Polyynes from *Toona ciliata* var. *ciliata* and Related Cytotoxic Activity

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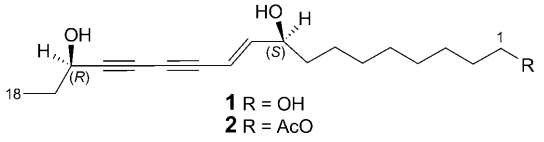
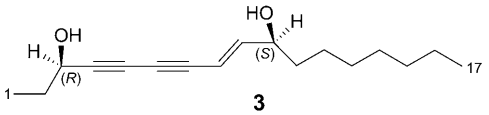
A phytochemical investigation of *Toona ciliata* var. *ciliata* afforded three new polyynes, **1**–**3**. Their structures were elucidated on the basis of spectroscopic analysis and chemical methods. Only compound **3** exhibited potent cytotoxicity against the HL-60 cell line with an IC_{50} value of 6.7 ± 0.27 μM.

Introduction. – *Toona ciliata* var. *ciliata* (Meliaceae), a good timber tree, is widely distributed in the south of China, such as Yunnan, Sichuan, Guangdong, Hainan provinces [1]. Previous chemical investigations on *Toona ciliata* and its varieties have led to the isolation of a series of bioactive compounds, especially limonoids [2]. In the course of our search for structurally unique and potentially bioactive natural products from the Meliaceae family, three new polyynes, (9*S*,10*E*,16*R*)-octadec-10-ene-12,14-diyne-1,9,16-triol (**1**), (9*S*,10*E*,16*R*)-9,16-dihydroxyoctadec-10-ene-12,14-diyn-1-yl acetate (**2**), and (3*R*,8*E*,10*S*)-heptadec-8-ene-4,6-diyne-3,10-diol (**3**), were isolated from the leaves of *Toona ciliata* var. *ciliata*. Here, we report the isolation and structure elucidation of the new compounds, and their cytotoxicity.



1 R = OH
2 R = AcO



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Results and Discussion. – The AcOEt extract of the leaves of *Toona ciliata* var. *ciliata* was subjected to SiO_2 and *Sephadex LH-20* column chromatography, as well as semi-preparative HPLC to afford three new compounds, **1**–**3**.

Compound **1** was obtained as a colorless oil. The molecular formula was determined as $C_{18}H_{28}O_3$ by a *pseudo*-molecular-ion peak in the HR-ESI-MS (*m*/*z* 315.1935 ($[M + Na]^+$; calc. 315.1936)). The IR absorption band at 3424 cm^{-1} implied the presence of an OH group. Its UV spectrum exhibited absorption maxima at 215, 241, 254, 268, and

284 nm, suggesting a typical ene-diyne system [3]. Obvious signals in the ^{1}H-NMR spectrum (*Table 1*) were those of two olefinic H-atoms (δ(H) 6.31 (*dd*, J = 6.4, 16.0, H–C(10)); 5.75 (*d*, J = 16.0, H–C(11)), two O-bearing CH groups (δ(H) 4.20 (*dd*, J = 6.4, 12.4, H–C(9)); 4.42 (*t*, J = 9.2, H–C(16)), one O-bearing CH_2 group (δ(H) 3.64 (*t*, J = 6.4, CH_2(1)), and one terminal Me group (δ(H) 1.01 (*t*, J = 3.6, Me(18)). The ^{13}C-NMR (*Table 2*) and DEPT spectra further showed signals for four quaternary acetylenic C-atoms (δ(C) 77.3 (*s*, C(12)), 73.7 (*s*, C(13)), 69.5 (*s*, C(14)), 83.0 (*s*, C(15))), as well as for eight non-O-bearing CH_2 groups. Detailed information on the structure was provided by HMBC and ^{1}H,^{1}H-COSY data (*Fig. 1*). A detailed analysis of the ^{1}H,^{1}H-COSY spectrum of **1** established the three fragments **1a** (C(1)–C(2)), **1b** (C(8) to C(11)) and **1c** (C(16) to C(18)). Further HMBCs of H–C(11) and H–C(16) to C(13) established the connection between fragments **1b** with **1c** *via* the conjugated diynes, and the connection from **1a** to **1b** *via* five CH_2 groups was deduced by the observed HMBCs of H–C(8) and H–C(2) to the relative CH_2 signals. Thus, **1** was determined as octadec-10-ene-12,14-diyne-1,9,16-triol. The C(10)=C(11) bond was assigned the (*E*)-configuration on the basis of the large vicinal coupling constant (J(10,11) = 16.0 Hz).

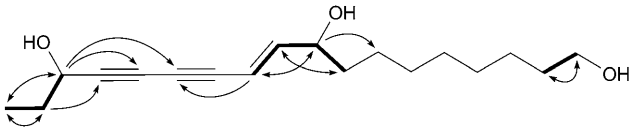


Fig. 1. *Key ^{1}H,^{1}H-COSY correlations* (—) *and HMBCs* (→) *of* **1**

Table 1. *^{1}H-NMR Data of Compounds* **1** (400 MHz), **2** (500 MHz), *and* **3** (400 MHz). Measured in $CDCl_3$; δ in ppm

	1	**2**	**3**
CH_2(1) or Me(1)	3.64 (*t*, J = 6.4)	4.05 (*t*, J = 6.8)	1.01 (*t*, J = 7.2)
CH_2(2)	1.54–1.58 (*m*)	1.60 (*dd*, J = 6.8, 13.5)	1.72–1.76 (*m*)
CH_2(3) or H–C(3)	1.30 (br. *s*)	1.30 (br. *s*)	4.42 (*t*, J = 6.8)
CH_2(4)	1.30 (br. *s*)	1.30 (br. *s*)	
CH_2(5)	1.30 (br. *s*)	1.30 (br. *s*)	
CH_2(6)	1.30 (br. *s*)	1.30 (br. *s*)	
CH_2(7)	1.30 (br. *s*)	1.30 (br. *s*)	
CH_2(8) or H–C(8)	1.51–1.55 (*m*)	1.53 (*t*, J = 6.5)	5.75 (*d*, J = 16.0)
H–C(9)	4.20 (*dd*, J = 6.4, 12.4)	4.19 (*dd*, J = 6.5, 12.0)	6.31 (*dd*, J = 16.0, 5.6)
H–C(10)	6.31 (*dd*, J = 6.4, 16.0)	6.31 (*dd*, J = 6.5, 16.0)	4.17 (*ddd*, J = 12.8, 5.6, 1.6)
H–C(11) or CH_2(11)	5.75 (*d*, J = 16.0)	5.76 (*d*, J = 16.0)	1.51–1.55 (*m*)
CH_2(12)			1.26 (br. *s*)
CH_2(13)			1.26 (br. *s*)
CH_2(14)			1.26 (br. *s*)
CH_2(15)			1.26 (br. *s*)
H–C(16) or CH_2(16)	4.42 (*t*, J = 9.2)	4.42 (*t*, J = 9.2)	1.26 (br. *s*)
CH_2(17) or Me(17)	1.73–1.77 (*m*)	1.74–1.78 (*m*)	0.87 (*t*, J = 6.4)
Me(18)	1.01 (*t*, J = 3.6)	1.01 (*t*, J = 3.8)	
AcO		2.05 (*s*)	

[a]) Assignments may be interchanged.

Table 2. *^{13}C-NMR Data of Compounds* **1**–**3**. At 100 MHz, δ in $CDCl_3$, in ppm.

C-Atom	**1**	**2**	**3**
1	63.0 (*t*)	64.6 (*t*)	9.4 (*q*)
2	32.6 (*t*)	28.5 (*t*)	30.6 (*t*)
3	29.4 (*t*)[a])	25.8 (*t*)[a])	64.1 (*d*)
4	29.3 (*t*)[a])	29.1 (*t*)[a])	83.0 (*s*)
5	29.2 (*t*)[a])	29.3 (*t*)[a])	69.5 (*s*)
6	25.6 (*t*)[a])	29.3 (*t*)[a])	73.7 (*s*)
7	25.1 (*t*)	25.1 (*t*)	77.3 (*s*)
8	36.8 (*t*)	36.8 (*t*)	108.1 (*d*)
9	72.0 (*d*)	72.0 (*d*)	149.6 (*d*)
10	149.6 (*d*)	149.6 (*d*)	72.1 (*d*)
11	108.1 (*d*)	108.1 (*d*)	36.8 (*t*)
12	77.3 (*s*)	77.3 (*s*)	25.2 (*t*)
13	73.7 (*s*)	73.7 (*s*)	29.4 (*t*)[a])
14	69.5 (*s*)	69.5 (*s*)	29.2 (*t*)[a])
15	83.0 (*s*)	82.9 (*s*)	31.8 (*t*)
16	64.1 (*d*)	64.1 (*d*)	22.6 (*t*)
17	30.6 (*t*)	30.6 (*t*)	14.1 (*q*)
18	9.3 (*q*)	9.3 (*q*)	
AcO		21.0 (*q*), 171.3 (*s*)	

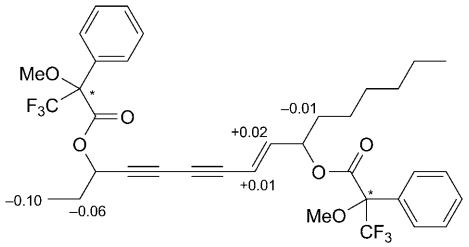
[a]) Assignments may be interchanged.

Compound **2** was isolated as a colorless oil. The molecular formula was established as $C_{20}H_{30}O_4$ deduced by the *pseudo*-molecular-ion peak in the HR-ESI-MS (*m*/*z* 357.2041 ($[M+Na]^+$); calc. 357.2041). The IR spectrum showed absorptions for OH (3419 cm^{-1}) and CO (1718 cm^{-1}) groups. The UV spectrum and 1D-NMR data were quite similar to those of **1**, except for the occurrence of an additional AcO group (δ(H) 2.05 (*s*); δ(C) 21.0 (*q*) and 171.3 (*s*)) in **2**. The downfield shift of CH_2(1) (δ(H) 4.05 (*t*, $J=6.8$)) indicated acylation of the OH group at C(1), which was identified by the HMBC of H–C(1) to ester CO (δ(C) 171.3 (*s*)). Further 2D-NMR (HSQC, HMBC, and ^{1}H,^{1}H-COSY) data confirmed the structure of **2** as 9,16-dihydroxyoctadec-10-ene-12,14-diyn-1-yl acetate. The (*E*)-configuration of the C(10)=C(11) bond was also deduced from the *J*(10,11) value (16.0 Hz).

Compound **3** was obtained as a colorless oil. The molecular formula was deduced as $C_{17}H_{26}O_2$ by a *pseudo*-molecular-ion peak in the HR-ESI-MS (*m*/*z* 285.1830 $[M+Na]^+$; calc. 285.1830). Comparison of its spectroscopic data with those of **1** and **2** revealed an overall similarity, except for the presence of an additional terminal Me group (δ(H) 1.01 (*t*, $J=7.2$)), and the absence of two CH_2 groups (one O-bearing) in **3**. Extensive 2D-NMR (HSQC, HMBC, and ^{1}H,^{1}H-COSY) data identified the planar structure of **3** as (*E*)-heptadec-8-en-4,6-diyne-3,10-diol [4]. However, the obvious different chemical shift of C(2) (δ(C) 30.6 (*t*)) in **3** indicated that **3** was an epimer of the latter, with the only difference being the different absolute configuration at C(3). The *Mosher*'s method was applied for determining its absolute configuration [5].

To this end, **3** was treated with (−)-(*R*)- and (+)-(*S*)-MTPA (=α-methoxy-α-(trifluoromethyl)phenylacetic acid) chloride to give (*S*)- and (*R*)-MTPA diesters of **3**,

3a and **3b**, respectively. ^{1}H,^{1}H-COSY Data were used for the assignment of H-atom signals of **3a** and **3b**. Analysis of the chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) of the H-atoms neighboring the O-bearing CH groups according to the *Mosher* model allowed the assignment of the (*R*)- and (*S*)-configuration at C(3) and C(10) [5], respectively (*Fig. 2*). Accordingly, compound **3** reported here was a new compound, (3*R*,8*E*,10*S*)-heptadec-8-ene-4,6-diyne-3,10-diol, while the compound reported previously should be (3*S*,8*E*,10*R*)-heptadec-8-ene-4,6-diyne-3,10-diol [4].



3a (*S*)-MTPA ester
3b (*R*)-MTPA ester

Fig. 2. *Application of the modified* Mosher*'s method for secondary alcohols on the MTPA esters of* **3** (**3a** and **3b**). $\Delta\delta$ ($\delta_S - \delta_R$) are given in ppm.

Due to the little amount of **1** and **2**, the absolute configuration of these two compounds could not be determined directly by *Mosher*'s method, but considering that compounds **1** and **2** were isolated from the same extract, and their chemical shifts and optical rotations were quite similar to those of **3**. Thus, the absolute configurations of **1** and **2** are assumed to be the same as **3**.

Compounds **1**–**3** were tested for *in vitro* inhibitory activities against HL-60, SMMC-7721, A549, SK-BR-3, and PANC-1 human tumor cell lines (details are available as *Supplementary Material*[1])), using DDP (*cis*-diammineplatinum(II) dichloride) as a positive control. Significant cytotoxicity was only observed for compound **3** against the HL-60 cells with an IC_{50} value of 6.7 ± 0.27 μM.

Polyacetylenes are uncommon in Meliaceae. To our knowledge, they had been found within this plant family only in *Swietenia mahagoni* [6]. In this context, an endophytic origin of the compounds cannot be excluded.

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Experimental Part

General. CC: silica gel *H* (SiO_2, 10–40 μm; *Qingdao Marine Chemical Ltd. Co.*); *MCI* gel *CHP 20P* (75–150 μm, *Mitsubishi Chemical Industries Ltd.*); *Sephadex LH-20* (40–70 μm, *Pharmacia*), or *RP-18* gel (40–63 μm, *Merck*). TLC: silica-gel plates (size: 50 × 100 mm, thickness: 0.20–0.25 mm, *Qingdao Marine Chemical Ltd. Co.*), detection by UV illumination and spraying with 10% H_2SO_4 in EtOH, followed by heating. HPLC: *Zorbax SB-C-18* column (i.d. 9.4 × 250 mm; *Agilent Co. Ltd.*). Optical rotations: *Perkin–Elmer* model *241* polarimeter. UV Spectra: *Shimadzu UV-2401* spectrophotometer. IR

[1]) *Supplementary Material* may be obtained upon request from the authors.

Spectra: *Bio-Rad FTS-135* spectrometer, with KBr pellets. 1D- and 2D-NMR spectra: *Bruker AM-400* (400 and 100 MHz, resp.) or *DRX-500* (500 and 125 MHz, resp.) instrument with TMS as an internal standard. ESI-MS: *Finnigan MAT 90* instrument. HR-ESI-MS: *API Qstar Pulsar LC/TOF* instrument.

Plant Material. The leaves of *Toona ciliata* var. *ciliata* were collected from the area of Gaoligongshan, Yunnan Province, P. R. China, in July 2008, and were identified by Prof. *H. Li* (Kunming Institute of Botany, Chinese Academy Sciences). A voucher specimen (KUN No. 080426) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

Extraction and Isolation. The air-dried and powdered leaves of the plant (3.5 kg) were extracted with EtOH 95% (3 × 6 l, 5 h each). The extracts were then suspended in H_2O and further extracted with petroleum ether (PE; 3 × 2 l) and AcOEt (4 × 3 l). The AcOEt extracts (92 g) were first subjected to CC (SiO_2; gradient of PE/acetone 10:0, 8:1, 7:3, and 6:4) to afford *Fractions 1–10*. *Fr. 3* (6.8 g) was first subjected to CC (*MCI* gel; gradient of MeOH/H_2O (60:40 to 100:0 (*v*/*v*)) to afford eight fractions, *Fr. A1–A8*. *Fr. A4* (654 mg) was subjected to CC (*Sephadex LH-20*; acetone 100%) to afford *Fr. B1–B3*. *Fr. B1* (32 mg) was further purified by HPLC (MeOH/H_2O 70:30; flow rate: 3.0 ml/min; detection: UV 254, 230, 210 nm; t_R: 9 min (**2**), 14 min (**1**)) at 30°, yielding compounds **1** (8 mg) and **2** (6 mg). *Fr. B2* (60 mg) was subjected to CC (SiO_2; gradient of $CHCl_3$/acetone 100:1) to afford **3** (20 mg).

*(9*S*,10*E*,16*R*)-Octadec-10-ene-12,14-diyne-1,9,16-triol* (**1**). Colorless oil. $[\alpha]_D^{25} = -25.8$ ($c = 0.070$, MeOH). UV (MeOH): 208 (1.5), 215 (1.8), 228 (0.2), 241 (0.3), 254 (0.5), 268 (0.7), 284 (0.6). IR (KBr): 3425, 2931, 2856, 1629. ^{1}H- and ^{13}C-NMR: see *Table 1* and *2*, resp. ESI-MS: 583 ($[2M-1]^-$). HR-ESI-MS: 315.1935 ($[M+Na]^+$, $C_{18}H_{28}NaO_3^+$; calc. 315.1936).

*(9*S*,10*E*,16*R*)-9,16-Dihydroxyoctadec-10-ene-12,14-diyn-1-yl Acetate* (**2**). Colorless oil. $[\alpha]_D^{25} = -12.8$ ($c = 0.2$, MeOH). UV (MeOH): 208 (1.4), 215 (1.8), 228 (0.2), 241 (0.3), 254 (0.5), 268 (0.7), 284 (0.6). IR (KBr): 3419, 2932, 2857, 1738, 1718, 1248, 1048. ^{1}H- and ^{13}C-NMR: see *Table 1* and *2*, resp. ESI-MS: 357 ($[M+Na]^+$). HR-ESI-MS: 357.2050 ($[M+Na]^+$, $C_{20}H_{30}NaO_4^+$; calc. 357.2041).

*(3*R*,8*E*,10*S*)-Heptadec-8-ene-4,6-diyne-3,10-diol* (**3**). Colorless oil. $[\alpha]_D^{25} = -10.9$ ($c = 0.2$, MeOH). UV (MeOH): 208 (1.9), 215 (2.4), 229 (0.2), 241 (0.4), 254 (0.7), 268 (1.0), 284 (0.8). IR (KBr): 3405, 2929, 2857, 1016, 956. ^{1}H- and ^{13}C-NMR: see *Table 1* and *2*, resp. ESI-MS: 285 ($[M+Na]^+$). HR-ESI-MS: 285.1830 ($[M+Na]^+$, $C_{17}H_{26}NaO_2^+$; calc. 285.1830).

Determination of the Absolute Configuration of Compound **3** *by the* Mosher*'s Method.* Compound **3** (2.0 mg) was dissolved in 250 µl of dry pyridine and treated with 4-(dimethylamino)pyridine (DMAP; a spatula tip) and (−)-(*R*)-MTPA (= α-methoxy-α-(trifluoromethyl)phenylacetic acid) chloride (10 µl). The mixture was stirred at r.t. for 1 h. After removal of the solvent, the mixture was purified by CC (*RP-18*; acetone/H_2O 50:50) to afford the (*S*)-MTPA diester **3a** (4.6 mg). The same procedure afforded the (*R*)-MTPA diester **3b** (5.1 mg).

*(3*R*,8*E*,10*S*)-Heptadec-8-ene-4,6-diyne-3,10-diyl (2*S*,2′S)-Bis[3,3,3-trifluoro-2-methoxy-2-phenylpropanoate]* (**3a**). Colorless oil. ^{1}H-NMR (500 Hz, $CDCl_3$): 7.53, 7.49, 7.41 (MTPA H-atoms); 6.20 (*ddd*, $J = 39.0, 16.0, 7.5$, H–C(9)); 5.72 (*dd*, $J = 62.5, 16.0$, H–C(8)); 5.59 (*t*, $J = 6.5$ H–C(3)); 5.48 (*t*, $J = 7.5$, H–C(10)); 3.58, 3.53 (MTPA MeO, overlapped); 1.83–1.87 (*m*, CH_2(2)); 1.64–1.72 (*m*, CH_2(11)); 1.28 (*d*, $J = 6.6$, H_a–C(12)); 1.25 (H_b–C(12), overlapped); 1.25 (CH_2(13), overlapped); 1.20 (CH_2(14), overlapped); 1.20 (CH_2(15), overlapped); 1.20 (CH_2(16), overlapped); 0.94 (*t*, $J = 7.0$, Me(1)); 0.87 (*t*, $J = 3.0$, Me(17)). The assignments of CH_2(13), CH_2(14), CH_2(15), and CH_2(16) may be interchanged.

*(3*R*,8*E*,10*S*)-Heptadec-8-ene-4,6-diyne-3,10-diyl (2*R*,2′R)-Bis[3,3,3-trifluoro-2-methoxy-2-phenylpropanoate]* (**3b**). Colorless oil. ^{1}H-NMR (500 Hz, $CDCl_3$): 7.53, 7.49, 7.41 (MTPA H-atoms); 6.18 (*ddd*, $J = 39.0, 16.0, 7.0$, H–C(9)); 5.71 (*dd*, $J = 62.5, 16.0$, H–C(8)); 5.55 (*t*, $J = 6.5$, H–C(3)); 5.48 (*t*, $J = 7.0$, H–C(10)); 3.55 (MTPA MeO, overlapped); 1.89–1.93 (*m*, CH_2(2)); 1.64–1.74 (*m*, CH_2(11)); 1.28 (*d*, $J = 6.6$, H_a–C(12)); 1.25 (H_b–C(12), overlapped); 1.25 (CH_2(13), overlapped); 1.20 (CH_2(14), overlapped); 1.20 (CH_2(15), overlapped); 1.20 (CH_2(16), overlapped); 1.04 (*t*, $J = 7.0$, Me(1)); 0.87 (*t*, $J = 3.0$, Me(17)). The assignments of CH_2(13), CH_2(14), CH_2(15), and CH_2(16) may be interchanged.

Cytotoxicity Assays. IC_{50} Values of compounds **1**–**3** against HL-60, SMMC-7721, A549, SK-BR-3, and PANC-1 human tumor cell lines were determined by the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method [7]. Briefly, cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. The percentage

of viable cells was quantified at 595/630 nm with an ELISA reader. The cytotoxic concentration that caused the reduction of viable cells by 50% was determined from dose–response curve, and data were obtained from triplicate experiments.

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