

Two New Taccalonolides from Tropic Plant *Tacca subflaellata*

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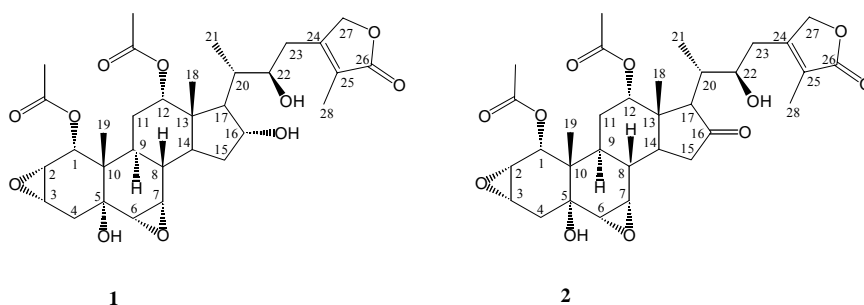
Abstract: Two new steroidal bitter principles, taccalonolide O, P have been isolated from *Tacca subflaellata* and their structures were established by spectroscopic methods.

Keywords: *Tacca subflaellata*, taccalonolides.

The plants of genus *Tacca* (Taccaceae) are distributed in tropical regions. In China there are five species and three of them have been long used as folk medicine for treatment of gastric ulcer, toothache and stomachache, incised wounds¹. Some of *Tacca* species have been chemically investigated thoroughly. From them some steroidal saponins, sapogenins, flavones, anthocyanins and amino acids have been isolated^{2,3}, the most promised compounds among them are some steroids with a kind of rare pentacycle skeleton in natural source⁴⁻⁸. The plant *Tacca subflaellata* P. P. Ling et C. T. Ting (1982) was collected in Hekou, Yunnan Province, for the searching of some bioactive compounds. This paper describes the structure elucidation of two new steroids, taccalonolides O (**1**), P (**2**) from the EtOH extract of rhizome of *T. subflaellata*.

Taccalonolide O (**1**) was obtained as white amorphous powder, mp 245°C, $[\alpha]_D^{12} +74.1$ (c 0.008, CHCl₃). In their IR spectrum signals at 1734.9, 1700.7 cm⁻¹ revealed the presence of α , β -unsaturated lactone, and the absorption at 3529.9 cm⁻¹ indicated the presence of an intramolecular hydrogen bond between two –OH groups at C-16, C-22. EI-MS spectrum exhibited the M⁺ peak at m/z 604. The molecular formula was then established as C₃₂H₄₄O₁₁ by ESI-HR-MS [(M-H+HCOOH)⁻: found 649.2860, calcd. 649.2862]. The ¹³C-NMR spectra showed 32 carbon signals, three ester carbonyls (169.9 s, 169.9 s, 166.5 s), one carbon carbon double bond (152.7 s, 125.9 s), four carbons belonging to two epoxy groups (50.8 d, 53.7 d, 54.8 d, 56.0 d), and six carbons connected to oxygen atoms (57.2 t, 69.9 s, 70.8 d, 71.2 d, 75.2 d, 79.3 d). The DEPT-¹³CNMR indicated the presence of five methylene groups.

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Figure 1 The structures of **1**, **2**

The $^1\text{H-NMR}$ spectrum showed six methyl signals, five of them connected to quaternary carbons. The $^1\text{H-}^1\text{H}$ 2D COSY, revealed that two H-4 –proton signals at δ 2.40 (m), 2.05 (m) were coupled only to the H-3 signal at δ 3.4, it suggested that one OH group was attached to C-5 position, which was also proved by the one H-6 signal at 2.82 (d, $J=3.5$) was coupled only to the H-7 signal at 3.1 (m). Therefore an epoxide-link between C-6 and C-7 was supposed which was supported by ^{13}C chemical shifts of C-6 at δ 56.0 and C-7 at δ 53.7. In the $^1\text{H-NMR}$ spectrum the H-27 signal appeared as single signal at 4.38, with integral of two protons also indicated a methylene connecting to an oxygen atom. To establish the relative stereostructure of **1**, the ROESY experiment was carried out. Observed correlations between H-2, H-3, H-1 and H-19 (CH_3); H-6, H-7, H-8 and H-19, indicated that 2,3-epoxide ring and 6,7-epoxide ring were both in α configuration. This was also confirmed by direct comparison of the chemical shifts of C-2, C-3, C-6, C-7 (54.8, 50.8, 53.7, 56.0 ppm) with those of the known taccalonolid compounds⁴⁻⁸. Detailed analysis of the ROESY spectrum found that the correlations between H-16, H-20 and H-18 (CH_3), which suggested the hydroxyl group at C-16 to be α configuraton. All the data confirmed the proposed structure **1**.

Taccalonolide P (**2**) was obtained as pale yellow needles, mp 207°C, $[\alpha]_{\text{D}}^{11} +12.2$ (c 0.004, CHCl_3). EI-MS spectrum exhibited the M^+ peak at m/z 602. The molecular formula was then established as $\text{C}_{32}\text{H}_{42}\text{O}_{11}$ by ESI-HR-MS [found, 647.2704 (M-H +HCOOH)⁻, calcd. 647.2704]. The $^{13}\text{C-NMR}$ spectra showed 32 carbon signals, except for a keto group in C-16 (214.3), the other signals were similar to taccalonolides O (**1**). Besides the $^1\text{H-NMR}$ spectra of **1** and **2** were very similar to each other, the ^1H signal for H-16 is missing in **2**. Thus it was assumed that the hydroxy group at C-16 in **1** was oxidized to a carbonyl group in **2**, which is supported by the mass difference of $\Delta m/z = 2$. Also HMQC, HMBC and ROESY experiments confirmed the proposed structure **2**.

Table 1 ^{13}C -NMR(DEPT) data for **1**, **2** (δ in ppm)

| | 1 | 2 | | 1 | 2 |
|-------|----------|----------|-------|----------|----------|
| C(1) | 71.5 d | 71.2 d | C(17) | 49.7 d | 39.2 d |
| C(2) | 54.8 d | 54.7 d | C(18) | 13.0 q | 14.2 q |
| C(3) | 50.8 d | 50.7 d | C(19) | 16.1 q | 16.1 q |
| C(4) | 32.4 t | 31.8 t | C(20) | 32.9 d | 34.3 d |
| C(5) | 69.9 s | 69.6 s | C(21) | 12.1 q | 13.3 q |
| C(6) | 56.0 d | 55.9 d | C(22) | 79.3 d | 76.9 d |
| C(7) | 53.7 d | 53.4 d | C(23) | 31.5 t | 31.6 t |
| C(8) | 35.6 d | 34.9 d | C(24) | 152.7 s | 152.1 s |
| C(9) | 27.9 d | 28.1 d | C(25) | 125.9 s | 125.7 |
| C(10) | 39.9 s | 39.8 s | C(26) | 166.5 s | 166.2 s |
| C(11) | 23.9 t | 23.9 t | C(27) | 57.2 t | 57.2 t |
| C(12) | 75.2 d | 73.5 d | C(28) | 19.8 q | 19.9 q |
| C(13) | 46.1 s | 46.1 s | Ac | 169.9 s | 169.9 s |
| C(14) | 42.4 d | 39.5 d | | 169.9 s | 169.8 s |
| C(15) | 35.4 t | 37.1 t | | 20.0 q | 19.9 q |
| C(16) | 70.8 d | 214.3 s | | 21.2 q | 21.2 q |

Table 2 ^1H -NMR data for **1-3**. (δ in ppm, J in Hz.)

| | 1 | 2 |
|----------------------|-----------------------------|------------------------------|
| CH(1) | 4.62(d, $J=5.0$, 1H) | 4.64(d, $J=5.0$, 1H) |
| CH(2) | 3.54(m, 1H) | 3.55(m, 1H) |
| CH(3) | 3.73(dd, $J=4.4$, 4.2, 1H) | 3.74(dd, $J=3.9$, 4.2, 1H) |
| CH ₂ (4) | 2.05(m, 1H), 2.40(m, 1H) | 2.06(m, 1H), 2.42(m, 1H) |
| CH(6) | 2.84(d, $J=3.5$, 1H) | 2.88(d, $J=3.5$, 1H) |
| CH(7) | 3.11(m, 1H) | 3.06(m, 1H) |
| CH(8) | 1.82(m, 1H) | 1.94(m, 1H) |
| CH(9) | 2.06(m, 1H) | 2.26(m, 1H) |
| CH ₂ (11) | 1.56(m, 2H) | 1.62(m, 2H) |
| CH(12) | 4.96(m, 1H) | 5.00(m, 1H) |
| CH(14) | 1.95(m, 1H) | 2.50(m, 1H) |
| CH ₂ (15) | 2.54(m, 1H), 1.40(m, 1H) | 2.04(m, 1H), 2.53(m, 1H) |
| CH(16) | 4.36(m, 1H) | |
| CH(17) | 1.63(m, 1H) | 2.46(m, 1H) |
| CH ₃ (18) | 1.05(s, 3H) | 0.99(s, 3H) |
| CH ₃ (19) | 0.81(s, 3H) | 0.83(s, 3H) |
| CH(20) | 2.47(m, 1H) | 2.35(m, 1H) |
| CH(21) | 0.93(d, $J=6.9$, 3H) | 0.96(d, $J=7.0$, 3H) |
| CH ₂ (22) | 4.61(m, 1H) | 4.98(m, 1H) |
| CH ₂ (27) | 4.37(m, 2H) | 4.35(dd, $J=12.5$, 5.8, 2H) |
| CH ₃ (28) | 2.06(s, 3H) | 2.03(s, 3H) |
| CH ₃ (Ac) | 2.00(s, 3H) | 2.12(s, 3H) |
| | 2.05(s, 3H) | 2.03(s, 3H) |

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