52.3 | 3.73 (*s*) |

Compound **2** was obtained as a yellow gum. The HR-ESI-MS exhibited a molecular-ion peak at *m*/*z* 279.0829 ($[M + Na]^+$), corresponding to the same molecular formula, $C_{12}H_{16}O_6$, as that of compound **1**. The $^1$H- and $^{13}$C-NMR spectra of **2** showed similar chemical shifts and the same multiplicities of all C-atoms as those of **1**, indicating a gardiol-type backbone in **2**. The minor differences of $\delta$(H) and $\delta$(C) of C(3)[1]), C(4), and C(5) suggested that the configuration of C(3) was different from that of **1**. This relative configuration was determined by the NOESY correlations of $\delta$(H) 4.89 (H–C(3))/5.62 (H–C(1)), 3.43 (Me(13)) and 3.35–3.38 (H–C(5)), and 2.74 (H–C(9))/5.62 (H–C(1)) and 3.35–3.38 (H–C(5)) (*Fig. 2*). The structure of **2** was

finally determined as methyl (+)-*rel*-(1*R*,3*S*,4*S*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate[1]).

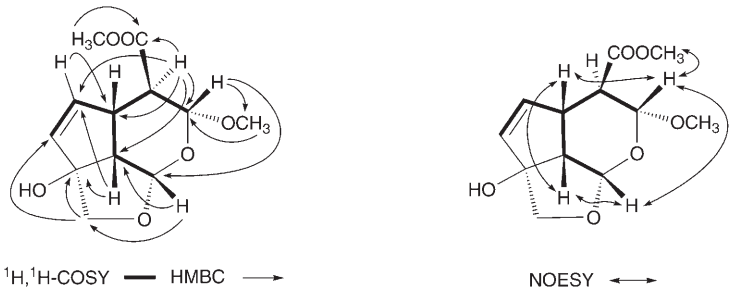



Fig. 2. *Key $^1$H,$^1$H-COSY, HMBC, and NOESY correlations of* **2**

Compounds **1** and **2** represent methyl acetals and methyl esters. MeOH was extensively used during the purification. To verify the original form of these compounds in the plant, the AcOEt extract was subjected to HPLC (208 nm, 3 ml/min, 20% MeCN), and *Frs. a–g* were obtained. These fractions were analyzed by ESI-MS. A ion peak at *m*/*z* 279 ($[M+Na]^+$), corresponding to the same molecular weight as that of compounds **1** and **2**, was found in *Fr. e*, in which compounds **1** and **2** were detected by HPLC (208 nm, 3 ml/min, 15% MeCN). Thus, it could be concluded that compounds **1** and **2** were present in the plant.

## Experimental Part

*General.* TLC: *Merck* precoated plates (silica gel ($SiO_2$), *60 F254*) of 0.25 mm thickness. HPLC: *Perkin-Elmer 600* prep. HPLC instrument, with a *Kromasil 100-10-C18* (250 × 20 mm) column. Column chromatography (CC): $SiO_2$ (200–300 mesh), *Sephadex* LH-20 (*Amersham*). Optical rotations: *Perkin-Elmer 341* automatic polarimeter. IR Spectra: *Perkin-Elmer* FT-IR spectrometer (KBr disk); in $cm^{-1}$. 1D- and 2D-NMR Spectra: *Bruker Advance-600* spectrometer; at 600 MHz; $\delta$ in ppm rel. to TMS as internal standard (at 25°), *J* in Hz. MS: *Bruker Daltonics Bio-TOF-Q* mass spectrometer; in *m*/*z*.

*Plant Material*. The stems of *Viburnum cylindricum* were collected in Yanbian County, Sichuan province, P. R. China in May 2004 and identified by Dr. *Fading Fu* (Chengdu Institute of Biology, the Chinese Academy of Science). A specimen was deposited with the Chengdu Institute of Biology, the Chinese Academy of Science, P. R. China.

*Extraction and Isolation*. The shade-dried, powdered stems (5 kg) were extracted with 95% EtOH (3 × 30 l, 10 d each) at r.t., and the extract was filtered. After evaporation of the filtrate, the residue was suspended in $H_2O$ and fractionated successively with petroleum ether, AcOEt, and BuOH. The AcOEt extract (97 g) was subjected to CC ($SiO_2$, 200–300 mesh; $CHCl_3$/MeOH 95:5 and 8:2): *Frs. 1–5*. *Fr. 1* (11 g) was subjected to CC ($SiO_2$, 200–300 mesh; $CHCl_3$/MeOH 100:1): **3** (13 mg), **4** (1.4 g), and **5** (3.0 g). *Fr. 2* (3 g) was subjected to CC ($SiO_2$, 200–300 mesh, $CHCl_3$/MeOH 80:1): **6** (8 mg) and **7** (23 mg). *Fr. 3* (2 g) was subjected to CC ($SiO_2$, 200–300 mesh, $CHCl_3$/MeOH 60:1; *Sephadex* LH-20, MeOH) and HPLC (208 nm, 3 ml/min, 30% MeOH): **1** (5 mg), **2** (3 mg), and **8** (35 mg). *Fr. 4* (35 g) was subjected to CC ($SiO_2$, 200–300 mesh; $CHCl_3$/MeOH 50:1 → 30:1): **9** (9 g), **10** (7.6 g), **11** (3.0 g), and **12** (35 mg). *Fr. 5* (13 g) was subjected to CC ($SiO_2$, 200–300 mesh; $CHCl_3$/MeOH 20:1): **13** (305 mg), **14** (478 mg), **15** (1.5 g), and **16** (16 mg).

*Methyl (+)*-rel-*(1*R,*3*S,*4*R,*5*R,*8*R,*9*R*)-1,3,4,5,8,9-Hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate* (= *Methyl* rel-*(2a*R,*4a*R,*5*R,*6*S,*7a*R,*7b*R*)-2a,4a,5,6,7a,7b-Hexahydro-2a-hydroxy-6-methoxy-2*H*-1,7-dioxacyclopent[*cd*]indene-5-carboxylate*; **1**). Yellow gum. $[\alpha]_D^{20} = +65.9$

($c = 0.29$, $CHCl_3$). IR: 3435, 2964, 1738, 1631, 1259, 1100, 1028, 956, 801. $^1$H- and $^{13}$C-NMR: see *Table*. ESI-MS: 279 ($[M + Na]^+$). HR-ESI-MS: 279.0829 ($[M + Na]^+$, $C_{12}H_{16}NaO_6^+$; calc. 279.0845).

*Methyl (+)-*rel-*(1*R*,3*S*,4*S*,5*R*,8*R*,9*R*)-1,3,4,5,8,9-Hexahydro-8-hydroxy-3-methoxy-2*H*-1a,2-dioxacyclopent[*cd*]indene-4-carboxylate* (= *Methyl* rel-*(2a*R*,4a*R, *5*S*,6*S*,7a*R*,7b*R*)-2a,4a,5,6,7a,7b-Hexahydro-2a-hydroxy-6-methoxy-2*H*-1,7-dioxacyclopent[*cd*]indene-5-carboxylate*; **2**). Yellow gum. $[\alpha]_D^{20} = +13.8$ ($c = 0.15$, $CHCl_3$). IR: 3435, 2957, 1736, 1631, 1259, 1100, 1024, 956, 800. $^1$H- and $^{13}$C-NMR: see *Table*. ESI-MS: 279 ($[M + Na]^+$). HR-ESI-MS: 279.0829 ($[M + Na]^+$, $C_{12}H_{16}NaO_6^+$; calc. 279.0845).

Compounds **3**–**16** were identified by comparison of their IR, $^1$H- and $^{13}$C-NMR, and MS spectral data with those reported or by comparison with authentic samples on TLC and by co-melting experiments.

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