New Diterpenoids from *Tinospora capillipes*

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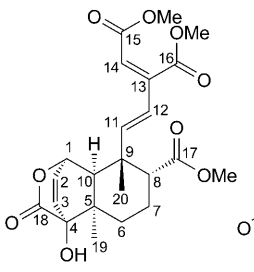
A phytochemical study of the roots of *Tinospora capillipes* resulted in the characterization of two new highly unsaturated diterpenoids, tinocapilactones A and B (**1** and **2**), as well as of six known ones. The structures of **1** and **2** were assigned on the basis of extensive 1D- and 2D-NMR experiments, combined with mass-spectrometric methods.

Introduction. – Species of the genus *Tinospora*, widely distributed over Asia and Africa, are well-known for their medicinal properties [1]. The distinctive constituents of this genus are the clerodane-based furonoid diterpenoids [2]. *Tinospora capillipes* GAGNEP, a member of the Menispermaceae family, is a special plant native in South China. Its rhizome (*Radix Tinosporae*, *R.T.*) has been used in traditional Chinese medicine (TCM) for treating sore throat, laryngitis, gastralgia, and diarrhea for thousands of years [3]. A series of clerodane-type diterpenes, botanic steroids, and alkaloids isolated from this species were reported [4–8]. In the present study, two new highly unsaturated diterpenoids, namely tinocapilactones A and B[1]) (**1** and **2**), were isolated from the roots of *T. capillipes*, together with six known ones, *i.e.*, columbin (**3**) [9], palmatoside C (**4**) [10], isocolumbin (**5**) [11], tinocallones A and B (**6** and **7**), and epitinophylloloside (**8**) [7]. Their structures were established by mass-spectrometric and spectroscopic analyses, especially 2D-NMR techniques (HMQC, HMBC, and NOESY).

Results and Discussion. – Tinocapilactone A (**1**) was obtained as an optically active, colorless, amorphous powder. The HR-ESI-MS (*m/z* 395.1473 ($[M+Na]^+$)) revealed a molecular formula $C_{23}H_{28}O_9$, suggesting the presence of ten degrees of unsaturation. Strong IR absorption bands at 1746 and 1716 cm^{-1} were attributable to δ-lactone and ester CO groups, respectively. The ^{13}C-NMR spectrum indicated the presence of 23 C-atoms comprising four CO groups (δ(C) 176.2, 175.0, 167.6, and 165.4), three olefinic C-atoms (δ(C) 147.9, 147.7, 136.8, 129.5, 125.2, and 118.6), an O-bearing CH group (δ(C) 75.2), one O-bearing quaternary C-atom (δ(C) 80.0), and three MeO groups (δ(C) 52.5, 52.0, and 51.7) (*Table*). Among the four CO groups, two relatively high-field C-atom signals (δ(C) 167.6 and 165.4) were assigned to two α,β-unsaturated ester CO groups, which was confirmed by the UV maximum at 264 nm (log ε 4.09). The

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



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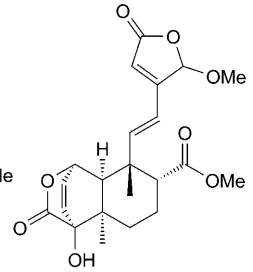

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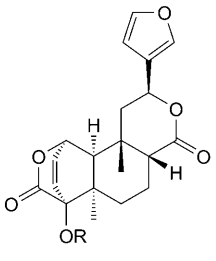

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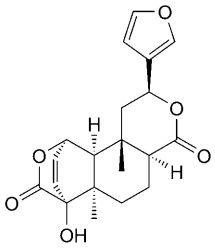
4 R = Glc



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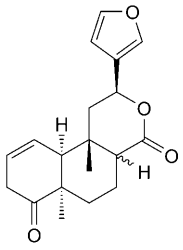
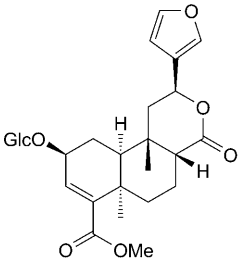


6 R = β-H
7 R = α-H



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^{1}H-NMR spectrum of **1** revealed signals for two Me groups (δ(H) 1.11 and 1.35 (2*s*)), five olefinic H-atoms (δ(H) 6.42 (*dd*, J = 7.7, 5.0), 6.32 (*dd*, J = 7.7, 1.2), 6.44 (*d*, J = 16.3), 6.12 (*d*, J = 16.3), and 5.82 (*s*)), three MeO groups (δ(H) 3.91, 3.76, and 3.63 (3*s*)), and an O-bearing CH group (δ(H) 5.24 (*d*, J = 5.4)). Seven out of the ten degrees of unsaturation were due to four CO groups and three C=C bonds; the remaining three degrees of unsaturation, thus, had to be accounted for by three rings. The data mentioned above disclosed **1** as a clerodane-type diterpene, containing no furane subunit.

The ^{1}H- and ^{13}C-NMR and HMQC data of **1** allowed the assignment of all H-atoms to the connected C-atoms. The scaffold of **1** was constructed by a HMBC experiment, which allowed the assemblage of all C-atoms, including quaternary C-atoms and hetero atoms (*Fig. 1*). The HMBC correlation H–C(1)/C(18) indicated that C(1) and C(18) were linked by an O-atom. The δ(H) of Me(19) correlated with the δ(C) at 37.0 (C(5)) and 80.0 (C(4)) revealing the presence of an OH at C(4). The quaternary-C-atom signal at δ(C) 175.0 was allocated to C(17) by the strong correlations between C(17) and H–C(8) (δ(H) 2.58 (*t*, J = 8.5)). The assemblage of C(13) and C(12) was established by the HMBC correlations H–C(14)/C(13) and H–C(14)/C(12). The key cross-peaks H–C(14)/C(15) and H–C(14)/C(16) allowed to identify the linkages C(14)–C(15) and C(13)–C(16), respectively. Thus, the constitutional formula of **1** was established.

The relative configuration of **1** was deduced by a NOESY analysis (*Fig. 2*). An NOE correlation between Me(20) and H–C(8) indicated that these were on the same face of the molecule, and were tentatively assumed as β-oriented; therefore the ester

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 500 and 125 MHz, resp.) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.20 (*d*, *J* = 4.5)	75.2	5.26 (*d*, *J* = 4.3)	74.9
H–C(2)	6.42 (*dd*, *J* = 7.7, 5.0)	129.5	6.42 (*dd*, *J* = 7.7, 5.2)	129.6
H–C(3)	6.32 (*dd*, *J* = 7.7, 1.2)	136.8	6.33 (*d*, *J* = 7.7)	136.8
C(4)	–	80.0	–	79.8
C(5)	–	37.0	–	36.8
CH_2(6)	1.62–1.65 (*m*, H_α), 1.55–1.60 (*m*, H_β)	27.5	1.64–1.68 (*m*, H_α), 1.54–1.60 (*m*, H_β)	28.1
CH_2(7)	1.85–1.92 (2*m*)	20.6	1.84–1.92 (2*m*)	20.9
H–C(8)	2.58 (*t*, *J* = 8.5)	47.0	2.63 (*t*, *J* = 8.0)	46.7
C(9)	–	40.6	–	40.6
H–C(10)	1.82 (*s*)	52.6	1.78 (*s*)	53.7
H–C(11)	6.44 (*d*, *J* = 16.3)	147.9	7.04 (*d*, *J* = 16.4)	149.8
H–C(12)	6.12 (*d*, *J* = 16.3)	125.2	6.29 (*d*, *J* = 16.4)	118.8
C(13)	–	147.7	–	159.1
H–C(14)	5.82 (*s*)	118.6	5.91 (*s*)	117.4
C(15)	–	165.4	–	170.4
C(16)	–	167.6	5.96 (*s*)	102.7
C(17)	–	175.0	–	175.4
C(18)	–	176.2	–	176.1
Me(19)	1.11 (*s*)	25.7	1.09 (*s*)	25.5
Me(20)	1.35 (*s*)	25.9	1.39 (*s*)	26.4
*Me*O–C(15)	3.91 (*s*)	52.5		
*Me*O–C(16)	3.76 (*s*)	52.0	3.57 (*s*)	56.0
*Me*O–C(17)	3.63 (*s*)	51.7	3.64 (*s*)	51.7

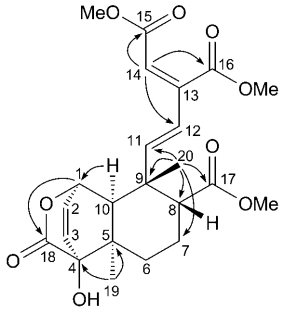

Fig. 1. *Selected HMBC* (H → C) *correlations of* **1**

group of C(17) must be α-oriented. The correlation H–C(8)/H_β–C(6) indicated that H_β–C(6) was also β-configured. The NOE correlations H_α–C(6)/Me(19), Me(19)/H–C(10), and H–C(10)/H–C(1) indicated that H–C(1), H–C(10), and Me(19) are on the α-face. 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 tinocapilactone A[1]) (**1**) was established.

Tinocapilactone B (**2**) had the molecular formula $C_{22}H_{26}O_8$ based on HR-ESI-MS analysis (*m*/*z* 419.1710 ($[M+H]^+$)). The compound was also obtained as colorless, amorphous powder. Its NMR spectra showed similarity with those of **1**, except for the

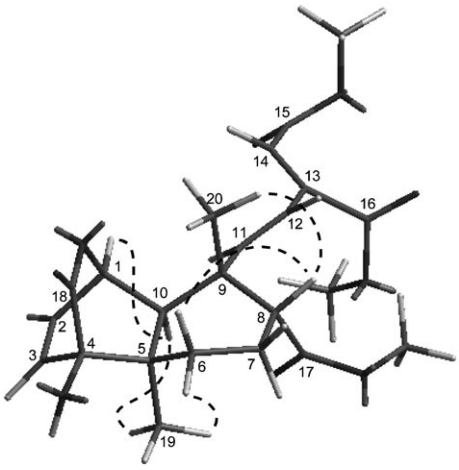

Fig. 2. *Key NOESY correlations and relative configuration of* **1**

signals of C(11) to C(16), which suggested that **2** possesses the same core (clerodane-type diterpene with δ-lactone) as **1** but differs in the side chain. The structure of this side chain was determined to be an ethenyl-linked α,β-unsaturated lactone moiety with a MeO group at C(16) by comparison of the NMR data of **2** with those of yunnancoronarin C [12]; this was confirmed by HMBC data (*Fig. 3*). The interpretation of the ^{1}H-NMR and NOESY data revealed that **2** has the same relative configuration as **1**. Thus, the structure of tinocapilactone B[1]) (**2**) was established, except for the relative configuration at C(16).

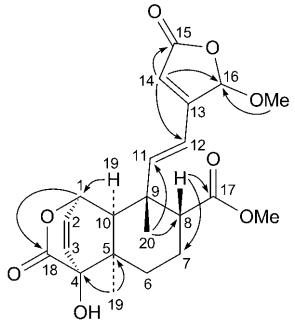

Fig. 3. *Selected HMBC* (H → C) *correlations of* **2**

The present study showed that the major constituent, tinocapilactone A (**1**), with significant anti-inflammatory activities may be the bioactive compound of *T. capillipes* [13]. The study also revealed that the constituents of this plant are similar to those of *T. saggitta* (especially concerning the major constituents, such as compounds **3**, **4**, and **8**) [14], which can explain why these two species are used for the same treatments in the traditional folk medicine in China.

Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*). TLC: precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): silica gel (SiO_2; 230–400 mesh), SiO_2 *H-60*, and *MCI-CHP20P* gel (75–150 μ; *Mitsubishi Chemical Industries Ltd.*). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-2450* spectrometer; λ_{max} (log ε). IR Spectra: *Thermo Nicolet-6700* spectrophotometer; in cm^{-1}. NMR spectra: *Bruker AM-500* apparatus; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Agilent 6210-Lc/Tof* mass spectrometer; in *m/z*.

Plant Material. The plant material used for this study was collected from Guangxi Province, P. R. China, in August 2007, and identified by Prof. *Yong-Hong Zhang* of the Fujian Medical University, P. R. China. A voucher specimen (No. 20070823T) was deposited with the Zhejiang University of Technology.

Extraction and Isolation. The air-dried roots of *T. capillipes* (3.2 kg) were coarsely powdered and percolated with 95% EtOH/H_2O. After solvent removal, the crude extract (252 g) was suspended in H_2O (3 l) and extracted with AcOEt (5 × 500 ml) to afford the AcOEt-soluble fraction. The AcOEt-soluble fraction (72 g) was subjected to CC (SiO_2, petroleum ether/acetone 4:1 → 0:1, then MeOH): to give three major fractions, *Frs. 1* (43 g), *2* (3.0 g), and *3* (10.1 g). Compound **3** (30 g) was purified by recrystallization of *Fr. 1* from petroleum ether/acetone 2:1. The filtrate was evaporated to give a yellow residue, which was further purified by CC (SiO_2, petroleum ether/AcOEt 4:1 → 2:1): **1** (1.6 mg), **2** (6.0 mg), and **5** (50 mg). *Fr. 2* was also separated by CC (SiO_2, $CHCl_3$/i-PrOH 20:1 → 10:1): **6** (230 mg) and **7** (34 mg). *Fr. 3* was subjected to CC (*MCI CHP20P* gel, MeOH/H_2O 0:10 → 4:6): **8** (2.1 g) and **4** (3.2 g).

Tinocapilactone A (= rel-*(2Z)-2-{(1E)-2-[1R,4R,4aR,7R,8S,8aS)-3,4,4a,5,6,7,8,8a-Octahydro-4-hydroxy-7-(methoxycarbonyl)-4a,8-dimethyl-3-oxo-1,4-etheno-1*H*-2-benzopyran-8-yl]ethenyl}but-2-enedioic Acid Dimethyl Ester*; **1**): Colorless amorphous powder. UV ($CHCl_3$): 264 (4.09). $[\alpha]_D^{20} = +34.9$ (c = 0.2, $CHCl_3$). IR (KBr): 3482 (OH), 2954, 1746 (C=O), 1716 (C=O), 1437, 1375, 1268, 1205, 1154, 927, 751. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 449 ($[M+H]^+$). HR-ESI-MS: 449.1815 ($[M+H]^+$, $C_{23}H_{29}O_9^+$; calc. 449.1812).

Tinocapilactone B (= rel-*(1R,4R,4aR,7R,8S,8aS)-8-[(1E)-2-(2,5-Dihydro-2-methoxy-5-oxofuran-3-yl)ethenyl]-3,4,4a,5,6,7,8,8a-octahydro-4-hydroxy-4a,8-dimethyl-3-oxo-1,4-etheno-1*H*-2-benzopyran-7-carboxylic Acid Methyl Ester*; **2**): Colorless amorphous powder. UV ($CHCl_3$): 262 (4.11). $[\alpha]_D^{20} = +57.3$ (c = 0.6, $CHCl_3$). IR (KBr): 3466 (OH), 2922, 2851, 1761 (C=O), 1728 (C=O), 1464, 1374, 1154, 972, 723. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 419 ($[M+H]^+$). HR-ESI-MS: 419.1710 ($[M+H]^+$, $C_{22}H_{27}O_8^+$; calc. 419.1706).

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