FULL PAPER

Two New Flavanols from *Glycosmis pentaphylla* and Their Cytotoxic Activities

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Two new flavanols, (8*S*,9*R*)-9,10-dihydro-5,9-dihydroxy-8-(3,4,5-trimethoxyphenyl)-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one (**1**) and (2*S*,3*R*)-3,4-dihydro-3,5-dihydroxy-2-(3,4,5-trimethoxyphenyl)-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-8-one (**2**), were isolated from the stems of *Glycosmis pentaphylla*. The structures of these compounds were determined by extensive spectroscopic (UV, IR, HR-ESI-MS, 1D- and 2D-NMR) analyses. The cytotoxic activities of these compounds were evaluated using the MTT method. The results showed that compounds **1** and **2** exhibited considerable cytotoxic activities against HL-60 and A549 cell lines.

Introduction. – The genus *Glycosmis* belongs to the family *Rutaceae*, which has been reported to be a rich source of various types of alkaloids and flavanols [1 – 3]. *G. pentaphylla* occurs in tropical forests at low altitudes, and is a shrub or small (1.5 – 5 m) tree which is mainly distributed in Xishuangbanna, Gengma, Shuangjiang, Mengding of Yunnan Province, P. R. China. It is widely used as a traditional medicine against fever, cough, rheumatism, anaemia, and liver disorders. The antioxidant, hepatoprotective, and anti-HCC activities of *G. pentaphylla* were already reported [4 – 6]. Earlier phytochemical studies have described the isolation of a variety of compounds belonging to flavanols, isoflavone, and hydroquinone diglycoside.

Herein, the isolation and structural elucidation of the two new flavanols from *G. pentaphyll* are described, as well as the cytotoxic activities against cancer cell lines (HL-60 and A549).

Results and Discussion. – *Chemistry.* Compound **1** was obtained as yellow solid with a molecular formula of $C_{21}H_{20}O_8$, as determined by HR-ESI-MS (*m*/*z* 401.1233 ($[M+H]^+$)), ^{13}C-NMR, and HSQC (*Table*). The ^{1}H-NMR signals displayed the presence of aromatic H-atoms at δ(H) 6.25 (*s*, 1 H) and δ(H) 6.22 (*s*, 2 H), two C=C H-atoms at δ(H) 7.94 (*d*, J = 9.4, 1 H) and 6.05 (*d*, J = 9.4, 1 H), two O-bearing CH H-atoms at δ(H) 4.62 (*d*, J = 5.4, 1 H) and 4.11 – 3.92 (*m*, 1 H), CH_2 H-atoms at δ(H) 2.72 (*dd*, J = 4.0, 15.6, 1 H) and 2.65 (*dd*, J = 5.2, 15.6, 1 H), as well as MeO H-atoms at δ(H) 3.80 (*s*, 3 H) and 3.67 (*s*, 6 H). The ^{13}C-NMR and HSQC spectra displayed 21 C-atom signals, including ten quaternary C-atoms, seven tertiary C-atoms, three MeO C-atoms, as well as one C=O C-atom. The ^{1}H- and ^{13}C-NMR spectral data showed the features of a flavan-3-ol skeleton. According to the HMBC between C(5), C(11), C(13), C(14), and δ(H) 6.25, the *singlet* signal at δ(H) 6.25 was assigned to H–C(6). Based on the HMBC spectrum, the C-atom signals at δ(C) 164.1, 108.3, and 140.1 were assigned to an α-pyrone moiety. The α-pyrone moiety was fused at C(11) and C(12) positions according to the HMBCs of H–C(3) with C(11) and H–C(4) with C(11) and C(12). Three MeO groups were fused at C(3′), C(4′), and C(5′). The heterocyclic *AMX*-system of the *C*-ring showed a coupling constant (J(8,9) = 5.4 Hz) typical of a 8,9-*trans* relative configuration [7]. In the CD spectrum, a positive CD at 320 nm agreed well with a (8*S*,9*R*) configuration [8]. The structure of compound **1** was established as shown in *Fig. 1*, and named as (8*S*,9*R*)-9,10-dihydro-5,9-dihydroxy-8-(3,4,5-trimethoxyphenyl)-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one.

Compound **2** was obtained as yellow solid. The molecular formula was deduced as $C_{21}H_{20}O_8$ by HR-ESI-MS (*m*/*z* 401.1233 ($[M+H]^+$)). The ^{1}H- and ^{13}C-NMR spectral data of **2** (*Table*) suggested that the structure of **2** was similar to that of compound **1**, except for the position of the α-pyrone ring. The α-pyrone ring was fused at C(11) and C(12) positions according to the HMBC spectrum (*Fig. 2*). In the CD spectrum, a positive CD at 320 nm agreed well with a (6*S*,7*R*) configuration [8][1]). Consequently, the structure of compound **2** was deduced as shown in *Fig. 1*, named as (2*S*,3*R*)-3,4-dihydro-3,5-dihydroxy-2-(3,4,5-trimethoxyphenyl)-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-8-one.

Biology. The isolated compounds **1** and **2** were evaluated for their cytotoxic activities against HL-60 and A549 cells using a modified MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Compounds **1** and **2** showed cytotoxic activity against HL-60 cells with IC_{50} values of 14.2 μM and 15.2 μM, respectively. The compounds were also subjected to a cytotoxic screening test using A549 cells. Compounds **1** and **2** exhibited

[1]) Arbitrary atom numbering, as shown in *Fig. 1*.

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Table. *^{1}H- and ^{13}C-NMR Data* (at 600 and 150 MHz, resp.; in (D_6)DMSO) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position[a])	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
2	–	164.1	–	162.9
3	6.05 (*d*, *J* = 9.4)	108.3	6.00 (*d*, *J* = 9.4)	107.5
4	7.94 (*d*, *J* = 9.4)	140.1	7.91 (*d*, *J* = 9.4)	139.4
5	–	153.1	–	–
6	6.25 (*s*)	98.2	4.62 (*d*, *J* = 5.4)	81.2
7	–	–	4.22 – 3.81 (*m*)	65.9
8	4.62 (*d*, *J* = 5.4)	81.2	2.72 (*dd*, *J* = 4.0, 15.6), 2.65 (*dd*, *J* = 4.0, 15.6)	25.8
9	4.11 – 3.92 (*m*)	65.3	–	155.3
10	2.72 (*dd*, *J* = 4.0, 15.6), 2.65 (*dd*, *J* = 4.0, 15.6)	24.2	6.36 (*s*)	95.6
11	–	103.9	–	102.2
12	–	154.3	–	153.0
13	–	158.5	–	152.6
14	–	99.8	–	102.2
1′	–	134.7	–	134.7
2′	6.22 (*s*)	105.6	6.22 (*s*)	105.6
3′	–	150.3	–	150.3
4′	–	135.2	–	135.2
5′	–	150.3	–	150.3
6′	6.22 (*s*)	105.6	6.22 (*s*)	105.6
*Me*O–C(3′)	3.67 (*s*)	60.5	3.71 (*s*)	60.9
*Me*O–C(4′)	3.80 (*s*)	61.8	3.80 (*s*)	61.8
*Me*O–C(5′)	3.67 (*s*)	60.5	3.71 (*s*)	60.9

[a]) Arbitrary atom numbering, as depicted in *Fig. 1.*

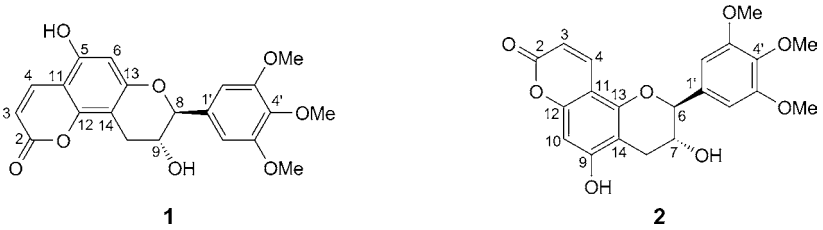

Fig. 1. *Chemical structures of compounds* **1** *and* **2**

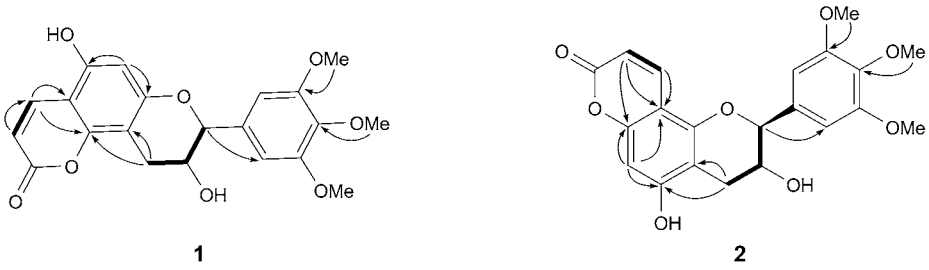

Fig. 2. *Selected HMBC* (H → C) *and ^{1}H,^{1}H-COSY* (—) *correlations of* **1** *and* **2**

cytotoxic activity against A549 cells with IC_{50} values of 22.4 μM and 21.1 μM, respectively.

In conclusion, two new flavanols were isolated from the whole plant *G. pentaphylla*. Furthermore, the two new compounds exhibited significant cytotoxic activity against HL-60 and A549 cells lines *in vitro*.

Experimental Part

General. All solvents used were of analytical grade. TLC: high-performance TLC plates precoated with silica gel GF_{254} (*Qingdao Haiyang Chemical Co., Ltd.*). Prep. TLC: glass plates (20 × 10 cm) precoated with silica gel GF_{254} (*Qingdao Haiyang Chemical Co., Ltd.*) of *ca.* 1.5 mm thickness, and the amount of sample applied to one layer was *ca.* 5 mg. Spots of TLC were visualized within I_2 vapor or by spraying with H_2SO_4/EtOH 1 : 9 followed by heating. Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Haiyang*

Chemical Co., Ltd., Qingdao, P. R. China) and *Sephadex LH-20* (25 – 100 μm; *Pharmacia Biotek*, Denmark). HPLC: *Agilent 1260*. Optical rotations: *GYROMAT-HP* polarimeter. UV Spectra: *Agilent 8453E* UV-Visible spectroscopy system; λ_{max} (log ε) in nm. CD Spectra: *Chirascan* spectropolarimeter. IR Spectra: *Thermo Nicolet NEXUS 470* FT-IR spectrometer in KBr discs; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Avance DRX-600* spectrometer operating at 600 (^{1}H) and 150 (^{13}C) MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *LTQ-Orbitrap XL*; in *m*/*z*.

Plant Material. G. pentaphylla were collected from Xishuangbanna Prefecture, Yunnan Province, P. R. China. The plant was identified, and authenticated at the Shandong University. The whole plants of *G. pentaphylla* were harvested and air-dried at r.t. in the dark. A voucher specimen (GP201308) has been deposited with the Herbarium of the School of Pharmaceutical Science, Shandong University.

Extraction and Isolation. The air-dried material (5 kg) was finely pulverized, then extracted with EtOH (5 l) for 24 h, and three times at r.t. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (74 g). The extract was dissolved and suspended in H_2O and partitioned with CH_2Cl_2 and BuOH. After evaporation of the solvents, 15.2 g of CH_2Cl_2 extract and 23.1 g of BuOH extract were obtained. The CH_2Cl_2 fraction (15.2 g) was initially subjected to CC over SiO_2, and then eluted in a step gradient with petroleum ether (PE)/AcOEt (200 : 1 – 1 : 1) to give four fractions. *Fr. 2* (2.1 g) was separated by CC over SiO_2 (PE/acetone 10 : 1 – 2 : 1), giving a mixture (310 mg). The mixture was further purified by *Sephadex LH-20* eluting with EtOH and HPLC (H_2O/MeCN 40 : 60) to afford **1** (21 mg) and **2** (22 mg).

*(8*S*,9*R*)-9,10-Dihydro-5,9-dihydroxy-8-(3,4,5-trimethoxyphenyl)-2*H*,8*H*-benzo[1,2-b:3,4-b′]dipyran-2-one* (**1**). Yellow powder. $[\alpha]_D^{25} = +97.2$ ($c = 0.3$, MeOH). UV (MeOH): 210 (3.24), 320 (4.33). CD (MeOH): $\Delta\varepsilon_{320}$ +5.65. IR (KBr): 3280, 1690, 1602, 1456, 1144. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 401.1233 ($[M+H]^+$, $C_{21}H_{21}O_8^+$; calc. 401.1236).

*(2*S*,3*R*)-3,4-Dihydro-3,5-dihydroxy-2-(3,4,5-trimethoxyphenyl)-2*H*,8*H-benzo[1,2-*b*:3,4-*b′*]dipyran-8-one* (**2**). Yellow powder. $[\alpha]_D^{25} = +75.0$ ($c = 0.2$, MeOH). UV (MeOH): 210 (2.98), 320 (3.51). CD (MeOH): $\Delta\varepsilon_{320}$ +6.22. IR (KBr): 3282, 1689, 1602, 1508, 1456, 1142. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 401.1233 ($[M+H]^+$, $C_{21}H_{21}O_8^+$; calc. 401.1236).

Biological Assays. HL-60 cells were maintained in an *RPMI-1640* medium, and A549 cells were maintained in MEM containing heat-inactivated 10% (*v*/*v*) FBS. The HL-60 (4×10^4 cells/ml) and A549 (1.2×10^4 cells/ml) cells were continuously treated with each compound for 72 h, and cell growth was evaluated using the MTT assay. Briefly, after terminating the cell culture, 20 μl of 5 mg/ml of MTT in PBS was added to every well, and the plate was re-incubated in 5% CO_2/air for 4 h at 37°. The plate was then centrifuged at 1500*g* for 5 min to precipitate the cells and MTT formazan. DMSO (200 μl) was added to dissolve the MTT formazan crystals. The plates were gently agitated until the color reaction was uniform and the OD_{570} was determined using a microplate reader. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay [9].

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