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Two new glycosides and one new neolignan from the roots of *Cynanchum stauntonii*

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ABSTRACT

Three new compounds including one C_{21}-steroidal glycoside, one methylglycoside, and one neolignan, named as Deoxyamplexicogenin A-3-O-yl-4-O-(4-O-α-L-cymaropyranosoyl-β-D-digitoxopyranosoyl)-β-D-canaropyranoside (**1**), Methyl-*O*-α-L-cymaropyranosoyl-(1 → 4)-β-D-digitoxopyranoside (**2**), and (+)-(7*S*, 8*R*, 7′*E*)-5-hydroxy-3, 5′-dimethoxy-4′, 7-epoxy-8, 3′-neolign-7′-ene-9, 9′-diol 9′-ethyl ether (**3**), respectively, were isolated from the roots of *Cynanchum stauntonii*. The structure elucidations were achieved by in-depth spectroscopic examination, mainly including the experiments and analyses of multiple 1D- and 2D-NMR and HRESIMS and CD analysis and qualitative chemical tests. Cytotoxicity activities of compounds **1**–**3** were evaluated against five tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) in cell based assays where they were found to be inactive.

1. Introduction

Cynanchum stauntonii (Decne.) Schltr. ex Levl. (Asclepiadaceae), known as a perennial medicinal herb, distributes broadly in south-central region of China. The roots, along with another species of the same genus, *C. glaucescens* (Decne.) Hand.-Mazz., have been used widely in traditional Chinese medicine (TCM) as antitussive and expectorant agent (China National Corp. of Traditional & Herbal Medicine, 1995). Both of them are documented in the Chinese Pharmacopoeia as 'Bai Qian'. Previous investigations of phytochemistry on this title plant have indicated the main native organic compounds isolated from *Cynanchum* species are steroidal glycosides, sharing aglycones assignable to either the normal four-ring C_{21} steroid skeleton or the aberrant 13,14:14,15-disecopregnane-type skeleton or the equally abnormal 14,15-secopregnane-type skeleton (Qiu et al., 1989; Sugama et al., 1986; Yu et al., 2013). These steroidal glycosides have been accepted, generally and chemotaxonomically, as the most effective and characteristic ingredients existing in the *Cynanchum* species. The potential medicinal importance and our interest in the chemistry of structurally unique natural products prompted us to investigate the roots of *C. stauntonii*. In order to find more structurally unique C_{21}-steroids and in continuation of our interest in the chemistry of genus *Cynanchum*, a detailed investigation of this species was carried out. One C_{21}-steroidal glycoside (**1**), one methylglycoside (**2**), and one neolignan (**3**), were isolated from the roots of *C. stauntonii*. In this article, cytotoxicity activities of compounds **1–3** were evaluated against five tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) by using the MTT *in vitro* assay, but with none being found to be active. Described herein is a full account of the isolation and structural elucidation of these new compounds, as well as the cytotoxic activities of the compounds tested.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter at 20 °C. IR spectra were recorded on a Nicolet 5700 spectrometer. CD spectrum was obtained using a Jasco J715 spectropolarimeter. 1D and 2D NMR spectra were taken on either a Bruker 500 spectrometer or a Varian 500 spectrometer with tetramethylsilane as internal standard. ESI-MS and HRESI-MS were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD system equipped with a SPD-10A detector, and a reversed-phase C_{18} column (YMC-Pack ODS-A U 20 × 250 mm, 10 μm) was employed. GC analyses were conducted on an Agilent 7890A instrument. Column chromatography (CC) was undertaken over

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silica gel (200–300 mesh). TLC was carried out with glass plate precoated silica gel G. Spots were visualized under UV light and by spraying with 10% H_2SO_4 in 95% EtOH, followed by heating at 100 °C. Acetonitrile and methanol used in preparative HPLC procedure were in HPLC grade, and other solvents were of analytical grade.

2.2. Plant material

The roots of *C. stauntonii* were collected from Hebei Province of China. It was identified by associate Prof. L Ma from Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (ID-S-2426) was deposited in the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, P. R. China.

2.3. Extraction and isolation

The dried-up and powdered roots of *C. stauntonii* (30 Kg) were extracted three times under reflux with 95% EtOH (2 h, 1 h, 1 h). The EtOH extract was evaporated under reduced pressure to yield a dark-brown residue (5000 g). The residue was suspended in 80% aqueous ethanol (10,000 mL) and then extracted with petroleum ether, and EtOAc, respectively and successively, each for three times in separatory funnel. The combined EtOAc solution was washed with 5% aqueous solution of $NaHCO_3$ (3 × 1000 mL) and then H_2O (2 × 1000 mL), respectively, to pH 7. After removal of the organic solvent, 190 g of brown residue was obtained. This resulting residue was fractionated by CC over silica gel eluted with gradient solvents of $CHCl_3$-MeOH (100:0 → 1:1) to yield 13 fractions (designated as F1 to F13) based on their TLC analysis. Fraction 3 (24.0 g) was applied to Flash C18 column chromatography eluted with CH_3OH/H_2O (40% → 100%) to give six subfractions (F3-1 to F3-6). Fraction 3-4 (2.0 g) was applied to Flash C18 column chromatography eluted with CH_3OH/H_2O (40% → 100%) to give five subfractions (F3-4-1 to F3-4-5). Fraction 3-4-5 (100 mg) was applied to preparative HPLC system [mobile phase: CH_3OH/H_2O (75:25, v/v); flow rate: 5 mL min^{-1}; UV detection at 210 nm] resulting in the isolation of compound **2** (35 mg). Fraction 5 (12.0 g) was applied to Flash C18 column chromatography eluted with CH_3OH/H_2O (40% → 100%) to give five subfractions (F5-1 to F5-5). Fraction 5-2 (2.5 g) was applied to Flash C18 column chromatography eluted with CH_3OH/H_2O (40% → 100%) to give five subfractions (F5-2-1 to F5-2-5). Fraction 5-2-3 (400 mg) was applied to preparative HPLC system [mobile phase: CH_3CN/H_2O (45:55, v/v); flow rate: 5 mL min^{-1}; UV detection at 280 nm] resulting in the isolation of compound **1** (80 mg). Fraction 6 (7.0 g) was further separated by CC over silica gel using gradient solvents of petroleum ether/acetone (25:1 → 1:1) as eluents to yield four further subfractions (F6-1 to F6-7). Fraction 6-3 (5.0 g) was applied to Flash C18 column chromatography eluted with CH_3OH/H_2O (40% → 100%) to give five subfractions (F6-3-1 to F6-3-5). Fraction 6-3-3 (90 mg) was applied to preparative HPLC system [mobile phase: CH_3CN/H_2O (38:62, v/v); flow rate: 5 mL min^{-1}; UV detection at 210 nm] resulting in the isolation of compound **3** (12 mg).

2.3.1. Deoxyamplexicogenin A-3-O-yl-4-O-(4-O-α-L-cymaropyranosoyl-β-D- digitoxopyranosoyl)-β-D-canaropyranoside(*1*)

White amorphous powder; $[\alpha]_D^{20}$ + 2.21 (*c* = 0.09, CH_3OH, 20 °C); IR (KBr) ν_{max}: 3432, 2934, 1679, 1452, 1379, 1163, 1063, 870 cm^{-1}; ESI-MS (positive mode) *m/z*: 769.4 $[M + Na]^+$; ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) for aglycone and sugar moiety, see Table 1; HRESI-MS (positive mode) *m/z*: 769.3775 $[M + Na]^+$ (calcd for $C_{40}H_{58}O_{13}Na$, 769.3770).

2.3.2. Methyl-O-α-L-cymaropyranosoyl-(1 → 4)-β-D-digitoxopyranoside (*2*)

White amorphous powder; $[\alpha]_D^{20}$ -11.6 (*c* = 0.12, CH_3OH, 20 °C); IR (KBr) ν_{max}: 3395, 2924, 2850, 1451, 1379, 1158, 1060, 992 cm^{-1}; ESI-MS (positive mode) *m/z*: 329.5 $[M + Na]^+$; ^1H NMR (500 MHz, pyridine-d_5): δ_H 5.41 (dd, *J* = 10.2, 1.8 Hz, H-1′), 5.06 (br s, H-1″), 4.46 (2H, overlapped, H-3′, 5′), 4.10 (m, H-5″), 3.70 (m, H-3″), 3.60 (m, H-4″), 3.54 (s, OMe-1′), 3.38 (s, OMe-3″), 3.41 (m, H-4′), 2.38 (m, H_a-2′), 2.35 (m, H_a-2″), 1.91 (m, H_b-2′), 1.82 (m, H_b-2″), 1.43 (d, *J* = 6.6 Hz, H-6′), 1.36 (d, *J* = 5.4 Hz, H-6″); ^{13}C NMR (125 MHz, pyridine-d_5): δ_C 98.6 (C-1′, d), 98.4 (C-1″), 80.9 (C-4′, d), 76.6 (C-3″, d), 72.8 (C-4″, d), 69.2 (C-5′, d), 67.9 (C-5″, d), 67.2 (C-3′, d), 57.5 (OMe-1′, q), 56.8 (OMe-3″, q), 38.6 (C-2′, t), 32.3 (C-2″, t), 18.6 (C-6″, q), 18.4 (C-6′, q); HRESI-MS (positive mode) *m/z*: 329.4577 $[M + Na]^+$ (calcd for $C_{14}H_{26}O_7Na$, 329.4575).

2.3.3. (+)-(7S, 8R, 7E′)-5-hydroxy-3, 5′-dimrthoxy-4′, 7-epoxy-8, 3′-neolign-7′-ene-9, 9′-diol 9′-ethyl ether(*3*)

Brown jelly; $[\alpha]_D^{20}$ + 1.4 (*c* = 0.10, CH_3OH, 20 °C); IR (KBr) ν_{max}: 3285, 2939, 1646, 1534, 1447, 1400, 1239, 1076 cm^{-1}; ESI-MS (positive mode) *m/z*: 409.1 $[M + Na]^+$; ^1H NMR (400 MHz, DMSO-d_6): 6.96 (br s, H-4), 6.95 (br s, H-2′), 6.89 (br s, H-2), 6.75 (d, *J* = 1.2 Hz, H-6′), 6.74 (d, *J* = 1.2 Hz, H-6), 6.50 (d, *J* = 16.0 Hz, H-7′), 6.19 (dt, *J* = 16.0, 6.0 Hz, H-8′), 5.45 (d, *J* = 6.6 Hz, H-7), 4.03 (dd, *J* = 6.0, 1.2 Hz, H-9′), 3.78 (s, OMe-5′), 3.73 (s, OMe-3), 3.70 (m, H_b-9), 3.62 (m, H_a-9), 3.44 (dd, *J* = 14.0, 7.2 Hz, H-11′), 3.43 (m, H-8), 1.13 (t, *J* = 6.0 Hz, H-12′); ^{13}C NMR (100 MHz, DMSO-d_6): 147.5 (C-3, s), 147.4 (C-4′, s), 146.4 (C-5, s), 143.7 (C-5′, s), 132.3 (C-1, s), 131.6 (C-7′, d), 130.0 (C-1′, s), 129.5 (C-3′, s), 124.0 (C-8′, d), 118.5 (C-6, d), 115.3 (C-4, d), 115.3 (C-6′, d), 110.4 (C-2′, d), 110.2 (C-2, d), 87.3 (C-7, d), 70.4 (C-9′, t), 64.6 (C-11′, t), 62.9 (C-9, t), 55.7 (OMe-3, t), 55.6 (OMe-5′, q), 53.0 (C-8, d), 15.2 (C-12′, q); HRESI-MS (positive mode) *m/z*: 409.1631 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_6Na$, 409.1622).

2.4. Acid hydrolysis of *1* and *2* and determination of the absolute configurations of the monosaccharides

Each solution of 6 mg of compounds **1** and **2** was refluxed within 10% HCl (3 ml) at 75 °C for 2.5 h. After cooling, the reaction mixture was extracted thoroughly with $CHCl_3$, the $CHCl_3$ layer was washed with water, and then the water fraction was combined with the original aqueous layer. The aqueous layer was evaporated under vacuum, then re-diluted with water and re-evaporated in vacuo repeatedly to eliminate the surplus HCl and furnish a final neutral residue. The monosaccharides obtained on acid hydrolysis, as described above, were dissolved in pyridine and reacted with L-cysteine methyl ester hydrochloride at 60 °C for 1 h. Equal volume of acetic anhydride was added and heating was carried out for another 1 h. Acetylated thiazolidine derivatives were injected into GC system. The absolute configurations of the sugars were determined by comparing the retention times with those of acetylated thiazolidine derivatives synthesized from the known sugars obtained through acid hydrolysis of the known compound Glaucogenin-C-3-O-α-L-cymaropyranosoyl- (1 → 4)-β-D-digitoxopyranosoyl-(1 → 4)-β-D-canaropyranoside (Fu et al., 2007). Also, the retention times of D-canarose, D-digitoxose, and L-cymarose were determined by interactive comparison among the known compound Glaucogenin-C-3-O-α-L- cymaropyranosoyl-(1 → 4)-β-D-digitoxopyranosoyl-(1 → 4)-β-D-canaropyranoside. GC conditions in the test: column, HP-5, 30 m × 0.25 mm, 0.25 μm; detection, FID; carrier gas, N_2; injection temperature, 250 °C, detection temperature, 280 °C, column temperature, 150 °C (0 min), 10 °C/min to 250 °C (20 min). Retention times of the authentic samples: tR D-digitoxose 13.09 min, tR L-cymarose 13.46 min, and tR D-canarose 16.51 min).

3. Results and discussion

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ + 2.21 (c = 0.09, CH_3OH, 20 °C) and showed positive Libermann–Burchard and Keller–Kiliani reactions, suggesting its glycosidic steroidal category with 2-deoxysugar units existing in the sugar moiety (Chen et al., 2008). The positive-ion mode HRESIMS experiment gave a $[M + Na]^+$ quasi-molecular ion peak at m/z 769.3775, consistent with the molecular formula of $C_{40}H_{58}O_{13}$ and indicative of twelve unsaturation degrees. The IR spectrum showed the absorption bands for hydroxy (3432 cm^{-1}) and olefin (1679 cm^{-1}) functionalities. The ^1H NMR spectrum, in conjunction with the ^1H, ^1H-COSY and HSQC experiments, revealed the diagnostic signals of a C_{21}-steroidal glycoside, with an aglycone of 14,15-secopregnane-type skeleton being exhibited typically by two angular methyl groups actually at δ_H 0.83 (3H, s, H-19) and 1.57 (3H, s, H-21), two oxy-methine groups at δ_H 4.53 (1H, m, H-3) and 4.80 (1H, m, H-16), and two oxy-methylenes at δ_H 3.82 (1H, dd, J = 11.0, 4.2 Hz, H_β-15) and 4.28 (1H, br d, J = 11.0 Hz, H_α-15), and at δ_H 4.01 (1H, d, J = 8.5 Hz, H-18_a) and 4.06 (1H, d, J = 8.5 Hz, H-18_b), as well as three anomeric proton signals at δ_H 4.91 (1H, d, J = 10.0 Hz, H-1′), 5.26 (1H, d, J = 10.0 Hz, H-1″), and 5.04 (1H, br s, H-1‴) correlating to the corresponding anomeric carbon signals at δ_C 99.0 (C-1′), 99.9 (C-1″), and 98.6 (C-1‴), respectively, and three secondary methyls at δ_H 1.36 (3H, d, J = 6.0 Hz, H-6′), 1.31 (3H, d, J = 6.0 Hz, H-6″), and 1.42 (3H, d, J = 6.0 Hz, H-6‴) (Table 1), which combined with the ^1H, ^1H-COSY and HSQC spectra suggested the presence of three 2,6-dideoxypyranoses (Fig. 1). In addition, one methoxyl at δ_H 3.36 (3H, s, OMe-3‴) was also determined in the ^1H NMR spectrum, which, along with the above mentioned three secondary methyls, was compatible with one methylated 2,6-dideoxypyranose among the aforementioned three 2,6-dideoxypyranoses when examining the ^{13}C and DEPT NMR data of **1** that exhibited forty carbon signals, with six methyls, nine methylenes, nineteen methines including three olefinic ones at δ_C 125.7 (d), 124.8 (d), and 122.8 (d), and six quaternary carbons being categorized (Table 1). The above mentioned three olefin methine carbons were corresponded to a 4,6-diene system at δ_H 5.73 (1H, s, H-4), 6.03 (1H, d, J = 9.5 Hz, H-6) and 6.69 (1H, d, J = 9.5 Hz, H-7) by the HSQC and HMBC spectra, which also provided solid evidence to unambiguously assign all signals of **1**. The ^1H and ^{13}C NMR resonances from the aglycone moiety of **1** were very superimposable to its counterparts in stauntoside C, a known steroidal glycoside isolated previously from *C. stauntoii* (Yu et al., 2013), confirming the presence of the same aglycone between **1** and stauntoside C, and demonstrating the attachment of an oligosaccharide chain to C-3 of the aglycone of **1** through oxygen atom. Then, in order to determine the sugar moiety, acid hydrolysis of **1** was carried out and one D-canaropyranose, one D-digitoxopyranose, and one L-cymaropyranose showing up the proportion of 1:1:1 were detected through derivatization reaction and GC analysis (Section 2). The ^1H, ^1H-COSY experiment, in combination with the HSQC spectrum, established the spin systems within the sugar moiety which were compatible with the identified one methylated 2,6-dideoxypyranose and two 2,6-dideoxypyranoses. The coupling states of the aforementioned three anomeric proton signals delivered the evidences that the D-canaropyranose and D-digitoxopyranose were β-linkages and the L-cymaropyranose was α-linkage. The sugar chain of **1** was substantiated unambiguously by the HMBC correlations from H-1‴ of α-L-cymaropyranose to C-4″ of β-D-digitoxopyranose, from H-1″ of β-D-digitoxopyranose to C-4′ of β-D-canaropyranose, and from H-1′ of β-D-canaropyranose to C-3. Therefore, compound **1** was elucidated unequivocally as Deoxyamplexicogenin A-3-O-yl-4-O-(4-O-α-L-cymaropyranosoyl-β-D-digitoxopyranosoyl)-β-D-canaropyranoside.

Compound **2** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ -11.6 (c = 0.12, CH_3OH, 20 °C). The pseudo-molecular ion peak at m/z 329.4577 $[M + Na]^+$ in the positive-ion mode HRESIMS experiment indicated the molecular formula of $C_{14}H_{26}O_7$ with two unsaturation degrees. The IR spectrum showed the absorption band for hydroxy (3395 cm^{-1}) group. The ^1H NMR spectrum of **2** displayed the diagnostic signals of two 2,6-dideoxypyranoses

Table 1
^1H and ^{13}C NMR data of the aglycone and sugar moieties for **1** (500/125 MHz, in pyridine-d_5) (δ in ppm, J values in Hz).

Aglycone moiety	**1**		Sugar moiety	**1**	
	δ_H	δ_C		δ_H	δ_C
1α	1.33 m[a]	33.6, t		β-D-can	
1β	1.53 m[a]		1′	4.91 d (10.0)	99.0, d
2α	1.92 m	27.7, t	2′	2.55 m, 2.01 m	40.1, t
2β	2.24 m		3′	3.98 m	70.1, d
3	4.53 m	74.7, d	4′	3.30 m	88.6, d
4	5.73 s	124.8, d	5′	3.54 m	71.0, d
5		144.9, s	6′	1.36 d (6.0)	18.1, q
6	6.03 d (9.5)	125.7, d		β-D-digt	
7	6.69 d (9.5)	122.8, d	1″	5.26 d (10.0)	99.9, d
8		108.1, s	2″	1.97 m, 2.43 m	38.2, t
9	2.14 dd (11.5, 6.0)	44.2, d	3″	4.49 m	67.6, d
10		35.6, s	4″	3.43 m	80.6, d
11α	1.48 m[a]	20.5, t	5″	4.18 m	69.4, d
11β	1.29 m[a]		6″	1.31 d (6.0)	18.1, q
12α	1.58 m[a]	30.8, t		α-L-cym	
12β	1.21 m[a]		1‴	5.04 br s	98.6, d
13		54.9, s	2‴	1.82 m, 2.33 m	32.2, t
14		155.4, s	3‴	3.69 m	76.4, d
15α	4.28 br d (11.0)	72.1, t	4‴	3.62 m	72.5, d
15β	3.82 dd (11.0, 4.2)		5‴	4.46 m	67.4, d
16	4.80 m	86.2, d	6‴	1.42 d (6.0)	18.4, q
17	2.78 d (7.5)	62.1, t	OMe-3‴	3.36 s	56.7, q
18a	4.01 d (8.5)	77.4, t			
18b	4.06 d (8.5)				
19	0.83 s	17.7, q			
20		118.4, s			
21	1.57 s	23.0, q			

[a] : Overlapped. can, canaropyranosyl; digt, digitoxopyranosyl; cym, cymaropyranosyl.

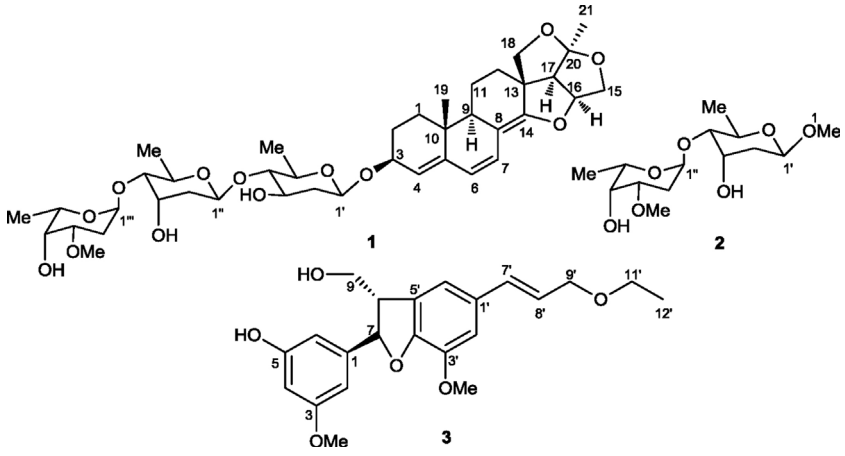

Fig. 1. The structures of compounds **1–3**.

being shown by two anomeric proton signals at δ_H 5.41 (1H, dd, J = 10.2, 1.8 Hz, H-1′) and 5.06 (1H, br s, H-1″), which correlated to the corresponding anomeric carbon signals at δ_C 98.6 (C-1′) and 98.4 (C-1″), respectively, in the HSQC spectrum, and two secondary methyls at δ_H 1.43 (3H, d, J = 6.6 Hz, H-6′), 1.36 (3H, d, J = 5.4 Hz, H-6″) (Section 2). According to the HMQC and HMBC spectra, the carbon signals were assigned respectively. In addition, the ^{1}H and ^{13}C NMR spectra also revealed the presence of two methoxyls at δ_H 3.54 (3H, s) and 3.38 (3H, s), which were determined to be linked at C-1′ and C-3″, respectively, by the HMBC correlations from δ_H 3.54 (OMe-1′) to δ_C 98.6 (C-1′) and from δ_H 3.38 (OMe-3″) to δ_C 76.6 (C-3″), indicating that compound **2** was a methyl disaccharide glycoside. Then, in order to determine the sugar moiety, acid hydrolysis of **2** was carried out and one D-digitoxopyranose, and one L-cymaropyranose showing up the proportion of 1:1 were detected through derivatization reaction and GC analysis (Section 2). The ^{1}H, ^{1}H-COSY experiment, in combination with the HSQC spectrum, established the spin systems within the sugar moiety which were compatible with the identified two 2,6-dideoxypyranoses. The coupling states of the aforementioned two anomeric proton signals delivered the evidences that the D-digitoxopyranose was β-linkage and the L-cymaropyranose was α-linkage. The sequence of the sugar chain was confirmed by the HMBC correlations from H-1″ of α-L-cymaropyranose to C-4′ of β-D-digitoxopyranose, from H-1′ of β-D-digitoxopyranose to OMe-1′. Therefore, compound **2** was identified as Methyl-*O*-α-L-cymaropyranosoyl-(1 → 4)-β-D-digitoxopyranoside.

Compound **3** was obtained as a brown jelly with $[\alpha]_D^{20}$ + 1.4 (c = 0.10, CH_3OH, 20 °C). The positive-ion mode HRESIMS experiment exhibited the pseudo-molecular ion peak at m/z 409.1631 $[M + Na]^+$, indicative of the molecular formula of $C_{22}H_{26}O_6$ with ten unsaturation degrees. The IR spectrum showed the absorption bands for hydroxy (3285 cm^{-1}) and aromatic ring (1646 cm^{-1}) groups. The ^{1}H NMR spectrum, in combination with the ^{1}H,^{1}H-COSY and HSQC experiments, allowed the identification of the diagnostic signals assignable to five aromatic protons, including three AB doublets at δ_H 6.74 (1H, d, J = 1.2 Hz, H-6), 6.89 (1H, br s, H-2), 6.96 (1H, br s, H-4) and two AB doublets at δ_H 6.95 (1H, br s, H-2′), 6.75 (1H, d, J = 1.2 Hz, H-6′), two *trans*-olefinic protons at δ_H 6.19 (1H, dt, J = 16.0, 6.0 Hz, H-8′) and 6.50 (1H, d, J = 16.0, H-7′), indicative of a *Z*-configuration, and dihydrofuran protons at δ_H 3.43 (1H, m, H-8) and 5.45 (1H, d, J = 6.6 Hz, H-7). In addition, three oxymethines at δ_H 3.62 (1H, m, H-9$_a$), 3.70 (1H, m, H-9$_b$), 4.03 (2H, dd, J = 6.0, 1.2 Hz, H-9′), and 3.44 (2H, dd, J = 14.0, 7.2 Hz, H-11′), and two methoxyls at δ_H 3.73 (3H, s, OMe-3), and 3.78 (3H, s, OMe-5′), and one methyl at δ_H 1.13 (3H, t, J = 6.0 Hz, H-12′), were also present in the ^{1}H NMR spectrum. Analysis of the ^{13}C NMR and DEPT spectra (Section 2) showed resonances for twenty-two carbons: seven aromatic quaternary carbons (including four oxygenated ones), nine methines (including five aromatic and two olefinic ones), three oxygenated methylenes, and three methyls (including two methoxyls). The proton and carbon signals in the ^{1}H and ^{13}CNMR spectra of **3** were superimposable on those of (+)-(7*S*, 8*R*, 7′*E*)-4-hydroxy-3, 5′-dimethoxy-4′, 7-epoxy-8, 3′-neolign-7′-ene-9, 9′-diol 9′-ethyl ether, a known neolignan isolated previously from *Sinocalamus affinis* (Xiong et al., 2011), except that the hydroxyl group of **3** was proposed to be linked at C-5 instead of C-4, which was supported by the long-range ^{1}H-^{13}C correlations from δ_H 6.89 (H-2) to δ_C 115.3 (C-4), 118.5 (C-6), 87.3 (C-7); δ_H 6.96 (H-4) to δ_C 110.2 (C-2), 118.5 (C-6); δ_H 6.74 (H-6) to δ_C 110.2 (C-2), 115.3 (C-4), 87.3 (C-7) in the HMBC spectrum (Fig. 2). In addition, the absolute configurations of C-7 and C-8 were established by analysis of the CD spectrum of **3**, which displayed a typical Cotton effect, positive at 230 nm and negative at 258 nm and 283 nm, suggesting compound **3** had the 7*S* and 8*R* configurations (Xiong et al., 2011). On the basis of the evidences above, compound **3** was concluded to be (+)-(7*S*, 8*R*, 7′*E*)-5-hydroxy-3, 5′-dimethoxy-4′, 7-epoxy-8, 3′-neolign-7′-ene-9, 9′-diol 9′-ethyl ether.

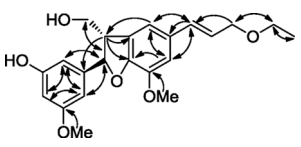

Fig. 2. Key HMBC correlations in **3**.

Compounds **1–3** were tested for cytotoxicity activities against HCT-8 (human colon cancer cell line), Bel-7402 (human hepatoma cancer cell line), BGC-823 (human gastric cancer cell line), A549 (human lung epithelial cell line), and A2780 (human ovarian cancer cell line) by means of the MTT method as described in the literature (Ni et al., 2009) with fluorouracil as the positive control. But none of them showed obvious activities ($IC_{50} > 10$ μM for all subjects, Table 2).

Table 2
Cytotoxic activities of compounds 1-3 by the MTT method.

Sample	IC_{50} (μM)				
	HCT-8	**Bel-7402**	**BGC-823**	**A549**	**A2780**
1	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10
3	>10	>10	>10	>10	>10
Fluorouracil[a]	3.07	4.06	1.12	1.41	1.71

[a] Positive control.

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