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TACCALONOLIDE C AND D, TWO PENTACYCLIC STEROIDS OF *TACCA PLANTAGINEA*

ZHONG-LIANG CHEN, BAO-DE WANG and JI-HUI SHEN

Shanghai Institute of Materia Medica, Academia Sinica, Shanghai 200031, China

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Key Word Index—*Tacca plantaginea*; Taccaceae; pentacyclic steroid; bitter principles; taccalonolides.

Abstract—Two pentacyclic steroidal bitter principles, taccalonolide C and D have been isolated from *Tacca plantaginea* and the structures were established by spectroscopic methods.

INTRODUCTION

The plants of *Tacca* genus are distributed in tropical regions. In China there are only three species and these have all been used as folk medicine [1]. Some *Tacca* species have been investigated chemically and found to contain amino acids [2], flavones and anthocyanins [3], steroidal saponins and sapogenins [4, 5] and an amorphous bitter compound taccalin $C_{18}H_{26}O_7$ [6]. In a previous paper we reported the isolation of the two bitter principles, taccalonolide A(**1**) and B(**2**), from the rhizome of *T. plantaginea* [7]. Taccalonolide A and B have a pentacyclic steroidal skeleton which was isolated from a natural source for the first time. Now we report the isolation and structure determination of the two related compounds, taccalonolide C(**3**) and D(**4**).

RESULTS AND DISCUSSION

Taccalonolide C, mp 305° FDMS: 703 $[M+1]^+$, molecular formula $C_{36}H_{46}O_{14}$. IR ν_{max}^{KBr} cm^{-1}, 3450 (OH), 1744, 1728 (ester and lactone carbonyls), 1704 (keto carbonyl). By comparison of **3** with **1** and **2**, taccalonolide C does not show the $-\overset{|}{C}=\overset{|}{C}-O-CO-$ group in the IR absorption at 1820 cm^{-1}. The ^{13}C NMR spectrum of **3** reveals two keto carbonyl carbons (δ 209.16 *s* and 202.42 *s*), five ester and/or lactone carbonyl groups, no olefinic carbon, five $-\overset{|}{C}H-O-$, one $-\overset{|}{C}-O$, nine $-Me$, two $-CH_2-$, nine $-\overset{|}{C}-H$ and three quaternary carbons (by off-resonance and DEPT techniques).

The ^1H NMR spectrum of **3** was measured in several solvents, because some proton signals were overlapped by acetyl methyl protons and/or solvent signals. All the signals were assigned by proton-proton spin decoupling and comparison with **1** and **2** (Table 1).

Taccalonolide C has the same molecular formula as taccalonolide A(**1**) and the majority of the proton signals are similar, but it does not contain an olefinic proton, and the chemical shift of H-7 was shifted downfield to δ 5.22 *d* (H-7 was at 4.22 *d* in **1**) and the chemical shift of H-15 was shifted upfield to 5.02 *dd*. In the comparison of **3** to **1** and **2** (H-15, 5.47 *dd*, in **1** and 4.00 *dd* in **2**), it seems to be lactonized at the C-15 position and we propose structure **3** for taccalonolide C.

The stereochemistry of **3** was determined with the NOE difference technique. It showed 1α, 7β, 11α and 12α-tetraacetoxy-2α, 3α-epoxy-24β-methyl-25β-methyl and 25α-hydroxy groups and the NOE enhancement data were as shown in Fig. 1.

Taccalonolide D, mp 284°, FDMS: *m/z* 703 $[M+1]^+$, molecular formula $C_{36}H_{46}O_{14}$, the same as **1**. By comparison of the ^1H NMR spectrum of **4** with that of **1**, the H-7 (δ 4.00 *br d*) and H-15 (5.47 *dd*) of **1** shifted to H-7 (5.16 *d*) and H-15 (4.47 *dd*) for **4**. Therefore it is obvious that taccalonolide D contains C-7 acetoxy and C-15 hydroxy groups.

Taccalonolide D is not very stable and it is partly transformed to **1** on silica gel column chromatography by an acetyl migration. The similar acetyl migrations under mild condition are well-known in *Aconitum* alkaloids [8] and other compounds [9].

Upon examination of the Dreiding stereo molecular model, the distance between the C-7 and C-15 oxygen atoms of taccalonolide analogues approximate 2.5 Å. It is quite possible for **4** to form a seven-membered ring semi-*ortho* ester intermediate and then transform to the C-15 acetoxy compound **1** (Scheme 1). But **1** does not show this acetyl migration under the same conditions, perhaps due to the hydrogen bonding formation of the C-7 hydroxy group with the C-6 carbonyl.

Owing to the presence of an enol γ-lactone partial structure, taccalonolide A, B and D show absorptions at 1820 and 1750 cm^{-1} in their spectra and they are very easy to confuse with a six-membered ring anhydride [6]. Taccalonolide A, B and D taste very bitter and the less bitter counterpart taccalonolide C shows no cytotoxicity to a P-388 cell culture and no antimalaria activity. Therefore the enol-γ-lactone portion may be important for the biological activities.

Table 1. 400 MHz ^{1}H NMR spectral data of taccalonolide C (**3**) and D (**4**)

H	Taccalonolide C Acetone-d_6	Taccalonolide C $CDCl_3$	Taccalonolide D $CDCl_3$
1	4.85 *d* (5.5)	4.73 *d* (5.5)	4.72 *d* (5.5)
2	3.41 *dd* (4, 5)	3.46 *dd* (4, 5)	3.52 *dd* (4, 5)
3	3.34 *m*	3.37 *m*	3.34 *m*
4	2.01 *ddd*	*	2.19 *dd*
	2.18 *ddd*	*	2.28 *ddd*
5	2.91 *dd* (7, 10.5)	2.84 *dd* (6, 11)	2.77 *dd* (6, 11)
7	5.10 *d* (12)	5.22 *d* (12)	5.16 *d* (11)
8	2.27 *ddd* (12, 12, 12)	*	1.75 *dd* (11, 11)
9	3.08 *dd* (12, 12)	2.82 *dd* (12, 11)	2.83 *dd* (11, 13)
11	5.41 *dd* (12, 2)	5.36 *dd* (12, 3)	5.32 *dd* (12, 3)
12	5.31 *d* (3)	5.28 *d* (3)	5.22 *d* (3)
14	2.61 *dd* (8, 11)	2.18	2.24 *dd* (9, 10)
15	5.13 *dd* (9, 9)	5.02 *dd* (9, 9)	4.47 *dd* (9, 9)
16	2.40 *dd* (10, 12)	2.55 *dd* (10, 13)	2.42 *dd* (10, 12)
17	2.23 *dd* (13, 11)	*	1.87 *dd* (11, 13)
Me-18	1.07 *s*	0.99 *s*	0.91 *s*
Me-19	0.86 *s*	0.82 *s*	0.78 *s*
20	1.85 *m*	1.74 *m*	*
Me-21	0.82 *d* (7)	0.83 *d* (7)	0.89 *d* (7)
22	2.40 *dd* (4.5, 13)	2.51 *dd* (5, 13)	5.05 *d* (2)
	3.19 *dd* (12, 13)	2.92 *dd* (12, 13)	
Me-27	1.37 *s*	1.45 *s*	1.66 *s*
Me-28	1.14 *s*	1.18 *s*	1.31 *s*
Ac	1.92 *s*	2.00 *s*	1.86 *s*
	2.05 *s*	2.14 *s*	2.02 *s*
	2.06 *s*	2.16 *s*	2.11 *s*
	2.15 *s*	2.27 *s*	2.12 *s*

*Overlapped by signals of acetyl methyl groups.

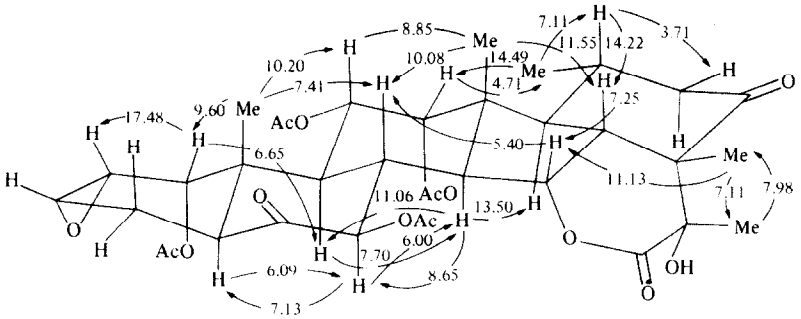


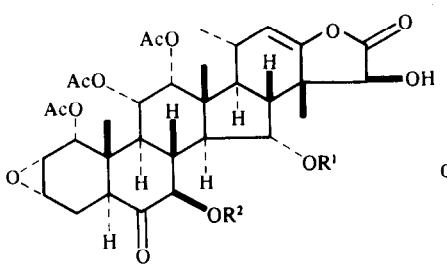
Fig. 1. NOE of taccalonolide C (enhancement data %).

EXPERIMENTAL

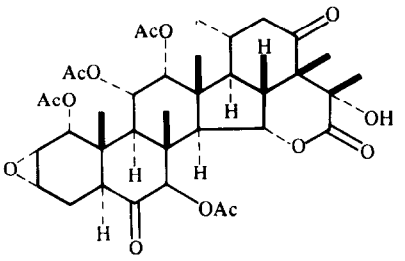
The plant material used in this study was collected in Kwangsi province, near Liu-Zhou county during June 1985. A voucher specimen has been deposited at Kwangsi Institute of Botany(Gui-Lin, China). The dry powdered rhizome material (6 kg) was extracted with EtOH and the resulting extract was dissolved in Et_2O, and the Et_2O soluble part was first separated by silica gel CC with the solvent systems: petrol, petrol–ether (9 : 1, 3 : 1, 1 : 1) and then Et_2O, Et_2O–MeOH (9 : 1). It afforded ceryl alcohol (petrol–Et_2O, 3 : 1), sitosterol (petrol–Et_2O, 1 : 1) and crude bitter substances (Et_2O). The crude amorphous bitter substances were separated further with silica gel rechromatography ($CHCl_3$, $CHCl_3$–EtOH 99 : 1, 9 : 1, 3 : 1 and 1 : 1) and **1**, 7.5 g, **2**, 90 mg, **3**, 190 mg and **4**, 40 mg were obtained.

Taccalonolide A, mp 215°, MS *m/z*: 702 $[M]^+$ $C_{36}H_{46}O_{14}$; IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1827, 1745, 1730 (CO).

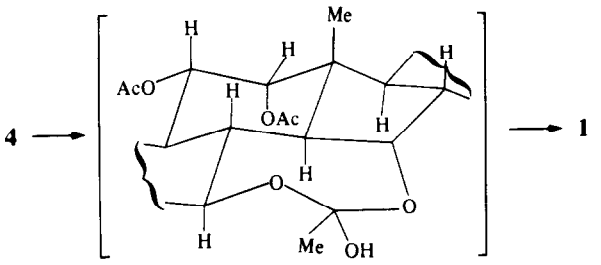
Taccalonolide B, mp 266°C, MS *m/z*: 660 $[M]^+$ $C_{34}H_{44}O_{13}$; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1825, 1750, 1730 (CO).



1 R^1 = Ac, R^2 = H
2 R^1 = H, R^2 = H
4 R^1 = H, R^2 = Ac



3



Scheme 1.

Taccalonolide C, mp 305°C, $[\alpha]_D^{14}$ + (but very small), FDMS: *m/z* 703 $[M+1]^+$ $C_{36}H_{46}O_{14}$, IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1744, 1728, 1704 (CO). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 209.16 *s*, 202.42 *s*, 169.58 *s*, 169.46 *s*, 169.15 *s*, 168.95 *s*, 168.20 *s*, 78.97 *s*, 76.61 *d*, 73.91 *s*, 73.11 *d*, 71.95 *d*, 70.32 *d*, 56.10 *d*, 51.47 *d*, 51.34 *d*, 49.24 *d*, 45.97 *d*, 45.96 *d*, 43.59 *s*, 42.11 *s*, 42.11 *s*, 41.97 *d*, 40.30 *d*, 37.40 *d*, 30.40 *d*, 21.06 *q*, 20.96 *t*, 20.78 *q*, 20.69 *q*, 20.44 *q*, 20.18 *q*, 20.12 *q*, 19.49 *q*, 12.34 *q*, 12.14 *q*.

Taccalonolide D, mp 284° $[\alpha]_D^{24}$ +31° ($CHCl_3$, *c* 0.032), FDMS *m/z*: 703 $[M+1]^+$ $C_{36}H_{46}O_{14}$, IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1810, 1740 (CO).

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REFERENCES

1. How, F. (1982) *A Dictionary of the Families and Genera of Chinese Seed Plants* 2nd Edn, p. 477. Sciences Press (Chinese).
2. Tiwari, K. P. and Tripathi, R. D. (1980) *Vijnana Parishad Anusandhan Patrika* **23**, 157; *Chem. Abst.* **94** 80285a.
3. Tripathi, R. D. and Tiwari, K. P. (1981) *Planta Med.* **41**, 414.
4. Kelginbaev, A. N., Gorovits, M. B. and Abubakirov, N. K. (1980) *Khim. Prir. Soedin.* 352.
5. Zhou, J., Chen, C., Liu, R. and Yang C. (1983) *Acta Bot. Sinica* **25**, 588.
6. Scheuer, P. J., Swanholn, C. E. and Madamba L. A. (1963) *Lloydia* **26**, 133.
7. Chen, Z., Wang, B. and Chen, M. (1987) *Tetrahedron Letters* **28** 1673.
8. Reinecke, M. G., Minter, D. E., Chen, D. and Yan, W. (1986) *Tetrahedron* **24** 6621.
9. Flowers, H. M. (1971) *The Chemistry of the Hydroxy Group* Part 2, Chap. 18, (Patai, S., *et al.*, eds). Interscience, New York.