Available online at www.sciencedirect.com
SCIENCE DIRECT
Phytochemistry 67 (2006) 486–491
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*

Junsong Wang, Yingtong Di, Xianwen Yang, Shunlin Li, Yuehu Wang, Xiaojiang Hao *

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Heilong tan, Kunming 650204, PR China

Received 1 August 2005; received in revised form 30 October 2005
Available online 19 January 2006

Abstract

Four hydroquinone diglycoside acyl esters, glypentosides A–C (**1**–**3**) and seguinoside F (**4**), were isolated from the stems of *Glycosmis pentaphylla*. Glypentosides A–B (**1**–**2**) were identified as compounds and designated as methoxyquinol 4-*O*-[(5-*O*-*trans*-*p*-coumaroyl)-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (**1**) and 4-demethylantiarol 4-*O*-[(3-methoxy-4-hydroxy-benzoyl)-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (**2**). Glypentoside C (**3**) is a hydroquinone diglycoside acyl ester with a neolignan moiety in the acyl unit. Their structures were elucidated by the combination of one- and two-dimensional NMR analysis, mass spectrometry and chemical evidences.

Keywords: *Glycosmis pentaphylla*; Rutaceae; Glypentosides A–C; Hydroquinone glycosides; Acyl esters; Apiosyl-(1 → 2)-glucosides

1. Introduction

The genus *Glycosmis* of the family Rutaceae is represented in China by nearly 11 species (Huang, 1997). *Glycosmis pentaphylla* (Retz.) DC. is a shrub or small (1.5–5 m) tree widely distributed from India, Malaysia and southern China to the Philippine Islands where it occurs in tropical forests at low altitudes. It has been used as a folk medicine in the treatment of fever, liver complaints and certain other diseases (Sastri, 1956). Phytochemical researches of this species were mainly focused on hydrophobic alkaloids, including those of the quinolone (Bhattacharyya and Chowdhury, 1985), quinazoline (Muthukrishnan et al., 1999; Sarkar and Chakraborty, 1979), acridone (Quader et al., 1999) and carbazole (Jash et al., 1992; Chowdhury et al., 1987) types, of leaves, root and stem bark. No study on glycosidic constituents of *G. pentaphylla* has been reported. In this paper, we present results of an study on the polar constituents of the stem wood of the plant.

2. Results and discussion

The EtOAc-soluble fraction of the MeOH extract was subjected to a succession of chromatographic procedures and finally by preparative ODS-HPLC to give three new hydroquinone diglycosides, namely glypentoside A (**1**), glypentoside B (**2**) and glypentoside C (**3**), besides the known compound seguinoside F (**4**). The known compound was identified by comparing its spectral data with those previously reported (Zhong et al., 1998).

Compound **1** was isolated as amorphous powder. Its molecular formula $C_{27}H_{32}O_{14}$ was deduced from the negative HRFABMS spectrum and ^{13}C NMR spectral data. The IR spectrum showed absorption bands due to hydroxyl (3420 cm^{-1}) and carbonyl groups (1690 cm^{-1}). The ^{1}H NMR signals due to aromatic and olefinic protons at δ 7.54 (1H, *d*, $J = 15.7$ Hz), 7.37 (2H, *d*, $J = 8.4$ Hz), 6.79

* Corresponding author. Tel.: +86 871 522 3263; fax: +86 871 515 0227.
E-mail address: haoxj@mail.kib.ac.cn (X. Hao).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.11.025

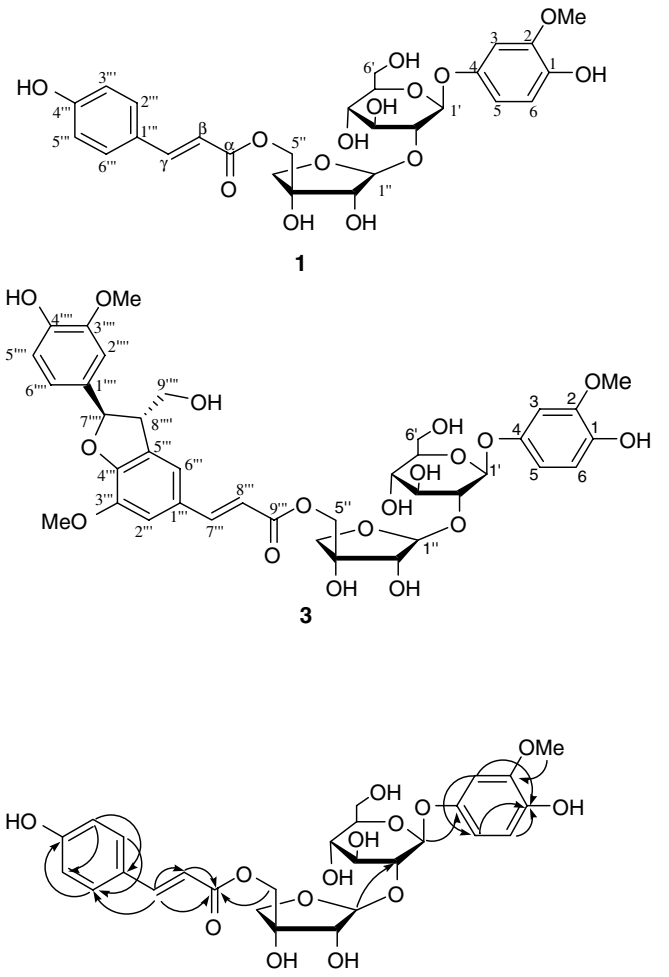

Fig. 1. Selected HMBC correlations of **1**.

Table 1
The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of **1** and **2** in CD_3OD (*J* in Hz within parentheses)

No.	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		142.9 *s*		155.5 *s*
2		149.2 *s*	5.99 *s*	94.5 *d*
3	6.70 *d* (2.4)	103.4 *d*		154.8 *s*
4		152.6 *s*		128.5 *s*
5	6.48 *dd* (2.4, 8.2)	109.6 *d*		154.8 *s*
6	6.62 *d* (8.2)	116.1 *d*	5.99 *s*	94.5 *d*
1′	4.80 *d* (7.3)	102.0 *d*	4.94 *d* (7.6)	102.7 *d*
2′	3.58–3.65	78.8 *d*	3.62–3.72	78.8 *d*
3′	3.58–3.65	78.5 *d*	3.62–3.72	78.5 *d*
4′	3.33–3.36	71.7 *d*	3.33–3.37	71.7 *d*
5′	3.33–3.36	78.1 *d*	3.33–3.37	78.1 *d*
6′	3.58–3.65, 3.85[a]	62.6 t	3.62–3.72, 3.82[a]	62.6 *t*
1″	5.48 *d* (1.8)	110.5 *d*	5.50 *d* (2.2)	110.5 *d*
2″	4.02 *d* (1.8)	78.7 *d*	4.00 *d* (2.2)	78.7 *d*
3″		79.2 *s*		79.6 *s*
4″	Ha 3.88 *d* (9.5)	75.4 *t*	Ha 3.90 *d* (9.8)	75.7 *t*
	Hb 4.28 *d* (9.5)		Hb 4.38 *d* (9.8)	
5″	Ha 4.26 *d* (11.2)	67.6 *t*	Ha 4.36 *d* (10.8)	67.8 *t*
	Hb 4.37 *d* (11.2)		Hb 4.43 *d* (10.8)	
1‴		127.1 *s*		122.4 *s*
2‴	7.37 *d* (8.4)	131.2 *d*	7.44 *d* (1.8)	113.6 *d*
3‴	6.79 *d* (8.4)	116.9 *d*		152.9 *s*
4‴		161.3 *s*		148.6 *s*
5‴	6.79 *d* (8.4)	116.9 *d*	6.78 *d* (8.2)	115.9 *d*
6‴	7.37 *d* (8.4)	131.2 *d*	7.47 *dd* (8.2,1.8)	125.2 *d*
α		168.8 *s*		168.1 *s*
β	6.20 *d* (15.7)	114.8 *d*		
γ	7.54 *d* (15.7)	146.9 *d*		
OMe	3.86 *s*	56.4 *q*	3.83 *s* (MeO-3‴)	56.3 *q* (MeO-3‴)
			3.70 *s* (MeO-3,5)	56.7 *q* (MeO-3,5)

[a] Signal pattern unclear due to overlapping.

(2H, *d*, *J* = 8.4 Hz), 6.20 (1H, *d*, *J* = 15.7 Hz), as well as one ester carbonyl carbon at *δ* 168.8, suggested the presence of one *trans-p*-coumaroyl moiety. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) also revealed the presence of glucopyranose and apiofuranose moieties. The β-anomeric configuration for the glucopyranose was determined from a large coupling constant value (7.3 Hz) of the anomeric proton (Agrawal, 1992; Ishii and Yanagisawa, 1998). The β-anomeric configuration for the apiofuranose was indicated from the anomeric signals at δ_C 110.5 (Kitagawa et al., 1993) with δ_H 5.48 (1H, *d*, *J* = 1.8 Hz) (Mbaïraroua et al., 1994; Otsuka et al., 1994). On acid hydrolysis, **1** afforded D-glucose and D-apiose as component sugars, which was identified by TLC and GLC analysis. The apiosyl-(1 → 2)-glucosyl linkage of the glycosidic moiety was assigned from the cross-peaks observed between apiose H-1 and glucose H-2 in the NOESY spectrum. Also in the HMBC spectrum (Fig. 1) of **1**, a correlation was evident between apiose H-1 (*δ* 5.48) and glucose C-2 (*δ* 78.8). The position was confirmed by the chemical shift (*δ* 78.8) of glucose C-2, as compared with a nonsubstituted C-2, which is ca. *δ* 74.0. All chemical shifts of this sugar moiety are in good agreement with the literature data (Zhong et al., 1998). The significant deshielding of H-5 of apiose (4.26 and 4.37 ppm) and the HMBC cross-peak between the proton at 4.37 ppm and the carbonyl carbon at 168.8 ppm confirmed that the coumaroyl unit was attached to position 5 of apiose.

The ^{13}C NMR spectrum of **1** showed, for the aglycon portion, seven signals. These were assigned to a methoxy group, three to aromatic CH, and three to phenolic functions. The ^{1}H NMR spectrum contained the signals for three aromatic protons at *δ* 6.70 (1H, *d*, *J* = 2.4 Hz), 6.62 (1H, *d*, *J* = 8.2 Hz), and 6.48 (1H, *dd*, *J* = 2.4, 8.2 Hz), along with a signal for a methoxy group at *δ* 3.86, which correlated in the HMBC spectrum with a signal at δ_C 149.2, corresponding to a typical methoxyquinol. The site of glycosidation was revealed to be C-4 by HMBC experiment, which showed a long-range correlation between C-4 (*δ* 152.6) and the anomeric proton (*δ* 4.80) of glucose, which was further supported by the NOE cross-peaks (Fig. 2) observed from the anomeric proton (*δ* 4.80) of glucose to two aromatic protons at δ_H 6.48 (1H, *dd*, *J* = 2.4, 8.2 Hz) and 6.70 (1H, *d*, *J* = 2.4 Hz). Based on the above results, the structure of glycopentoside A (**1**) was established as methoxyquinol 4-*O*-(5-*O*-*trans-p*-coumaroyl)-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside.

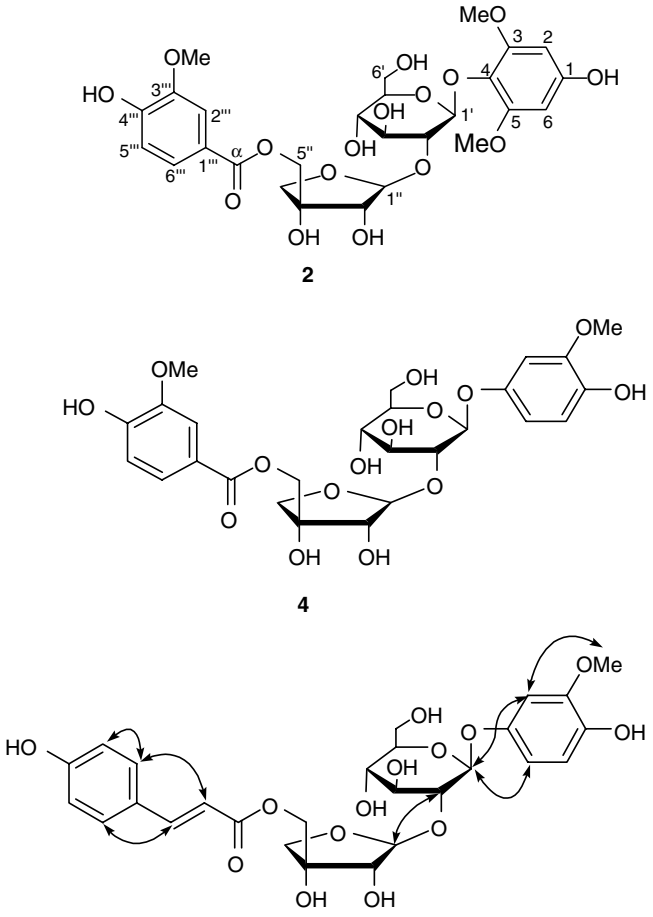

Fig. 2. Key NOEs correlations of **1**.

Glycopentoside B (**2**) was isolated as amorphous powder, whose elemental composition was determined to be $C_{27}H_{34}O_{16}$. Its IR spectrum showed hydroxyl groups (3424 cm^{-1}), a conjugated ester group (1706 cm^{-1}), and aromatic ring (1605; 1513 cm^{-1}). Comparison of its ^1H NMR and ^{13}C NMR data (Table 1) with those of compound **1** suggested the same sugar portion. Furthermore, the signals attributable to a ABX-coupling system were observed at δ 7.44 (1H, *d*, $J = 1.8$ Hz), 6.78 (1H, *d*, $J = 8.2$ Hz), and 7.47 (1H, *dd*, $J = 1.8$, 8.2 Hz), in combination with the signal for a carbonyl carbon at δ 168.1 in the ^{13}C NMR spectrum, suggested a 1,3,4-substituted benzoyl moiety in **2**. HMBC correlations (Fig. 3) were observed between the proton signals at δ 7.44 (H-2‴) and 7.47 (H-6‴) and the carbonyl carbon, as well as methoxy signals at δ 3.83 and the carbon signal at 152.9, which confirmed the presence of a vanillic acid unit in the compound. The HMBC spectrum of **2** confirmed that the ester linkage is on the hydroxyl group on C-5 of apiose, since significant cross-peaks were observed between δ_C 168.1 and δ_H 4.36 and 4.43.

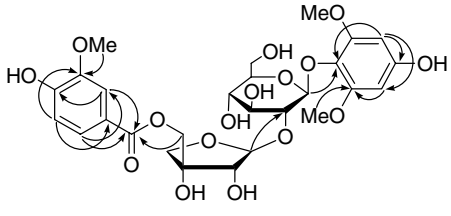

Fig. 3. Selected HMBC correlations of **2**.

In the ^1H NMR spectrum of **2**, the only features for the aglycon moiety were a signal at δ 5.99 (2H, s) and a signal for a methoxy group at δ 3.70 (6H, s). The shielded aromatic protons allowed us to propose the presence of isolated aromatic protons in symmetrical relationship with the rest of the molecule, positioned in an electron donor environment. The ^{13}C NMR spectrum of **2** exhibited, for the aglycon moiety, five signals. These were indicative for two methoxy group, two aromatic CH, and four phenolic functions.

The location of the methoxy groups at C-3,5 and of the disaccharide chain at C-4 were deduced from the HMBC correlations between the proton signal at δ 3.70 (OMe) and the carbon resonance at δ 154.8 (C-3,5), and between the anomeric signal of the glucose unit at δ 4.94 and the carbon resonance at δ 128.5 (C-4). Thus, the structure 4-demethylantiarol 4-*O*-(3-methoxy-4-hydroxy-benzoyl)-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside was assigned to **2**.

Glycopentoside C (**3**) was isolated as an amorphous powder. The molecular formula was established as $C_{38}H_{44}O_{18}$ by HRFABMS. In comparison of the ^1H and ^{13}C NMR spectra (Table 2) of **3** with those of **1**, the signals due to sugars and the aglycon were superimposable. On acid hydrolysis, **3** afforded D-glucose and D-apiose as component sugars. These suggested that **3** was also methoxyquinol apiosyl-(1 → 2)-glucoside acyl ester. The ^1H NMR

Table 2
The ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of **3** in CD_3OD (*J* in Hz within parentheses)

No	δ_H (*J* in Hz)	δ_C	No	δ_H (*J* in Hz)	δ_C
1		142.9 *s*	3‴		145.8 *s*
2		149.2 *s*	4‴		152.1 *s*
3	6.68 *d* (2.2)	103.2 *d*	5‴		131.7 *s*
4		152.7 *s*	6‴	7.08 *s*	119.3 *d*
5	6.46 *d* (8.2, 2.2)	109.5 *d*	7‴	7.55 *d* (15.8)	147.1 *d*
6	6.62 *d* (8.2)	116.1 *d*	8‴	6.25 *d* (15.8)	115.5 *d*
1′	4.81 *d* (7.8)	102.0 *d*	9‴		168.7 *s*
2′	3.54–3.65	78.8 *d*	1⁗		134.1 *s*
3′	3.54–3.65	78.3 *d*	2⁗	6.94 *d* (2.0)	110.6 *d*
4′	3.34 *m*	71.7 *d*	3⁗		149.2 *s*
5′	3.34 *m*	78.1 *d*	4⁗		147.8 *s*
6′	3.86[a], 3.54–3.65	62.6 *t*	5⁗	6.77 *d* (8.2)	116.2 *d*
1″	5.53 *d* (2.0)	110.5 *d*	6⁗	6.82 *dd* (2.0, 8.2)	119.9 *d*
2″	4.01 *d* (2.0)	78.7 *d*	7⁗	5.57 *d* (6.4)	89.9 *d*
3″		79.2 *s*	8⁗	3.54 *m*	54.7 *d*
4″	Ha 3.78 *d* (10.2)	75.4 *t*	9⁗	3.83[a]	64.6 *t*
	Hb 4.24 *d* (10.2)				
5″	Ha 4.20 *d* (11.0)	67.6 *t*	2-OMe	3.81 *s*	56.4 *q*
	Hb 4.28 *d* (11.0)				
1‴		129.6 *s*	3‴-OMe	3.88 *s*	56.8 *q*
2‴	7.04 *s*	113.6 *d*	3⁗-OMe	3.75 *s*	56.4 *q*

[a] Signal pattern unclear due to overlapping.

spectrum revealed the remaining signals of five aromatic protons enclosed in two aromatic systems: an AX system corresponding to a 1,2,3,5-tetrasubstituted ring and an AMX system corresponding to a 1,2,4-trisubstituted ring. The aromatic region of the ^{1}H NMR spectrum of **3** showed one AB set of signals at δ 6.25 and 7.55 (1H, *d*, $J = 15.8$ Hz). The coupling constants of the vinylic system indicated that they have a *trans* configuration. HMBC correlations (Fig. 4) between the proton resonances of the vinylic system with the carbon resonance of the ester group, suggested that compound **3** have one α,β-unsaturated ester moieties in its structure. The presence of these moieties was also confirmed by the presence of a band at 1699 cm^{-1} in the IR spectrum of **3**. In the ^{1}H NMR spectrum of **3**, an ABC set of signals can also be found. They are due to proton resonances of a methine group [δ_H 3.54 (m); δ_C 54.7], one benzylic methine group [δ_H 5.57 (*d*, $J = 6.4$ Hz); δ_C 89.9] and also a methylene group (δ_H 3.83; δ_C 64.6). The ^{13}C NMR spectrum of **3** displayed characteristic signals for three methoxy groups. Besides these substituent signals, 18 skeletal carbon resonances appeared in the ^{13}C NMR spectrum of the acyl unit of **3**, which, in combination with its ^{1}H NMR data, suggested that the acyl moiety of **3** is a dihydrobenzo[b]furan neolignan (Chakravarty et al., 1996; Li et al., 1997). HMBC correlations (Fig. 4) led to the planar structure of the acyl moiety. The NOE interactions (Fig. 5) between OMe/H-3, OMe/H-2‴ and OMe/ H-2⁗ allowed the locations of the methoxy at C-2, C-3‴ and C-3⁗. Since the coupling constant ($J = 6.4$ Hz) of H-7⁗ was similar to $J = 6.2$ Hz of H-7 in *trans*-dehydrodiconiferyl alcohol (Wang et al., 1992), the relative configuration of C-7⁗ and C-8⁗ in **3** was determined as *trans* form which was also confirmed by a lack of correlation between H-7⁗ and H-8⁗ and a pronounced coupling between the two protons at C-9⁗ and C-7⁗ from the NOESY spectrum (Fig. 5).

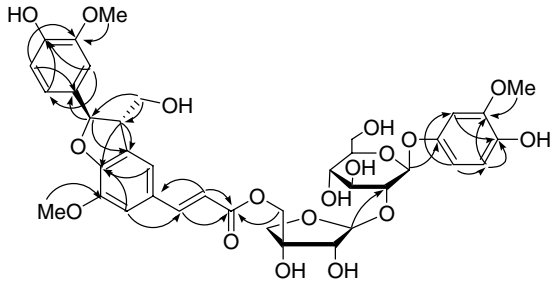

Fig. 4. Selected HMBC correlations of **3**.

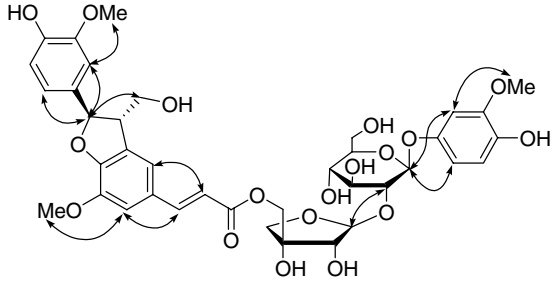

Fig. 5. Key NOEs correlations of **3**.

The absolute configuration of the dihydrofuran ring was determined using CD spectroscopic evidence. The CD spectrum of **3** showed a negative Cotton effect at 287 nm, providing evidence that the configuration in **3** must be 7⁗*S*, 8⁗*R* (Lynn et al., 1987; Wang and Jia, 1997). The linkage of acyl moiety with sugar moiety was solved by analysis of the HMBC spectrum. In the HMBC spectrum, the carbonyl group (δ_C 168.7) not only showed correlation with protons assigned to double bond but also had correlation with methylene protons at δ 4.20 (1H, *d*, $J = 11.0$) and 4.28 (1H, *d*, $J = 11.0$ Hz), suggesting the acyl moiety is connected to the hydroxyl group on C-5 of apiose. This confirmed the structure of glycopentoside C is shown as that of **3**.

The four hydroquinone diglycoside acyl esters identified in this investigation of glycosidic metabolites from the genus *Glycosmis* is the first report of a compound of this type in the Rutaceae. However, since no studies on the polar constituents of the stems of other species have been carried out, whether this has taxonomic value remains unsure.

These phenolic glycoside esters may store in the plant as precursors of 'post-inhibitin' and has a defensive ecological role. The sugar moiety appears to stabilize the molecules, preventing dehydrogenation to give hydroquinones which have been reported to show antimicrobial (Jin and Sato, 2003; Ma et al., 1999; Perry and Brennan, 1997) and cytotoxic activities (Perry and Brennan, 1997) and to be allelopathic agents (Weidenhamer and Romeo, 2004). However, glycoside form of these hydroquinone generally exhibited low activities (Jin and Sato, 2003; Ma et al., 1999; Perry and Brennan, 1997; Weidenhamer and Romeo, 2004). Active phenolic toxins are released from the corresponding glycosides by enzymic hydrolysis caused by microbial invasion or herbivore attack on foliage against the invasion of pathogens under environmental conditions (Harborne, 1988).

3. Experimental

3.1. General procedures

^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker AM 400 NMR and a DRX-500 spectrometer with TMS as internal standard. MS data were obtained on a VG AutoSpec 3000 spectrometers. UV spectra were obtained on a Shimadzu double-beam 210A spectrophotometer. The IR (KBr) spectra were obtained on a Bio-Rad FTS-135 spectrometer. HPLC separations were performed on a HP 1100 apparatus equipped with Diode array UV detector and XTERRA® C18 (Waters, 10 μm, 15 × 200 mm, flow rate: 15 mL/min) column. GC–MS

was run on a FISONS MD-800 instrument. The CD spectra were measured with a JASCO-715 spectropolarimeter. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in MeOH solution.

3.2. Plant material

The stems of *G. pentaphylla* were collected in Xishuangbanna, Yunnan, China., in March 2003. The plant material was identified by Prof. De-Ding Tao, and a voucher specimen (BN20030412) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried material (10 kg) was finely pulverized and extracted by percolation with MeOH for one month at room temperature. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (350 g). The extract was partitioned between water and $CHCl_3$ and then further extracted with EtOAc. The EtOAc-soluble fraction (25 g) was fractionated by column chromatography over D101 porous resin using gradient aqueous ethanol to give six fractions (fractions I–VI).

Fraction II (5 g) was subjected to column chromatography over D101 porous resin and eluted with 5–30% Me_2CO in H_2O, yielding fractions II-1–II-5. Fraction II-2 (956 mg) was subjected to medium pressure chromatography (MPLC) over C18 Si gel and eluted with MeOH–H_2O (1:9–5:5) under gradient conditions yielding fractions II-2-1–II-2-6. Fraction II-2-4 (66 mg) was applied to Sephadex LH-20 with MeOH. The major component eluted as a yellow band (32 mg) and was further purified by RP-18 preparative HPLC with MeOH–H_2O (2:8), yielded compound **2** (7 mg) at 26.4 min. Fraction II-3 (3 g) was applied to RP-18 MPLC and eluted with Me_2CO–H_2O (1:9–3:7) under gradient conditions yielding fractions II-3-1–II-3-6. Fraction II-3-1 (1.3 g) was again applied to RP-18 MPLC and eluted with MeOH–H_2O (1:9–5:5) yielding fractions II-3-1-1–II-3-1-6. Fraction II-3-1-1 (600 mg) and Fraction II-3-1-5 (90 mg) were chromatographed on Sephadex LH-20 with MeOH and further purified by successive RP-18 preparative HPLC with 40% MeOH to obtain **1** (5 mg, t_R 26.1 min), and with 30% MeOH to afford **3** (10 mg, t_R 41.4 min), respectively.

3.3.1. Methoxyquinol 4-O-[(5-O-trans-p-coumaroyl)-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (Glycopentoside A), 1

Amorphous powder; $[\alpha]_D^{25}$: −7.5° (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (logε): 313 (4.12), 289 (4.15), 206 (4.55); IR ν_{max} (KBr) cm^{-1}: 3420, 2937, 2076, 1690, 1605, 1514, 1454, 1361, 1271, 1200, 1168, 1110, 1074, 1030, 945, 833; ^1H and ^{13}C NMR spectral data: see Table 1; HRFABMS (negative-ion mode) *m/z*: 579.1726 $[M-H]^-$ ($C_{27}H_{31}O_{14}$ requires 579.1714).

3.3.2. 4-Demethylantiarol 4-O-[(3-methoxy-4-hydroxybenzoyl)-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside] (Glycopentoside B), 2

Amorphous powder; $[\alpha]_D^{25}$:−60.7° (*c* 0.48, MeOH); UV (MeOH) λ_{max} nm (logε): 267 (4.03), 206 (4.62); IR ν_{max} (KBr) cm^{-1}: 3424, 2940, 2850, 2075, 1706, 1605, 1513, 1465, 1429, 1336, 1284, 1219, 1119, 1073, 1029, 817; ^1H and ^{13}C NMR spectral data: see Table 1; HRFABMS (negative-ion mode) *m/z*: 613.1773 $[M-H]^-$ ($C_{27}H_{33}O_{16}$ requires 613.1769).

3.3.3. Glycopentoside C, 3

Amorphous powder; $[\alpha]_D^{25}$:−49.7° (*c* 0.84, MeOH); UV (MeOH) λ_{max} nm (logε): 329 (4.34), 290 (4.14), 224 (4.44), 205 (4.70); IR ν_{max} (KBr) cm^{-1}: 3424, 2937, 2885, 2059, 1699, 1630, 1606, 1514, 1454, 1432, 1336, 1274, 1144, 1072, 1030, 943, 837, 803; ^1H and ^{13}C NMR spectral data: see Table 2; HRFABMS (negative-ion mode) *m/z*: 787.2462 $[M-H]^-$ ($C_{38}H_{43}O_{18}$ requires 787.2449). CD (MeOH): $[\theta]_{287} = -12400°$.

3.4. Acid hydrolysis, TLC and GC analysis of 1–3

Each solution of **1**–**3** (each 2 mg), in 1 M HCl (dioxane–H_2O, 1:1, 2 mL) was heated at 95 °C for 2 h in a water bath. After removing the solution under a stream of nitrogen, the residue was suspended with H_2O and extracted with EtOAc three times. The aqueous layer was then neutralized with $NaHCO_3$ and concentrated to dryness under reduced pressure.

The residue was compared with standard sugars by cothin layer chromatography ($CHCl_3$–MeOH–H_2O–HOAc, 16:9:2:2; detection with spray agent: 4% α-naphthol–EtOH–5% H_2SO_4). Hexoses gave purple spots and pentoses blue spots. The R_f values of each sugar are as follows: glucose, 0.42 and apiose, 0.52.

The residue was dried and dissolved in pyridine (0.5 mL). Then trimethylchlorosilane (0.5 mL) was added and the reaction mixture was kept at ambient temperature for 20 min. After concentrated to dryness under reduced pressure, the residue was dissolved in diethyl ether and then directly subjected to GC–MS [column: 30 m × 0.32 mm (30QC2/AC5)] analysis under the following conditions: electron-impact (EI) mode (70 eV), temperature programming from 180 to 240 °C at 5 °C/min; carrier N_2 gas. In the acid hydrolysate of **1**–**3**, D-glucose and D-apiose were confirmed by comparison of the retention times of their TMSi derivatives with those of D-glucose and D-apiose derivatives prepared in a similar way, which showed retention times of 6.86 and 3.08 min, respectively.

Acknowledgments

The authors gratefully acknowledge financial support from the National Natural Science Foundation of China (NSFC) for Distinguished Young Scientists to X.-J. Hao

(39525025, 30070087). We are grateful to members of the analytical group in the Laboratory of Phytochemistry, Kunming Institute of Botany for the spectral measurements.

References

Agrawal, P.K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307–3330.

Bhattacharyya, P., Chowdhury, B.K., 1985. Glycolone, a quinolone alkaloid from *Glycosmis pentaphylla*. Phytochemistry 24, 634–635.

Chakravarty, A.K., Mukhopadhyay, S., Saha, S., Pakrashi, S.C., 1996. A neolignan and sterols in fruits of *Solanum sisymbrifolium*. Phytochemistry 41, 935–939.

Chowdhury, B.K., Mustapha, A., Garba, M., Bhattacharyya, P., 1987. Carbazole and 3-methylcarbazole from *Glycosmis pentaphylla*. Phytochemistry 26, 2138–2139.

Harborne, J.B., 1988. In Introduction to Ecological Biochemistry, third ed. Academic, London, pp. 312–315.

Huang, C.C., 1997. Flora of China, 43 (2), Science Press, pp. 117–126.

Ishii, T., Yanagisawa, M., 1998. Synthesis, separation and NMR spectral analysis of methyl apiofuranosides. Carbohydr. Res. 313, 189–192.

Jash, S.S., Biswas, G.K., Bhattacharyya, S.K., Bhattacharyya, P., Chakraborty, A., Chowdhury, B.K., 1992. Carbazole alkaloids from *Glycosmis pentaphylla*. Phytochemistry 31, 2503–2505.

Jin, S.G., Sato, N., 2003. Benzoquinone, the substance essential for antibacterial activity in aqueous extracts from succulent young shoots of the pear *Pyrus* spp. Phytochemistry 62, 101–107.

Kitagawa, I., Hori, K., Sakagami, M., Hashiuchi, F., Yoshikawa, M., Ren, J., 1993. Saponin and Sapogenol. XLIX. On the constituents of the roots of *Glycyrrhiza inflate* Batalin from Xinjiang, China. Characterization of two sweet oleanane-type triterpene oligoglycosides, apioglycyrrhizin and araboglycyrrhizin. Chem. Pharm. Bull. 41, 1350–1357.

Li, S., Iliefski, T., Lundquist, K., Wallis, A.F.A., 1997. Reassignment of relative stereochemistry at C-7 and C-8 in arylcoumaran neolignans. Phytochemistry 46, 929–934.

Lynn, D.G., Chen, R.H., Manning, K.S., Wood, H.N., 1987. The structural characterization of endogenous factors from *Vinca rosea* crown gall tumors that promote cell division of tobacco cells. Proc. Natl. Acad. Sci. USA 84, 615–619.

Ma, W.G., Fukushi, Y., Ducrey, B., Hostettmann, K., Tahara, S., 1999. Phenolic glycosides from *Eriosema tuberosum*. Phytochemistry 51, 1087–1093.

Mbaïraroua, O., Ton-That, T., Tapiéro, C., 1994. Synthèses de 6-*O*-β-D-apiofuranosyl-β-D-glucopyranosides de monoterpényle. Carbohydr. Res. 253, 79–99.

Muthukrishnan, J., Seifert, K., Hoffmann, K.H., Lorenz, M.W., 1999. Inhibition of juvenile hormone biosynthesis in Gryllus bimaculatus by *Glycosmis pentaphylla* leaf compounds. Phytochemistry 50, 249–254.

Otsuka, H., Kamada, K., Ogimi, C., Hirata, E., Takushi, A., Takeda, Y., 1994. Alangionosides A and B, ionol glycosides from leaves of *Alangium premnifolium*. Phytochemistry 35, 1331–1334.

Perry, N.B., Brennan, N.J., 1997. Antimicrobial and cytotoxic phenolic glycoside esters from the New Zealand Tree *Toronia toru*. J. Nat. Prod. 60, 623–626.

Quader, M.A., Nutan, M.T.H., Rashid, M.A., 1999. Antitumor alkaloid from *Glycosmis pentaphylla*. Fitoterapia 70, 305–307.

Sarkar, M., Chakraborty, D.P., 1979. Glycophymoline, a new minor quinazoline alkaloid from *Glycosmis pentaphylla*. Phytochemistry 18, 694–695.

Sastri, B.N., 1956. The wealth of India: Raw Materials, vol. IV. CSIR, New Delhi, p. 150.

Wang, C.Z., Jia, Z.J., 1997. Lignan, phenylpropanoid and iridoid glycosides from *Pedicularis Torta*. Phytochemistry 45, 159–166.

Wang, H.B., Yu, D.Q., Liang, X.T., 1992. The structure of a new neolignan glycoside from *Stauntonia chinensis*. J. Nat. Prod. 55, 214–216.

Weidenhamer, J.D., Romeo, J.T., 2004. Allelochemicals of *Polygonella myriophylla*: chemistry and soil degradation. J. Chem. Ecol. 30, 1067–1082.

Zhong, X.N., Otsuka, H., Ide, T., Hirata, E., Takushi, A., Takeda, Y., 1998. Hydroquinone glycosides from leaves of *Myrsine seguinii*. Phytochemistry 49, 2149–2153.