# Glycopentosides D – F, Three New Phenolic Glycosides from *Glycosmis pentaphylla*

by **Yu Chen**[a]), **Er-Li Tian**[a]), **Xin Hu**[b]), **Jun Li**[b]), and **Guang-Zhong Yang***[b])

[a]) College of Chemistry and Material Sciences, South-Central University for Nationalities, Wuhan 430074, P. R. China

[b]) Laboratory for Natural Product Chemistry, College of Pharmacy, South-Central University for Nationalities, Wuhan 430074, P. R. China

(phone/fax: +86-27-67841196; e-mail: yanggz888@126.com)

A phytochemical investigation of the BuOH-soluble fraction of the EtOH extract from the stems of *Glycosmis pentaphylla* resulted in the isolation of three new phenolic glycosides, glycopentosides D – F (**1** – **3**, resp.). Their structures were determined by using spectroscopic analysis including UV, $^1$H- and $^{13}$C-NMR, DEPT, COSY, ROESY, HMBC, HSQC, HR-ESI-MS, and acid hydrolysis.

**Introduction.** – Plants of the genus *Glycosmis* (family: Rutaceae) are a rich source of various types of alkaloids with fascinating structures and significant bioactivities [1]. *Glycosmis pentaphylla* (RETZ.) DC. is a shrub or small tree, *ca.* 1.5 – 5.0 m high, and is widely distributed from India, Malaysia, and Southern China to the Philippine Islands. It is used for treatment of cough, rheumatism, anaemia, and jaundice. Stems and roots of plant are used for treatment of ulcer [2]. Several phytochemical studies revealed that the extracts from this plant contained secondary metabolites such as alkaloids including compounds of the acridone [3], carbazole [4], quinolone [5], and quinazoline type [6], as well as isoflavone diglycosides [7] and hydroquinone diglycoside acyl esters [8]. As part of an ongoing program to search for bioactive compounds from Rutaceae plants [9], we have carried out a chemical study on the stems of *G. pentaphylla*, resulting in the isolation of three new carbazole alkaloids [10], two new flavanols [11], and four new phenolic glycosides [12]. Furthermore, we also demonstrated that glycoborinine induced apoptosis in HepG2 cells through a mitochondrial-dependent pathway [13]. In the current work, we report the isolation and structural elucidation of three new phenolic glycosides (*Fig. 1*) on the basis of extensive spectroscopic analysis, including 2D-NMR and ESI-MS spectra.

**Results and Discussion.** – Compound **1** was obtained as white amorphous powder. Its molecular formula was determined as $C_{28}H_{36}O_{17}$ on the basis of the *pseudo*-molecular-ion peak at *m*/*z* 643.1875 ($[M-H]^-$, $C_{28}H_{35}O_{17}^-$; calc. 643.1874) by HR-ESI-MS. The $^1$H-NMR spectrum (*Table 1*) of **1** showed three MeO groups at $\delta$(H) 3.83 (*s*, 3 H) and 3.70 (*s*, 6 H), one 1,3,4-trisubstituted benzene ring at $\delta$(H) 7.45 (*d*, $J = 1.5$, 1 H), 6.84 (*d*, $J = 8.3$, 1 H), and 7.43 (*dd*, $J = 1.5$, 8.3, 1 H), a symmetrical 1,3,4,5-tetrasubstituted benzene ring at $\delta$(H) 6.42 (*s*, 2 H), and two anomeric H-atoms at $\delta$(H) 4.76 (*d*, $J = 8.0$, 1 H) and 4.77 (*d*, $J = 8.0$, 1 H). Acid hydrolysis of **1** gave D-glucose

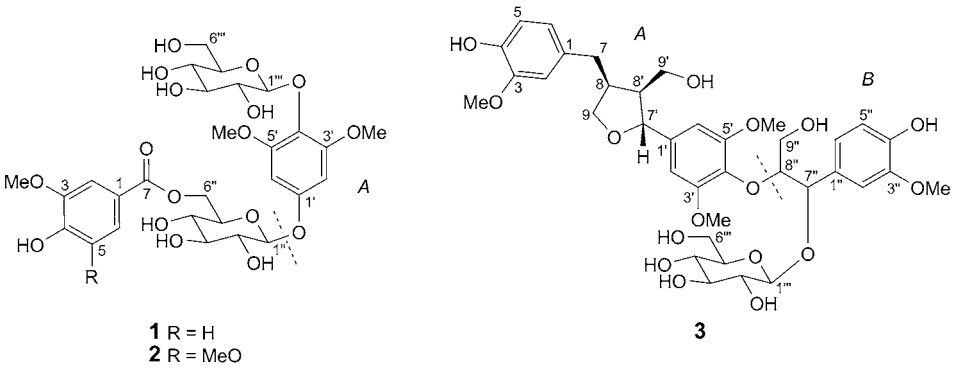


Fig. 1. *Structures of compounds* **1**–**3**

which was identified by HPLC analysis after derivatization with L-cysteine methyl ester hydrochloride. The large coupling constant ($J = 8.0$) of the anomeric H-atom indicated that the glucose was $\beta$-configurated. Comparison of the NMR data of **1** with those of 4-hydroxy-2,6-dimethoxyphenyl $\beta$-D-glucopyranoside [14], indicated that they possessed the same 4-*O*-$\beta$-D-glucopyranosyl-3,5-dimethoxyphenoxy moiety (*Part A*) which was further supported by the HMBC between the anomeric H-atom H–C(1‴) ($\delta$(H) 4.76 (*d*, $J = 8.0$)) and C(4′) ($\delta$(C) 131.5). In addition to the $^1$H- and $^{13}$C-NMR signals of *Part A* mentioned above, the $^1$H- and $^{13}$C-NMR spectra also showed evident signals attributed to a vanilloyl group ($\delta$(H) 7.45 (*d*, $J = 1.5$, 1 H), 6.84 (*d*, $J = 8.3$, 1 H), 7.43 (*dd*, $J = 1.5$, 8.3, 1 H), and 3.83 (*s*, 3 H); $\delta$(C) 122.7 (C(1)), 113.7 (C(2)), 148.9 (C(3)), 153.9 (C(4)), 116.3 (C(5)), 125.2 (C(6)), 168.2 (C(7)), 56.7 (MeO)) and a $\beta$-D-glucopyranosyl group ($\delta$(H) 4.77 (*d*, $J = 8.0$, 1 H); $\delta$(C) 103.3, 75.1, 78.0, 72.1, 75.9, 65.1). The HMBC between the anomeric H-atom H–C(1″) ($\delta$(H) 4.77 (*d*, $J = 8.0$) and C(1′) ($\delta$(C) 156.6) indicated the second $\beta$-D-glucopyranosyl group to be attached to C(1′) of the partial structure *A*. The attachment of the vanilloyl group to C(6″) was deduced from the HMBC between $CH_2$(6″) and C(7), which was also supported by the down-field shift of chemical shift of the $CH_2$(6″) H-atoms due to the esterification by the C=O C-atom of the vanilloyl group. Therefore, compound **1** was identified as 4-($\beta$-D-glucopyranosyloxy)-3,5-dimethoxyphenyl 6-*O*-(4-hydroxy-3-methoxybenzoyl)-$\beta$-D-glucopyranoside, and named as glycopentoside D.

Compound **2** was obtained as white amorphous powder. Its molecular formula was assigned as $C_{29}H_{38}O_{18}$ by HR-ESI-MS (*m*/*z* 673.1982 ($[M-H]^-$, $C_{29}H_{37}O_{18}^-$; calc. 673.1980)). Comparison of the NMR data of **2** with those of **1** indicated that **2** was similar to **1** except for a syringoyl group ($\delta$(H) 7.14 (*s*, 2 H), and 3.77 (*s*, 6 H); $\delta$(C) 119.6 (C(1)), 107.3 (C(2)), 147.9 (C(3)), 141.5 (C(4)), 147.9 (C(5)), 107.3 (C(6)), 166.0 (C(7)), 56.5 (2 × MeO)) instead of a vanilloyl group. This assumption was further confirmed by the HMBCs of H–C(1‴) ($\delta$(H) 4.77 (*d*, $J = 6.0$) with C(4′) ($\delta$(C) 129.5), of H–C(1″) ($\delta$(H) 4.75 (*d*, $J = 7.5$) with C(1′) ($\delta$(C) 154.6), and of $CH_2$(6″) with C(7) ($\delta$(C) 166.0). Accordingly, the structure of **2** was determined to be 4-($\beta$-D-glucopyranosyloxy)-3,5-dimethoxyphenyl 6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-$\beta$-D-glucopyranoside, and named as glycopentoside E.

Table 1. *$^1$H- and $^{13}$C-NMR Data of* **1** (in $CD_3OD$) *and* **2** (in ($D_6$)DMSO). Arbitrary atom numbering indicated in *Fig. 1*; $\delta$ in ppm, $J$ in Hz.

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| 1 | | 122.7 | | 119.6 |
| 2 | 7.45 (*d*, *J* = 1.5) | 113.7 | 7.14 (*s*) | 107.3 |
| 3 | | 148.9 | | 147.9 |
| 4 | | 153.9 | | 141.5 |
| 5 | 6.84 (*d*, *J* = 8.3) | 116.3 | | 147.9 |
| 6 | 7.43 (*dd*, *J* = 1.5, 8.3) | 125.2 | 7.14 (*s*) | 107.3 |
| 7 | | 168.2 | | 166.0 |
| 3- or 3, 5-MeO | 3.83 (*s*, 3 H) | 56.7 | 3.77 (*s*, 6 H) | 56.5 |
| 1′ | | 156.6 | | 154.6 |
| 2′ | 6.42 (*s*) | 96.5 | 6.34 (*s*) | 95.4 |
| 3′ | | 154.9 | | 153.4 |
| 4′ | | 131.5 | | 129.5 |
| 5′ | | 154.9 | | 154.6 |
| 6′ | 6.42 (*s*) | 96.5 | 6.34 (*s*) | 95.4 |
| 3′, 5′-MeO | 3.70 (*s*, 6 H) | 57.1 | 3.64 (*s*, 6 H) | 56.5 |
| 7-Glc | | | | |
| 1″ | 4.77 (*d*, *J* = 8.0) | 103.3 | 4.75 (*d*, *J* = 7.5) | 101.3 |
| 2″ | 3.39–3.50 (*m*) | 75.1 | 3.19–3.26 (*m*) | 73.5 |
| 3″ | 3.38–3.48 (*m*) | 78.0 | 3.23–3.35 (*m*) | 76.2 |
| 4″ | 3.39–3.47 (*m*) | 72.1 | 3.26–3.31 (*m*) | 70.2 |
| 5″ | 3.39–3.50 (*m*) | 75.9 | 3.36–3.42 (*m*) | 74.3 |
| 6″ | 4.56 (*d*, *J* = 11.5), 4.32 (*dd*, *J* = 6.5, 12.0) | 65.1 | 4.52 (*d*, *J* = 11.0), 4.15 (*dd*, *J* = 5.5, 11.5) | 64.1 |
| 4′-Glc | | | | |
| 1‴ | 4.76 (*d*, *J* = 8.0) | 105.5 | 4.77 (*d*, *J* = 6.0) | 103.5 |
| 2‴ | 3.39–3.50 (*m*) | 75.7 | 3.19–3.26 (*m*) | 74.4 |
| 3‴ | 3.38–3.48 (*m*) | 78.2 | 3.23–3.35 (*m*) | 76.7 |
| 4‴ | 3.28–3.35 (*m*) | 71.8 | 3.08–3.13 (*m*) | 70.3 |
| 5‴ | 3.38–3.48 (*m*) | 78.5 | 3.23–3.35 (*m*) | 77.4 |
| 6‴ | 3.89 (*dd*, *J* = 1.6, 11.9), 3.60–3.67 (*m*) | 62.9 | 3.69 (*d*, *J* = 12.5), 3.39–3.44 (*m*) | 61.2 |

Compound **3** was obtained as colorless oil. Its molecular formula was established as $C_{37}H_{48}O_{16}$ by HR-ESI-MS at *m*/*z* 747.2855 ($[M-H]^-$). The NMR data indicated that **3** was a sesquilignan constituted by a 5′-methoxylariciresinol (*Part A*) and guaiacylglycerol unity (*Part B*). The $^1$H- and $^{13}$C-NMR data (*Table 2*) revealed that *Part A* of **2** resembled those of the known compound 5′-methoxylariciresinol [15]. The $^1$H-NMR spectrum showed two CH groups ($\delta$(H) 2.60–2.68 (*m*, 1 H) and 2.24–2.32 (*m*, 1 H)), one benzylic O–CH moieties ($\delta$(H) 4.75 (*d*, *J* = 6.0, 1 H)), two O-bearing $CH_2$ groups ($\delta$(H) 3.94 (*dd*, *J* = 6.8, 8.0, 1 H) and 3.68 (*dd*, *J* = 6.8, 8.0, 1 H), 3.58–3.60 (*m*, 1 H) and 3.78–3.84 (*m*, 1 H)), one $CH_2$ group ($\delta$(H) 2.83 (*dd*, *J* = 4.8, 13.2, 1 H) and 2.43 (*dd*, *J* = 11.2, 13.2, 1 H)), one 1,3,4-trisubstituted benzene ring ($\delta$(H) 6.73 (*d*, *J* = 1.2, 1 H), 6.65 (*d*, *J* = 7.8, 1 H), and 6.58 (*dd*, *J* = 1.2, 7.8, 1 H)), and a symmetrical 1,3,4,5-tetrasubstituted benzene ring ($\delta$(H) 6.55 (*s*, 2 H)). Accordingly, *Part A* of **3** was

Table 2. *$^1$H- and $^{13}$C-NMR Data* (in $CD_3OD$) *of* **3**. Arbitrary atom numbering indicated in *Fig. 1*; $\delta$ in ppm, $J$ in Hz.

| Position | $\delta$(H) | $\delta$(C) | Position | $\delta$(H) | $\delta$(C) |
|---|---|---|---|---|---|
| 1 | | 133.6 | 3′, 5′-MeO | 3.63 (*s*) | 56.6 |
| 2 | 6.73 (*d*, *J* = 1.2) | 113.4 | 3, 3″-MeO | 3.78 (*s*) | 56.5 |
| 3 | | 149.1 | 1″ | | 130.9 |
| 4 | | 146.0 | 2″ | 7.17 (*d*, *J* = 1.2) | 113.2 |
| 5 | 6.65 (*d*, *J* = 7.8) | 116.3 | 3″ | | 148.8 |
| 6 | 6.58 (*dd*, *J* = 1.2, 7.8) | 122.3 | 4″ | | 147.2 |
| 7 | 2.83 (*dd*, *J* = 4.8, 13.2), | 33.8 | 5″ | 6.71 (*d*, *J* = 8.0) | 115.6 |
| | 2.43 (*dd*, *J* = 11.2, 13.2) | | 6″ | 6.84 (*dd*, *J* = 1.2, 8.0) | 122.2 |
| 8 | 2.60 – 2.68 (*m*) | 43.9 | 7″ | 5.21 (*d*, *J* = 3.2) | 77.7 |
| 9 | 3.94 (*dd*, *J* = 6.8, 8.0), | 73.9 | 8″ | 4.16 – 4.22 (*m*) | 86.9 |
| | 3.68 (*dd*, *J* = 6.8, 8.0) | | 9″ | 3.37 (*dd*, *J* = 4.4, 11.2), | 61.5 |
| 1′ | | 141.4 | | 3.78 – 3.84 (*m*) | |
| 2′ | 6.55 (*s*) | 103.9 | 7″-Glc | | |
| 3′ | | 154.5 | 1‴ | 4.14 (*d*, *J* = 7.2) | 101.0 |
| 4′ | | 136.2 | 2‴ | 3.19 – 3.29 (*m*) | 75.3 |
| 5′ | | 154.5 | 3‴ | 3.19 – 3.29 (*m*) | 77.8 |
| 6′ | 6.55 (*s*) | 103.9 | 4‴ | 3.19 – 3.29 (*m*) | 72.0 |
| 7′ | 4.75 (*d*, *J* = 6.0) | 84.2 | 5‴ | 3.02 – 3.08 (*m*) | 77.9 |
| 8′ | 2.24 – 2.32 (*m*) | 54.3 | 6‴ | 3.75 – 3.81 (*m*), | 62.8 |
| 9′ | 3.58 – 3.60 (*m*), | 60.7 | | 3.58 – 3.64 (*m*) | |
| | 3.78 – 3.84 (*m*) | | | | |

assigned to be a 7′,9-epoxylignane with three MeO groups, one O-functional group, and one OH group. Comparing its $^{13}$C-NMR data with those of 5′-methoxylariciresinol [15] disclosed that they had almost the same chemical shift except for down-field shifts at C(3′), C(4′), and C(5′) ($\Delta\delta$(C) +7.5, +2.2, and +7.5, resp.). This suggested that HO–C(4′) of sinapylic moiety of *Part A* should be connected to *Part B* of **3** through an ether linkage. Therefore, the constitutional structure of *Part A* of **3** was identified as that of 5′-methoxylariciresinol, except for one O-functional group at C(4′) in **3** instead of a OH group, which was further supported by the HMBCs (*Fig. 2*). The relative configuration of *Part A* of **3** was determined on the basis of a ROESY experiment. The ROESY correlations of H–C(7′)/$CH_2$(9′) and $CH_2$(7), and H–C(8)/H–C(8′) indicated that H–C(8) and H–C(8′) were *cis*, and H–C(7′) and H–C(8′) were *trans* (*Fig. 3*). In addition to the $^1$H- and $^{13}$C-NMR signals of *Part A* mentioned above, the $^1$H- and $^{13}$C-NMR spectra also showed evident signals attributed to a *ABX* system ($\delta$(H) 7.17 (*d*, *J* = 1.2, 1 H), 6.71 (*d*, *J* = 8.0, 1 H), and 6.84 (*dd*, *J* = 1.2, 8.0, 1 H)), together with three aromatic C-atoms ($\delta$(C) 130.9, 148.8, 147.2), three aromatic CH ($\delta$(C) 113.2, 115.6, 122.2), two O–CH ($\delta$(H)/$\delta$(C) 4.16 – 4.22 (*m*, 1 H)/86.9 (C(8″)), 5.21 (*d*, *J* = 3.2, 1 H)/77.7 (C(7″))), one $CH_2O$ ($\delta$(H)/$\delta$(C) 3.37 (*dd*, *J* = 4.4, 11.2, 1 H), 3.78 – 3.84 (*m*, 1 H)/61.5 (C(9″))), and an anomeric CH ($\delta$(H)/$\delta$(C) 4.14 (*d*, *J* = 7.2, 1 H)/101.0 (C(1‴))). Acid hydrolysis of **3** gave D-glucose which was determined by HPLC analysis. The $\beta$-configuration of glucose was determined from the coupling constant ($J$ = 7.2) of the anomeric H-atom. All the NMR data mentioned above suggested that *Part B* of **3** was a guaiacylglycerol derivative. In comparison of the $^{13}$C-NMR data of **3**

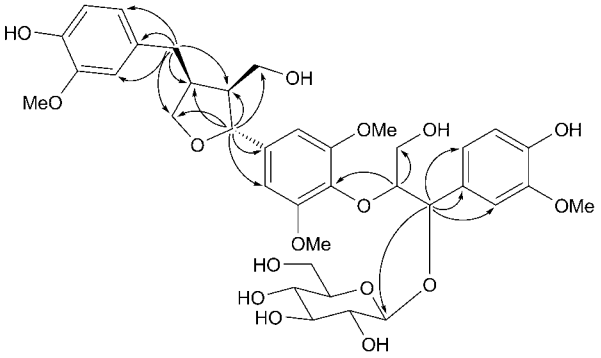


Fig. 2. *Key HMBCs of* **3**

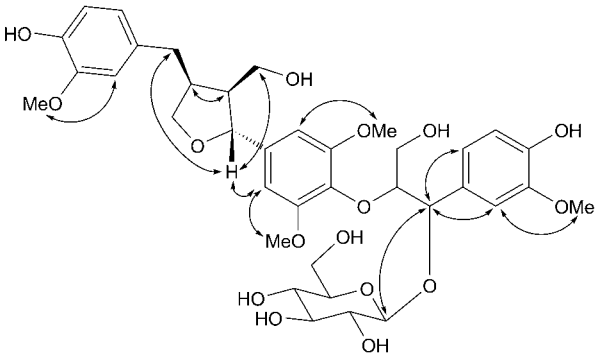


Fig. 3. *Key ROESY correlations of* **3**

with those of the guaiacylglycerol moiety of ficusesquiligan A [16], a majority of $^{13}$C-NMR data were almost the same except for the difference at C(7″) ($\delta$(C) 77.7 (C(7″)) in **3**; 72.5 (C(7″)) in ficusesquiligan A). Thus, the glycoside linkage was suggested to be at C(7″) which was further confirmed by the HMBC of H–C(1‴) ($\delta$(H) 4.14 (*d*, $J = 7.2$)) with C(7″) ($\delta$(C) 77.7). However, the relative configuration of the guaiacylglycerol moiety could not be determined due to the possible free rotation of C(7″)–C(9″) C-atom chain. The HMBC of H–C(8″) ($\delta$(H) 4.16 – 4.22 (*m*, 1 H)) with C(4′) ($\delta$(C) 136.2) indicated that compound **3** was a sesquilignan resulting from coupling between C(4′) of 5′-methoxylariciresinol unit and C(8″) of guaiacylglycerol moiety *via* an ether linkage. From the above data, the structure of **3** was elucidated as depicted in *Fig. 1*, and was given the trivial name glycopentoside F.

This work was financially supported by the *Natural Science Foundation of Hubei Province of China* (2013CFB450; 2013CFA013) and the *National Key Technology R&D Program of the 12th Five-Year Plan of China* (2012BAI27B06).

## Experimental Part

*General*. TLC: Precoated silica gel $GF_{254}$ plates ($SiO_2$; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). Column Chromatography (CC): $SiO_2$ (200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China) and $C_{18}$ reversed-phase $SiO_2$ (*YMC Co., Ltd.*, Japan). HPLC: *Ultimate 3000* HPLC system (*Dionex Co.*, California, USA); *Ultimate 3000* pump; *Ultimate 3000* variable wavelength; column *Waters* $5C_{18}$*-MS-II* (10 mm × 250 mm). Optical rotations: *P-1020* digital polarimeter in MeOH soln. (*JASCO*, Tokyo, Japan). UV: *210A* double-beam spectrophotometer in MeOH soln. (*Shimadzu Co.*, Kyoto, Japan); $\lambda_{max}$ (log $\varepsilon$) in nm. $^1$H-, $^{13}$C-, and 2D-NMR spectra: *Bruker-AM-400* instrument; $\delta$ in ppm rel. to $Me_4Si$ as internal standard, *J* in Hz. ESI-MS: *QSTAR i API Pulsar System* spectrometer (*ABI Co.*, Foster, California USA).

*Plant Material*. The stems of *Glycosmis pentaphylla* were picked from Xishuangbanna Prefecture, Yunnan Province, P. R. China, in September 2010. The plant material identification was verified by Prof. *Ying-Hong Zhao*, Xishuangbanna Prefecture National Medicine Research Institute. A voucher specimen (No. 20100901) has been deposited with the Herbarium of College of Pharmacy, South-Central University for Nationalities.

*Extraction and Isolation.* The air-dried material (24.5 kg) was finely pulverized, then extracted with EtOH (80 l, 24 h) for three times at r.t. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (586 g). The EtOH extract was suspended in 90% $H_2O$/MeOH and then successively partitioned with petroleum ether (3 × 4 l), AcOEt (3 × 4 l), and BuOH (3 × 4 l). The BuOH extract (101 g) was chromatographed ($SiO_2$; $CHCl_3$/MeOH 95 : 5, 9 : 1, 8 : 2, 7 : 3, 1 : 1, 3 : 7, and 0 : 1) to give ten main fractions, *Frs. 1–10*. *Fr. 5* (9.6 g) was subjected to CC (octadecylsilane; $H_2O$/MeOH 9 : 1 → 0 : 1) and further purified by semi-prep. HPLC (MeCN/$H_2O$ 5 : 95, 3 ml/min) to afford compound **3** (11 mg, $t_R$ 14.1 min). *Fr. 6* (11.1 g) was subjected to CC (octadecylsilane; $H_2O$/MeOH 9 : 1 → 0 : 1) to give twelve fractions, *Frs. 6.1–6.12*. *Fr. 6.10* (1.38 g) was subjected to CC ($SiO_2$; $CHCl_3$/MeOH 95 : 5 → 1 : 1) and further purified by semi-prep. HPLC (MeCN/$H_2O$ 12 : 88, 3 ml/min) and (MeCN/$H_2O$ 17 : 83, 3 ml/min) to afford compound **2** (6 mg, $t_R$ 19.9 min) and **1** (2 mg, $t_R$ 12.9 min), resp.

*Glycopentoside D* (= *4-(β-*D*-Glucopyranosyloxy)-3,5-dimethoxyphenyl 6-*O*-(4-Hydroxy-3-methoxybenzoyl)-β-*D*-glucopyranoside*; **1**). White powder. $[\alpha]_D^{20} = +4.0$ ($c = 0.23$, MeOH). UV (MeOH): 208 (3.66), 270 (2.94), 299 (2.70). $^1$H- and $^{13}$C-NMR: see *Table 1*. HR-ESI-MS: 643.1875 ($[M-H]^-$, $C_{28}H_{35}O_{17}^-$; calc. 643.1874).

*Glycopentoside E* (= *4-(β-*D*-Glucopyranosyloxy)-3,5-dimethoxyphenyl 6-*O*-(4-Hydroxy-3,5-dimethoxybenzoyl)-β-*D*-glucopyranoside*; **2**). White powder. $[\alpha]_D^{20} = -27.23$ ($c = 0.12$, MeOH). UV (MeOH): 208 (3.70), 281 (3.06). $^1$H- and $^{13}$C-NMR: see *Table 1*. HR-ESI-MS: 673.1982 ($[M-H]^-$, $C_{29}H_{37}O_{18}^-$; calc. 673.1980).

*Glycopentoside F* (= *3-Hydroxy-2-{4-[(2*R**,3*S**,4*S**)-tetrahydro-4-(4-hydroxy-3-methoxybenzyl)-3-(hydroxymethyl)furan-2-yl]-2,6-dimethoxyphenoxy}-1-(4-hydroxy-3-methoxyphenyl)propyl β-*D*-Glucopyranoside*; **3**). Colorless oil. $[\alpha]_D^{20} = -13.95$ ($c = 0.22$, MeOH). UV (MeOH): 233 (3.83), 281 (3.68). $^1$H- and $^{13}$C-NMR: see *Table 2*. HR-ESI-MS: 747.2855 ($[M-H]^-$, $C_{37}H_{47}O_{16}^-$; calc. 747.2864).

*Acidic Hydrolysis.* Compounds **1**–**3** (each 2 mg) were dissolved in 4N $CF_3COOH$ soln. (4 ml), resp., and heated at 95° for 3 h, then cooled to r.t. The acid hydrolysates of **1**–**3** were extracted with $CH_2Cl_2$ (2 ml × 3). The dried aq. layers and the reference D-glucose were derivatized by reacting with anh. pyridine (0.5 ml) and L-cysteine methyl ester hydrochloride (1.0 mg) for 1 h at 60°. After cooling, *o*-tolyl isothiocyanate (5 µl) was added, and the mixture was heated at 60° for 1 h. After cooling to r.t., the mixture was diluted twice with pyridine and analyzed with HPLC (MeCN/$H_2O$ 25 : 75, 0.8 ml/min). The samples (5 µl) were injected into a chromatographic column (4.6 mm × 250 mm, *Waters*) under 30°. The retention time of derivatized D-glucose was determined to be 18.85 min. By comparison of the retention times of the acid hydrolysate derivatives with that of the standard, the absolute configuration of sugar in each hydrolysis was established as D-glucose.

## REFERENCES


[1] J. Wang, Y. Zheng, T. Efferth, R. Wang, Y. Shen, X. Hao, *Phytochemistry* **2005**, *66*, 697.
[2] P. S. Sreejith, R. J. Praseeja, V. V. Asha, *J. Pharm. Res.* **2012**, *5*, 2723.
[3] M. A. Quader, M. T. H. Nutan, M. A. Rashid, *Fitoterapia* **1999**, *70*, 305; T. R. Govindachari, B. R. Pai, P. S. Subramaniam, *Tetrahedron* 1**966**, *22*, 3245.
[4] S. Mukherjee, M. Mukherjee, S. N. Ganguly, *Phytochemistry* **1983**, *22*, 1064; P. Bhattacharyya, B. K. Chowdhury, A. Mustapha, M. Garba, *Phytochemistry* **1987**, *26*, 2138; P. Bhattacharyya, P. K. Chakrabartty, B. K. Chowdhury, *Phytochemistry* **1985**, *24*, 882; P. Bhattacharyya, B. K. Chowdhury, *J. Nat. Prod.* **1985**, *48*, 465.
[5] P. Bhattacharyya, B. K. Chowdhury, *Phytochemistry* **1985**, *24*, 634.
[6] M. Sarkar, D. P. Chakraborty, *Phytochemistry* **1977**, *16*, 2007.
[7] J. Wang, X. Yang, Y. Di, Y. Wang, Y. Shen, X. Hao, *J. Nat. Prod.* **2006**, *69*, 778.
[8] J. Wang, Y. Di, X. Yang, S. Li, Y. Wang, X. Hao, *Phytochemistry* **2006**, *67*, 486.
[9] Y. Hu, Y. Chen, Y. Shang, G. Yang, *Bull. Korean Chem. Soc.* **2009**, *30*, 1884; G.-Z. Yang, Y. Hu, B. Yang, Y. Chen, *Helv. Chim. Acta* **2009**, *92*, 1657; D.-R. Zhu, D. Zhu, Y. Chen, Z. Mei, G. Yang, *Chin. J. Org. Chem.* **2013**, *33*, 1345.
[10] G.-Z. Yang, Y. Wu, Y. Chen, *Helv. Chim. Acta* **2012**, *95*, 1449.
[11] Y. Wu, X. Hu, G.-Z. Yang, Z.-N. Mei, Y. Chen, *J. Asian Nat. Prod. Res.* **2012**, *14*, 738.
[12] E.-L. Tian, Y.-Y. Cui, G.-Z. Yang, Z.-N. Mei, Y. Chen, *J. Asian Nat. Prod. Res.* **2014**, *16*, 1119; E. Tian, G. Yang, Z. Mei, Y. Chen, *Chin. Tradit. Herb. Drugs* **2014**, *45*, 1358.
[13] H. Yang, S.-T. Tian, R.-Y. Wu, Y. Chen, Z.-N. Mei, C.-Y. Wang, G.-Z. Yang, *J. Asian Nat. Prod. Res.* **2014**, *16*, 991.
[14] Y. Xiao, J. Li, Y. Ding, *Chin. Tradit. Herb. Drugs* **2003**, *34*, 109.
[15] C.-Y. Duh, C. H. Phoebe Jr., J. M. Pezzuto, A. D. Kinghorn, N. R. Farnsworth, *J. Nat. Prod.* **1986**, *49*, 706; W. Huang, D. Kong, P. Yang, *Chin. J. Nat. Med.* **2003**, *1*, 199.
[16] Y.-C. Li, Y.-H. Kuo, *Chem. Pharm. Bull.* **2000**, *48*, 1862.


*Received February 16, 2015*