# Phenolic Derivatives from the Root Bark of *Oplopanax horridus*

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Four new phenolic derivatives, including two phenylpropanoid glycosides, one benzoate glycoside, and one lignan glycoside, together with one known glyceride, were isolated from the root bark of *Oplopanax horridus*. The structures of the new compounds were elucidated as 3-{4-[(6-*O*-acetyl-*β*-D-glucopyranosyl)oxy]-3,5-dimethoxyphenyl}propanoic acid (**1**), (+)-[5,6,7,8-tetrahydro-7-(hydroxymethyl)-10,11-dimehoxydibenzo[*a*,*c*][8]annulen-6-yl]methyl *β*-D-glucopyranoside (**2**), (+)-methyl 4-[6-*O*-{3-hydroxy-3-methyl-5-(1-methylpropyl)oxy]-5-oxopentanoyl}-4-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)oxy]-3-methoxybenzoate (**3**), and 2-methoxy-4-[(1*E*)-3-methoxy-3-oxoprop-1-en-1-yl]phenyl 6-*O*-{3-hydroxy-3-methyl-5-[(1-methylpropyl)oxy]-5-oxopentanoyl-4-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranoside (**4**) on the basis of spectroscopic techniques including NMR and MS analyses. The known compound was identified as glycer-2-yl ferulate (**5**) by comparing its physical and spectral data with those reported in the literature.

**Introduction.** – Phenolic constituents, including phenylpropanoids and lignans, were recently isolated from the genus *Oplopanax* [1–4]. Phenylpropanoids have been shown to possess activities in protecting against biotic stress such as infections, wounding, pollutants, and UV irritation [5]. Lignans from natural resources exhibit anticancer [6], antioxidant, antiviral, and anti-inflammatory activities [7][8]. A previous phytochemical investigation on the root bark of *Oplopanax horridus* led to the isolation and identification of some polyynes from the hydrophobic extract [9]. As part of our research work on bioactive compounds from *O. horridus*, we studied the hydrophilic extract and isolated three phenylpropanoid glycosides and one lignan glycoside along with one known glyceride. Herein, we report the isolation and structure elucidation of these phenolic compounds.

**Results and Discussion.** – After a series of column chromatography (silica gel) and preparative HPLC, an 80%-EtOH extract of air-dried root bark of *O. horridus* yielded four new natural products **1**–**4** (for the names, see the *Exper. Part*) together with one known compound, glycer-2-yl ferulate (**5**). The structures of **1**–**5** (*Fig. 1*) were

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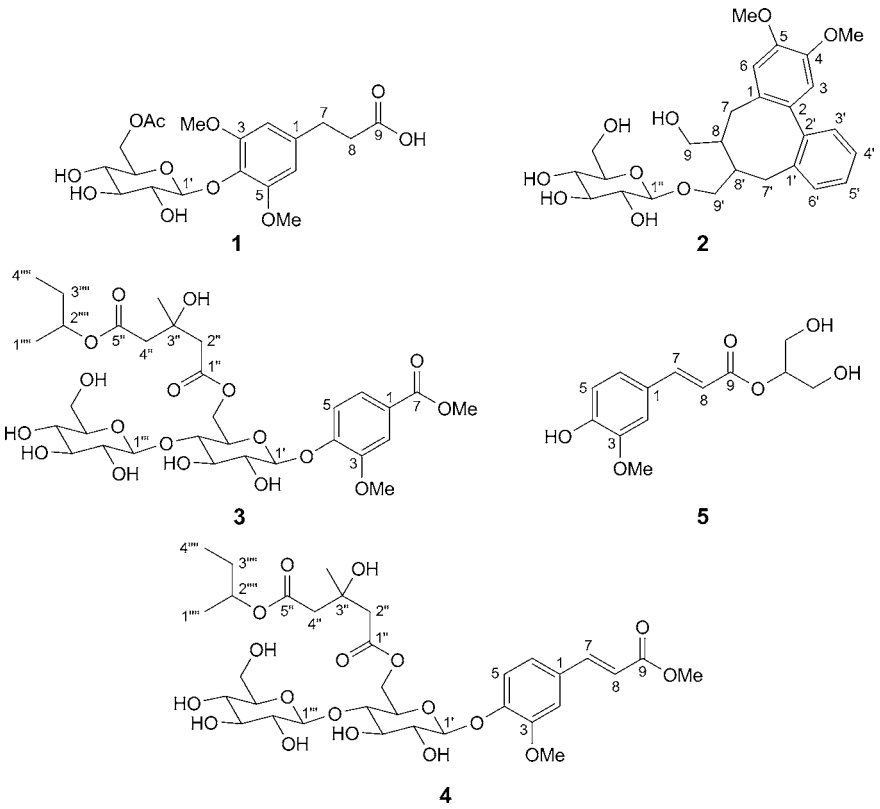


Fig. 1. *Structures of* **1**–**5**

elucidated by their physicochemical properties and spectroscopic data. The known compound, glycer-2-yl ferulate (**5**), was also confirmed by comparing its physical and spectral data with those reported in the literature.

Compound **1** was obtained as white powder. Its molecular formula was deduced as $C_{19}H_{26}O_{11}$ from the *quasi*-molecular-ion peak at *m*/*z* 453.1359 ($[M+Na]^+$, $C_{19}H_{26}NaO_{11}^+$; calc. 453.1373) in the HR-ESI mass spectrum. The UV absorption maxima displayed at 228 and 256 nm implied the presence of a conjugated C=C bond system in this compound. The IR spectrum of **1** indicated the presence of an aromatic ring based on the absorptions at 1601, 1512, 1432, and 897 $cm^{-1}$. Acidic hydrolysis of **1** yielded D-glucose, which was identified by GC/MS analysis. The $^1$H-NMR spectrum of **1** exhibited signals of two symmetrical aromatic H-atoms ($\delta$(H) 7.34 (*s*)), two symmetrical MeO groups (3.89 (*s*)), the Me group of AcO (2.50 (*s*)), as well as of an anomeric H-atom (5.04 (*d*, $J=7.6$)). The corresponding coupling constant ($J=7.6$) suggested that the D-glucopyranosyl unit possessed $\beta$-configuration (*Table 1*). The $^{13}$C-NMR spectrum (*Table 1*) displayed signals of four different sp$^2$ C-atoms ($\delta$(C) 154.5, 140.0, 127.3, and 105.5) due to a symmetrical 1,3,4,5-tetrasubstituted aromatic ring, of six different O-bearing sp$^3$ C-atoms (101.7, 77.8, 75.7, 75.6, 71.7, and 64.5),

Table 1. *$^1$H- and $^{13}$C-NMR Data* (500 and 125 MHz, resp., in $CD_3OD$) *of* **1**[a]). $\delta$ in ppm, $J$ in Hz.

| Position | $\delta$(H) | $\delta$(C) | Position | $\delta$(H) | $\delta$(C) |
|---|---|---|---|---|---|
| 1 | – | 127.3 | 2′ | 3.51 (*dd*, *J* = 7.9, 8.0) | 75.7 |
| 2 | 7.34 (*s*) | 105.5 | 3′ | 3.41 – 3.44 (*m*) | 77.8 |
| 3 | – | 154.5 | 4′ | 3.37 – 3.41 (*m*) | 71.7 |
| 4 | – | 140.0 | 5′ | 3.39 – 3.42 (*m*) | 75.6 |
| 5 | – | 154.5 | 6′ | 4.33 (*dd*, *J* = 5.5, 10.9), | 64.5 |
| 6 | 7.34 (*s*) | 105.5 | | 4.18 (*dd*, *J* = 2.0, 10.9) | |
| 7 | 2.61 (*t*, *J* = 7.2) | 46.4 | *Me*C=O | 2.50 (*s*) | 27.0 |
| 8 | 2.52 (*t*, *J* = 7.2) | 46.0 | MeC=*O* | – | 168.1 |
| 9 | – | 172.3 | 3,5-MeO | 3.89 (*s*) | 57.0 |
| 1′ | 5.04 (*t*, *J* = 7.6) | 101.7 | | | |

[a]) Assignments based on 2D-NMR spectra.

assignable to a D-glucosyl moiety, of one MeO group (57.0), of AcO C-atoms (168.1 and 27.0) as well as of a COOH group signal (172.3), and two more $sp^3$ C-atom signals at 46.4 and 46.0.

Based on HMQC and HMBC experiments, the downfield chemical-shift value of C(6′) from $\delta$(C) 62.6 to 64.5 suggested that the AcO group was attached to C(6′) of the glucopyranosyl group, on the basis of the comparison with the chemical-shift value of C(6) of a glucopyranosyl moiety without any substituent [10]. However, the correlation between $CH_2$(6′) ($\delta$(H) 4.33 and 4.18) and the Me*C*=*O* C-atom ($\delta$(C) 168.1) was not observed in the HMBC spectra. Even so, the experimental data were sufficient to elucidate the structure of **1**, as depicted in *Fig. 1*.

Compound **2** was obtained as amorphous powder with an optical rotation value of $[\alpha]_D^{20} = +18.1$ ($c = 0.5$, MeOH), and its IR spectrum exhibited strong absorption bands due to a OH group at 3210 $cm^{-1}$ and aromatic rings at 1622, 1570, and 1220 $cm^{-1}$. Compound **2** displayed a $[M+H]^+$ ion peak at *m*/*z* 491.2274 ($C_{26}H_{35}O_9^+$; calc. 491.2281) in the HR-ESI mass spectrum, indicating the molecular formula $C_{26}H_{34}O_9$. Acidic hydrolysis of **2** also yielded D-glucose identified by GC/MS analysis. The $^1$H-NMR spectrum (*Table 2*) showed signals of H-atoms at $\delta$(H) 7.03 (*dd*, *J* = 8.1, 8.1, 1 H), 6.69 (*dd*, *J* = 8.0, 1.4, 1 H), 6.61 (*ddd*, *J* = 8.0, 8.0, 1.2, 1 H), and 6.53 (*dd*, *J* = 8.0, 2.0, 1 H), which were ascribable to an *ortho*-substituted benzene ring, of two aromatic H-atoms at 6.67 and 6.65, (2*s*) assignable to a 1,2,4,5-tetrasubstituted benzene ring. The H-atom signals of a glucosyl moiety were detected at $\delta$(H) 3.30 – 3.80. Additionally, two more MeO signals at $\delta$(H) 3.81 (*s*) and 3.82 (*s*) overlapped with these signals.

The $^{13}$C-NMR spectrum displayed 26 signals, including those assignable to two aromatic rings ($\delta$(C) 150.6, 148.8, 146.2, 145.5, 137.5, 133.9, 122.9, 122.8, 117.9, 115.9, 114.4, and 113.6), a glucose moiety (103.2, 78.2, 77.9, 75.0, 71.4, and 62.6), and two MeO groups at 56.4 and 56.6; *Table 2*). Based on the $^1$H,$^1$H-COSY, HMQC, DEPT, and HMBC data (*Fig. 2*), the aglycone was similar to 1,4-dibenzyl butane [11]. The coupling constant between the anomeric H-atom H–C(1″) and H–C(2″) ($J = 7.8$) indicated the $\beta$-configuration of the glucose. The $^1$H,$^1$H-COSY spectrum provided the correlations $CH_2$(7)/H–C(8) as well as H–C(8)/H–C(8′), indicated that C(8) and C(8′) were connected. Thus, structure of **2** was determined as shown in *Fig. 1*. The absolute

Table 2. *$^{1}$H- and $^{13}$C-NMR Data* (500 and 125 MHz, resp., in $CD_3OD$) *of* **2**[a]). $\delta$ in ppm, $J$ in Hz.

| Position | $\delta$(H) | $\delta$(C) | Position | $\delta$(H) | $\delta$(C) | Position | $\delta$(H) | $\delta$(C) |
|---|---|---|---|---|---|---|---|---|
| 1 | – | 133.9 | 1′ | – | 145.5 | 1″ | 4.79 (overlapped) | 103.2 |
| 2 | – | 137.5 | 2′ | – | 146.2 | 2″ | 3.21 (*dd*, $J$ = 2.6, 7.8) | 75.0 |
| 3 | 6.65 (*s*) | 122.9 | 3′ | 6.53 (*dd*, $J$ = 2.0, 8.0) | 113.6 | 3″ | 3.19 – 3.22 (*m*) | 78.2 |
| 4 | – | 150.6 | 4′ | 6.61 (*ddd*, $J$ = 1.2, 8.0, 8.0) | 117.9 | 4″ | 3.24 – 3.27 (*m*) | 71.4 |
| 5 | – | 148.8 | 5′ | 7.03 (*dd*, $J$ = 8.1, 8.1) | 122.8 | 5″ | 3.29 – 3.31 (*m*) | 77.9 |
| 6 | 6.65 (*s*) | 115.9 | 6′ | 6.69 (*dd*, $J$ = 1.4, 8.0) | 114.4 | 6″ | 3.81 – 3.85 (*m*, $H_b$), | 62.6 |
| 7 | 2.54 – 2.57 (*m*) | 36.1 | 7′ | 2.66 – 2.68 (*m*) | 36.2 | | 3.63 – 3.65 (*m*, $H_a$) | |
| 8 | 1.89 – 1.92 (*m*) | 44.1 | 8′ | 1.90 – 1.94 (*m*) | 44.3 | MeO | 3.82 (*s*) | 56.6 |
| 9 | 3.84 (*dd*, $J$ = 6.3, 8.2, $H_a$), | 62.2 | 9′ | 3.80 – 3.83 (*m*, $H_b$), | 62.1 | MeO | 3.81 (*s*) | 56.4 |
| | 3.75 (*dd*, $J$ = 7.8, 8.5, $H_b$) | | | 3.75 – 3.78 (*m*, $H_a$) | | | | |

[a]) Assignments based on 2D-NMR spectra.

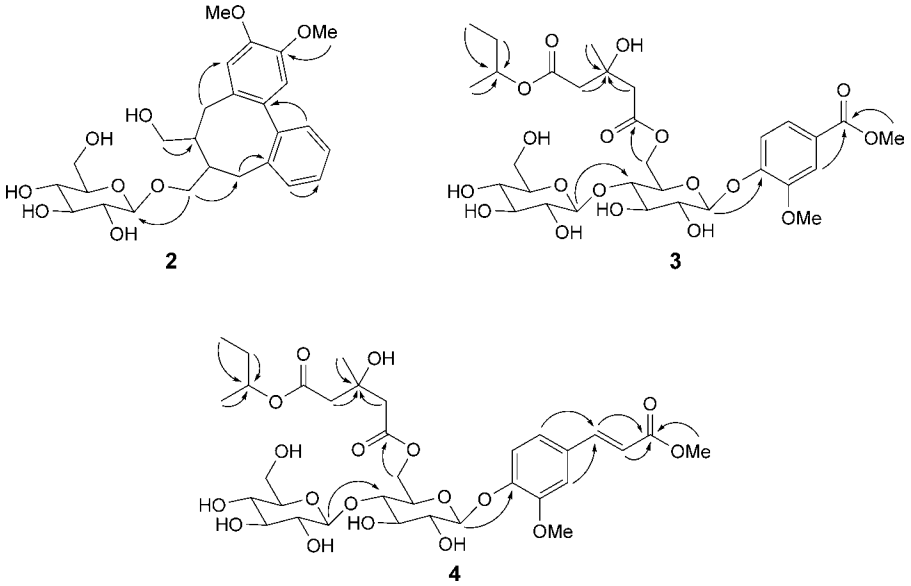


Fig. 2. *Key HMBCs* (H → C) *of* **2**–**4**

configuration of this compound could not be determined due to the shortage of material.

Compound **3** was obtained as colorless gum with an optical rotation of $[\alpha]_D^{20} = +9.4$ ($c = 0.5$, MeOH). The UV spectrum showed absorption maxima at 220 and 265 nm. The IR spectrum displayed absorption bands for OH (3409 $cm^{-1}$) and C=O (1720 $cm^{-1}$) groups and an aromatic ring (1597 and 1513 $cm^{-1}$). The molecular-ion peak at *m*/*z* 707.2446 ($[M+H]^+$, $C_{31}H_{47}O_{18}^+$; calc. 707.2762) in HR-ESI mass spectrum provided the molecular formula $C_{31}H_{46}O_{18}$. Acidic hydrolysis of **3** gave D-glucose. The $^1$H-NMR spectrum of **3** displayed signals of three aromatic H-atoms ($\delta$(H) 7.63 (*dd*, $J = 8.5, 2.0$), 7.60 (*d*, $J = 2.0$), and 7.21 (*d*, $J = 8.5$)) suggesting the presence of a 1,3,4-trisubstituted aromatic ring, and of a MeO group (3.91). Compared to those of oplopanpheside A [2], the NMR data of **3** revealed the presence of an additional sugar moiety ($\delta$(C) 103.9, 75.1, 78.3, 75.3, 71.8, and 62.5), and of a *sec*-Bu group ($\delta$(H) 3.80–3.83 (*m*, 1 H), 1.58–1.61 (*m*, 1 H), 1.44–1.47 (*m*, 1 H), 1.20 (*d*, $J = 6.3$, 3 H); and 0.94 (*t*, $J = 7.5$, 3 H); and $\delta$(C) 79.1, 30.3, 21.5, and 9.9; *Table 3*). The *sec*-Bu group was linked *via* an O-atom to C(5″) of the 3-hydroxy-3-methylglutaryl moiety on the basis of the HMBC H–C(2‴)/C(5″). The chemical-shift and coupling-constant values ($\delta$(H) 4.33 (*d*, $J = 7.8$)) indicated the relative configuration of the additional sugar moiety as $\beta$. The HMBCs (*Fig. 2*) correlations between $CH_2$(6′) of glucosyl ($\delta$(H) 4.20, 4.45) and C(1″) ($\delta$(C) 172.4) of the *sec*-butyl 3-hydroxy-3-methylglutaryl moiety revealed that C(6′) and C(1″) were connected *via* a glycosidic bond. Though a few relevant HMBCs were not observed, the downfield shift of the C(6′) resonance of the glucosyl moiety from $\delta$(C) 62.6 to 64.8 suggested that the *sec*-butyl 3-hydroxy-3-methylglutaryl moiety was attached to C(6′) of the glucopyranosyl group [11]. The downfield shift of the C(4′)

Table 3. *$^1$H- and $^{13}$C-NMR Data* (500 and 125 MHz, resp., in $CD_3OD$) *of* **3** *and* **4**[a]). $\delta$ in ppm, $J$ in Hz.

| Position | **3** | | **4** | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| 1 | – | 125.4 | – | 127.8 |
| 2 | 7.60 (*d*, *J* = 2.0) | 114.2 | 7.17 (*d*, *J* = 2.0) | 111.8 |
| 3 | – | 150.5 | – | 150.4 |
| 4 | – | 152.2 | – | 149.6 |
| 5 | 7.21 (*d*, *J* = 8.5) | 116.5 | 7.05 (*d*, *J* = 8.5) | 116.6 |
| 6 | 7.63 (*dd*, *J* = 2.0, 8.5) | 124.5 | 7.61 (*dd*, *J* = 2.0, 8.5) | 124.1 |
| 7 | – | 168.3 | 7.19 (*d*, *J* = 16.0) | 130.3 |
| 8 | – | – | 6.78 (*d*, *J* = 16.0) | 115.2 |
| 9 | – | – | – | 168.2 |
| 1′ | 5.02 (*d*, *J* = 7.5) | 101.9 | 5.03 (*d*, *J* = 7.6) | 101.9 |
| 2′ | 3.63–3.65 (*m*) | 74.8 | 3.66–3.69 (*m*) | 74.7 |
| 3′ | 3.45 (*dd*, *J* = 8.0, 8.1) | 77.9 | 3.46–3.48 (*m*) | 77.9 |
| 4′ | 3.40 (*dd*, *J* = 8.8, 9.5) | 74.6 | 3.41–3.44 (*m*) | 74.7 |
| 5′ | 3.66–3.68 (*m*) | 75.3 | 3.69–3.71 (*m*) | 75.2 |
| 6′ | 4.45 (*dd*, *J* = 2.2, 11.8, $H_\alpha$), 4.20 (*dd*, *J* = 6.1, 11.8, $H_\beta$) | 64.8 | 4.44 (*dd*, *J* = 2.1, 11.6), 4.20 (*dd*, *J* = 6.0, 11.6) | 64.7 |
| 1″ | – | 172.4 | – | 172.5 |
| 2″ | 2.74 (*d*, *J* = 14.6, $H_\alpha$), 2.68 (*d*, *J* = 14.6, $H_\beta$) | 46.5 | 2.75 (*d*, *J* = 14.6), 2.66 (*s*) | 46.4 |
| 3″ | – | 70.8 | – | 70.7 |
| 4″ | 2.66 (*s*) | 46.0 | 2.66 (*s*) | 45.9 |
| 5″ | – | 175.3 | – | 174.9 |
| 1‴ | 4.33 (*d*, *J* = 7.8) | 103.9 | 4.33 (*d*, *J* = 7.2) | 103.9 |
| 2‴ | 3.64–3.67 (*m*) | 75.1 | 3.68–3.72 (*m*) | 75.2 |
| 3‴ | 3.35 (*dd*, *J* = 7.8, 8.1) | 77.8 | 3.38–3.41 (*m*) | 77.9 |
| 4‴ | 3.28 (*dd*, *J* = 7.7, 8.0) | 71.3 | 3.32–3.34 (*m*) | 71.4 |
| 5‴ | 3.56–3.58 (*m*) | 78.1 | 3.52–3.55 (*m*) | 78.1 |
| 6‴ | 4.24 (*dd*, *J* = 2.0, 11.8, $H_\alpha$), 3.80–3.83 (*m*, $H_\beta$) | 62.5 | 4.20 (*dd*, *J* = 2.0, 11.7), 3.79–3.82 (*m*) | 62.4 |
| 1⁗ | 1.20 (*d*, *J* = 6.3) | 21.5 | 1.19 (*d*, *J* = 6.2) | 21.4 |
| 2⁗ | 3.80–3.83 (*m*) | 79.1 | 3.78–3.82 (*m*) | 79.1 |
| 3⁗ | 1.58–1.61 (*m*, $H_\alpha$), 1.44–1.47 (*m*, $H_\beta$) | 30.3 | 1.59–1.61 (*m*), 1.45–1.47 (*m*) | 30.2 |
| 4⁗ | 0.94 (*t*, *J* = 7.5) | 9.9 | 0.93 (*d*, *J* = 7.5) | 10.0 |
| 3-MeO | 3.91 (*s*) | 56.7 | 3.89 (*s*) | 56.6 |
| 7-MeO | 3.88 (*s*) | 52.6 | 3.85 (*s*) | 52.6 |
| 3″-Me | 1.38 (*s*) | 27.8 | 1.38 (*s*) | 27.7 |

[a]) Assignments based on 2D-NMR spectra.

resonance of the glucosyl moiety from $\delta$(C) 71.5 to 74.6 evidenced that one more sugar moiety was connected with C(4′). Accordingly, the structure of **3** was identified as depicted in *Fig. 1*.

Compound **4** was purified as colorless gum with a specific rotation $[\alpha]_D^{20} = +7.5$ ($c$ = 0.4, MeOH). The HR-ESI-MS displayed the $[M+Na]^+$ ion peak at $m/z$ 755.2755 ($C_{33}H_{48}NaO_{18}^+$; calc. 755.2738), indicating that the molecular formula $C_{33}H_{48}O_{18}$. The

UV spectrum showed absorption maxima at 207 and 213 nm. The IR spectrum of **4** evidenced the presence of OH (3418 $cm^{-1}$) and conjugated C=O (1711 $cm^{-1}$) groups, and an aromatic ring (1601 and 1514 $cm^{-1}$) groups. Compared with **3**, signals of an additional pair of (*E*)-olefinic H-atoms ($\delta$(H) 7.19 (*d*, *J* = 16.0) and 6.78 (*d*, *J* = 16.0)) were observed in the $^1$H-NMR spectrum of **4** (*Table 3*). Based on the combination of $^1$H,$^1$H-COSY, HSQC, HMBC, and ROESY experiments, the NMR signals of **4** were assigned as compiled in *Table 3*. The HMBCs (*Fig. 2*) H–C(7)/C(2), C(6), and C(9) indicated the presence of a ferulic acid moiety. The 3-hydroxy-3-methylglutaryl moiety was at C(6′) of the glucopyranosyl group based on the downfield shift of the C(6′) resonance of the glucosyl moiety from $\delta$(C) 62.6 to 64.7 [11]. The connection of another sugar moiety and a *sec*-Bu group could be deduced as for compound **3**. Thus, the structure of **4** was elucidated as shown in *Fig. 1.*

Compound **5** was isolated as light-yellowish gum and deduced as glycer-2-yl ferulate by spectroscopic analyses. The NMR (in ($D_6$)DMSO) data were similar to those (in $C_5D_5N$ and $CD_3OD$) reported in [12][13] because different solvents were used for NMR experiments [13].

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## Experimental Part

*General*. HPLC-Grade MeOH and MeCN were purchased from *Merck* (Germany). The deionized $H_2O$ used for HPLC was purified by a *Milli-Q* purification system (*Millipore*, USA). All anal.-grade org. solvents were purchased from *Uni-Chem* (USA). The glassware was from *Büchi* (Switzerland). The glass columns packed with separation materials were purchased from *Xiamei* (Shanghai, P. R. China). Macroporous resin (pre-treated type, *D-101*) was purchased from *Haiguang Chemical Industrial Company* (Tianjin, P. R. China). Supercritical fluid extraction: supercritical fluid extractor *SFT-250* (*Supercritical Fluid Technologies, Inc.*, USA). TLC: Precoated silica gel $GF_{254}$ plates ($SiO_2$; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China). Column chromatograpy (CC): $SiO_2$ (100 – 200 and 200 – 300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*) and reversed-phase $C_{18}$ ($RP\text{-}C_{18}$) $SiO_2$ (40 – 63 μm; *Alltech*, USA). Anal. HPLC: *Agilent 1100* liquid chromatograph with an *Agilent Zorbax SB* $RP\text{-}C_{18}$ column (250 mm × 4.6 mm i.d., 5 μm; *Alltech*, USA). Prep. HPLC: *Agilent 1100* liquid chromatograph with a *Phenomenex Luna* $RP\text{-}C_{18}$ column (250 mm × 22 mm i.d., 5 μm). Optical rotations: *PerkinElmer Model 341* polarimeter. UV Spectra: *Beckman Coulter DU 640* spectrophotometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *PerkinElmer Spectrum 100* FT-IR spectrometer; KBr pellets; $\tilde{\nu}$ in $cm^{-1}$. $^1$H- and $^{13}$C-NMR spectra: *Bruker AV-500* spectrometer (*Bruker*, Germany); $\delta$ in ppm rel. to $Me_4Si$ as internal standard, *J* in Hz. ESI-MS: *Agilent 1100* series LC/MSD *Trap VL* mass spectrometer (*Agilent*, USA); in *m*/*z*. HR-ESI-MS: *Agilent* time-of-flight (TOF) mass spectrometer; in *m*/*z*.

*Plant Material*. The dried root bark of *O. horridus* was collected and authenticated by *C.-Z. W.* from Chicago, IL, USA, in March, 2009. A voucher specimen (OH-20090312-1) has been deposited with the Laboratory of Quality Control, State Key Laboratory for Quality Research in Chinese Medicine and Institute of Chinese Medical Sciences, University of Macau, Macao, P. R. China.

*Extraction and Isolation*. After removal of the volatile oil from the plant material by supercritical fluid extraction, pressurized liquid extraction and HPLC methods were applied to analyze the main components in order to optimize the best ratio between EtOH and $H_2O$ as the extraction solvent. The results suggested that the most effective solvent, 80% EtOH/$H_2O$, should be selected as extraction solvent with pressure refluxing. Then, the 80%-EtOH/$H_2O$ extract was diffused into pure $H_2O$ (5 l) and

extracted with petroleum ether (PE; 60–90°), AcOEt, and BuOH. The three org. solvents used for extraction were saturated with $H_2O$, and the extractions were carried out three times with a volume of 5 l for each solvent.

The BuOH extract was subjected to a macroporous resin column (EtOH/$H_2O$ 0:100, 40:60, and 95:5) to afford *Fr. 1* (210 g), *Fr. 2* (160 g), and *Fr. 3* (80 g). A 105-g portion of *Fr. 2* was then subjected to CC ($SiO_2$; 100–200 mesh; $CHCl_3$/MeOH 10:1 to 0:1) to give ten subfractions, *Frs. 2.1–2.10*. *Fr. 2.3* (13 g) was separated by CC (*RP-$C_{18}$*; $SiO_2$; MeOH/$H_2O$ 40:60), then further by prep. HPLC (MeCN/$H_2O$ 25:75) to afford **1** (8 mg). *Fr. 2.5* (18 g) was subjected to CC ($SiO_2$; 200–300 mesh; $CHCl_3$/MeOH 5:1), to afford five subfractions, *Frs. 2.5.1–2.5.5*. *Fr. 2.5.2* (2 g) was purified by CC (*RP-$C_{18}$*; $SiO_2$; MeOH/$H_2O$ 40:60), then by prep. HPLC (MeCN/$H_2O$ 22:78) to afford **2** (12 mg). *Fr. 2.5.3* (1.7 g) was separated by CC (*RP-$C_{18}$*; $SiO_2$; MeOH/$H_2O$ 38:62), then by prep. HPLC (MeCN/$H_2O$ 18:82) to afford **3** (5 mg) and **4** (4 mg). *Fr. 2.5.4* (1.9 g) was subjected to CC (*RP-$C_{18}$*; $SiO_2$; MeOH/$H_2O$ 35:65), then to prep. HPLC (MeCN/$H_2O$ 16:84) to afford **5** (11 mg).

*3-{4-[(6-*O*-Acetyl-β-*D*-glucopyranosyl)oxy]-3,5-dimethoxyphenyl}propanoic Acid* (**1**). Amorphous powder. $[\alpha]_D^{20} = +8.5$ ($c = 0.6$, MeOH). UV (MeOH): 256 (2.76), 228 (2.21), 210 (1.43). IR: 3252, 2876, 1740, 1601, 1512, 1432, 897. $^1$H- and $^{13}$C-NMR: see *Table 1*. ESI-MS: 453 ($[M+Na]^+$). HR-ESI-MS: 453.1359 ($[M+Na]^+$, $C_{19}H_{26}NaO_{11}^+$; calc. 453.1373).

*[7-(Hydroxymethyl)-10,11-dimethoxy-5,6,7,8-tetrahydrodibenzo[*a,c*][8]annulen-6-yl]methyl β-*D*-Glucopyranoside* (**2**). Amorphous powder. $[\alpha]_D^{20} = +18.1$ ($c = 0.5$, MeOH). UV (MeOH): 228 (1.88), 216 (1.43), 208 (0.78). IR: 3408, 3210, 2936, 1622, 1597, 1570, 1509, 1463, 1422, 1220, 1075, 827, 625. $^1$H- and $^{13}$C-NMR: see *Table 2*. ESI-MS: 491 ($[M+H]^+$). HR-ESI-MS: 491.2274 ($[M+H]^+$, $C_{26}H_{35}O_9^+$; calc. 491.2281).

*Methyl 4-[(6-*O*-{3-Hydroxy-3-methyl-5-[(1-methylpropyl)oxy]-5-oxopentanoyl}-4-*O*-(β-*D*-glucopyranosyl)-β-*D*-glucopyranosyl)oxy]-3-methoxybenzoate* (**3**). Colorless gum. $[\alpha]_D^{20} = +9.4$ ($c = 0.5$, MeOH). UV (MeOH): 194 (0.40), 220 (2.67), 265 (1.43), 358 (0.03). IR: 3459, 3409, 2926, 1720, 1597, 1513, 1468, 1430, 1383, 1239, 1128, 829, 667. $^1$H- and $^{13}$C-NMR: see *Table 3*. ESI-MS: 729 ($[M+Na]^+$). HR-ESI-MS: 707.2446 ($[M+H]^+$, $C_{31}H_{47}O_{18}^+$; calc. 707.2762).

*2-Methoxy-4-[(1*E*)-3-methoxy-3-oxoprop-1-en-1-yl]phenyl 6-*O*-{3-Hydroxy-3-methyl-5-[(1-methylpropyl)oxy]-5-oxopentanoyl}-4-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranoside* (**4**). Colorless gum. $[\alpha]_D^{20} = +7.5$ ($c = 0.4$, MeOH). UV (MeOH): 193 (0.90), 207 (2.63), 213 (2.72), 218 (2.65), 244 (1.56), 298 (1.74), 327 (1.94). IR: 3418, 2930, 1711, 1631, 1601, 1514, 1435, 1384, 1275, 1219, 1181, 1128, 1075, 1027, 893, 765. $^1$H- and $^{13}$C-NMR: see *Table 3*. ESI-MS: 755 ($[M+Na]^+$). HR-ESI-MS: 755.2755 ($[M+Na]^+$, $C_{33}H_{48}NaO_{18}^+$; calc. 755.2738).

*Glycer-2-yl Ferulate* (*=1,3-Dihydroxypropan-2-yl (2*E*)-3-(4-Hydroxy-3-methoxyphenyl)prop-2-enoate*; **5**). Light-yellowish gum. $[\alpha]_D^{20} = +52.1$ ($c = 0.5$, MeOH). UV (MeOH): 264 (2.75), 226 (1.84), 210 (0.91). IR: 3396, 2965, 1695, 1634, 1593, 1518, 1376, 1273, 1180, 1127, 1033, 975, 841. $^1$H-NMR ((D$_6$)DMSO, 500 MHz): 7.52 (*d*, $J = 15.8$, H–C(7)); 7.29 (*d*, $J = 1.5$, H–C(2)); 7.07 (*dd*, $J = 7.8$, 1.5, H–C(6)); 6.80 (*d*, $J = 7.8$, H–C(5)); 6.43 (*d*, $J = 15.8$, H–C(7)); 5.20–5.22 (*m*, H–C(2′)); 3.80 (*s*, MeO); 3.50 (*d*, $J = 6.0$, H–C(1′,3′)). $^{13}$C-NMR ((D$_6$)DMSO, 125 MHz): 166.3 (C(9)); 149.3 (C(3)); 147.9 (C(4)); 144.5 (C(7)); 125.6 (C(1)); 122.9 (C(8)); 115.5 (C(5)); 115.2 (C(6)); 111.1 (C(2)); 73.1 (C(2′)); 71.3 (C(1′,3′)); 56.4 (MeO). ESI-MS: 269 ($[M+H]^+$, $C_{13}H_{17}O_6^+$).

*Acidic Hydrolysis.* Compounds **1** (1.4 mg), **2** (1.6 mg), and **3** (1.1 mg) were hydrolyzed with 2M $CF_3COOH$ (1 ml) in a sealed glass tube with screw cap, which was filled with $N_2$ at 100° for 2 h, resp. The hydrolyzed soln. was evaporated to dryness under 50°, and then MeOH (2 ml) was added for further evaporation and complete removal of $CF_3COOH$. The hydrolysate was used for derivatization.

*Sugar Analysis.* The stock soln. of standard monosaccharides (1 ml) was treated with $NH_2OH \cdot HCl$/pyridine soln. (1 ml) in a sealed glass tube at 90° for 30 min. $Ac_2O$ (1 ml) was added, and heating was continued for another 30 min before the soln. was cooled to r.t. The cooled soln. was evaporated to dryness under reduced pressure at 50°. The residue was dissolved in dry MeOH (2 ml). The mixture was filtered through a 0.45-μm syringe filter (*Agilent Technologies*) prior to injection into the GC/MS system. The hydrolysate was reacted with $NH_2OH \cdot HCl$ and $Ac_2O$ to form the derivatives by the procedures mentioned above for sugar determination. GC/MS was performed on an *Agilent 6890* gas chromatograph coupled with an *Agilent 5973* mass spectrometer (*Agilent Technologies*, Palo Alto, CA, USA). A *HP-*

*5 MS* cap. column (30 m × 0.25 mm i.d.) coated with 0.25 μm film 5% phenylmethylsiloxane was used for separation. The column temp. was set at 175° and held for 7 min, then programmed at 5°/min to 185°, held for 5 min, and then at 4°/min to 230°. Split injection (2 μl) with a split ratio of 1:50 was applied. High-purity He was used as carrier gas with a flow rate of 1.0 ml $min^{-1}$. The mass spectrometer was operated in electron-impact (EI) mode, the scan range was 40–550 amu, the ionization energy was 70 eV, and the scan rate was 2.89 s per scan. The inlet and ionization source temp. were 250 and 280°, resp.

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