Chantriolides A and B, Two New Withanolide Glucosides from the Rhizomes of *Tacca chantrieri*

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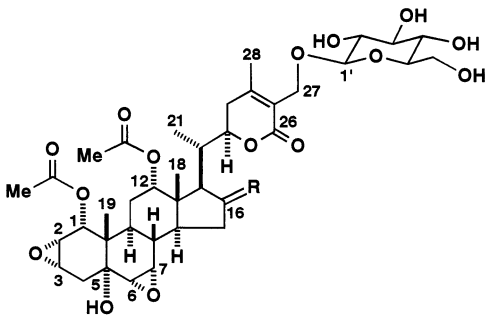
Two new withanolide glucosides, chantriolides A (**1**) and B (**2**), were isolated from the rhizomes of *Tacca chantrieri*. Their structures were determined on the basis of extensive spectroscopic studies and by acid hydrolysis as (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α-hydroxy-16-oxowith-24-enolide (**1**) and (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α,-16β-dihydroxywith-24-enolide (**2**), respectively. It is notable that withanolides, which have been almost exclusively isolated from plants of the family Solanaceae to date, have been found in a species in the family Taccaceae in the present study.

Tacca chantrieri André belongs to the family Taccaceae and is indigenous to the southeast region of the People's Republic of China. The rhizomes of *T. chantrieri* have been used in traditional Chinese medicine for the treatment of gastric ulcer, enteritis, and hepatitis.[1] Previously, we reported the isolation and structural characterization of two new diarylheptanoids and seven diarylheptanoid glucosides from *T. chantrieri* rhizomes, as well as their cytotoxic activities against cultured tumor and normal cells.[2] Phytochemical investigations were also carried out on the rhizomes, with particular inference to the steroidal constituents, which resulted in the isolation of a variety of steroidal glycosides of the spirostan, furostan, pseudofurostan, and pregnane types.[3,4] In a continuation of study on the chemical constituents of *T. chantrieri*, we have further examined the steroidal glycoside-enriched fraction prepared from a MeOH extract of its rhizomes. As a result, two new withanolide glucosides, chantriolides A (**1**) and B (**2**), have been isolated. In this paper, we describe the structural determination of **1** and **2** on the basis of extensive spectroscopic studies and the results of acid hydrolysis.



	R
1	=O
2	α-H, β-OH

The fraction enriched with steroidal glycosides, which was prepared by passing the MeOH extract of *T. chantrieri* rhizomes through a porous-polymer polystyrene resin (Diaion HP-20) column eluted with MeOH−H_2O mixtures followed by MeOH, was subjected to multiple chromatographic steps over Si gel and octadecylsilanized (ODS) Si gel, giving compounds **1** (33 mg) and **2** (43 mg).

Chantriolide A (**1**), obtained as an amorphous solid, was shown to have a molecular formula of $C_{38}H_{52}O_{16}$ on the basis of its HRESITOFMS (*m/z* 787.3157 $[M + Na]^+$), ^{13}C NMR, and DEPT spectral data. Preliminary inspection of the ^1H and ^{13}C NMR spectra of **1** and the results of acid hydrolysis, which gave D-glucose, suggested that **1** was a C_{28} steroid monoglucoside with a six-membered α,β-unsaturated lactone ring in the side chain, and thus a withanolide glucoside. The signals due to the following functional groups were assigned by comparison with the ^1H and ^{13}C NMR spectra of **1** with those of previously reported withanolide glucosides:[5−7] a secondary methyl group [δ_H 0.96 (3H, d, J = 7.1 Hz, Me-21)], an olefinic methyl group [δ_H 2.02 (3H, s, Me-28)], an oxymethine proton [δ_H 5.12 (1H, m, H-22)], a methine proton [δ_H 2.43 (1H, m, H-20)] coupled with Me-21 and H-22, a methylene group attached to a deshielded moiety [δ_H 2.22 (2H, m, H_2-23)], an isolated oxymethylene group [δ_H 5.00 and 4.74 (each 1H, ABq, J = 10.9 Hz, H_2-27)], and an α,β-unsaturated carbonyl group [δ_C 165.5 (C=O, C-26), 156.8 (C-24), and 123.4 (C-25)]. The anomeric proton signal of a β-D-glucopyranosyl moiety at δ 4.99 (d, J = 7.7 Hz) showed a long-range correlation with the C-27 carbon resonance at δ 63.3 in the HMBC spectrum. Thus, the structure of the C_9 side chain moiety was shown to be the same as that of the 27-glucosyloxywithanolide derivatives reported from certain species in the Solanaceae.[5−7] Furthermore, two acetyl groups (δ_H 2.17/δ_C 170.5 and 21.2; δ_H 2.13/δ_C 170.3 and 20.4), a carbonyl group (δ_C 215.6), and a tertiary hydroxyl group [δ_H 3.15 (1H, s) in DMSO-d_6, disappeared on the addition of the vapor of HCl/δ_C 70.2 (C)] in **1** were identified; however, the tetracyclic basic steroid moiety (C-1−C-19) could not be determined by conventional spectral data comparison with the known withanolides.[5−7] The gross structure of **1** was confirmed by detailed interpretation of various 2D NMR spectra such as its $^1H-^1H$ COSY, HMQC, HMBC, and ROESY spectra. The oxymethine proton signal at δ 3.87 (dd, J = 5.3, 3.6 Hz, H-2) was correlated to the other two oxymethine protons at δ 4.89 (d, J = 5.3 Hz, H-1) and 3.55 (br d, J = 3.6 Hz, H-3) in the $^1H-^1H$ COSY spectrum. The signal at δ 3.55 had spin-coupling links with a geminal pair of the protons appearing at δ 2.36 and 2.01

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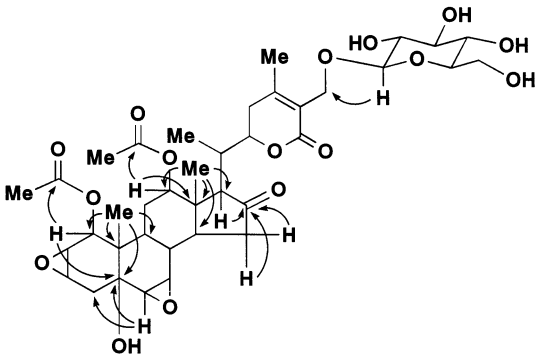

Figure 1. Important HMBC correlations of **1**.

(ABq-like, J = 15.5 Hz, H_2-4). In the HMBC spectrum, the δ 4.89 resonance showed correlation peaks with the signals of the quaternary carbon at δ 40.5 (C-10) and the tertiary methyl carbon at δ 16.3 (C-19). The H-2 and H-3 oxymethine proton signals were correlated to the one-bond coupled carbon signals at δ 51.6 and 55.5, respectively, in the HMQC spectrum, which was indicative of a C-2/C-3 epoxy ring. An HMBC correlation from H-1 to the acetyl carbonyl carbon signal at δ 170.3 indicated that an acetoxyl group was attached to C-1. Long-range HMBC correlations from H-1, H-4eq (δ 2.36), and Me-19 to the quaternary carbon signal at δ 70.2 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.

The spin-coupling correlations of the B, C, and D rings were also disclosed by analysis of the ^{1}H−^{1}H COSY spectrum, starting from the oxymethine proton signal at δ 5.10 (br s, H-12), which showed an HMBC correlation with the quaternary carbon signal at δ 46.4 (C-13). The signals for the two mutually coupled oxymethine protons at δ 2.96 (d, J = 3.6 Hz) and 3.14 (m) were assigned to H-6 and H-7, respectively, which were correlated to the δ 56.7 and 53.9 resonances in the HMQC spectrum. This was indicative of a C-6/C-7 epoxy ring. The presence of an acetoxyl group at C-12 was evident from an HMBC correlation between the signals of the carbonyl carbon at δ 170.5 and the H-12 oxymethine proton. The proton spin-coupling correlation ended at H_2-15, and the signals for H_2-15 at δ 2.49 (dd, J = 17.8, 7.8 Hz) and 2.12 (dd, J = 17.8, 13.1 Hz) and H-17 at δ 2.65 (d, J = 8.6 Hz) displayed $^2J_{C,H}$ correlations with the carbonyl carbon signals at δ 215.6, consistent with the presence of a carbonyl group at C-16. The ring junction of the A and B rings was assigned by long-range correlations from H-6 to C-4 (δ 33.1) and C-5, and from Me-19 to C-1 (δ 72.3) and C-9 (δ 28.7). Accordingly, the planar structure of **1** was determined as shown in Figure 1.

ROE correlations from H-8 to Me-18 and Me-19, and from H-14 to H-9 and H-17, in the ROESY spectrum indicated the B/C *trans* and C/D *trans* ring junctions. Further NOEs from H-1, H-2, H-3, H-6, and H-7 to Me-19, and from H-12 to Me-18, were consistent with the 1α, 2α, 3α, 5α, 6α, 7α, and 12α configurations. The large J value between H-17 and H-20 (J = 8.6 Hz) indicated that the H_{17}−C_{17}−C_{20}−H_{20} part was preferably *trans*-oriented, and ROE correlations from H-20 to both H-12 and Me-18 made it possible to confirm the 17β and 20*S* configurations. The absolute configuration at the C-22 chiral center was elucidated as *R* by a positive Cotton effect at 252.6 nm ($\Delta\epsilon$ 11.1) in the CD spectrum.[8,9] Accordingly, the structure of **1** was formulated as (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α-hydroxy-16-oxo-with-24-enolide.

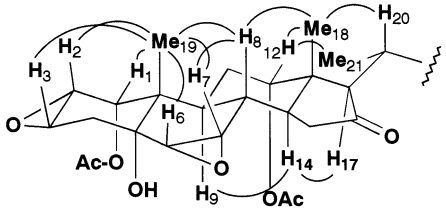

Figure 2. Important ROE correlations of **1**.

Chantriolide B (**2**) was isolated as an amorphous solid. The HRESITOFMS showed an accurate $[M + Na]^+$ ion at m/z 789.3322, corresponding to the empirical molecular formula $C_{38}H_{54}O_{16}$. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** showed their considerable structural similarity. However, the molecular formula of **2** had two additional hydrogen atoms and the C-16 carbonyl carbon signal was not observed in the ^{13}C NMR spectrum of **2**. The ^{1}H NMR spectrum of **2** measured in DMSO-d_6 showed two exchangeable proton signals at δ 4.78 (br d, J = 4.6 Hz) and 3.10 (br s, H-5) arising from the aglycon moiety, as well as five protons from a glucosyl moiety. The δ 4.78 resonance was shown to be coupled with the multiplet signal centered at δ 4.13, which was changed to a doublet of doublets of doublets (J = 7.6, 7.4, 3.4 Hz) by addition of the vapor of HCl and was assigned to H-16. Thus, it was revealed that **2** differed from **1** in having a hydroxyl group at C-16 instead of a carbonyl group. A key ROE between H-14 and H-16 indicated that the configuration of the C-16 hydroxyl group was β-oriented. The structure of **2** was established as (22*R*)-1α,12α-diacetoxy-2α,3α;6α,7α-diepoxy-27-[(β-D-glucopyranosyl)oxy]-5α,16β-dihydroxywith-24-enolide.

Chantriolides A (**1**) and B (**2**) were found to be minor components relative to the spirostanol and furostanol saponins and the diarylheptanoids previously isolated from *T. chantrieri* rhizomes. However, it is notable that withanolides, which have been almost exclusively isolated from plants of the family Solanaceae previously,[10,11] have been now found in a species of the family Taccaceae.

Experimental Section

General Experimental Procedures. The instruments and experimental procedures were the same as described in the previous paper,[2] except for HRESITOFMS measurements, which were recorded on a Micromass LCT (Manchester, UK) mass spectrometer.

Plant Material. The rhizomes of *T. chantrieri* were collected in Si Mao City, Yunnan Province, People's Republic of China, in October 1996, and identified by one of the authors (Y.S.). A voucher specimen has been deposited in the laboratory of Y.S. (voucher No. TC-96-003, Laboratory of Medicinal Plant Science).

Extraction and Isolation. The plant material (dry wt, 7.3 kg) was extracted with hot MeOH (3 L × 2). The MeOH extract (630 g) was passed through a Diaion HP-20 (2.2 kg, Mitsubishi-Chemical, Tokyo, Japan) column, eluting with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (4 L of each). The 50% MeOH of the eluate portion (70 g) was chromatographed on a Si gel (200−400 mesh, Fuji-Silysia Chemical, Aichi, Japan) column, eluting 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 finally with MeOH. The $CHCl_3$−MeOH (4:1) eluate portion (10 g) was subjected to column chromatography on ODS Si gel, eluting with MeOH−H_2O (1:2), and Si gel with $CHCl_3$−MeOH−H_2O (60:10:1, 3 L) to give **1** (33 mg) and **2** (43 mg).

Compound 1: amorphous solid; $[\alpha]^{25}_D$ −4.0° (*c* 0.10, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 251.7 nm (+11.1), 295.5 nm (−20.0); IR (film) ν_{max} 3454 (OH), 2927 (CH), 1732 and 1715 (C=O), 1652 (C=C), 1246, 1030 cm^{-1}; ^{1}H and ^{13}C NMR

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** in C_5D_5N

	1				**2**		
position	^{1}H (ppm)	*J* (Hz)	^{13}C	position	^{1}H (ppm)	*J* (Hz)	^{13}C
1	4.89 d	5.3	72.3	1	4.89 d	5.1	72.4
2	3.87 dd	5.3, 3.6	51.6	2	3.87 dd	5.1, 3.6	51.6
3	3.55 br d	3.6	55.5	3	3.55 br d	3.6	55.5
4eq	2.36 br d	15.5	33.1	4eq	2.35 br d	15.6	33.1
ax	2.01 br d	15.5		ax	2.01 m		
5			70.2	5			70.3
6	2.96 d	3.6	56.7	6	2.95 d	3.3	56.6
7	3.14 m		53.9	7	3.14 m		54.2
8	1.95 m		35.3	8	1.87 m		36.1
9	2.42 m		28.7	9	2.42 m		28.7
10			40.5	10			40.4
11eq	1.82 m		24.2	11eq	1.77 m		24.4
ax	1.57 m			ax	1.52 m		
12	5.10 br s		74.4	12	5.09 br s		76.1
13			46.4	13			46.2
14	2.60 m		40.4	14	2.07 m		43.6
15a	2.49 dd	17.8, 7.8	37.4	15a	2.53 m		36.6
b	2.12 dd	17.8, 13.1		b	1.69 m		
16			215.6	16	4.26 m		69.3
17	2.65 d	8.6	56.1	17	1.73 m		48.6
18	0.93 s		14.0	18	1.17 s		13.4
19	0.79 s		16.3	19	0.76 s		16.2
20	2.43 m		35.0	20	2.89 m		33.3
21	0.96 d	7.1	12.7	21	1.06 d	6.7	11.8
22	5.12 m		77.3	22	5.22 m		77.7
23 (2H)	2.22 m		31.7	23eq	2.42 m		30.3
				ax	2.23 m		
24			156.8	24			157.1
25			123.4	25			123.4
26			165.5	26			165.9
27a	5.00 d	10.9	63.3	27a	5.02 d	10.7	63.3
b	4.74 d	10.9		b	4.76 d	10.7	
28	2.02 s		20.3	28	2.12 s		20.6
Ac	2.13 s		20.4	Ac	2.13 s		20.3
			170.3				170.3
	2.17 s		21.2		2.07 s		21.1
			170.5				170.3
1′	4.99 d	7.7	104.7	1′	4.99 d	7.8	104.7
2′	4.04 dd	7.9, 7.7	75.2	2′	4.04 dd	7.8, 7.8	75.1
3′	4.26 m		78.4	3′	4.27 m		78.5
4′	4.25 m		71.7	4′	4.26 m		71.6
5′	3.97 m		78.6	5′	3.96 m		78.6
6′a	4.56 dd	11.8, 2.2	62.8	6′a	4.56 dd	11.8, 2.0	62.7
b	4.39 dd	11.8, 5.3		b	4.40 dd	11.8, 5.3	

(C_5D_5N), see Table 1; HRESITOFMS *m*/*z* 787.3157 $[M + Na]^+$ (calcd for $C_{38}H_{52}O_{16}Na$, 787.3153).

Acid Hydrolysis of 1. A solution of **1** (14 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Si gel eluting with $CHCl_3$−MeOH (9:1 to 1:1, 100 mL of each) to give a sugar fraction (1.5 mg). The sugar fraction was dissolved in H_2O (1 mL) and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 SG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN−H_2O (17:3); flow rate, 0.9 mL/min; detection, RI and OR. Identification of D-glucose present in the sugar fraction was carried out by comparison of its retention time and optical rotation with that of an authentic sample; t_R (min) 18.74 (D-glucose, positive optical rotation).

Compound 2: amorphous solid; $[\alpha]_D^{25}$ +54.0° (*c* 0.10, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 252.6 nm (+16.6); IR (film) ν_{max} 3443 (OH), 2938 (CH), 1732 and 1714 (C=O), 1652 (C=C), 1249, 1030 cm^{-1}; ^{1}H and ^{13}C NMR (C_5D_5N), see Table 1; HRESITOFMS *m*/*z* 789.3322 $[M + Na]^+$ (calcd for $C_{38}H_{54}O_{16}$-Na, 789.3310).

Acid Hydrolysis of 2. A solution of **2** (15 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (2.0 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose; t_R (min) 18.68 (D-glucose, positive optical rotation).

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