SOME CHEMICAL REACTIONS OF TACCALONOLIDE A - A BITTER SUBSTANCE FROM *TACCA PLANTAGINEA*

Zhong-liang Chen, Ji-hui Shen, and Yi-sheng Gao

Shanghai Institute of Materia Medica, Academia Sinica
319 Yue-yang Road, Shanghai 200031, China

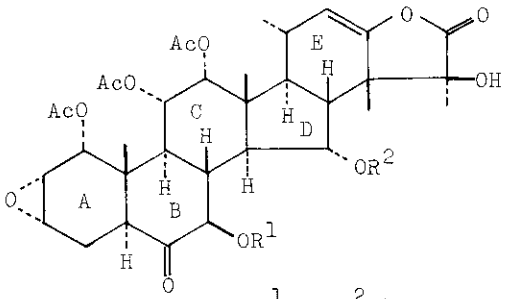
Abstract — Taccalonolide A, a pentacyclic steroidal bitter principle from Chinese medicinal plant *Tacca plantaginea* (Hance) Drenth, was studied by chemical reactions.

Tacca is a genus of the family Taccaceae. They are herbaceous plants and found predominantly in tropic zones.[1] In the previous papers we have reported the structure determination of taccalonolides A **1**, B **2**,[2] C **3** and D **4**,[3] the new biologically active bitter substances from *Tacca plantaginea* (Hance) Drenth, by spectroscopic methods. These rather unusual pentacyclic steroids are closely related to physalins,[4] a type of highly oxygenated steroids that exhibit the unique structural feature of a seco C/D steroid nucleus at which an additional carbocyclic ring E and an extra methyl group C-28 are present.

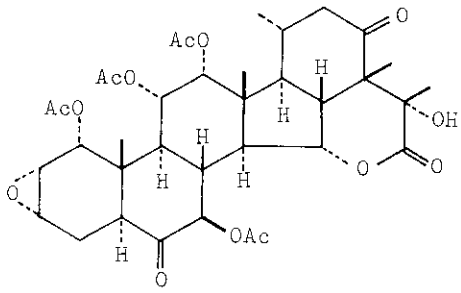
In continuation of our investigations we desired to study its chemical characteristics by chemical reactions. Herein we report the result of some chemical reactions carried out with taccalonolide A **1**.

1 is very unstable in alkaline solutions even at room temperature. Alkaline degradation of **1** gave taccalonol **5**. The ir spectrum of **5** presents two keto carbonyls at 1730 and 1705 cm^{-1}. ^{13}C-Nmr spectrum reveals 25 carbons, among which two are keto carbonyls. We elucidated the structure of **5** by ^{1}H-nmr spectrum and spin-decoupling techniques. The stereochemistry of **5** was established by NOE difference spectroscopy for indicating the 24-β-methyl configuration, in case of saturation of H-21 the ^{1}H-nmr of **5** gave NOE with H-24 (5.69%) and saturation of H-24 it gave NOE with H-21 (5.23%) also.

By the reduction with $LiAlH_4$, **1** also suffered the split of C_{24}-C_{25} bond to produce compound **6**, which was somewhat like **5** in the spectral and chemical characteristics, but it has one keto, seven hydroxy groups but no C-2, C-3 epoxy.



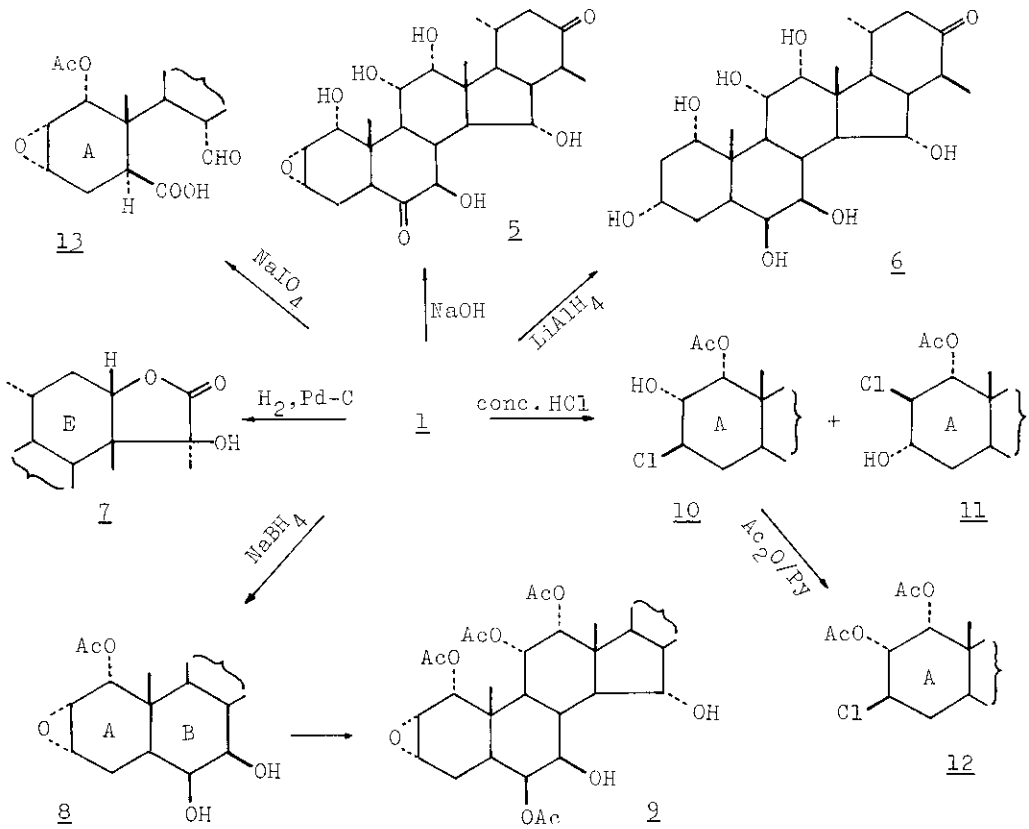
1 R^1=H, R^2=Ac
2 R^1=R^2=H
4 R^1=Ac, R^2=H



3

The structure of 6 was established by spectroscopic data, and the assignment of ^{1}H-nmr spectrum was accomplished by the spin-decouping method.

Catalytic hydrogenation of 1 afforded a main product 7, its ir spectrum presents a typical γ-lactone absorption at 1770 cm^{-1} instead of the enol γ-lactone



1820 cm^{-1} in 1.

The reduction of 1 with $NaBH_4$ formed 8, which was easily converted to 9 in $CHCl_3$ solution. Both 8 and 9 were lack of C-6 keto carbonyl. ^{1}H-Nmr of 9 showed the presence of a 6-β-acetoxy group, owing to its smaller coupling constants, $J_{5,6}$ = 2.5 Hz and $J_{6,7}$ = 2.5 Hz. The formation of 9 may be resulted from a two-step acetyl migration and this kind of migration has also been found in the case of taccalonolide D.[3]

Opening the epoxy ring of 1 with HCl afforded chloro compounds 10 and 11, their C-OH and C-Cl bonds in such two compounds are both equatorial in 10 and both axial in 11 in accordance with the ^{1}H-nmr spectra analysis.

The above chemical reactions provide further supports for the proposed structure of taccalonolide A 1, which had been deduced by spectroscopic methods and confirmed by X-ray crystallographic method.[2]

EXPERIMENTAL

Ir spectra were recorded on a PE-599B spectrophotometer; ^{13}C and ^{1}H nmr spectra on Bruker AM-400 and AC-100 spectrometer and mass spectra on a Varian MAT-711, fdms on a Hitachi M-80.

Reaction with NaOH To a solution of 20 ml of 5% aqueous NaOH, 600 mg of taccalonolide A was added. After 1 was dissolved, the reaction mixture was neutralized with 5% aqueous HCl, and concentrated to a small amount in vacuo, and then cooled in a refrigerator to give a white precipitate, which was recrystallized from MeOH to yield 250 mg (63%) of 5. 5 mp 252-253 °C, ms: 464 $C_{25}H_{36}O_8$, 446 $[M-18]^+$, 428 $[446-18]^+$, 410 $[428-18]^+$, 392 $[410-18]^+$. Ir:(KBr) 3540, 3340 (OH), 1730, 1705 (C=O). ^{13}C-Nmr:(DMSO-d_6, 100 MHz) 212.06s, 209.71s, 76.97d, 74.85d, 70.13d, 69.50d, 68.15d, 55.91d, 51.59d, 51.22d, 49.79t, 49.79d, 49.30d, 45.05d, 44.66s, 43.93s, 42.11d, 41.86d, 41.36d, 32.42d, 21.43t, 18.79q, 12.92q, 12.77q, 12.53q. ^{1}H-Nmr (DMSO-d_6, 400 MHz): 4.06d(J=5.2, H-1), 3.21dd (J=4, 5, H-2), 3.28ddd(J=4, 2, 2, H-3), 1.96m(H-4), 1.92m(H-4'), 2.65dd(J=4.8, 10.8, H-5), 3.95d(J=10.8, H-7), 1.55ddd(J=10.5, 10.5, 10, H-8), 2.23dd(J=10.8, 10.8, H-9), 3.82dd(J=10.8, 3, H-11), 3.64d(J=3, H-12), 2.00dd(J=10, 10, H-14), 4.00dd(J=9, 9, H-15), 1.68dd(J=10, 12, H-16), 2.02dd(J=12, 10, H-17), 0.66s (H-18), 0.64s(H-19), 1.62m(H-20), 1.02d(J=5.6, H-21), 2.18m(H-22), 2.44m(H-24), 1.00d(J=6, H-25).

Reduction with $LiAlH_4$ A solution of 20 mg of taccalonolide A in 2 ml of anhydrous tetrahydrofuran was added to 100 mg of $LiAlH_4$ in 2 ml of anhydrous tetrahydrofuran, the mixture was stirred and refluxed for 5 h. Then the reaction mixture was evaporated in vacuo and extracted with EtOAc, the EtOAc solution was concentrated to dryness. The residue was crystallized in MeOH to afford 10 mg (75%) of 6. 6 mp 286-289 °C, ms: 468 $C_{25}H_{40}O_7$, hrms: 450.2593 $C_{25}H_{38}O_6$, $[M-H_2O]^+$. Ir(KBr): 3530, 3350 (OH), 1690 (C=O). ^{13}C-Nmr(DMSO-d_6, 100 MHz): 212.14s, 75.85d, 74.46d, 74.12d, 73.75d, 71.36d, 67.58d, 65.79d, 53.64d, 49.94d, 49.94t, 49.51d, 45.16d, 45.16d, 45.01s, 40.39s, 34.77d, 34.03d, 33.62t, 33.25t, 32.57d, 19.90q, 14.95q, 12.55q, 12.55q. ^1H-Nmr(DMSO-d_6, 400 MHz): 4.13br(H-1), 3.88br(H-3), 1.85m(H-5), 3.17dd(J=2, 2, H-6), 3.22dd(J=2, 10, H-7), 1.87dd(J=11, 11, H-8), 1.53dd(J=10.5, 10.5, H-9), 3.74dd(J=10.5, 4, H-11), 3.58d(J=4, H-12), 2.04dd(J=11, 11, H-14), 3.92dd(J=10, 10, H-15), 1.74dd(J=9, 10, H-16), 1.99dd(J=11, 12, H-17), 0.96s(H-18), 0.68s(H-19), 1.63m(H-20), 1.01d(J=7, H-21), 2.18dd(J=4, 14, H-22), 2.07dd(J=14, 12, H-22′), 2.44m(H-24), 1.03d(J=6, H-25).

Hydrogenation of taccalonolide A 200 mg of 1 in 10 ml of MeOH were hydrogenated over 5% Pd-C (ca. 20 mg) for 12 h. After filtration, the filtrate was concentrated in vacuo to afford crude crystals, and then recrystallization from MeOH gave 150 mg of dihydrotaccalonolide A 7 (yield 75%). 7 mp 230 °C, fdms: 704 $C_{36}H_{48}O_{14}$. Ir(KBr): 3450 (OH), 1770, 1740 (C=O), 1375. ^1H-Nmr ($CDCl_3$, 400 MHz): 4.69d(J=5.5, H-1), 3.46dd(J=3.5, 5.5, H-2), 3.36ddd(J=2, 2, 3.5, H-3), 3.95brd(J=10.5, H-7), 5.31dd(J=11.5, 2.5, H-11), 5.26d(J=2.5, H-12), 5.60dd(J=8.5, 8.5, H-15), 4.09dd(J=11.5, 6.5, H-23), 0.76s(H-19), 0.92s(H-18), 0.84d(J=6.5, H-21), 1.70s(H-27), 1.28s(H-28), 1.99s, 1.97s, 2.17s, 2.15s(CH_3COO).

Reduction with $NaBH_4$ 50 mg of $NaBH_4$ were added to a solution of 50 mg of 1 in 5 ml of MeOH at 0 °C for 5 min, then 20 ml of ice-cold water were added, the aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ extracts were evaporated and separated with tlc (silica gel, $CHCl_3$-EtOH 95:5), 35 mg (70%) of 8 was obtained as a white powder. 8 kept in $CHCl_3$ solution at room temperature for 3 days, 9 was formed and separated in pure state with tlc (yield about 60%). 8 mp 220 °C, $C_{36}H_{48}O_{14}$. ^1H-Nmr ($CDCl_3$, 400 MHz): 4.56d(J=5.5, H-1), 3.40dd(J=5.5, 3.5, H-2), 3.31ddd(J=3.5, 2, 2, H-3), 3.59brd(J=2, H-6 or H-7), 3.29br (H-6 or H-7), 5.25dd(J=11.5, 2.5, H-11), 5.20d(J=2.5, H-12), 5.49dd(J=9.5, 9.5,

H-15), 5.01brs(H-22), 0.94s(H-18 or H-19), 0.96s(H-19 or H-18), 0.86d(J=7, H-21), 1.57s(H-27), 1.27s(H-28), 1.90s, 2.00s, 2.02s, 2.07s(CH_3COO). 9 mp 227-230°C, fdms: 705 $[M+1]^+$, $C_{36}H_{48}O_{14}$; 677 $[(M+1)-28]^+$, $C_{35}H_{48}O_{13}$. Ir(KBr): 3400 (OH), 1810, 1740, 1680 (C=O). ^{1}H-Nmr ($CDCl_3$, 400 MHz): 4.62d(J=5.5, H-1), 3.44dd(J=5.5, 4, H-2), 3.32ddd(J=4, 2, 2, H-3), 5.05dd(J=2.5, 2.5, H-6), 3.76dd(J=2.5, 10.5, H-7), 5.34dd(J=11.5, 3, H-11), 5.20d(J=3, H-12), 4.35brdd(J=10, 7.5, H-15), 4.97d(J=2, H-22), 0.99s(H-18 or H-19), 1.01s(H-19 or H-18), 0.89d(J=7, H-21), 1.63s(H-27), 1.30s(H-28), 1.95s, 2.05s, 2.11s, 2.20s(CH_3COO).

Opening the epoxy ring with HCl A solution of 50 mg of 1 in 2 ml of concentrated HCl was kept at 0°C for 30 min, and the reaction mixture was diluted with water to 20 ml, and extracted with $CHCl_3$, the organic extracts were washed with water and evaporated to dryness. Separation of the residue by tlc (silica gel, $CHCl_3$-EtOH 95:5) yielded pure 10, 25 mg (47%) and 11, 10 mg (19%). 10 mp 235-240°C, fdms: 739 $[M+1]^+$, $C_{36}H_{47}O_{14}Cl$. Ir(KBr): 3450 (OH), 1790, 1760, 1740, 1720, 1680 (C=O), 1370. ^{1}H-Nmr (acetone-d_6, 400 MHz): 5.60d(J=2.5, H-1), 3.83dd(J=10, 2.5, H-2), 4.01ddd(J=10, 11.5, 6, H-3), 3.29dd(J=12, 4.5, H-5), 4.28dd(J=10, 2, H-7), 5.23dd(J=11, 2.5, H-11), 5.27d(J=2.5, H-12), 5.53dd(J=10, 10, H-15), 5.01d(J=1.5, H-22), 0.95s(H-18 or H-19), 1.08s(H-19 or H-18), 0.91d (J=7, H-21), 1.65s(H-27), 1.34s(H-28), 1.92s, 2.05s, 2.15s, 2.19s(CH_3COO). 11 mp 225°C, fdms: 739 $[M+1]^+$, $C_{36}H_{47}O_{14}Cl$. Ir(KBr): 3450 (OH), 1800, 1730 (C=O), 1370. ^{1}H-Nmr ($CDCl_3$, 400 MHz): 4.89d(J=1.5, H-1), 4.17m(2H, H-2 and H-3), 3.23brd(J=12, H-5), 4.09dd(J=10, 3, H-7), 2.75dd(J=11, 11, H-9), 5.27dd(J=11, 2.5, H-11), 5.24d(J=2.5, H-12), 5.53dd(J=10, 10, H-15), 5.06d(J=1.5, H-22), 0.99s(H-18 or H-19), 1.09s(H-19 or H-18), 0.88d(J=7, H-21), 1.62s(H-27), 1.34s (H-28), 1.98s, 1.98s, 2.09s, 2.13s(CH_3COO).

Acetylation of 10 10 mg of 10 were acetylated in usual manner to afford 5 mg of 12 (yield 46%). 12 mp 282-284°C, fdms: 781 $[M+1]^+$, $C_{38}H_{49}O_{15}Cl$. ^{1}H-Nmr (acetone-d_6, 400 MHz): 5.68d(J=2.5, H-1), 4.82dd(J=11, 2.5, H-2), 4.22ddd(J=11, 11.5, 6, H-3), 3.34dd(J=11.5, 5, H-5), 4.26brd(J=10, H-7), 5.21dd(J=11.5, 2.5, H-11), 5.24d(J=2.5, H-12), 5.50dd(J=10, 10, H-15), 4.98d(J=1.5, H-22), 0.98s (H-18 or H-19), 1.06s(H-19 or H-18), 0.88d(J=7, H-21), 1.62s(H-27), 1.31s(H-28), 1.89s, 1.93s, 1.94s, 2.09s, 2.20s(CH_3COO).

Oxidation with $NaIO_4$ 50 mg of 1 and 100 mg of $NaIO_4$ were dissolved in the solution of acetone-H_2O (1:1, 5 ml) and kept in room temperature for 2 weeks,

and then the solution was evaporated in vacuo and the residue was dissolved in $CHCl_3$. The organic solution was evaporated and separated by tlc (silica gel, $CHCl_3$-EtOH 95:5) to give 13, recrystallization from MeOH afforded 10 mg (19.5%) of 13. 13 mp 225-227°C, fdms: 719 $(M+1)^+$, $C_{36}H_{46}O_{15}$. Ir(KBr): 3300-3500 (OH), 1810, 1740 (C=O), 1230, 1040. ^{1}H-Nmr (acetone-d_6, 400 MHz): 4.77d(J=5.5, H-1), 3.41dd(J=5.5, 3, H-2), 3.33ddd(J=3, 2, 2, H-3), 6.00dd(J=11.5, 2.7, H-11), 5.25d(J=2.7, H-12), 5.14dd(J=10, 10, H-15), 5.00d(J=1.5, H-22), 9.71d(J=5.5, CHO), 1.06s(H-18 or H-19), 1.12s(H-18 or H-19), 0.91d(J=7, H-21), 1.59s(H-27), 1.22s(H-28), 1.92s, 1.92s, 2.11s, 2.14s(CH_3COO).

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