Two New Furanoid Diterpenoids from *Tinospora sagittata*

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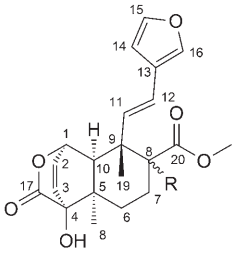
Two new clerodane-based furanoid diterpenoids, tinosagittones A and B (**1** and **2**, resp.), were isolated from the roots of *Tinospora sagittata*, together with five known diterpenoids, *i.e.*, columbin (**3**), its glucoside palmatoside C (**4**), isocolumbin (**5**), 6-hydroxycolumbin (**6**), and tinophylloloside (**7**). Their structures were established by mass spectrometry and spectroscopic methods, especially 2D-NMR techniques.

Introduction. – Species of the genus *Tinospora* are widely employed as medicinal plants throughout a large part of Asia and Africa [1]. The roots of *Tinospora sagittata* (OLIV.) GAGNEP (Menispermaceae), known as Jin-Guo-Lan in China, constitute a traditional Chinese medicine (TCM), and are commonly applied to relieve sore throat, expel superficial infection, and stop diarrhea [2]. A previous investigation on this species resulted in the isolation of a number of alkaloids, clerodane-type diterpenes, and botanic steroids [3]. Modern pharmacological studies have shown that ethanolic extracts of Jin-Guo-Lan prepared from the roots of *T. sagittata* and *T. capillipes* possess broader bioactivities, such as antibiotic, anti-inflammatory, and antiviral effects [4][5]. In the present study, two new clerodane-based furanoid diterpenoids, namely tinosagittones A and B (**1** and **2**, resp.), were isolated from the roots of *T. sagittata*, together with five known diterpenoids, *i.e.*, columbin (**3**) [6], and its glycoside palmatoside C (**4**) [6][7], isocolumbin (**5**) [8][9], 6-hydroxycolumbin (**6**) [9], and tinophylloloside (**7**) [9][10]. Their structures were established by mass spectrometry and spectroscopic analyses, especially 2D-NMR techniques (HMQC, HMBC, and NOESY).

Results and Discussion. – Tinosagittone A (**1**) was obtained as an optically active, colorless, amorphous powder ($[\alpha]_D^{20} = +51.9$). Its molecular formula was assigned $C_{21}H_{24}O_6$ as deduced from its HR-ESI-MS (*m/z* 395.1473 ($[M+Na]^+$; calc. 395.1471), inferring ten degrees of unsaturation. Strong IR absorption bands at 1748 and 1730 cm^{-1} were attributable to δ-lactone and ester C=O groups, respectively, as confirmed by ^{13}C-NMR signals at δ(C) 176.4 and 175.7 (*Table*). The ^{1}H-NMR spectrum of **1** displayed signals for two Me groups (δ(H) 1.12 (*s*); 1.37 (*s*)), a 3-substituted furan (δ(H) 6.51 (*d*, *J* = 1.5); 7.36 (*d*, *J* = 1.5); 7.39 (*s*)), and four olefinic H-atoms (δ(H) 6.42 (*dd*, *J* = 7.5, 5.0); 6.32 (*dd*, *J* = 7.5, 1.5); 6.31 (*d*, *J* = 15.8); 6.27 (*d*, *J* = 15.8)), a MeO



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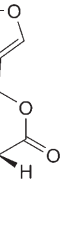
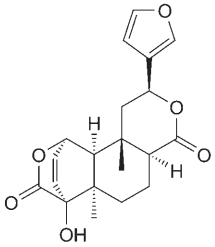
2[1]) R = α-H



3 R = H
4 R = Glc



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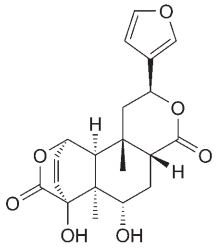

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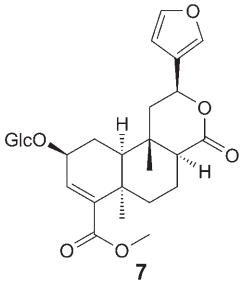

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singlet (δ(H) 3.62 (*s*)), and a signal for an oxygenated CH group (δ(H) 5.24 (*dd*, *J* = 5.0, 1.5)) (*Table*). Consistent with the molecular formula of **1**, a total of 21 ^{13}C-NMR signals were observed, corresponding to six quaternary, ten tertiary, two secondary C-atoms, as well as three Me groups. Among them, one CH-group and one quaternary C-atom signal (δ(C) 75.5, δ(H) 5.24; δ(C) 80.2) were ascribed to the C-atoms bearing O, and the two downfield C-atom signals at δ(C) 176.4 and 175.7 were due to ester CO functions. Six out of the ten degrees of unsaturation were due two CO groups and four C=C bonds; the remaining four degrees of unsaturation, thus, had to be accounted for by four rings. The data mentioned above disclosed **1** as a typical furanoid clerodane-type diterpenoid.

Analysis of the ^{1}H- and ^{13}C-NMR, and HMQC spectra of **1**[1]) enabled us to assign all the H-atoms to the connected C-atoms. The assemblage of all C-atoms, including quaternary C-atoms and heteroatoms, was mainly achieved by interpretation of a well-resolved HMBC spectrum (*Fig. 1*). The linkage of C(13) and C(12) was established by the HMBC correlations of H–C(14)/C(13) and H–C(14)/C(12), which was corroborated by the UV maximum at 243 nm (log ε 4.34). The HMBC correlation of H–C(1)/C(17) indicated C(1) and C(17) to be linked by an O-atom. The H-atom signal of H–C(18) correlated with the C-atom signals at δ(C) 37.2 C(5) and 80.2 C(4), revealing the presence of a OH group at C(4). The quaternary C-atom signal at δ(C) 175.7 was allocated to C(20) by the strong correlations between C(20) and H–C(8) (δ(H) 2.59 (*dd*, *J* = 9.5, 6.5)). Thus, the structure of **1** was deduced as shown in *Fig. 1*.

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[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.

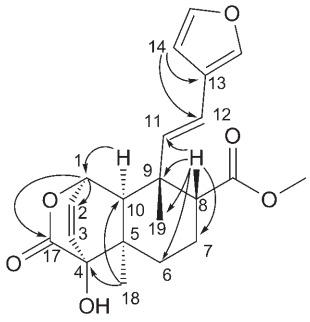

Fig. 1. *Selected HMBC correlations* (H → C) *of* **1**[1])

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[1]) ($CDCl_3$, 400 MHz and 100 MHz, resp.). δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.24 (*dd*, *J* = 5.0, 1.5)	75.5	5.00 (*dd*, *J* = 5.0, 1.5)	74.5
H–C(2)	6.42 (*dd*, *J* = 7.5, 5.0)	129.5	6.40 (*dd*, *J* = 7.5, 3.0)	129.6
H–C(3)	6.32 (*dd*, *J* = 7.5, 1.5)	136.6	6.29 (*dd*, *J* = 7.5, 1.2)	136.1
C(4)	–	80.2	–	80.8
C(5)	–	37.2	–	37.6
H_α–C(6)	1.67 – 1.72 (*m*)	27.4	1.77 – 1.81 (*m*)	24.8
H_β–C(6)	1.60 – 1.64 (*m*)		1.42 – 1.48 (*m*)	
H_α–C(7)	1.94 – 1.98 (*m*)	20.6	2.08 – 2.13 (*m*)	19.2
H_β–C(7)	1.89 – 1.93 (*m*)		1.91 – 1.95 (*m*)	
H–C(8)	2.59 (*dd*, *J* = 9.5, 6.5)	47.3	2.63 (*dd*, *J* = 11.5, 8.5)	47.5
C(9)	–	40.0	–	42.3
H–C(10)	1.97 (*s*)	51.9	1.50 (*s*)	54.7
H–C(11)	6.31 (*d*, *J* = 15.8)	136.3	6.28 (*d*, *J* = 16.5)	137.9
H–C(12)	6.27 (*d*, *J* = 15.8)	118.7	5.78 (*d*, *J* = 16.5)	118.9
C(13)	–	124.1	–	123.8
H–C(14)	6.51 (*d*, *J* = 1.5)	107.3	6.51 (*d*, *J* = 1.5)	107.3
H–C(15)	7.36 (*d*, *J* = 1.5)	143.6	7.38 (*d*, *J* = 1.5)	143.6
H–C(16)	7.39 (*s*)	140.1	7.42 (*s*)	140.3
C(17)	–	176.4	–	175.8
Me(18)	1.12 (*s*)	25.7	1.16 (*s*)	25.6
Me(19)	1.37 (*s*)	26.4	1.24 (*s*)	15.3
C(20)	–	175.7	–	174.1
MeO	3.62 (*s*)	51.5	3.53 (*s*)	51.4

The relative configuration of **1** was deduced by a NOESY analysis (*Fig. 2*). The NOEs for H–C(19)/H–C(8), H–C(8)/H_β–C(7), and H–C(8)/H_β–C(6) indicated that the H_β–C(6), H_β–C(7), H–C(8), and H–C(19) are on the same side of the molecular plane, tentatively assumed as β-orientation. As a consequence, the NOE correlations of H_α–C(6)/H–C(18), H–C(18)/H–C(10), and H–C(10)/H–C(1) indicated that the H–C(1), H–C(10), and H–C(18) were α-configured. Complete ^{1}H- and ^{13}C-NMR assignments (*Table*) were accomplished by a combination of 2D-NMR techniques, including HMQC, HMBC and NOESY. Thus, the structure of tinosagittol A (**1**)[1]) was established, but its absolute configuration remains to be determined.

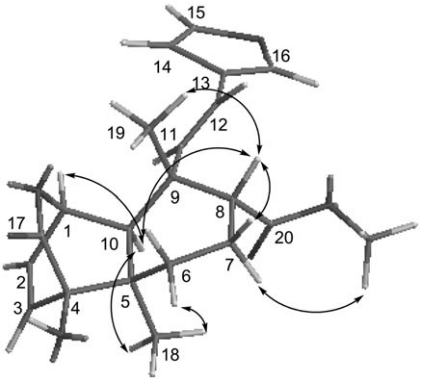

Fig. 2. *Key NOESY correlations and relative configuration of* **1**[1])

Tinosagittol B (**2**) has the molecular formula $C_{21}H_{24}O_6$, which was identical with that of **1**, based on by HR-ESI-MS analysis (*m/z* 395.1474 ($[M+Na]^+$; calc. 395.1471). The compound was obtained as colorless, amorphous powder. The IR, UV, and ^{1}H- and ^{13}C-NMR spectra were very similar with those of **1**, except that the chemical shift of the signal of C(19) was upfield in comparison with that of **1**. An extensive analysis of the 2D-NMR spectra (HMQC and HMBC) showed that **2** has the same constitution as **1**, which indicated that they might be diastereoisomers.

The relative configuration of **2**[1]) was deduced by a NOESY analysis (*Fig. 3*). NOE correlations of H–C(19)/H_β–C(7), H_β–C(7)/H_β–C(6) indicated that the H–C(19), H_β–C(7), H_β–C(6) are on the same side and presumably in β-orientation. As a consequence, the correlations of H_α–C(7)/H–C(8), H_α–C(6)/H–C(18), H–C(18)/H–C(10), and H–C(10)/H–C(1) indicated that the H–C(1), H–C(8), H–C(10), and H–C(18) are in the α-configuration. The structure of **2** was thus established as 8-epitinosagittol A, and named as tinosagittol B[1]).

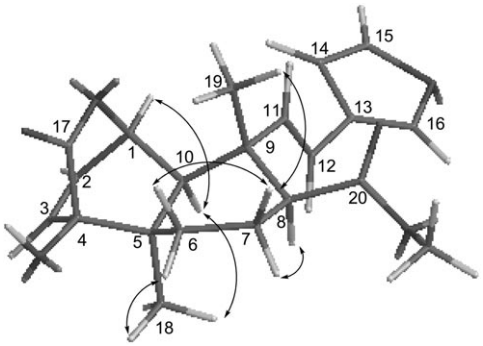

Fig. 3. *Key NOESY correlations and relative configuration of* **2**[1])

The structures of the five known diterpenoids also isolated from *T. sagittata*, *i.e.*, columbin (**3**), palmatoside C (**4**), isocolumbin (**5**), 6-hydroxycolumbin (**6**), and tinophylloloside (**7**), were corroborated by comparison of their spectroscopic data with those reported in the literature [6–10].

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Experimental Part

General. All solvents used were of analytical grade (*Shanghai Chemical Plant*). Silica gel (230–400 mesh), and *MCI CHP20P* gel (75–150 μ; *Mitsubishi Chemical Industries Ltd.*) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Plant*) were used for thin layer chromatography (TLC). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Varian Cary Bio* spectrometer. IR Spectra: *Perkin-Elmer 577* spectrophotometer; in cm^{-1}. NMR Spectra: *Bruker AM-500*; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Finnigan LCQ^{DECA}* mass spectrometer; in *m*/*z*.

Plant Material. The roots of *T. sagittata* were collected from Guangxi Province, P. R. China, in June, 2006, and authenticated by Prof. *Yong-Hong Zhang* of the Fujian Medical University, P. R. China. A voucher specimen (No. 200607014T) was deposited at the Zhejiang Chinese Medical University.

Extraction and Isolation. The powdered roots of *T. sagittata* (5.1 kg) were percolated with aq. 95% EtOH. After solvent removal, the crude extract (472 g) was suspended in H_2O (5 l) and extracted with $CHCl_3$ and BuOH (each 5×500 ml) to afford two extracts. The $CHCl_3$-soluble fraction (102 g) was subjected to CC (SiO_2; petroleum ether/acetone $4:1 \rightarrow 2:1$) to afford two major fractions, *Fr. 1* (73 g) and *Fr. 2* (5.1 g). *Fr. 1* was recrystallized from petroleum ether/acetone (2:1) to afford **3** (53 g). The filtrate was condensed to give a yellow residue, which was then separated by CC (SiO_2; petroleum ether/AcOEt $10:1 \rightarrow 4:1$) to afford **1** (27 mg), **2** (7.0 mg), and **5** (110 mg). *Fr. 2* was also purified by CC (SiO_2; $CHCl_3$/MeOH $20:1 \rightarrow 10:1$) to afford **6** (157 mg). The BuOH-soluble fraction (48 g) was subjected to CC (*MCI CHP20P* gel; MeOH/H_2O $0:10 \rightarrow 3:7$) to give **7** (5.1 g) and **4** (10.2 g).

Tinosagittone A (= *Methyl* rel-*(1*R*,4*R*,4a*R*,7*R*,8*S*,8a*S*)-8-[(*E*)-2-(3-Furyl)vinyl]-1,4,4a,5,6,7,8,8a-octahydro-4-hydroxy-4a,8-dimethyl-9-oxo-1,4-(epoxymethano)naphthalene-7-carboxylate*; **1**). Colorless amorphous powder. $[\alpha]_D^{20} = +51.9$ ($c = 0.9$, $CHCl_3$). UV ($CHCl_3$): 243 (4.34). IR (KBr): 3448 (OH), 2952, 1748 (C=O), 1730 (C=O), 1458, 1375, 1278, 1157, 1052, 752. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS (pos.): 395 ($[M+Na]^+$). HR-ESI-MS: 395.1473 ($[M + Na]^+$, $C_{21}H_{24}NaO_6^+$; calc. 395.1471).

Tinosagittone B (= *Methyl* rel-*(1*R*,4*R*,4a*R*,7*S*,8*S*,8a*S*)-8-[(*E*)-2-(3-Furyl)vinyl]-1,4,4a,5,6,7,8,8a-octahydro-4-hydroxy-4a,8-dimethyl-9-oxo-1,4-(epoxymethano)naphthalene-7-carboxylate*; **2**). Colorless amorphous powder. $[\alpha]_D^{20} = -42.3$ ($c = 0.1$, $CHCl_3$). UV ($CHCl_3$): 244 (4.32). IR (KBr): 3448 (OH), 2925, 1745 (C=O), 1730 (C=O), 1437, 1371, 1157, 988, 752. ^{1}H- and ^{13}C- NMR: see *Table*. ESI-MS (pos.): 395 ($[M+Na]^+$). HR-ESI-MS: 395.1474 ($[M + Na]^+$, $C_{21}H_{24}NaO_6^+$; calc. 395.1471).

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