Tirucallane-Type Triterpenoids from *Celastrus stylosus* WALL.

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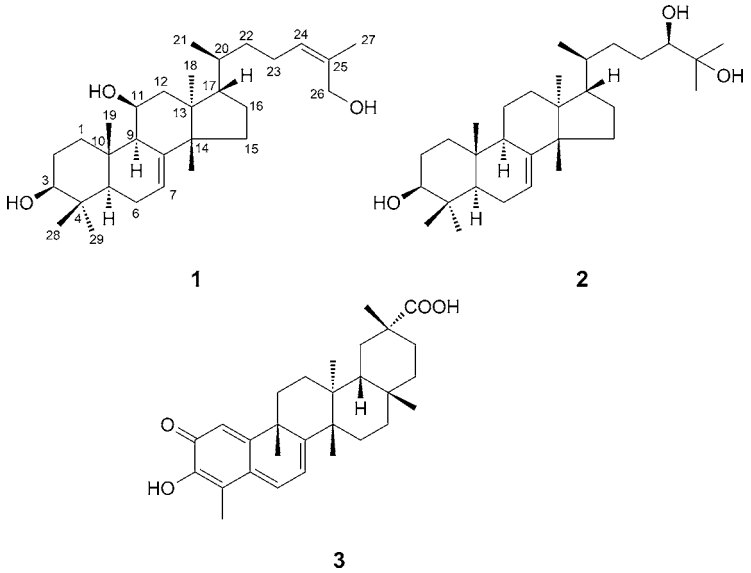
Two new tirucallane-type triterpenoids, (24*Z*)-tirucalla-7,24-diene-3β,11β,26-triol (**1**) and (3*S*,24*R*)-tirucall-7-ene-3,24,25-triol (**2**), along with the known compound celastrol (**3**), were isolated from the root barks of *Celastrus stylosus* WALL. The structures of **1**–**3** were established by spectroscopic methods, including extensive 2D NMR and MS analyses. It is the first report on tirucallane-type triterpenoids from the genus *Celastrus* and may be of vital chemotaxonomic significance.

Introduction. – The genus *Celastrus* comprises *ca*. 50 species throughout the world. They are widely distributed in Asia, especially in China [1]. Plants of this genus have been used as natural insecticides, and folk medicine to treat fever, chill, joint pain, edema, rheumatoid arthritis, and bacterial infection in China for a long time [2][3]. Studies on the chemical constituents of *Celastrus* in recent years have disclosed that sesquiterpenoids and triterpenoids are the important active components [4]. In the present work, we report the isolation and identification of two new tirucallane-type triterpenoids, (24*Z*)-tirucalla-7,24-diene-3β,11β,26-triol (**1**) and (3*S*,24*R*)-tirucall-7-ene-3,24,25-triol (**2**), along with the known compound celastrol (**3**), from the root barks of *C. stylosus* WALL.

Results and Discussion. – A $CHCl_3$ extract of the root bark of *C. stylosus* WALL. was subjected to extensive column chromatography to afford the two new tirucallane-type triterpenoids **1** and **2**, as well as the known metabolite **3** (*Fig. 1*).

Compound **1** was obtained as white amorphous powder with a molecular formula of $C_{30}H_{52}O_3$, as deduced from the $[M+H]^+$ peak at *m*/*z* 459.3826 in the HR-ESI-MS, corresponding to six degrees of unsaturation. The IR spectrum displayed a broad absorption band for OH groups (3359 cm^{-1}). The ^{13}C-NMR and DEPT spectra (*Table*) indicated that compound **1** possesses a C_{30}-triterpenoid structure. The olefinic signals at δ(C) 142.9 (C(8)), 134.0 (C(25)), 128.9 (C(24)), and 119.8 (C(7)) were ascribed to two C=C bonds. The ^{1}H-NMR spectrum showed one Me *doublet* at δ(H) 0.90 (*d*, *J* =



1 2 3

Fig. 1. *Structures of compounds* **1**–**3**

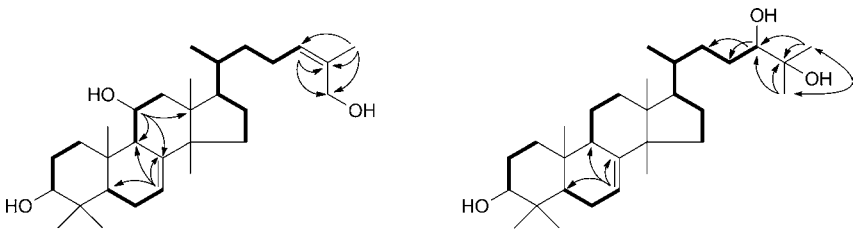
6.5, Me(21)), six Me *singlet*s at δ(H) 1.79 (*s*, Me(27)), 0.97 (*s*, Me(29)), 0.89 (*s*, Me(18)), 0.87 (*s*, Me(30)), 0.86 (*s*, Me(28)), and 0.85 (*s*, Me(19)), signals of a CH_2OH group at δ(H) 4.13 (*s*, CH_2(26)), two olefinic signals at δ(H) 5.31 (*m*, H–C(7)) and 5.28 (*dd*, $J = 7.5, 7.5$, H–C(24)), and two CH–O groups at δ(H) 4.17 (*ddd*, $J = 14.0, 9.5, 4.5$, H–C(11)) and 3.26 (*dd*, $J = 11.0, 4.5$, H–C(3)), corresponding to the O-bearing C-atom signals at δ(C) 67.4 (C(11)) and 78.8 (C(3)), respectively. These data of **1** provided evidence for a tirucallane or euphane system with two C=C bonds and three OH groups [5][6]. A detailed comparison of the NMR data of **1** with those reported in literature suggested that **1** should be a hydroxylated analog of the known compound (24*Z*)-tirucalla-7,24-diene-3β,27-diol [7], which was verified by elucidating the 2D-NMR spectra. Specifically, ^{1}H,^{1}H-COSY plots (*Fig. 2*) of H–C(9) (δ(H) 2.22–2.38) and CH_2(11) (δ(H) 4.17), as well as HMBCs (*Fig. 2*) from H–C(11) (δ(H) 4.17) to C(8), C(9), and C(13) indicated that the additional OH group was at C(11). As to the relative configuration at C(11), an NOE correlation H–C(11)/Me(18) indicated the α-orientation of H–C(11). Compound **1** was thus identified as (24*Z*)-tirucalla-7,24-diene-3β,11β,26-triol.

Compound **2** was obtained as white amorphous powder and assigned the molecular formula $C_{30}H_{52}O_3$ according to the $[M+H]^+$ peak at *m*/*z* 461.3985 in the HR-ESI-MS, implying five degrees of unsaturation. The NMR data of **2** showed similarities with those of **1**. However, compound **2** did not exhibit signals for a C=C bond in the side chain as in the case of **1**. Instead, it possessed an additional CH–O group and quaternary C-atom (δ(C) 79.5 and 73.2, resp.). This implied that **2** possesses a side chain containing two OH groups at the expense of the C(24)=C(25) bond in compound **1**. The two *singlet*s assignable to Me(26) and Me(27) were shifted downfield (δ(H) 1.16 and 1.22), suggesting that one of the OH groups in the side chain is located at C(25)

Table. *NMR Data of Compounds* **1** *and* **2**. In $CDCl_3$; δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1a	2.00–2.07 (*m*)	38.3	1.61–1.73 (*m*),	37.2
1b	1.43–1.50 (*m*)		1.07–1.19 (*m*)	
2a	1.61–1.70 (*m*)	27.7	1.54–1.71 (*m*)	27.6
2b	1.58–1.62 (*m*)			
3	3.26 (*dd*, *J* = 11.0, 4.5)	78.8	3.24 (*dd*, *J* = 11.5, 4.0)	79.2
4	–	39.3	–	39.0
5	1.35–1.41 (*m*)	50.5	1.27–1.34 (*m*)	50.6
6a	2.15–2.21 (*m*)	24.1	2.09–2.20 (*m*),	23.9
6b	1.95–2.01 (*m*)		1.90–2.02 (*m*)	
7	5.31 (*m*)	119.8	5.26 (*m*)	117.9
8	–	142.9	–	145.8
9	2.22–2.28 (*m*)	56.7	2.14–2.26 (*m*)	48.9
10	–	36.6	–	34.9
11	4.17 (*ddd*, *J* = 14.0, 9.5, 4.5)	67.4	1.45–1.56 (*m*)	18.1
12a	2.33–2.41 (*m*)	48.0	1.78–1.86 (*m*)	33.7
12b	1.46–1.51 (*m*)		1.60–1.79 (*m*)	
13	–	44.2	–	43.6
14	–	51.0	–	51.3
15a	1.49–1.57 (*m*)	34.1	1.48–1.57 (*m*)	33.9
15b	1.44–1.51 (*m*)		1.38–1.52 (*m*)	
16a	1.90–1.98 (*m*)	28.0	1.89–1.99 (*m*)	28.4
16b	1.26–1.32 (*m*)		1.24–1.33 (*m*)	
17	1.41–1.59 (*m*)	52.5	1.49–1.54 (*m*)	53.0
18	0.89 (*s*)	20.9	0.83 (*s*)	22.1
19	0.85 (*s*)	14.2	0.75 (*s*)	13.1
20	1.33–1.42 (*m*)	35.8	1.36–1.54 (*m*)	36.4
21	0.90 (*d*, *J* = 6.5)	18.4	0.86 (*d*, *J* = 6.5)	18.8
22a	1.39–1.49 (*m*)	36.4	1.86–1.96 (*m*)	32.3
22b	1.01–1.09 (*m*)		0.92–1.01 (*m*)	
23a	2.08–2.13 (*m*)	24.5	1.55–1.64 (*m*)	29.0
23b	1.90–1.99 (*m*)		1.07–1.16 (*m*)	
24	5.28 (*dd*, *J* = 7.5, 7.5)	128.9	3.28 (*dd*, *J* = 9.5, 2.0)	79.5
25	–	134.0	–	73.2
26	4.13 (*s*)	61.5	1.16 (*s*)	23.2
27	1.79 (*s*)	21.2	1.22 (*s*)	26.6
28	0.86 (*s*)	14.7	0.86 (*s*)	14.7
29	0.97 (*s*)	27.6	0.97 (*s*)	27.3
30	0.87 (*s*)	27.4	0.87 (*s*)	27.7

[8][9]. This assumption was supported by the HMBC from the signals at δ(H) 1.16 and 1.22 to the resonances at δ(C) 73.2 and 79.5. The other OH group was determined to be located at C(24) by resorting to the HMBC from H–C(24) (δ(H) 3.28) to C(22), C(23), C(25), C(26), and C(27). These data established a constitutional formula tirucall-7-ene-3,24,25-triol for **2**. Search of the related literature provided one hit, *i.e.*, (3*S*,24*S*)-tirucall-7-ene-3,24,25-triol, whose ^{1}H-NMR data were consistent with those of **2**, except for the data ascribed to H–C(24). Specially, H–C(24) gave rise to a double



1 **2**

Fig. 2. *Selected* $^1H,^1H$*-COSY* (—) *and HMBC* (H → C) *features of* **1** *and* **2**

doublet at δ(H) 3.28 (*dd*, $J = 9.5, 2.0$) in **2**, while it resonated at δ(H) 3.36 as a *multiplet* in (3*S*,24*S*)-tirucall-7-ene-3,24,25-triol [10], suggesting that the two compounds most likely are stereoisomers at C(24). Similar deviations were also observed in a series of 3,4-secotirucallane triterpenoids possessing the same C_8 side chain and their corresponding C(24) stereoisomers, in which, the (24*R*)- and (24*S*)-isomers exhibited the H–C(24) resonances as double *doublet*s at δ(H) 3.28 and *triplet*s at δ(H) 3.34, respectively [9]. Hence, compound **2** was elucidated as (3*S*,24*R*)-tirucall-7-ene-3,24,25-triol.

The known compound was identified as celastrol (**3**) by comparing its spectroscopic data with those in the literature [11].

Studies on the chemical constituents of the genus *Celastrus* resulted in the discovery of many triterpenoids [4][12], though no tirucallane triterpenoid had been identified as yet from this genus. Compounds **1** and **2** represent the first two tirucallane triterpenoids occurring in the genus *Celastrus*. In addition, to the best of our knowledge, there has been no report concerning a chemical investigation of the species *Celastrus stylosus* before. The isolation and identification of compounds **1** and **2** may be of considerable significance for the chemotaxonomy of the species *Celastrus stylosus* and the genus *Celastrus*.

Experimental Part

General. All solvents used were of anal. grade and obtained from commercial sources. TLC: Precoated silica gel GF_{254} plates (*Qingdao Marine Chemical Inc.*, Qingdao, P. R. China); visualized with UV light and 10% H_2SO_4/EtOH. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China) and *ODS C-18* gel (50 μm; *YMC Co. Ltd.*, Kyoto, Japan). Optical rotations: *Rudolph Research Autopol III* automatic polarimeter. IR Spectra: *Thermo-Nicolet-6700 FT-IR* microscope instrument (FT-IR microscope transmission). NMR Spectra: *Bruker-AM-500* apparatus; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI- and HR-ESI-MS: *Agilent-6210-LC/TOF* mass spectrometer; in m/z.

Plant Material. Celastrus stylosus was collected in Hubei Province, P. R. China, in July 2010, and was identified by Prof. *Fa-Song Wang* of the Hubei University for Nationalities, P. R. China. A voucher specimen (No. HUN201007) was deposited with the Hubei University for Nationalities.

Extraction and Isolation. The shade-dried root barks of *C. stylosus* WALL. (9.6 kg) were coarsely powdered and extracted with 95% aq. MeOH (4×80 l). After solvent removal, the crude extract (1450 g) was suspended in H_2O (2 l) and extracted with $CHCl_3$ (5×2 l) to afford the $CHCl_3$-soluble fraction (652 g), which was subjected to CC (SiO_2; petroleum ether/AcOEt 10 : 1 → 1 : 2) to furnish three

main fractions *Frs. 1–3*. *Fr. 2* was purified by CC (*ODS C18*; MeOH/H_2O 1:1 → 9:1) to afford **3** (30 mg). *Fr. 3* was separated by CC (SiO_2; petroleum ether/acetone 5:1 → 1:2) to give four *Subfrs. 3A–3D*. *Fr. 3C* was subjected to CC (*ODS C18*; MeOH/H_2O 3:1 → 9:1) to afford **2** (20 mg) and **1** (15 mg).

(24Z)-Tirucalla-7,24-diene-3β,11β,26-triol (=*(3β,11β,13α,14β,17α,20S,24Z)-Lanosta-7,24-diene-3,11,26-triol*; **1**). White amorphous power. $[\alpha]_D^{20} = -39.9$ ($c = 0.53$, $CHCl_3$). IR: 3359, 2938, 1718, 1456, 1380, 1031. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 459 ($[M+H]^+$). HR-ESI-MS (pos.): 459.3826 ($[M+H]^+$, $C_{30}H_{51}O_3^+$; calc. 459.3833).

(3S,24R)-Tirucall-7-ene-3,24,25-triol (=*(3β,13α,14β,17α,20S,24R)-Lanost-7-ene-3,24,25-triol*; **2**). White amorphous powder. $[\alpha]_D^{20} = -28.9$ ($c = 0.45$, $CHCl_3$). IR: 3409, 2920, 2852, 1738, 1463, 1375, 1241, 1157, 1069, 1032. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 461 ($[M+H]^+$). HR-ESI-MS (pos.): 461.3985 ($[M+H]^+$, $C_{30}H_{53}O_3^+$; calc. 461.3989).

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