Two Unusual Rearranged Flavan Derivatives from *Narcissus tazetta* var. *chinensis*

by **Ka-Li Fu**[a]), **Yun-Heng Shen***[a]), **Lu Lu**[a]), **Bo Li**[c]), **Yi-Ren He**[a]), **Bo Li**[a]), **Xian-Wen Yang**[a])[d]), **Juan Su**[a]), **Run-Hui Liu**[a]), and **Wei-Dong Zhang***[a])[b])

[a]) Department of Phytochemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China (phone/fax: +86-21-81871245 (*Y.-H. S.*), +86-21-81871244 (*W.-D. Z.*); e-mail: shenyunheng9217018@yahoo.com.cn, wdzhangy@hotmail.com)

[b]) School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200030, P. R. China

[c]) Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai 200040, P. R. China

[d]) Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, P. R. China

Two unusual rearranged flavan derivatives with a rare bicyclo[3.3.1]non-3-ene-2,9-dione ring, tazettone A (**1**) and tazettone B (**2**), together with five known flavans, **3**–**7**, were isolated from the bulbs of *Narcissus tazetta* var. *chinensis* ROEM. The structures of two new compounds were elucidated by spectroscopic analyses, including 1D- and 2D-NMR spectroscopy. All of the isolated compounds were evaluated for their cytotoxicities against four human tumor cell lines A549, HCT116, SK-BR-3, and HepG2. Compounds **1** and **2** were almost inactive against all tested cell lines, while compounds **3**–**7** exhibited moderate or weak cytotoxicities against the tested cell lines.

Introduction. – *Narcissus tazetta* var. *chinensis* ROEM. (Amaryllidaceae), a species of the *Narcissus* genus, is a popular ornamental flower in China, and widely cultivated in southern China. In China, the dried bulbs of *N. tazetta* var. *chinensis* have been long used for the treatment of mumps, carbuncles, mastitis, and insect bites [1]. Due to their remarkable antitumor activities, Amaryllidaceae alkaloids have become targets as antitumor leads [2]. Discovery of the promising antitumor lead compound narciclasine greatly stimulated the research of *Narcissus* species, and resulted in the isolation of several alkaloids during the past decades [3]. However, so far, few studies have been reported about the chemical constituents of *N. tazetta* var. *chinensis* [4–6]. In our continuing efforts to discover antitumor leads from Amaryllidaceae plants growing in China, two novel flavan derivatives, tazettones A and B (**1** and **2**, resp.; *Fig. 1*), together with five known flavans, **3**–**7**, which were isolated from *N. tazetta* var. *chinensis* for the first time. Herein, we describes the isolation and structure elucidation of the two unusual flavan derivatives, as well as the cytotoxicity of all isolated flavans against four human tumor cell lines A549, HCT116, SK-BR-3, and HepG2.

Results and Discussion. – The $CHCl_3$-soluble fraction of 90% EtOH extract of the bulbs of *N. tazetta* var. *chinensis* was subjected to repeated column chromatography on silica gel (SiO_2), *RP-18*, and *Sephadex LH-20* to afford two unusual compounds, **1** and **2**, along with five known flavans, **3**–**7** (*Fig. 1*). The structures of the known flavans were

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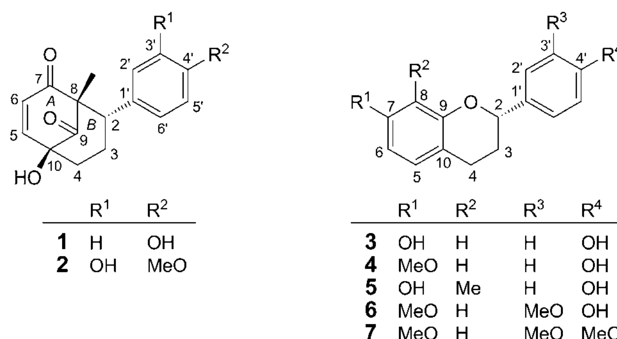


Fig. 1. *Chemical structures of compounds* **1**–**7**

identified as (2*S*)-7,4′-dihydroxyflavan (**3**) [7][8], (2*S*)-4′-hydroxy-7-methoxyflavan (**4**) [7], (2*S*)-7,4′-dihydroxy-8-methylflavan (**5**) [9], (2*S*)-7,3′-dimethoxy-4′-hydroxyflavan (**6**) [7][10], and (2*S*)-7,3′,4′-trimethoxyflavan (**7**) [7], by comparing their spectroscopic data and physical properties with those reported in the literature.

Tazettone A (**1**) was obtained as a white powder. Its molecular formula was determined as $C_{16}H_{16}O_4$ by positive-ion HR-ESI-MS, which exhibited a pseudomolecular-ion peak at *m*/*z* 295.0945 ($[M+Na]^+$). The IR spectrum of **1** indicated the presence of OH (3384 cm^{-1}), non-conjugated C=O (1736 cm^{-1}), and α,β-unsaturated ketone C=O groups (1660 cm^{-1}).

The ^{1}H-, ^{13}C-, and DEPT-NMR spectra (*Table 1*) revealed the presence of one Me group, two CH_2, and seven CH groups (including four aromatic CH and two olefinic CH groups), six quaternary C-atoms (including two C=O groups, two aromatic C-atoms), and two OH groups. In the ^{1}H-NMR and ^{1}H,^{1}H-COSY spectra, the A_2B_2 spin coupling system at δ(H) 6.77 (*d*, *J* = 8.6, 2 H) and δ(H) 6.64 (*d*, *J* = 8.6, 2 H) indicated the presence of a 1,4-disubstituted phenyl group (δ(C) 128.8, 129.9 (2 C), 114.6 (2 C), and 156.6). Considering that seven of nine unsaturations have been accounted for by one benzene ring, two C=O groups, and a C=C bond, it was concluded that **1** contained two additional rings. The ring *A* was established by key ^{1}H,^{1}H-COSY correlation between H–C(5)[1]) (δ(H) 7.08) and H–C(6) (δ(H) 6.41), in combination with key HMBCs (*Fig. 2*) of H–C(5) with C(7) (δ(C) 197.6) and C(9) (δ(C) 207.7), of H–C(6) with C(8) (δ(C) 68.0) and C(10) (δ(C) 77.4), and of Me–C(8) (δ(H) 0.84) with C(7) and C(9). The ^{1}H,^{1}H-COSY correlations H–C(2) (δ(H) 2.72)/CH_2(3) (δ(H) 1.67–1.72, 1.94–2.01)/CH_2(4) (δ(H) 1.89, 1.94–2.01), together with the HMBCs from H–C(4) to C(9) and C(10), from H–C(2) to C(8) and Me–C(8) (δ(C) 14.9), revealed the presence of the ring *B*. The above data also indicated that the rings *A* and *B* were fused along C(8)–C(10), and formed a rare bicyclo[3.3.1]non-3-ene-2,9-dione core. The 4-hydroxyphenyl group was attached to C(2) (δ(C) 54.3) of **1** based on the key HMBCs of H–C(2′) (δ(H) 6.77) with C(2), and of H–C(2) (δ(H) 2.72) with C(2′) (δ(C) 129.9). A OH group was at C(10) of **1** deduced from HMBCs of H–C(6) with CH_2(3), and of CH_2(4) with C(10). Therefore, the planar structure of **1** was determined

[1]) Atom numbering as indicated in *Fig. 1*. For systematic names, see the *Exper. Part*.

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in *Fig. 1*.

Position	1		2	
	δ(H)[a])	δ(C)[b])	δ(H)[c])	δ(C)[d])
H–C(2)	2.72 (*dd*, J = 12.7, 4.0)	54.3 (*d*)	2.71 (*dd*, J = 13.0, 4.4)	57.4 (*d*)
H_a–C(3)	1.67 – 1.72 (*m*)	26.3 (*t*)	1.81 – 1.86 (*m*)	28.3 (*t*)
H_b–C(3)	1.94 – 2.01 (*m*)		2.05 – 2.15 (*m*)	
H_a–C(4)	1.89 (*dd*, J = 14.0, 4.4)	36.7 (*t*)	2.07 (*dd*, J = 15.2, 4.2)	38.6 (*t*)
H_b–C(4)	1.94 – 2.01 (*m*)		1.96 – 1.99 (*m*)	
H–C(5)	7.08 (*d*, J = 10.1)	152.7 (*d*)	7.06 (*d*, J = 10.1)	153.3 (*d*)
H–C(6)	6.41 (*d*, J = 10.1)	130.7 (*d*)	6.45 (*d*, J = 10.1)	132.8 (*d*)
C(7)		197.6 (*s*)		200.2 (*s*)
C(8)		68.0 (*s*)		70.0 (*s*)
C(9)		207.7 (*s*)		209.5 (*s*)
C(10)		77.4 (*s*)		79.3 (*s*)
C(1′)		128.8 (*s*)		133.0 (*s*)
H–C(2′)	6.77 (*d*, J = 8.6)	129.9 (*d*)	6.47 (*d*, J = 2.4)	117.5 (*d*)
H–C(3′)	6.64 (*d*, J = 8.6)	114.6 (*d*)		
C(3′)				147.4 (*s*)
C(4′)		156.6 (*s*)		148.9 (*s*)
H–C(5′)	6.64 (*d*, J = 8.6)	114.6 (*d*)	6.80 (*d*, J = 8.8)	112.6 (*d*)
H–C(6′)	6.77 (*d*, J = 8.6)	129.9 (*d*)	6.46 (*dd*, J = 8.8, 2.4)	122.1 (*d*)
Me–C(8)	0.84 (*s*)	14.9 (*q*)	0.99 (*s*)	15.9 (*q*)
HO–C(10)	6.16 (*s*)			
HO–C(3′)				
HO–C(4′)	9.45 (*s*)			
*Me*O–C(4′)			3.82 (*s*)	56.8 (*q*)

[a]) Recorded at 400 MHz in (D_6)DMSO. [b]) Recorded at 100 MHz in (D_6)DMSO. [c]) Recorded at 400 MHz in CD_3OD. [d]) Recorded at 100 MHz in CD_3OD.

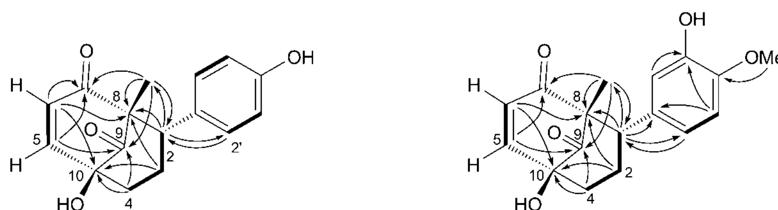


Fig. 2. *^{1}H,^{1}H-COSY* (bold lines) *and key HMBC* (arrows) *correlations of* **1** *and* **2**

as depicted in *Fig. 1*. A similar compound, acutifolin A, with a 3-methylbut-2-enyl group instead of a Me group at C(8) has been reported from Brazilian medicinal plant *Brosimum acutifolium* (Moraceae) [11]. Comparison of the NMR data of acutifolin A [11] with those of **1** confirmed that two compounds share the same skeleton except for the substituent at C(8). The ROSEY correlations Me(8)/H–C(2)/H_a–C(4), as well as the ROSEY correlation between H_b–C(4) and H–C(5), indicated β-orientation of 8-Me and 10-OH groups, and of H–C(2) (*Fig. 3*).

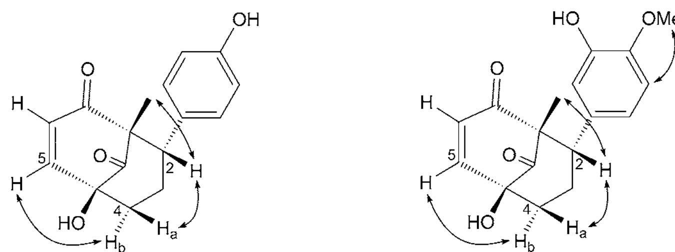


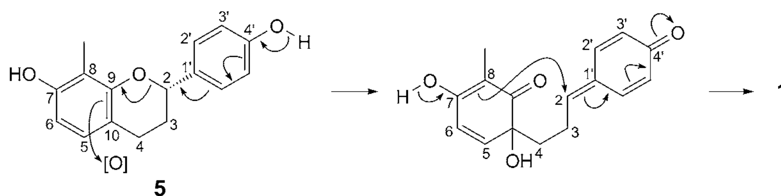
Fig. 3. *Key ROESY correlations of* **1** *and* **2**

Tazettone B (**2**), a white powder, had the molecular formula $C_{17}H_{18}O_5$ as deduced from positive-ion HR-ESI-MS (*m/z* 325.1054 ($[M+Na]^+$)). The IR spectrum showed characteristic absorption bands at 1735 and 1666 cm^{-1}, for one non-conjugated and one α,β-unsaturated C=O group, respectively, as well as at 3419 cm^{-1} for a OH group.

The NMR data of **2** (*Table 1*) revealed structural features similar to those of **1**, including two C=O groups (δ(C) 200.2, 209.5), one C=C bond (δ(H) 7.06, 6.45; δ(C) 153.3, 132.8), one Me group (δ(H) 0.99; δ(C) 15.9), one oxygenated quaternary C-atom (δ(C) 79.3), one sp^3 quaternary C-atom (δ(C) 70.0), one sp^3 CH group (δ(H) 2.71; δ(C) 57.4), two sp^3 CH_2 groups (δ(C) 28.3, 38.6), and one phenyl group (δ(H) 6.47, 6.80, 6.46; δ(C) 133.0, 117.5, 147.4, 148.9, 112.6, 122.1). The only difference between compounds **2** and **1** is that a 1,3,4-trisubsituted phenyl group, based on an *ABX* spin coupling system of the aromatic H-atoms in compound **2**, replaced the 1,4-disubstituted phenyl group, based an A_2B_2 spin coupling system in **1**. A OH group and a MeO group were the substituents at C(3′) and C(4′) of the phenyl group, respectively, as revealed by the HMBCs (*Fig. 2*) of MeO–C(4′) (δ(H) 3.82) with C(4′) (δ(C) 148.9), of H–C(2′) (δ(H) 6.47) with C(3′) (δ(C) 147.4), and of H–C(6′) (δ(H) 6.46) with C(4′), as well as the ROESY correlation between MeO–C(4′) and H–C(5′) (δ(H) 6.80). The relative configuration of **2** was characterized to be identical to that of **1** due to their similar REOSY correlations (*Fig. 3*). Therefore, the structure of **2** was determined as shown in *Fig. 1*, and named tazettone B.

Compounds **1** and **2** belongs to a small group of natural products with a rare bicyclo[3.3.1]non-3-ene-2,9-dione core. To this day, only one similar natural product was reported from the Brazilian medicinal plant *Brosimum acutifolium* [11]. Biogenetically, compounds **1** and **2** might be derived from the rearrangement of an 8-methylflavan (*Scheme*). *Simpkins* and co-workers have reported the synthesis of several compounds with a bicyclo[3.3.1]nonane-2,4,9-trione core [12], indicating that

Scheme. *Plausible Biogenetic Pathway for Tazettone A* (**1**)



this rearrangement reaction of flavans is possible. Considering that the plant samples were extracted at room temperature, and no acid or base was employed during the extraction and separation procedure, it can be assumed that **1** and **2** are genuine metabolites of the plant.

Cytotoxicity. – All the isolated compounds were tested for their cytotoxicities against human lung cancer (A549), colon cancer (HCT116), breast cancer (SK-BR-3), and hepatoma (HepG2) cell lines using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-terazolium bromide) colorimetric assay. As can been seen from *Table 2*, compound **5** exhibited moderate-to-weak cytotoxicities against the tested cell lines with IC_{50} values in the range of 10.50 – 31.62 µg/ml. Compound **4** showed weak cytotoxicity against A549, HCT116, SK-BR-3, and HepG2 cell lines with IC_{50} values of 36.47, 28.48, 16.82, and 28.71 µg/ml, respectively. Compound **6** exhibited weak cytotoxicity against SK-BR-3 and HepG2 with IC_{50} values of 26.81 and 26.50 µg/ml, respectively. Compounds **3** and **7** exhibited weak cytotoxicities against HCT116 and HepG2 with an IC_{50} values 28.96 and 34.36 µg/ml, respectively, while compounds **1** and **2** were almost inactive against all cell lines. From these data, it can be concluded that flavans with a regular skeleton, *i.e.*, **3** – **7**, are more active than those with a rearranged scaffold, *i.e.*, **1** and **2**. In addition, the comparison of the activities of **3** and **5** indicates that the presence of Me group at C(8) enhances the cytotoxicity.

Table 2. *Cytotoxicties of Compounds* **1** – **7**

Compounds	IC_{50} [µg/ml][a])			
	A549	HCT116	SK-BR-3	HepG2
1	> 100	86.74 ± 2.20	51.08 ± 2.00	> 100
2	> 100	> 100	> 100	> 100
3	> 100	28.96 ± 3.60	> 100	29.18 ± 2.00
4	36.47 ± 2.10	28.48 ± 3.60	16.82 ± 2.10	28.71 ± 2.80
5	31.62 ± 2.40	17.12 ± 2.00	10.50 ± 1.80	20.39 ± 2.60
6	45.08 ± 2.70	40.06 ± 3.80	26.81 ± 2.00	26.50 ± 2.40
7	> 100	34.36 ± 2.40	> 100	38.51 ± 2.50
Doxicyclin[b])	0.0183 ± 0.0001	0.0207 ± 0.0003	0.0938 ± 0.0004	0.0284 ± 0.0005

[a]) Data are given in mean ± SD. [b]) Positive control.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh), *RP-18* silica gel (*ODS*, 25 – 40 µm, *Merck*), and *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA). Optical

rotation: *Perkin-Elmer 341* digital polarimeter (*Perkin-Elmer*, Norwalk, CT, USA), at 589 nm. UV: *Shimadzu UV-2550*; λ_{max} (log ε) in nm. IR Spectra: *Bruker Vector-22* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Bruker DRX-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Agilent-1100-LC/MSD-Trap* (ESI-MS) and *Agilent Micro-Q-Tof* (HR-ESI-MS) spectrometer; in *m/z*.

Plant Material. The bulbs of *Narcissus tazetta* var. *chinensis* Roem were collected from Zhangzhou county, Fujian province, P. R. China, in August 2010, and authenticated by Prof. *Han-Ming Zhang* of Second Military Medical University. A voucher specimen (No. 2010081008) was deposited with the School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The air-dried bulbs of *N. tazetta* var. *chinensis* ROEM. (20.0 kg) were extracted with 90% EtOH (4 × 80 l, each 24 h) at r.t. The pooled extract was concentrated *in vacuo* to afford a residue, which was suspended in H_2O (4 l) and extracted with $CHCl_3$ (4 × 4 l). The $CHCl_3$ extract (280 g) was subjected to CC (SiO_2 (200–300 mesh, 1000 g); petroleum ether PE/AcOEt 100:0 → 0:100) to give eight fractions, *Fr. A–H*. *Fr. A* (53.0 g) was submitted to CC (*RP-18*; 40 → 100% MeOH/H_2O; and SiO_2; PE/$CHCl_3$/MeOH 100:50:1; PE/acetone 100:1 → 10:1) to yield compounds **4** (55.0 mg), **6** (6.0 mg), and **7** (12.0 mg). Compounds **3** (14.3 mg) and **5** (3.0 mg) were obtained from *Fr. D* (20.0 g) by repeated CC (*RP-18*; 40 → 100% MeOH/H_2O; and SiO_2; $CHCl_3$/MeOH 100:0 → 20:1). *Fr. E* (9.5 g) was separated by a similar procedure, and finally purified by CC (*Sephadex LH-20*; PE/$CHCl_3$/MeOH 5:5:1) to afford compounds **1** (17.8 mg) and **2** (19.0 mg).

Tazettone A (*=(1S,5S,8S)-5-Hydroxy-8-(4-hydroxyphenyl)-1-methylbicyclo[3.3.1]non-3-ene-2,9-dione*; **1**). White amorphous powder. $[\alpha]_D^{22} = +118.6$ ($c = 0.425$, MeOH). UV (MeOH): 217 (4.34), 228 (4.33). IR (KBr): 3384, 2943, 1736, 1660, 1614, 1516, 1444, 1373, 1271. ^{1}H- and ^{13}C-NMR ((D_6)DMSO): see *Table 1*. ESI-MS (pos.): 295 ([M + Na]$^+$), 567 ([2 M + Na]$^+$). HR-ESI-MS (pos.): 295.0945 ([M + Na]$^+$, $C_{16}H_{16}NaO_4^+$; calc. 295.0946).

Tazettone B (*=(1S,5S,8S)-5-Hydroxy-8-(3-hydroxy-4-methoxyphenyl)-1-methylbicyclo[3.3.1]non-3-ene-2,9-dione*; **2**). White amorphous powder. $[\alpha]_D^{22} = +447.1$ ($c = 0.225$, MeOH). UV (MeOH): 217 (4.08), 280 (4.59). IR (KBr): 3419, 2931, 1735, 1666, 1618, 1510, 1444, 1373, 1275 cm^{-1}. ^{1}H- and ^{13}C-NMR (CD_3OD): see *Table 1*. ESI-MS (pos.): 325 ([M + Na]$^+$), 603 ([2 M + Na]$^+$). HR-ESI-MS (pos.): 325.1054 ([M + Na]$^+$, $C_{17}H_{18}NaO_5^+$; calc. 325.1052).

Cytotoxicity. A MTT colorimetric assay was performed in 96-well plates. Cells culture were diluted with fresh medium consisting of *Dulbecco*'s modified eagle's medium (DMEM), 10% fetal bovine serum (FBS), and penicillin, as well as streptomycin, to 4–6 × 10^4 cells/ml, and placed in 96-well microplates at 100 μl/well. After 24 h incubation at 37° in a 5% CO_2 atmosphere, the tested compounds at different concentrations were added to the microplates in 10-μl amounts. The four tumor cell lines, A549, HCT116, SK-BR-3, and HepG2, were exposed to the drugs for another 72 h. Then, 20 μl of MTT soln. (5 mg/ml) were added to each well, and the plate was incubated for 4 h. DMSO (100 μl) was added to each well later. The OD of each well was measured on a *Wellscan* reader (*Varioskan Flash*, *Thermo Scientific*) at 570 nm. Doxicyclin was used as positive control. The assay was performed in triplicate. The data were represented as mean ± S.D. The cell lines were all preserved in Shanghai Institute for Pharmaceutical Industrial, P. R. China.

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