Chantriolide C, a New Withanolide Glucoside and a New Spirostanol Saponin from the Rhizomes of *Tacca chantrieri*

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A new withanolide, chantriolide C (1) and a new spirostanol saponin, chantrieroside A (2) were isolated from the rhizomes of *Tacca chantrieri*, together with another five known steroidal compounds. Their structures were established as (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α-hydroxy-with-24-enolide (1) and (25*R*)-spirost-5-en-3-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside (2). The structures of the new saponins were determined by detailed analysis of their 1 dimensional (1D) and 2D NMR spectra, and chemical evidences.

Key words *Tacca chantrieri*; Taccaceae; withanolide; spirostanol saponin

Tacca chantrieri ANDRÉ (Taccaceae) is a perennial plant that grows in southeastern China. Its rhizomes have been employed in traditional Chinese medicine for the treatment of gastric ulcer, enteritis, and hepatitis.[1)] Previously phytochemical investigations revealed some new diarylheptanoids, steroidal constituents including the spirostan, furostan, pseudofurostan, withanolide and pregnane types from *T. chantrieri* rhizomes.[2—5)] Besides family Solanaceae, withanolides have been first found to distribute in a species of the family Taccaceae. As part of our investigation of bioactive constituents from Dai medicine, herein we report the isolation and structure elucidation of steroidal compounds from the rhizomers of this plant. A new withanoside, chantriolide C (**1**) and a new spirostanol saponin, chantrieroside A (**2**) were isolated out, and their structures were established as (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α-hydroxywith-24-enolide (**1**) and (25*R*)-spirost-5-en-3-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside (**2**), on the basis of detailed analysis of their 1 dimensional (1D) and 2D NMR spectra and chemical evidences. Another five known steroidal compounds, chantriolide A (**3**),[6)] taccalonolide O (**4**),[7)] taccalonolide P (**5**),[7)] polyphyllin C (**6**)[8)] and collettiside IV(**7**)[8)] were also isolated and identified by comparison of their spectra data with references.

Results and Discussion

The EtOH extract of *T. chantrieri* rhizomes was suspended in water, followed by partition between petrol ester, $CHCl_3$ and *n*-BuOH successively. The $CHCl_3$ fraction was enriched with steroidal ingredients, which was subjected to multiple chromatographic steps over Si gel and octadecylsilanized (ODS) Si gel, giving compounds **1** (30 mg) and **2** (11 mg), together with other known compounds **3**—**7**.

Compound **1** was isolated as white plates. The IR spectrum of **1** displayed absorption bands of hydroxyl (3440 cm^{-1}), ketone (1733 cm^{-1}), and α,β-unsaturated δ-ketone (1701, 1690 cm^{-1}) functions. The UV spectrum of **1** showed absorption at λ_{max} (MeOH) 217 nm, which also implied the presence of α,β-unsaturated δ-ketone moieties. The molecular formula of **1** was determined to be $C_{38}H_{54}O_{15}$ by high-resolution (HR)-electrospray ionization (ESI)-MS at *m*/*z* 773.3342 ($[M+Na]^+$, Calcd 773.3360).

The ^1H- and ^{13}C-NMR spectral data (Table 1) showed three methyl singlets (δ_H 0.81×2, 2.01; δ_C 12.4, 16.3, 20.2), a secondary methyl (δ_H 0.86, d, J=6.6 Hz; δ_C 12.4), an α,β-unsaturated δ-lactone [δ_C 157.0 (C-24), 122.9 (C-25), 166.2 (C-26)], two epoxy group (δ_H 2.80, 3.11; δ_C 56.2, 54.1, and δ_H 3.54, 3.73; δ_C 55.0, 51.5), two acetoxyl group (δ_H 2.06, 2.11; δ_C 20.7, 21.4, 170.1, 171.0), and a set of hexose group (δ_H 4.42, d, J=7.8 Hz, 3.35—3.82, 5H; δ_C 102.6, 62.0—76.4), revealed **1** to be a typical withanolide glycoside bearing two acetoxyl group and two epoxy group.

The oxymethine proton signal at δ 3.73 (1H, dd, J=4.8, 3.6 Hz, H-2) was correlated to the other two oxymethine protons at δ 4.62 (1H, d, J=4.8 Hz, H-1) and 3.54 (m, H-3) in the ^1H–^1H correlation spectroscopy (COSY) spectrum, suggested a 2α,3α-epoxy moiety. A heteronuclear multiple bond connectivity (HMBC) correlation from H-1 to an acetyl carbonyl carbon signal at δ 170.1 indicated that an acetoxyl group was attached to C-1. Long-range HMBC correlations from H-2, H-4eq (δ 2.38), and Me-19 to the quaternary carbon signal at δ 70.1 gave evidence for the presence of a hydroxyl group at C-5. Thus, the C-1 acetoxy, C-2/C-3 epoxy, and C-5 hydroxy functionalities were assigned for ring A. Long-range HMBC correlations from H-6 (δ 2.80) to C-5 at δ 70.1 indicated another C-6/C-7 epoxy ring. The presence of another acetoxyl group was atributed to C-12 from the HMBC correlation between the signals of the H-12 oxymethine proton at δ 4.96 (1H, br s) and the carbonyl carbon at δ 170.5.

The anomeric proton signal of a β-D-glucopyranosyl moiety at δ 4.42 (d, J=7.8 Hz) showed a long-range correlation with the C-27 carbon resonance at δ 63.0 in the HMBC spectrum, and the presence of D-glucose was also evidenced by results of acid hydrolysis. Accordingly, the planar structure of **1** was determined as shown in Fig. 1.

The stereo configuration of **1** was further convinced by nuclear Overhauser effect (NOEs). NOE correlations from H-1,

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Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (125 MHz) Chemical Shift Assignments of Compounds **1** (in $CDCl_3$) and **2** (in C_5D_5N)

Position	**1**		Position	**2**	
	δ_C	δ_H		δ_C	δ_H
1	71.7	4.62(1H, d, J=4.8 Hz)	1	37.5	1.70, 0.95 (1H each, m)
2	51.5	3.73 (1H, dd, J=4.8, 3.6 Hz)	2	30.1	2.06, 1.84 (1H each, m)
3	55.0	3.54 (1H, m)	3	77.8	3.90 (1H, m)
4-eq	32.6	2.38 (1H, br d, J=15.0 Hz)	4	38.7	2.74, 2.68(1H, br d)
ax		2.03 (1H, m)			
5	70.1		5	140.7	
6	56.2	2.80 (1H, d, J=3.6 Hz)	6	121.8	5.30 (1H, d, J=3.6 Hz)
7	54.1	3.11 (1H, br s)	7	32.3	1.43 (1H, m, overlap)
8	36.0	1.74 (1H, m)	8	31.8	1.64 (1H, m)
9	28.0	2.03 (1H, m)	9	50.3	0.88 (1H, m)
10	39.8		10	37.1	
11-eq	24.6	1.57 (1H, m)	11	21.1	1.42 (1H, m)
ax		1.51 (1H, m,)			
12	75.3	4.96 (1H, br s)	12	39.8	1.68 (1H, m, overlap)
13	46.1		13	40.5	
14	44.3	2.02 (1H, m)	14	56.6	1.05 (1H, m)
15-eq	22.7	1.90 (1H, m)	15	32.2	1.43 (2H, m, overlap)
ax		1.33 (1H, m)			
16-eq	26.5	1.76 (1H, m)	16	81.1	4.82 (1H, m)
ax		1.43 (1H, m)			
17	43.5	1.72 (1H, m)	17	62.2	1.80 (1H m)
18	12.4	0.81 (3H, s)	18	16.3	0.81 (3H, s)
19	16.3	0.81 (3H, s)	19	19.4	1.09 (3H, s)
20	38.1	1.95 (1H, m)	20	42.0	1.94 (1H, m)
21	12.4	0.86 (3H, d, J=6.6 Hz)	21	15.0	0.69 (3H, d, J=4.8 Hz)
22	78.2	4.42 (1H, m)	22	109.2	
23-eq	29.8	2.46 (1H, m)	23	31.7	2.01, 1.86 (1H each, m)
ax		2.03 (1H, m)			
24	157.0		24	29.3	1.56 (2H, m)
25	122.9		25	30.6	1.57 (1H, m)
26	166.2		26	66.9	3.59, 3.50 (1H each, m)
27a	63.0	4.62 (1H, d, J=10.8 Hz)	27	17.3	1.30 (3H, d, J=7.2 Hz)
27b		4.42 (1H, d, J=10.8 Hz)			
28	20.2	2.01 (3H, s)			
Glc-1′	102.6	4.42 (1H, d, J=7.8 Hz)		99.9	4.90 (1H, d, J=7.8 Hz)
2′	73.3	3.37 (1H, dd, J=8.8, 7.8 Hz)		78.6	4.08 (1H, dd, J=9.1, 7.8 Hz)
3′	76.4	3.54 (1H, m)		86.4	4.19 (1H, m)
4′	70.1	3.54 (1H, m)		69.7	4.08 (1H, m)
5′	75.8	3.35 (1H, m)		78.0	3.74 (1H, m)
6′	62.0	3.82 (2H, m)		62.6	4.44 (2H, m)
Rha-1″				102.6	5.81 (1H, br s)
2″				72.5	4.72 (1H, br s)
3″				72.8	4.51 (1H, m)
4″				73.8	4.33 (1H, m)
5″				69.9	4.86 (1H, m)
6″				18.7	1.75 (3H, d, J=6.6 Hz)
Rha-1‴				103.2	5.75 (1H, br s)
2‴				72.1	4.80 (1H, m)
3‴				72.4	4.57 (1H, m)
4‴				84.6	4.44 (1H, m)
5‴				68.7	4.83 (1H, m)
6‴				18.3	1.68 (3H, d, J=6.6 Hz)
Glc-1⁗				106.6	5.24 (1H, d, J=7.8 Hz)
2⁗				76.4	4.08 (1H, m)
3⁗				78.6	4.19 (1H, m)
4⁗				71.4	4.33 (1H, m)
5⁗				78.4	3.74 (1H, m)
6⁗				62.9	4.33 (2H, m)
*CO	171.0				
	170.1				
*CH_3CO	20.7	2.11(3H, s)			
	21.4	2.06 (3H, s)			

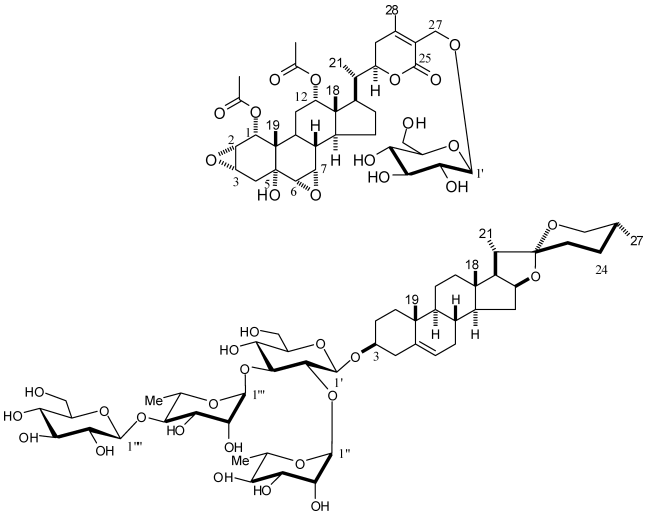

Fig. 1. Structures of Chantriolide C (**1**) and Chantrieroside A (**2**)

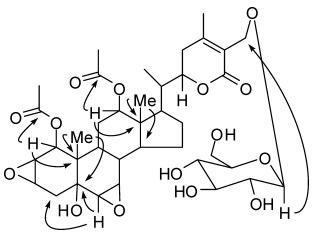

Fig. 2. Key HMBC Correlations Observed in **1**

H-2, H-3, H-6, and H-7 to Me-19, from H-12 to Me-18 were consistent with the 1α, 2α, 3α, 6α, 7α and 12α. The correlations from H-14, H-17 to Me-21 indicated C-20 to be *S* configuration, and the conclusion was also confirmed by comparisons of chemical shift data at C-17, C-20, C-21, C-22 of compound **1** with those of analogues ((+)-6*a*,7*a*-epoxy-5*a*-hydroxy-1-oxowitha-2,24-dienolide).[5] The absolute configuration at the C-22 chiral center was elucidated as *R* by a positive Cotton effect at 251.7 nm in the CD spectrum.[6] The fully assignments of all NMR signals of **1** were carried out by ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY experiments (Table 1), in agreements with those of previously reported withanolide glucosides chantriolides A (**3**) and B,[6] the fully assignments of all NMR signals of **1** were carried out by ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY experiments (Table 1). They all process the similar structure, with slight differences in C-16 substitute group.

Finally, the structure of **1** was established as (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α-hydroxywith-24-enolide.

Compound **2** was obtained as white needles. The IR spectrum of **2** displayed absorption bands of hydroxyl (3347 cm^{-1}) and C–O bands (1047 cm^{-1}) functions. The molecular formula of **2** was determined to be $C_{51}H_{82}O_{21}$ by HR-ESI-MS at *m*/*z* 1053.5208 ([M+Na]$^+$, Calcd 1053.5410).

The ^{1}H- and ^{13}C-NMR spectral data of **2** (Table 1) showed two methyl singlets (δ_H 0.81, 1.09; δ_C 16.3, 19.4), four secondary methyl [(δ_H 0.69, d, J=4.8 Hz; δ_C 15.0), (δ_H 1.68, d, J=6.6 Hz; δ_C 18.3), (δ_H 1.75, d, J=6.6 Hz; δ_C 18.7), and (δ_H 1.30, d, J=7.2 Hz; δ_C 17.3)], a methene [δ_H 5.30, d, J=3.6 Hz; δ_C 121.8 (C-6), δ_C 140.7 (C-5)], and four

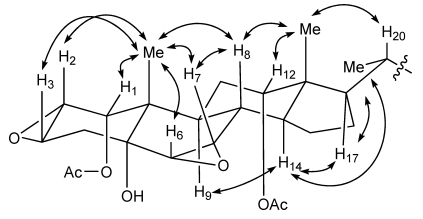

Fig. 3. Important NOE Correlations of **1**

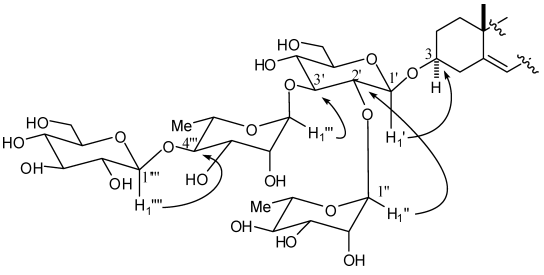

Fig. 4. Key HMBC Correlations Observed in **2**

anomeric protons and carbons of four monosaccharides (δ_H 4.90, d, J=7.8 Hz, δ_C 99.9; δ_H 5.24, d, J=7.8 Hz, δ_C 106.6; δ_H 5.75, s, δ_C 103.2; δ_H 5.81, s, δ_C 102.6), suggested **2** to be a steroidal glycoside. By comparisons of the ^{1}H- and ^{13}C-NMR data with those reported,[3,9,10] the signals of C-24, C-25, C-26, C-27 (δ_C 29.3, 30.6, 66.9, 17.3) revealed Me-27 in an equatorial position rather than in axial position (δ_C 25.8, 26.0, 65.0, 16.1). Thus, the aglycone of **2** was deduced as diosgenin ((25*R*)-spirost-5-en-3β-ol).[9,11]

Results of acid hydrolysis gave only D-glucose and L-rhamnose. In HMBC spectrum, correlations were observed from δ_H 4.90 (H-1′) to δ_C 77.8 (C-3), from δ_H 5.81 (H-1″) to δ_C 78.6 (C-2′), from δ_H 5.75 (H-1‴) to δ_C 86.4 (C-3′), and from δ_H 5.24 (H-1⁗) to δ_C 84.6 (C-4‴). By comparision its NMR data with (25*S*)-spirost-5-en-3-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside,[3] **2** has the same sugar sequences with the known one. Thus, **2** was identified as (25*R*)-spirost-5-en-3-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside.

Experimental

General Optical rotation was measured on a JASCO DIP-360 digital polarimeter (l=5 cm). UV spectra were measured on a Hitachi 200 spectrophotometer; IR spectra were recorded on a Perkin Elmer 781 infrared spectrophotometer; CD spectra were recorded with a Jasco J-720 spectropolarimeter; ^{1}H-NMR (600 MHz), ^{13}C-NMR (125 MHz), and 2D-NMR spectra were recorded on a Inova-600 spectrometer. ESI-MS and HR-ESI-MS were recorded on a JMS-700 mass spectrometer; Column chromatography was performed on silica gel 60 (Merck, 70—230 mesh), MPLC was performed on a BÜCHI B-688 type instrument, and preparative HPLC was performed using an ODS column (YMC-ODS, 20 mm i.d.×250 mm).

Plant Material The rhizomes of *T. chantrieri* were collected in Jing Hong City, Yunnan Province, People's Republic of China, in October 2003, and identified by Prof. Zai-Lin Li, Yunnan Branch of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen has been deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College (voucher No. TC-2003-10-08).

Extraction and Isolation The plant material (dry weight, 13.0 kg) was extracted with 95% EtOH (100 l×2) and 50% EtOH (100 l×1) under reflux. The EtOH extract (1.5 kg) was suspended in water, extracted with petrol ester, $CHCl_3$, *n*-BuOH successively. The $CHCl_3$ part (220 g) was chromatographed on a silica gel column with a stepwise gradient mixture of $CHCl_3$–MeOH (9 : 1, 4 : 1, 3 : 1, 2 : 1, and 1 : 1; 4 l of each), and each fraction was monitored by TLC, and combined to 10 fractions.

Fraction 6 was further chromatographed on a silica gel MPLC column with gradient $CHCl_3$–MeOH (100 : 0—90 : 10) as eluent, and further purified with Sephadex LH-20 column to give **4** (22 mg) and **5** (39 mg). Fraction 7 was further chromatographed on an ODS column with gradient MeOH–H_2O (4 : 6—6 : 4) as eluent, and further purified with Sephadex LH-20 column to give **1** (30 mg). Fraction 8 was recrystalized with 95% EtOH to give **3** (25 mg). Fraction 9 was separated by preparative HPLC using CH_3CN–H_2O (25 : 75) to give **6** (11 mg) and **7** (21 mg). Fraction 10 was separated by preparative HPLC using CH_3CN–H_2O (25 : 75) to give **2** (11 mg).

Compound **1**: White plates, mp 224—226 °C (MeOH); $[\alpha]_D^{25}$ +66.8° (c=0.10, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 251.7 nm (+15.0); IR (film) ν_{max} 3440 (OH), 1733 ((C=O), 1701, 1690 (α,β-unsaturated δ-ketone); ^{1}H- and ^{13}C-NMR ($CDCl_3$), see Table 1; HR-ESI-MS *m*/*z*: 773.2224 (Calcd for $C_{38}H_{54}O_{15}Na$, 773.3360).

Compound **2**: White needle, mp 260—261 °C (MeOH); $[\alpha]_D^{25}$ −93.6° (c=0.10, MeOH); IR (film) ν_{max} 3440 (OH), 2934 (CH), 1635 (C=C); ^{1}H- and ^{13}C-NMR (C_5D_5N), see Table 2; HR-ESI-MS *m*/*z*: 1053.5208 $[M+Na]^+$ (Calcd for $C_{51}H_{82}O_{21}Na$, 1053.5210).

Acid Hydrolysis of 1 and 2 Compounds **1** and **2** (3 mg, each) were dissolved in 2 M CF_3COOH (2 ml) and heated to 120 °C in a sealed tube for 2 h. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in H_2O (1 ml) and filtrated, which was then analyzed by HPLC under the following conditions: column, Cosmosil sugar-D (4.6 mm i.d.×250 mm, 5 μm); solvent, MeCN–H_2O (80 : 20); flow rate, 1.0 ml/min; detection, ELSD and OR. Identification of D-glucose for **1**, L-rhamnose and D-glucose for **2** were carried out by comparison of their retention time and optical rotation with those of authentic samples: t_R (min) 6.3 (L-rhamnose, negative optical rotation), 11.7 (D-glucose, positive optical rotation).

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