

WITHANOLIDE GLUCOSIDES FROM THE RHIZOMES OF *Tacca chantrieri*

Vu Thi Quynh Chi, Pham Hai Yen, Duong Thi Dung, Nguyen Xuan Nhiem, Hoang Le Tuan Anh,
Dan Thi Thuy Hang, Chau Van Minh, Phan Van Kiem*

Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST)

Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

By various chromatographic separations, three withanolide glucosides, chantriolides A-C were isolated from the rhizomes of *Tacca chantrieri*. Their structures were elucidated by 1D-, 2D-NMR, and MS spectra and in comparison with the reported data.

Keywords. *Tacca chantrieri*, chantriolide A, chantriolide B, chantriolide C.

1. INTRODUCTION

Tacca chantrieri André (Taccaceae) is perennial plant that grows in Vietnam and southeastern China. Its rhizomes have been used in traditional medicine for the treatment of gastric ulcer, enteritis, and hepatitis. The phytochemical investigations of this plant confirmed the presence of diarylheptanoids [1], steroidal saponins [2-4], sterol saponins [5]. In addition, these compounds showed cytotoxic activity [1]. This paper deals with the isolation and structure determination of compounds from the rhizomes of *T. chantrieri*.

2. MATERIAL AND METHODS

2.1. Plant materials

The rhizomes of *Tacca chantrieri* were collected in Tamdao National park, Vinhphuc on December 10, 2013. The scientific name was identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources, VAST. A voucher specimen (NCCT-TC.02) was deposited at Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

Chemical shifts are reported in parts per million from TMS. All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ¹H-NMR and 125 MHz for ¹³C-NMR). NMR measurements, including ¹H-NMR, ¹³C-NMR, HSQC, HMBC, and COSY experiments, were carried out using 5-mm probe tubes at temperature

of 22.2 °C in CD₃OD solutions, with TMS as the internal standard. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Column chromatography was performed using a silica-gel (Kieselgel 60,70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F₂₅₄ (0.25 mm, Merck) and RP-18 F₂₅₄S plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried rhizomes of *T. chantrieri* (4.5 kg) were extracted with MeOH three times using sonicator to yield 300.0 g of a dark solid extract, which was then suspended in water and successively partitioned with CH₂Cl₂ and ethyl acetate (EtOAc) to obtain CH₂Cl₂ (TCRA, 70.0 g), ethyl acetate (TCRB, 120.0 g), and water (TCRC, 110.0 g) layers after removal solvent *in vacuo*. The ethyl acetate layer (TCRB) was chromatographed on a silica gel column eluting gradient solvent of CH₂Cl₂:methanol (100:1 ~ 0:1, v/v) to obtain four fractions, TCRB1-TCRB4. The TCRB3 fraction was chromatographed on a RP-18 column eluting with MeOH:water (1:2, v/v) to give three smaller fractions, TCRB3.1-TRCB3.3. The TCRB3.1 fraction was chromatographed on a silica gel column eluting with ethyl acetate:methanol (13:1, v/v), then further purified by a silica gel column, using CH₂Cl₂:methanol (9:1, v/v) as eluents, to obtain compounds **2** (21.0 mg) and **3** (18.0 mg). The TCRB3.2 was chromatographed on a silica gel column eluting with ethyl acetate:methanol (6:1, v/v) to yield compound **1** (32.0 mg).

Chantriolide A (1): White amorphous powder; $[\alpha]_D^{25}$: -20.0 (c 0.1, MeOH), ESI-MS m/z 787 $[M+Na]^+$, 1H - and ^{13}C -NMR (CD_3OD), see table 1.

Chantriolide B (2): White amorphous powder; $[\alpha]_D^{25}$: $+47.0$ (c 0.1, MeOH), ESI-MS m/z 789

$[M+Na]^+$, 1H - and ^{13}C -NMR (CD_3OD), see table 1.

Chantriolide C (3): White amorphous powder; $[\alpha]_D^{25}$: $+62.0$ (c 0.1, MeOH), 1H - and ^{13}C -NMR (CD_3OD), see table 1.

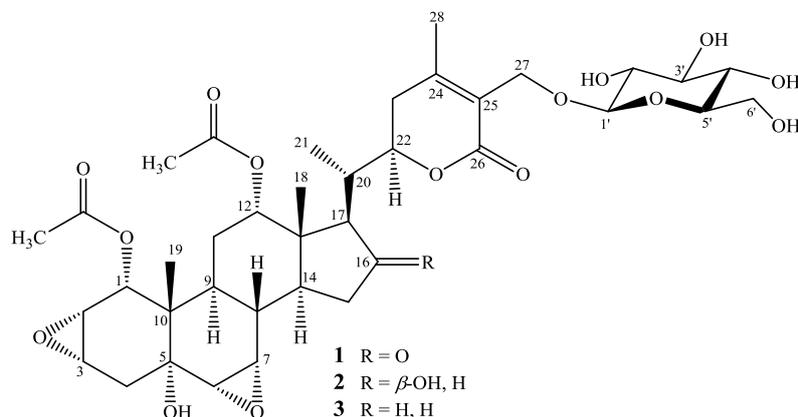


Figure 1: Chemical structure of compounds 1-3 from *T. chantrieri*

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. Molecular formula was determined to be $C_{38}H_{52}O_{16}$ in the basis of its ESI-MS (m/z at 787 $[M+Na]^+$) and ^{13}C -NMR spectrum. The 1H -NMR of compound **1** showed methyl groups at δ_H 0.91 (s), 1.07 (s), 2.04 (s), 2.14 (s), 2.19 (s), and 1.00 (d, $J = 7.0$ Hz) (each, 3H), assigned to a withanolide skeleton and two acetyl groups; one anomeric proton at δ_H 4.35 (d, $J = 7.5$ Hz), assigned to a sugar moiety. The ^{13}C -NMR and DEPT spectra of **1** showed the signal of 36 carbons, including four carbonyl at δ_C 167.89, 171.71, 172.34, and 217.95, five non-protonated at δ_C 41.20, 47.60, 71.66, 123.71, and 159.70, seventeen methine at δ_C 29.55, 35.92, 36.31, 41.12, 52.40, 54.44, 56.50, 57.18, 57.50, 71.59, 73.22, 75.05, 75.71, 77.96, 77.94, 78.75, and 103.87, six methylene at δ_C 24.84, 32.76, 33.49, 38.12, 62.76, and 63.52, six methyl carbons at δ_C 13.20, 14.50, 16.40, 20.50, 20.67, and 21.57. The 1H and ^{13}C -NMR data of compound **1** were similar to those of chantriolide A [6]. The HMBC correlations between H-19 (δ_H 0.91) and C-1 (δ_C 73.22)/C-5 (δ_C 71.66)/C-9 (δ_C 29.55)/C-10 (δ_C 41.20); H-1 (δ_H 4.72) and acetyl carbonyl (δ_C 171.71); H-18 (δ_H 1.07) and C-12 (δ_C 75.71)/C-13 (δ_C 47.60)/C-14 (δ_C 41.12)/C-17 (δ_C 57.18); H-12 (δ_H 5.03) and acetyl carbonyl (δ_C 172.34) suggested two acetyl groups at C-1 and C-12. The sugar in **1** was determined to be β -D-glucopyranosyl by its NMR data (δ_C 103.87, 75.05, 77.96, 71.59, 77.94 and 62.76) and coupling constant between glc H-1 and glc H-2, $J = 7.5$ Hz. In addition, the position of

this sugar at C-27 was confirmed by the HMBC correlations between H-27 (δ_H 4.48 and 4.65) and glc C-1' (δ_C 103.87) as well as glc H-1' (δ_H 4.35) and C-27 (δ_C 63.62). Thus, the structure of **1** was elucidated to be chantriolide A [6].

Compound **2** was also obtained as a white amorphous powder. The ^{13}C -NMR and DEPT spectra of **2** exhibited the presence of three carbonyl, five non-protonated, eighteen methine, six methylene, and six methyl carbons. The analytical NMR data of compound **2** indicated the structure of **2** was similar to those of **1** except for the difference of a hydroxyl group at C-16. The position of this hydroxyl group was confirmed by the HMBC correlations between H-14 (δ_H 1.90)/H-15 (δ_H 1.46 and 2.48)/H-17 (δ_H 1.76) and C-16 (δ_C 70.89). Consequently, the structure of compound **2** was elucidated to be chantriolide B [6].

Comparison of the 1H and ^{13}C -NMR spectra of compound **3** with to those of compounds **1** and **2** showed the similar. However, the ^{13}C -NMR spectrum of compound **3** showed an addition of C-16 methylene carbon, replaced to C-16 carbonyl in **1**. The position of all functional groups in **3** was confirmed by the HMBC correlations. Thus, the structure of **3** was determined to be chantriolide C [7].

Acknowledgments. This research was supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2012.22. The authors would like to thank Mr Dang Vu Luong, Institute of Chemistry for recording NMR.

Table 1: The NMR data for compounds **1-3** and reference compounds

C	1			2			3		
	$^{\#}\delta_{\text{C}}$	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (mult, J, Hz)	$^{\%}\delta_{\text{C}}$	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (mult, J, Hz)	$^{\text{S}}\delta_{\text{C}}$	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (mult, J, Hz)
1	72.3	73.22	4.72 (d, 5.5)	72.4	73.36	4.70 (d, 5.5)	71.7	73.37	4.70 (d, 5.5)
2	51.6	52.40	3.72 (dd, 4.0, 5.5)	51.6	52.44	3.71 (dd, 4.0, 5.5)	51.5	52.43	3.70 (dd, 4.0, 5.5)
3	55.5	56.50	3.61 (br d, 4.0)	55.5	56.52	3.61 (br d, 4.0)	55.0	56.51	3.61 (br d, 4.0)
4	33.1	33.49	2.24 (dd, 2.0, 15.0) 2.29 (d, 15.0)	33.1	33.55	2.21 (dd, 2.0, 14.0) 2.29 (d, 14.0)	32.6	33.52	2.21 (br d, 15.5) 2.29 (d, 15.5)
5	70.2	71.66	-	70.3	71.69	-	70.1	71.65	-
6	56.7	57.50	2.93 (d, 3.5)	56.6	57.50	2.90 (d, 3.5)	56.2	57.43	2.90 (d, 3.5)
7	53.9	55.44	3.18 (t, 3.0)	54.2	55.65	3.18*	54.1	55.69	3.17*
8	35.3	35.92	2.11 (m)	36.1	36.84	1.97*	36.0	37.11	1.90 (m)
9	28.7	29.55	2.18 (m)	28.7	29.54	1.99*	28.0	29.35	2.01 (m)
10	40.5	41.20	-	40.4	41.08	-	39.8	41.03	-
11	24.2	24.84	1.65 (br d, 14.5) 1.75 (dd, 12.5, 14.5)	24.4	25.02	1.58 (m) 1.68 (m)	24.6	25.43	1.57 (m) 1.65 (m)
12	74.4	75.71	5.03 (br s)	76.1	77.29	4.95 (br s)	75.3	77.12	4.99 (br s)
13	46.4	47.60	-	46.2	47.11	-	46.1	47.21	-
14	40.4	41.12	2.43 (m)	43.6	44.13	1.90 (m)	44.3	45.92	2.01 (m)
15	37.4	38.12	2.19 (m) 2.42 (m)	36.6	36.79	1.46 (dt, 4.0, 13.5) 2.48 (m)	22.7	23.67	1.45 (m) 1.89 (m)
16	215.6	217.95	-	69.3	70.89	4.28 (m)	26.5	27.40	1.49 (m) 1.92 (m)
17	56.1	57.18	2.71 (d, 8.0)	48.6	49.46	1.76 (dd, 7.5, 11.5)	43.5	44.73	1.89 (m)
18	14.0	14.50	1.07 (s)	13.4	13.49	1.10 (s)	12.4	12.32	0.91 (s)
19	16.3	16.40	0.91 (s)	16.2	16.34	0.89 (s)	16.3	16.37	0.89 (s)
20	35.0	36.31	2.37 (m)	33.3	34.24	2.50 (m)	38.1	39.68	1.99 (m)
21	12.7	13.20	1.00 (d, 7.0)	11.8	12.05	0.96 (d, 6.5)	12.4	12.74	0.93 (d, 6.5)
22	77.3	78.75	4.96 (m)	77.7	79.40	4.90 (m)	78.2	79.87	4.53 (m)
23	31.7	32.76	2.38* 2.54*	30.3	31.19	2.35* 2.61 (dd, 14.0, 17.5)	29.8	30.64	2.28* 2.58 (dd, 13.5, 17.5)
24	156.8	159.70	-	157.1	160.32	-	157.0	160.22	-
25	123.4	123.71	-	123.4	123.74	-	122.9	123.65	-
26	165.5	167.89	-	165.9	168.66	-	166.2	168.37	-
27	63.3	63.52	4.48 (d, 11.5) 4.65 (d, 11.5)	63.3	63.59	4.51 (d, 11.5) 4.64 (d, 11.5)	63.0	63.58	4.46 (d, 11.0) 4.64 (d, 11.0)
28	20.3	20.67	2.14 (s)	20.6	20.77	2.18 (s)	20.2	20.75	2.17 (s)
1-CH ₃ CO	170.3	171.71	-	170.3	171.69	-	170.1	171.69	-
1-CH ₃ CO	20.4	20.50	2.04 (s)	20.3	20.46	2.02 (s)	20.7	20.49	2.03 (s)
12-CH ₃ CO	170.5	172.34	-	170.3	172.17	-	171.0	172.20	-
12-CH ₃ CO	21.2	21.57	2.19 (s)	21.1	21.38	2.13 (s)	21.4	21.38	2.14 (s)
27-OGlc									
1'	104.7	103.87	4.35 (d, 7.5)	104.7	103.92	4.35 (d, 8.0)	102.6	103.93	4.35 (d, 8.0)
2'	75.2	75.05	3.19 (t, 7.5)	75.1	75.01	3.17 (t, 8.0)	73.3	74.97	3.19 (m)
3'	78.4	77.96	3.38*	78.5	78.06	3.39 (t, 8.0)	76.4	77.99	3.37 (t, 8.0)
4'	71.7	71.59	3.32 (m)	71.6	71.59	3.32 (m)	70.1	71.54	3.32 (m)
5'	78.6	77.94	3.31 (m)	78.6	78.03	3.29 (m)	75.8	77.97	3.30 (m)
6'	62.8	62.76	3.71 (dd, 5.5, 11.5) 3.89 (dd, 1.5, 11.5)	62.7	62.75	3.71 (dd, 5.5, 12.0) 3.88 (dd, 2.0, 12.0)	62.0	62.72	3.69 (dd, 5.0, 12.0) 3.88 (dd, 2.0, 12.0)

^{a)} recorded in CD₃OD, ^{b)} 125 MHz, ^{c)} 500 MHz, *overlapped signals, [#] δ_{C} of chantriolide A [6], [%] δ_{C} of chantriolide B [6], ^S δ_{C} of chantriolide C [7].

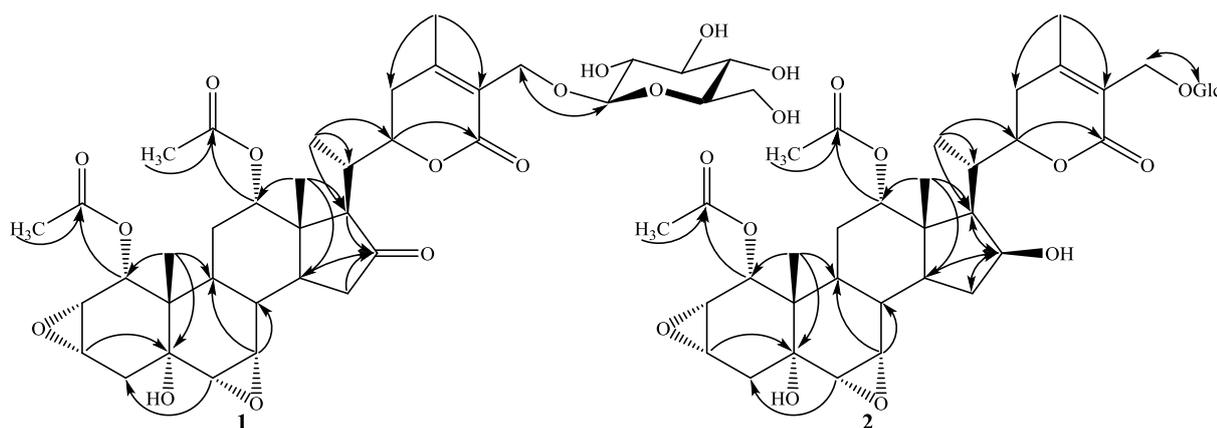


Figure 2: The key HMBC correlations of **1** and **2**

REFERENCES

1. A. Yokosuka, Y. Mimaki, H. Sakagami, Y. Sashida. *New diarylheptanoids and diarylheptanoid glucosides from the rhizomes of Tacca chantrieri and their cytotoxic activity*, J. Nat. Prod., **65**, 283-289 (2002).
2. T. L. Tinley, D. A. Randall-Hlubek, R. M. Leal, E. M. Jackson, J. W. Cessac, J. C. Quada, Jr., T. K. Hemscheidt, S. L. Mooberry. *Taccalonolides E and A: Plant-derived steroids with microtubule-stabilizing activity*, Cancer Res, **63**, 3211-3220 (2003).
3. H. H. Shwe, M. Aye, M. M. Sein, K. T. Htay, P. Kreitmeier, J. Gertsch, O. Reiser, J. Heilmann. *Cytotoxic steroidal saponins from the rhizomes of Tacca integrifolia*, Chem. Biodivers, **7**, 610-622 (2010).
4. A. Yokosuka, Y. Mimaki, Y. Sashida. *Taccasterosides A-C, Novel C28-sterol oligoglucosides from the Rhizomes of Tacca chantrieri*, Chemical and Pharmaceutical Bulletin, **52**, 1396-1398 (2004).
5. A. Yokosuka, Y. Mimaki, C. Sakuma, Y. Sashida. *New glycosides of the campesterol derivative from the rhizomes of Tacca chantrieri*. Steroids, **70**, 257-265 (2005).
6. A. Yokosuka, Y. Mimaki, Y. Sashida. *Chantriolides A and B, two new withanolide glucosides from the rhizomes of Tacca chantrieri*, J. Nat. Prod., **66**, 876-878 (2003).
7. L. Zhang, J. -Y. Liu, L. -Z. Xu, S. -L. Yang. *Chantriolide C, a new withanolide glucoside and a new spirostanol saponin from the rhizomes of Tacca chantrieri*, Chemical and Pharmaceutical Bulletin, **57**, 1126-1128 (2009).

Corresponding author: **Phan Van Kiem**

Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: phankiem@yahoo.com.