Taccalonolides W – Y, Three New Pentacyclic Steroids from *Tacca plantaginea*

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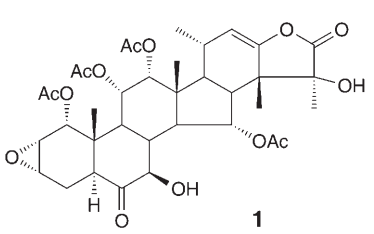
Three new pentacyclic steroids, taccalonolides W – Y (**2** – **4**, resp.), have been isolated from the whole plants of *Tacca plantaginea*. Their structures were elucidated on the basis of spectroscopic methods including extensive 1D- and 2D-NMR experiments.

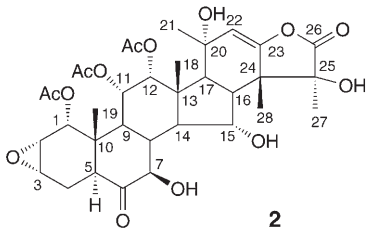
Introduction. – Plants of the genus *Tacca* are phenomenal resources of taccalonolide steroids, which possess a special pentacyclic steroidal skeleton, and some of which show antitumor activity [1][2]. Up to now, the 22 taccalonolides A – V have been isolated from *T. plantaginea*, *T. subflaellata*, and *T. paxiana* [1][3 – 9]. The rhizome of *Tacca plantaginea* has long been used in China as folk medicine for analgesic, antipyretic, anti-inflammatory, and incised wounds [10]. Previous chemical investigation of this plant led to the isolation of four new steroidal saponins, and five new withanolides, plantagiolides A – E [11 – 13], as well as 13 taccalonolides A – M [1][3 – 6]. As part of our continuing work to search for novel compounds, three new taccalonolides W – Y (**2** – **4**, resp.) were isolated from the species besides the known taccalonolide A. The isolation and structure elucidation of compounds **2** – **4** are the subject of this report.

Results and Discussion. – The $CHCl_3$-soluble part of the extract from the whole plants of *T. plantaginea* using 95% EtOH was subjected to repeated column chromatography on silica gel and semi-preparative HPLC to afford taccalonolides A, W, X, and Y (**1** – **4**).

Taccalonolide W (**2**) was obtained as a white powder. The molecular formula of **2** was deduced to be $C_{34}H_{44}O_{14}$ from HR-ESI-MS at *m*/*z* 699.2618 ($[M+Na]^+$; calc. 699.2629), indicating 13 degrees of unsaturation. The IR spectrum showed absorptions at 3420, 1818, 1745, and 1693 cm^{-1}, which implied the presence of OH groups, an enol γ-lactone, Ac groups, and C=C bonds, respectively. The ^{1}H-NMR spectrum of **2** (*Table 1*) exhibited characteristic resonances similar to those of taccalonolides, including five Me groups at δ(H) 0.83, 1.05, 1.35, 1.37, and 1.74, and an olefinic H-atom at 5.26. A detailed comparison of the ^{1}H- and ^{13}C-NMR and DEPT spectroscopic data (*Tables 1* and *2*) of **2** with those of taccalonolide B [1] revealed the absence of a *doublet* for Me(21) in the ^{1}H-NMR spectrum, and a missing CH signal for C(20) in the



1



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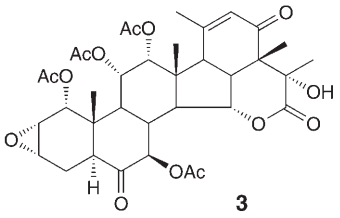

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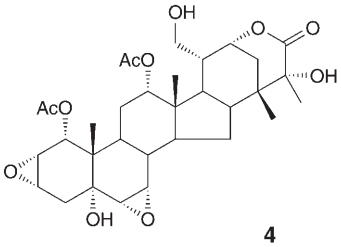

4

^{13}C-NMR spectrum, but instead the presence of a Me *singlet* at δ(H) 1.35 for Me(21) in the ^{1}H-NMR spectrum, and a quaternary oxygenated C-atom at δ(C) 72.7 in the ^{13}C-NMR spectrum for C(20) in **2**. This was confirmed by HMBC correlations of the C-atom at δ(C) 72.7 (*s*, C(20)) with the H-atoms at δ(H) 2.48–2.52 (*m*, H–C(16)), 2.44–2.48 (*m*, H–C(17)), 1.35 (*s*, Me(21)), and 5.26 (br. *s*, H–C(22)). The relative configuration of **2** was determined by the analysis of a ROESY experiment, in which correlations of Me(18)/H–C(11) and H–C(12), Me(19)/H–C(1) and H–C(11) indicated that the AcO groups at C(1), C(11), and C(12) are in α-orientation, while the correlations of H–C(1)/H–C(2) and H–C(2)/H–C(3) confirmed the β-orientation of H–C(2) and H–C(3). The observed ROESY correlations for H–C(14)/H–C(7) and H–C(17), Me(18)/H–C(16), Me(21), and Me(28), and H–C(17)/Me(27) suggested that HO–C(7), HO–C(25), Me(21), and Me(28) were in the β-orientation. The H–C(5) and HO–C(15) were assigned α on the basis of ROESY correlations for H–C(5)/H–C(7) and H–C(15)/Me(18). From the above analysis, the structure of **2** was unequivocally determined as shown and named taccalonolide W.

Taccalonolide X (**3**) was isolated as a colorless powder. Its molecular formula, $C_{36}H_{44}O_{14}$ was determined by HR-ESI-MS at *m*/*z* 723.2626 ($[M+Na]^+$; calc. 723.2629), corresponding to 15 degrees of unsaturation. The UV spectrum of **3** showed an absorption maximum at 244 nm, indicating the presence of a conjugated enone system, while the absorptions in the IR spectrum at 3439, 1813, 1745, and 1659 cm^{-1} suggested the presence of OH groups, a δ-lactone, an Ac group, and C=C bonds, respectively. The ^{13}C-NMR spectrum indicated 36 C-atom resonances as required by the HR-ESI-MS. There were two signals for C=O groups at δ(C) 202.4 and 198.0, five ester C=O groups at 170.9, 170.6, 169.7, 169.6, and 169.2, one trisubstituted C=C bond at 160.9 and 130.0, nine Me groups, one CH_2 group, 13 CH groups, thereof seven oxygenated, four quaternary C-atoms, thereof one oxygenated. The general NMR characteristics indicated that **3** is quite similar in structure to taccalonolide C [3]. The major

Table 1. *^{1}H-NMR Data of Compounds* **2**–**4**. δ in ppm, J in Hz.

	2[a])	**3**[a])	**4**[b])
H–C(1)	4.72 (*d*, *J* = 5.4)	4.73 (*d*, *J* = 5.5)	4.79 (*d*, *J* = 5.2)
H–C(2)	3.51 (*dd*, *J* = 3.7, 5.4)	3.46 (*dd*, *J* = 3.7, 5.5)	3.82 (*d*, *J* = 5.2)
H–C(3)	3.40 (br. *s*)	3.37–3.38 (*m*)	3.50 (br. *s*)
CH_2(4)	2.08–2.10 (*m*), 2.20–2.26 (*m*)	2.00–2.11 (*m*), 2.17–2.20 (*m*)	2.03–2.05 (*m*), 2.30–2.33 (*m*)
H–C(5)	2.79 (*dd*, *J* = 6.1, 11.0)	2.84–2.88 (*m*)	–
H–C(6)	–	–	2.88 (*d*, *J* = 3.4)
H–C(7)	4.16 (*d*, *J* = 10.8)	5.28 (*d*, 11.5)	3.13 (br. *s*)
H–C(8)	1.78–1.83 (*m*)	2.06–2.13 (*m*)	1.70–1.72 (*m*)
H–C(9)	2.72–2.77 (*m*)	2.87–2.92 (*m*)	2.34–2.36 (*m*)
H–C(11) or CH_2(11)	5.31 (*d*, *J* = 2.4)	5.38 (*dd*, *J* = 2.6, 11.5)	1.48–1.50 (*m*), 1.56–1.59 (*m*)
H–C(12)	5.28 (br. *s*)	5.43 (*d*, *J* = 2.6)	5.06 (br. *s*)
H–C(14)	2.08–2.11 (*m*)	2.50–2.53 (*m*)	2.60–2.62 (*m*)
H–C(15) or CH_2(15)	4.43 (*dd*, *J* = 8.0, 9.1)	5.06 (*dd*, *J* = 2.0, 8.5)	1.40–1.43 (*m*), 2.62–2.65 (*m*)
H–C(16)	2.48–2.52 (*m*)	2.48–2.50 (*m*)	1.90–1.93 (*m*)
H–C(17)	2.44–2.48 (*m*)	3.76 (*d*, *J* = 12.8)	2.21–2.25 (*m*)
Me(18)	1.05 (*s*)	1.06 (*s*)	0.87 (*s*)
Me(19)	0.83 (*s*)	0.83 (*s*)	0.71 (*s*)
H–C(20)	–	–	2.03–2.05 (*m*)
Me(21) or CH_2(21)	1.35 (*s*)	1.93 (*s*)	3.85–3.88 (*m*), 4.01–4.03 (*m*)
H–C(22)	5.26 (br. *s*)	5.84 (*s*)	5.30 (br. *s*)
CH_2(23)	–	–	1.43 (*d*, *J* = 4.8), 2.20–2.22 (*m*)
Me(27)	1.74 (*s*)	1.63 (*s*)	1.45 (*s*)
Me(28)	1.37 (*s*)	1.25 (*s*)	1.15 (*s*)
Ac	2.00 (*s*), 2.10 (*s*), 2.16 (*s*)	2.02 (*s*), 2.12 (*s*), 2.12 (*s*), 2.16 (*s*)	1.96 (*s*), 2.03 (*s*)

[a]) In $CDCl_3$. [b]) In (D_5)pyridine.

differences in the ^{13}C-NMR spectrum for **3** were the disappearance of signals for one CH and one CH_2 group each, and the presence of a signal for one trisubstituted C=C bond. The location of the C=C bond was deduced from the HMBC spectrum, which indicated important correlations of the CH H-atom at δ(H) 2.48–2.50 (*m*, H–C(16)) to the C-atom at δ(C) 160.9 (*s*, C(20)), of the methine H-atom at δ(H) 3.76 (*d*, H–C(17)) to the C-atoms at δ(C) 21.5 (*q*, C(21)), 160.9 (*s*, C(20)), and 130.0 (*d*, C(22)), and of the methine H-atom at δ(H) 5.84 (*s*, H–C(22)) to the C-atoms at δ(C) 21.5 (*q*, C(21)), 44.4 (*d*, C(17)), 198.0 (*s*, C(23)), and 47.6 (*s*, C(24)). These correlations indicated that the C=C bond connects C(20) with C(22). Extensive interpretation of the ROESY spectrum correlations, combined with comparison of the data with those of taccalonolide C, established the configuration of **3** as follows: H–C(1), H–C(2), H–C(3), H–C(11), H–C(12), H–C(15), Me(27), and Me(28) possess β-configuration, while H–C(5) and H–C(7) possess α-configuration. Consequently, the structure of **3** was unambiguously established and named taccalonolide X.

Table 2. *^{13}C-NMR Data of Compounds* **2**–**4**. δ in ppm.

	2[a])	**3**[a])	**4**[b])		**2**[a])	**3**[a])	**4**[b])
C(1)	72.5 (*d*)	72.4 (*d*)	72.6 (*d*)	C(19)	13.1 (*q*)	12.8 (*q*)	16.3 (*q*)
C(2)	49.8 (*d*)	49.5 (*d*)	51.8 (*d*)	C(20)	72.7 (*s*)	160.9 (*s*)	48.1 (*d*)
C(3)	52.0 (*d*)	52.1 (*d*)	55.6 (*d*)	C(21)	26.1 (*q*)	21.5 (*q*)	60.1 (*t*)
C(4)	21.4 (*t*)	21.2 (*t*)	33.6 (*t*)	C(22)	115.7 (*d*)	130.0 (*d*)	76.5 (*d*)
C(5)	42.4 (*d*)	42.7 (*d*)	70.6 (*s*)	C(23)	153.1 (*s*)	198.0 (*s*)	40.4 (*t*)
C(6)	208.5 (*s*)	202.4 (*s*)	56.9 (*d*)	C(24)	51.0 (*s*)	47.6 (*s*)	40.2 (*s*)
C(7)	75.6 (*d*)	76.7 (*d*)	54.7 (*d*)	C(25)	79.2 (*s*)	77.2 (*s*)	77.5 (*s*)
C(8)	42.5 (*d*)	38.0 (*d*)	36.3 (*d*)	C(26)	174.9 (*s*)	170.9 (*s*)	180.3 (*s*)
C(9)	39.3 (*d*)	40.8 (*d*)	29.7 (*d*)	C(27)	21.6 (*q*)	23.3 (*q*)	27.6 (*q*)
C(10)	43.2 (*s*)	42.7 (*s*)	40.6 (*s*)	C(28)	25.4 (*q*)	22.3 (*q*)	22.4 (*q*)
C(11)	70.2 (*d*)	70.2 (*d*)	25.4 (*t*)	Ac	170.9 (*s*)	170.6 (*s*)	170.9 (*s*)
C(12)	73.9 (*d*)	73.0 (*d*)	75.5 (*d*)		170.0 (*s*)	169.7 (*s*)	170.5 (*s*)
C(13)	44.5 (*s*)	44.0 (*s*)	46.5 (*s*)		170.0 (*s*)	169.6 (*s*)	20.8 (*q*)
C(14)	57.0 (*d*)	57.3 (*d*)	44.1 (*d*)		21.0 (*q*)	169.2 (*s*)	20.5 (*q*)
C(15)	71.5 (*d*)	78.4 (*d*)	25.6 (*t*)		21.3 (*q*)	21.1 (*q*)	
C(16)	44.8 (*d*)	46.0 (*d*)	52.4 (*d*)		20.5 (*q*)	20.8 (*q*)	
C(17)	49.6 (*d*)	44.4 (*d*)	41.1 (*d*)			20.6 (*q*)	
C(18)	15.2 (*q*)	14.8 (*q*)	12.9 (*q*)			20.5 (*q*)	

[a]) In $CDCl_3$. [b]) In (D_5)pyridine.

Taccalonolide Y (**4**) was obtained as a white powder. Its positive FAB-MS indicated the pseudomolecular ion at *m*/*z* 605 ($[M+H]^+$), and the HR-ESI-MS indicated the molecular formula $C_{32}H_{44}O_{11}$ with eleven degrees of unsaturation. The IR spectrum showed characteristic absorptions at 3448, 1730, and 1635 cm^{-1} which indicated the presence of OH and Ac groups, as well as a δ-lactone. The ^{1}H-NMR showed signals for six Me groups, four epoxy H-atoms, one HO–CH_2, and three downfield-shifted H-atoms. As for taccalonolides A, W, and X (**1**–**3**), the basic skeleton of taccalonolide Y (**4**) was that of a pentacyclic steroid, except that the side chain was enlarged from C_5 to C_6, and ring *B* contained an epoxy group. According to the ^{1}H- and ^{13}C-NMR spectral data, **4** was very similar to the known compound taccalonolide Q [8], except for the lack of a COOH group and the presence of a HO–CH_2 (δ(C) 60.1) connected to C(20) (δ(C) 48.1). Thus, it was supposed that the COOH group at C(20) of taccalonolide Q was reduced to a HO–CH_2 group, which was confirmed by the mass difference of 14 amu and the HMBC spectrum. In the HMBC spectrum, cross-peaks between δ(H) 2.21–2.25 (*m*, H–C(17)) with δ(C) 44.1 (*d*, C(14)), 52.4 (*d*, C(16)), 12.9 (*q*, C(18)), 48.1 (*d*, C(20)), 60.1 (*t*, C(21)), and 76.5 (*d*, C(22)), and between δ(H) 5.30 (br. *s*, H–C(22)) and δ(C) 41.1 (*d*, C(17)), 60.1 (*t*, C(21)), 40.4 (*t*, C(23)), 40.2 (*s*, C(24)), and 180.3 (*s*, C(26)) were observed. The relative configuration was determined by a ROESY experiment with cross-peaks between Me(18)/H–C(12) and H–C(16), H–C(16)/H–C(22), H–C(22)/H–C(20) and Me(28), Me(28)/Me(27), and Me(19)/H–C(1), H–C(2), H–C(3), H–C(6), and H–C(7). Therefore, the structure of **4** was assigned as shown and named taccalonolide Y.

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Experimental Part

General. Semiprep. HPLC: *Agilent 1100* apparatus equipped with a *Zorbax SB-C-18* column (*Agilent*, 9.4 mm × 25 cm). Column chromatography (CC): silica gel (SiO_2) (200–300 mesh; *Qingdao Marine Chemical Inc.*, China) or SiO_2 *H* (10–40 μm, *Qingdao Marine Chemical Inc.*), and *Lichroprep RP-18* (43–63 μm, *Merck*). Fractions were monitored by TLC, and spots were visualized by heating SiO_2 plates sprayed with 10% H_2SO_4 in EtOH. Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-2401 PC* spectrophotometer; λ_{max} in nm. IR Spectra: *Bio-Rad FTS-135* infrared spectrophotometer with KBr pellets. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* instruments using Me_4Si as the internal standard; δ in ppm rel. to solvent signals. ESI-MS and HR-ESI-MS Spectra: *API Qstar Pulsar LC/TOF* spectrometer; in *m/z* (rel. %).

Plant Material. The whole plants of *Tacca plantaginea* were collected in Guilin, Guangxi Zhuang Autonomous Region, P. R. China, in August 1999, and identified by Professor *De-Ding Tao*, Kunming Institute of Botany, the Chinese Academy of Sciences (CAS). The voucher was deposited with the Herbarium of Kunming Institute of Botany, CAS.

Extraction and Isolation. The powdered air-dried plants of *T. plantaginea* (30 kg) were exhaustively extracted three times with 400 l of 95% EtOH under reflux. After evaporation of the solvent, the resulting residue (1.5 kg) was successively extracted with $CHCl_3$ and BuOH. The $CHCl_3$ extract (700 g) was subjected to CC (SiO_2) eluting with a petroleum ether/AcOEt gradient (1:0, 10:1, 5:1, 7:1, 1:1) to give five fractions. *Fr. 4* (150 g) was repeatedly chromatographed on SiO_2 ($CHCl_3$/MeOH, 100:1) and semiprep. HPLC (MeCN/H_2O, 30:70) to afford **1** (1.1 g), **2** (20 mg), **3** (4 mg), and **4** (21 mg).

Taccalonolide W (=*(1*S*,5*S*,5a*S*,6*R*,7*R*,8a*S*,9a*S*,10a*S*,11*R*,11a*R*,12*S*,13*R*,13a*R*)-11,12,13-Tris(acetyloxy)-5,5a,5b,6,6a,6b,7,8a,9,9a,10a,11,11a,11b,12,13,13a,13b-octadecahydro-1,5,6,7-tetrahydroxy-1,5,5a,11a,13a-pentamethyl-1*H*-oxireno[6′,7′]naphtho[1′,2′:7,8]fluoreno[2,1-b]furan-4,8-dione*; **2**). White powder. $[\alpha]_D^{19} = +39.5$ ($c = 0.50$, $CHCl_3$). IR (KBr): 3420, 2975, 2930, 1818, 1745, 1693, 1434, 1375, 1250, 1126, 1091, 1041, 751. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 677 (4, $[M+H]^+$). HR-EI-MS: 699.2618 ($[M+Na]^+$, $C_{34}H_{44}NaO_{14}^+$; calc. 699.2629).

Taccalonolide X (=*(3a*S*,4*S*,6a*R*,7*R*,8a*S*,9a*S*,10a*S*,11*R*,11a*R*,12*S*,13*R*,13a*R*)-7,11,12,13-Tetrakis(acetyloxy)-3a,4,6a,6b,6c,7,8a,9,9a,10a,11,11a,11b,12,13,13a,13b,13c-octadecahydro-4-hydroxy-1,3a,4,11a,13a-pentamethyl-3*H*-oxireno[6′,7′]naphtho[1′,2′:7,8]fluoreno[9,1-bc]pyran-3,5,8-trione*; **3**). Colorless powder. $[\alpha]_D^{19} = +23.8$ ($c = 0.13$, MeOH). UV ($CHCl_3$): 244. IR (KBr): 3439, 2926, 2854, 1813, 1745, 1659, 1438, 1376, 1248, 1124, 1040, 761. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 723 (100, $[M+Na]^+$). HR-EI-MS: 723.2626 ($[M+Na]^+$, $C_{36}H_{44}NaO_{14}^+$; calc. 723.2629).

Taccalonolide Y (=*(1a*S*,1b*R*,2a*S*,3a*S*,4*R*,4a*S*,6*S*,6a*S*,7*R*,8*R*,11*R*,12*S*,13c*S*)-4,6-Bis(acetyloxy)icosahydro-1b,11-dihydroxy-7-(hydroxymethyl)-4a,6a,11,12-tetramethyl-8,12-methanobisoxireno[3′,4′:6′,7′]naphtho[2′,1′:4,5]indeno[1,2-d]oxocin-10(2*H*)-one*; **4**): White powder. $[\alpha]_D^{26} = +7.7$ ($c = 0.23$, MeOH). IR (KBr): 3448, 2976, 2941, 1730, 1635, 1378, 1254, 1131, 1032. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 605 (49, $[M+H]^+$), 545 (100, $[M-AcOH]^+$), 527 (70, $[M-AcOH-H_2O]^+$). HR-EI-MS: 627.2778 ($[M+Na]^+$, $C_{32}H_{44}NaO_{11}^+$; calc. 627.2781).

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