Three Novel Taccalonolides from the Tropical Plant *Tacca subflaellata*

by **Yue Huang** and **Ji-Kai Liu***

Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204 Yunnan, People's Republic of China

and

A. Mühlbauer* and **T. Henkel**

Bayer AG, Pharma Research Center Wuppertal, Life Science Center Natural Products, P.O. Box 101709, D-42096 Wuppertal

Three novel steroidal bitter principles, taccolanolide O (**1**), P (**2**), and Q (**3**), have been isolated from the tubers of *Tacca subflaellata* and their structures were established by spectroscopic methods.

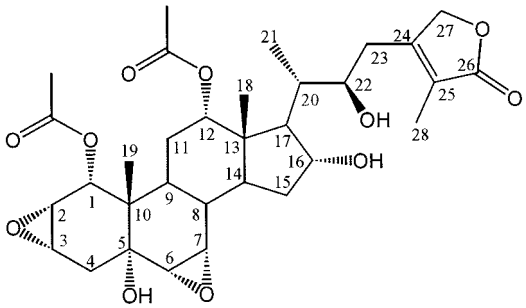
Introduction. – The plants of genus *Tacca* belong to the family of the Taccaceae. At present, *ca.* 50 species of the genus *Tacca* are known [1]. They originate from the tropics and subtropics, in particular from the Asiatic region. In African ethnobotanics, an EtOH extract of leaves from *Tacca leontopetaloides* is used against slugs and snails. Furthermore, an aqueous extract of tubers from *Tacca leontopetaloides* is used as an agent for controlling roundworms [2]. So far, 14 different compounds, which, owing to their origin, are referred to as taccalonolides, which have a rare pentacyclic skeleton, have already been isolated from *Tacca plantaginea* [3–9]. The species *Tacca subflaellata* is distributed in south of Yunnan Province, China. We have now succeeded in isolating three novel taccalonolides from the rhizomes of *T. subflaellata*. This paper describes the structure elucidation of the taccalonolides O (**1**), P (**2**), and Q (**3**).

Results and Discussion. – The fractions of the Et_2O-soluble part of the EtOH extract from the rhizome of *T. subflaellata* were subjected to repeated CC and PTLC to afford taccalonolides O (**1**), P (**2**), and Q (**3**).

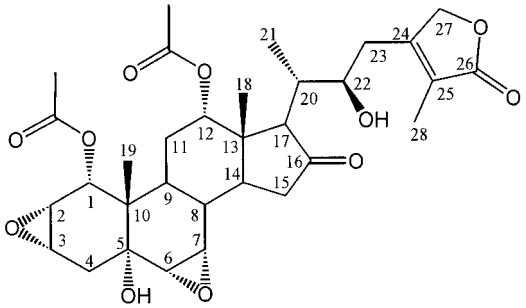
Taccalonolide O (**1**) was obtained as white amorphous powder. Its IR spectrum (3530, 1735, 1700 cm^{-1}) revealed the presence of OH groups and an α,β-unsaturated lactone. EI-MS exhibited the M^+ peak at m/z 604. The molecular formula was then established as $C_{32}H_{44}O_{11}$ by ESI-HR-MS (649.2862 ($[M+HCOO]^-$); calc. 649.2860) (*Table 1*).

The ^{13}C-NMR Spectra (*Table 2*) showed 32 ^{13}C signals, two ester C=O groups (169.90(*s*), 169.90 (*s*)), one lactone C=C (166.50 (*s*)), one C=C moiety (152.70 (*s*), 125.91 (*s*)), four C-atoms belonging to two epoxy groups (50.82 (*d*), 53.68 (*d*), 54.80 (*d*), 56.01 (*d*)), and six C-atoms connected to O-atoms (57.20 (*t*), 69.92 (*s*), 70.76 (*d*), 71.50 (*d*), 75.24 (*d*), 79.25 (*d*)). The DEPT indicated the presence of five CH_2 groups.

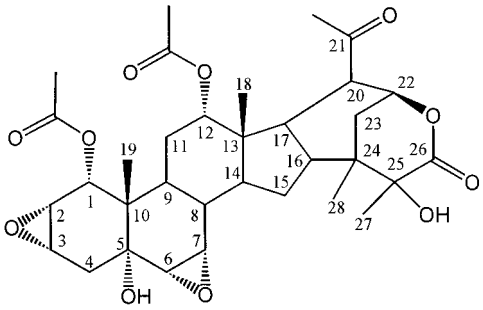
The ^1H-NMR spectra (*Table 3*) revealed six Me signals, five of them connected to quaternary C-atoms (0.81 (Me), 1.05 (Me), 2.00 (Ac), 2.05 (Ac), and 2.06 (Me)) and



Taccalonolide O (1)



Taccalonolide P (2)



Taccalonolide Q (3)

one *doublet* at 0.93 ($J = 6.9$ Hz). Besides eight signals for CH H-atoms between 5.00 and 2.8, one signal for CH_2 H-atoms at 4.3 and diverse *multiplets* between 0.8 and 2.6 were also identified.

The ^{1}H,^{1}H COSY spectrum revealed that two H–C(4) signals at 2.40 (*m*) and 2.05 (*m*) were correlated only to the H–C(3) signal at 3.54, indicating that one OH group was attached to C(5), which was also confirmed by the H–C(6) signal at 2.84 (*d*, $J =$ 3.5 Hz) correlating only to the H–C(7) signal at 3.11 (*m*). Therefore, an epoxide link between C(6) and C(7) was assumed, which was supported by ^{13}C shifts of C(6) at 56.01 and C(7) at 53.68. Substitution of ring D was elucidated by re-evaluation of ^{1}H,^{1}H-

Table 1. *HPLC/LC-MS for* **1**–**3**

	ESI-HR-MS	ESI (neg.)	EI-MS	t_R [min]
1	$C_{32}H_{44}O_{11}$ Calc.: 649.28602 Found: 649.28624 for $[M+HCOO]^-$	649 ($[M+HCOO]^-$), 603 ($[M-H]^-$)	604, 558, 542, 488, 207, 123, 107, 95, 81	13.00
2	$C_{32}H_{42}O_{11}$ Calc.: 647.27037 Found: 647.27043 for $[M+HCOO]^-$	647 ($[M+HCOO]^-$), 601 ($[M-H]^-$)	602, 584, 542, 524, 461, 402, 375, 150, 123, 107	13.62
3	$C_{32}H_{42}O_{12}$ Calc.: 617.25980 Found: 617.26002 for $[M-H]^-$	651 ($[M+HCOO]^-$), 617 ($[M-H]^-$)	618, 577, 558, 496, 452, 408, 318, 287, 205, 123, 109, 95, 83, 59	9.49

Table 2. *^{13}C-NMR Data* (100 MHz) *of* **1**–**3**. δ in ppm.

C-Atom	**1**[a])	**2**[a])	**3**[b])	C-Atom	**1**[a])	**2**[a])	**3**[b])
C(1)	71.50 (*d*)	71.24 (*d*)	71.50 (*d*)	C(17)	49.65 (*d*)	56.18 (*d*)	39.22 (*d*)
C(2)	50.82 (*d*)	50.74 (*d*)	50.95 (*d*)	C(18)	13.01 (*q*)	14.16 (*q*)	11.95 (*q*)
C(3)	54.80 (*d*)	54.75 (*d*)	54.79 (*d*)	C(19)	16.05 (*q*)	16.13 (*q*)	16.06 (*q*)
C(4)	32.43 (*t*)	22.29 (*t*)	32.48 (*t*)	C(20)	32.97 (*d*)	34.27 (*d*)	49.42 (*d*)
C(5)	69.92 (*s*)	69.57 (*s*)	69.85 (*s*)	C(21)	12.11 (*q*)	13.25 (*q*)	176.21 (*s*)
C(6)	56.01 (*d*)	55.98 (*d*)	55.94 (*d*)	C(22)	79.25 (*d*)	76.88 (*d*)	76.59 (*d*)
C(7)	53.68 (*d*)	53.35 (*d*)	54.05 (*d*)	C(23)	31.49 (*t*)	31.59 (*t*)	39.86 (*t*)
C(8)	35.55 (*d*)	34.88 (*d*)	35.42 (*d*)	C(24)	152.70 (*s*)	152.06 (*s*)	38.88 (*s*)
C(9)	27.92 (*d*)	28.06 (*d*)	28.46 (*d*)	C(25)	125.91 (*s*)	125.66 (*s*)	76.09 (*s*)
C(10)	39.92 (*s*)	39.88 (*s*)	39.70 (*s*)	C(26)	166.50 (*s*)	166.22 (*s*)	179.30 (*s*)
C(11)	23.98 (*t*)	23.97 (*t*)	23.76 (*t*)	C(27)	57.20 (*t*)	57.21 (*t*)	26.76 (*q*)
C(12)	75.24 (*d*)	73.51 (*d*)	73.27 (*d*)	C(28)	19.83 (*q*)	19.88 (*q*)	21.55 (*q*)
C(13)	46.12 (*s*)	46.12 (*s*)	46.00 (*s*)	Ac	169.90 (*s*)	169.86 (*s*)	170.09 (*s*)
C(14)	42.43 (*d*)	39.46 (*d*)	41.83 (*d*)		169.90 (*s*)	169.78 (*s*)	170.95 (*s*)
C(15)	35.35 (*t*)	37.13 (*t*)	24.18 (*t*)		20.03 (*q*)	19.88 (*q*)	20.03 (*q*)
C(16)	70.76 (*d*)	214.31 (*s*)	51.56 (*d*)		21.19 (*q*)	21.22 (*q*)	21.33 (*q*)

[a]) In $CDCl_3$. [b]) In D_2O.

COSY and ^{1}H,^{13}C-correlations spectra suggesting a OH group at C(16) and a C,C connection between C(17) and C(20). H–C(17) and H–C(20) showed correlation in the ^{1}H,^{1}H-COSY. In addition, H–C(20) showed correlation to the Me group at 0.93 (*d*, $J = 6.9$ Hz) and to the CH H-atom at 4.61 (*m*, H–C(22)). The H–C(22) correlated with H–C(23) at 2.12 (*m*) and 2.47 (*m*). Because of the low-field shift, a OH group was suggested at C(22).

^{1}H,^{13}C-Correlations between H–C(23) and C(24) (152.7), C(27) (57.2), and C(25) (125.91) indicated that a cyclic system was connected to C(23). ^{1}H,^{13}C-Correlations suggested a pentenolide moiety substituted with a Me group (2.05, *s*, H–C(28)) at C(25).

Taccalonolide P (**2**) was obtained as white amorphous powder. The EI-MS exhibited the M^+ peak at *m*/*z* 602. The molecular formula was established as $C_{32}H_{42}O_{11}$ by

Table 3. *^{1}H-NMR Data* (400 MHz) *of* **1**–**3**. δ in ppm, J in Hz.

	1[a])	**2**[a])	**3**[b])
H–C(1)	4.62 ($d, J = 5$)	4.64 ($d, J = 5$)	4.69 ($d, J = 5.1$)
H–C(2)	3.73 ($dd, J = 4.4, 4.2$)	3.74 ($dd, J = 3.9$)	3.68 (m)
H–C(3)	3.54 (m)	3.55 (m)	3.51 (br. s)
CH_2(4)	2.05 (m), 2.40 (m)	2.06 (m), 2.42 (m)	2.04 (m), 2.38 (m)
H–C(6)	2.84 ($d, J = 3.5$)	2.88 ($d, J = 3.5$)	2.82 ($d, J = 3.3$)
H–C(7)	3.11 (m)	3.06 (m)	3.08 (br. s)
H–C(8)	1.82 (m)	1.94 (m)	1.73 (m)
H–C(9)	2.06 (m)	2.26 (m)	2.03 (m)
CH_2(11)	1.56 (m)	1.62 (m)	1.50 (m)
H–C(12)	4.96 (m)	5.00 (m)	4.78 (m)
H–C(14)	1.95 (m)	2.50 (m)	2.32 (m)
CH_2(15)	2.54 (m), 1.40 (m)	2.04 (m), 2.53 (m)	1.35 (m), 2.44 (m)
H–C(16)	4.36 (m)		1.82 (m)
H–C(17)	1.63 (m)	2.46 (m)	2.33 (m)
Me(18)	1.05 (s)	0.99 (s)	0.92 (s)
Me(19)	0.81 (s)	0.83 (s)	0.87 (s)
H–C(20)	2.47 (m)	2.35 (m)	2.53 ($d, J = 11.8$)
Me(21)	0.93 ($d, J = 6.9$)	0.96 ($d, J = 7$)	
CH_2(22)	4.61 (m)	4.98 (m)	4.85 (m)
CH_2(23)	2.12 (m), 2.47 (m)	2.13 (m), 2.37 (m)	1.48 (m), 2.28 (m)
H at C(27)	4.37 (m)	4.35 ($dd, J = 12.5, 5.8$)	1.44 (s, 3 H)
Me(28)	2.06 (s)	2.03 (s)	1.13 (s)
Ac	2.00 (s), 2.05 (s)	2.12 (s), 2.03 (s)	1.95 (s), 2.01 (s)
HO–C(25)			2.92 (s)

[a]) In $CDCl_3$. [b]) In D_2O.

ESI-HR-MS (647.27043 ($[M + HCOO]^-$); calc., 647.27037). The ^{13}C-NMR spectra showed 32 ^{13}C signals; except for a keto group in C(16) (214.3), the other signals were similar to those of taccalonolide O (**1**). The ^{1}H-NMR spectra of **1** and **2** were very similar to each other; the ^{1}H signal for H–C(16) is missing in **2**. Thus, it was assumed that the OH group at C(16) in **1** was oxidized to a C=O group (δ 214.3) in **2**, which is supported by the mass difference of $\Delta m/z$ 2. HMQC and HMBC Experiments confirmed the proposed structure **2**.

Taccalonolide Q (**3**) was obtained as colorless crystals. The molecular formula was established as $C_{32}H_{42}O_{12}$ by ESI-HR-MS (617.2600 ($[M - H]^-$); calc. 617.2598) and HR-FAB$^+$-MS (619.2724 ($[M + 1]^+$); calc. 618.2754). Unlike taccalonolide O (**1**) and P (**2**), the basic skeleton of taccalonolide Q (**3**) was a pentacyclic steroid, more similar to the known taccalonolide-series compounds, except that the side chain was enlarged from C_5 to C_6, and ring B contained an epoxy group. The IR spectrum revealed the presence of a OH group at 3490.6, a C=O of COOH function at 1717.2, and a C=O of lactone at 732.8 cm^{-1}. The ^{13}C-NMR spectrum showed 32 ^{13}C signals, two ester C=O groups (170.09, 170.95), one COOH C=O group (176.21), and one lactone C=O group (179.30), four C-atoms involved in two epoxy groups (50.95 (d), 54.05 (d), 54.79 (d), 55.94 (d)), and five C-atoms connected to O-atoms (69.85 (s), 71.50 (d), 73.27 (d), 76.09 (d), 76.59 (s)). HMBC Experiments indicated that the H–C(22) (4.85) was correlated with the C=O of COO (C(26), 176.21 (s)) and the CH_2(23) (39.86 (t)), and

the signal at 76.59 was assigned to C(22); ^{1}H,^{1}H-COSY revealed two signals for C(23) at 2.28 (*m*) and 1.48 (*m*), coupled to the H–C(22) signal at 4.85, indicating that C(24) is a quaternary C-atom. In the ^{1}H-NMR spectrum, the signal at 7.2 (*s*) indicated the presence of a COOH fragment. The structure of compound **3** was confirmed also by the other HMBC, ^{1}H,^{1}H-COSY and HMQC evidence: one striking observation was that the *doublet* for the Me(21) group, which can be observed in all ^{1}H-NMR spectra of taccalonolides, was missing in the ^{1}H-NMR spectra of **3**. Therefore, it was assumed that C(20) had a different substitution pattern. Long-range correlations between both H–C(17) and H–C(22), and C(21) (176.21 (*s*)) established a COOH group as substituent at C(20). From the ^{1}H,^{1}H-COSY spectra, correlations between H–C(17) and H–C(20), H–C(20) and H–C(22), H–C(22) and H–C(23), and H–C(17) and H–C(16) were deduced. Because of the low-field shift of H–C(22) signal (4.85 (*m*)), it was assumed that an O-atom is connected to C(22). Long-range correlations between H–C(22) and C(20), C(17), C(21), and C(26) revealed a lacton ring moiety. This was confirmed by other long-range correlations shown in the *Figure.*

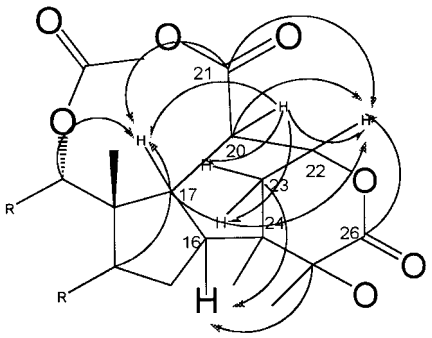


Figure. *Some selected ^{1}H,^{1}H-correlations derived from the ^{1}H,^{1}H-2-D-COSY spectra and some ^{1}H,^{13}C-correlations derived from the HMBC spectra of* **3**

Taccalonolides O (**1**), P (**2**), and Q (**3**) did not show any biological activity, neither in the nematicidal screening against *Meloidogyne incognita* nor in the insecticidal screening against *Phaedon cochleariae*, *Tetranychus urticae*, or *Plutella maculipennis.*

Experimental Part

General. Silica gel (200–300 mesh) for column chromatography (CC) and GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. M.p.: a *VEB Wägetechnik* (*PHMK*) apparatus, uncorrected. Optical rotations: *Horiba SEPA-300* polarimeter (*Horiba*, Tokyo, Japan). IR Spectra: *Bio-Rad FTS135* spectrophotometer, KBr pellets, $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer; δ in ppm, *J* in Hz. 2D-NMR Spectra: *Bruker DRX-500* spectrograph (500 MHz). MS: *VG Autospec-3000* spectrometer, at 70 eV for EI; *m*/*z* (rel. int.).

Plant Material. The plants of *T. subflaellata* P. P. LING *et* C. T. TING were collected at Hekou county of Yunnan Province, China, in May of 1999. The voucher specimen was deposited at the herbarium of Kunming Institute of Botany (No. KUN 0435286).

Extraction and Isolation. The air-dried and powdered rhizomes (12 kg) were extracted with EtOH three times. The residue obtained by evaporation was malaxated in Et_2O to dissolve in Et_2O. The Et_2O-soluble part was first fractionated by CC (silica gel; petroleum ether/acetone, 9:1, 8:2, 7:3, and 6:4, then $CHCl_3$/MeOH 99:1, 97:3, 95:5, 92:8, and 9:1) to afford several fractions. The fraction (0.9 g) eluted with petroleum ether/

acetone 7:3 was purified by repeated prep. TLC with $CHCl_3$/MeOH 95:5 to give one pure compound **1** (17 mg). The fraction (1.2 g) eluted with petroleum ether/acetone 8:2 was purified by repeated prep. TLC with $CHCl_3$/MeOH 97:3 to give the pure compound **2** (22 mg). The fraction (2.7 g) eluted with $CHCl_3$/MeOH 95:5 was purified by repeated prep. TLC with $CHCl_3$/MeOH 92:8 to give another pure compound **3** (15 mg).

Taccalonolide O (**1**). White amorphous powder. M.p. 245°. $[\alpha]_D^{12} = +74.1$ ($c = 0.008$, $CHCl_3$). IR (KBr): 3520, 2925, 2862, 1735, 1700, 1461, 1383, 1245, 1028, 992, 846. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 604 (5), 558 (4), 542 (63), 488 (16), 475 (12), 386 (10), 265 (19), 237 (24), 207 (33), 145 (35), 123 (50), 107 (64), 95 (98), 81 (63), 69 (53), 55 (100). HR-ESI-MS: *Table 1*.

Taccalonoid P (**2**). Pale yellow needles. M.p. 207°. $[\alpha]_D^{11} = +12.2$ ($c = 0.004$, $CHCl_3$). IR (KBr): 3499, 2978, 1734, 1465, 1380, 1246, 1029, 970, 851. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 602 (2), 584 (5), 542 (3), 524 (7), 461 (11), 402 (11), 375 (16), 169 (28), 150 (58), 123 (87), 95 (100), 69 (61). HR-ESI-MS: *Table 1*.

Taccalonoid Q (**3**). Colorless crystals. M.p. 305°. $[\alpha]_D^{11} = +31.9$ ($c = 0.003$, CH_3OH). IR (KBr): 3491, 2972, 1733, 1717, 1378, 1260, 1132, 1030, 896, 856. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 618 (0.5), 558 (3), 318 (27), 287 (51), 273 (14), 217 (16), 205 (33), 191 (28), 135 (26), 123 (39), 109 (43), 95 (54), 83 (64), 59 (100). HR-FAB$^+$-MS: 619.2724 ($[M+1]^+$, $C_{32}H_{42}O_{12}$; calc. 619.2754).

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