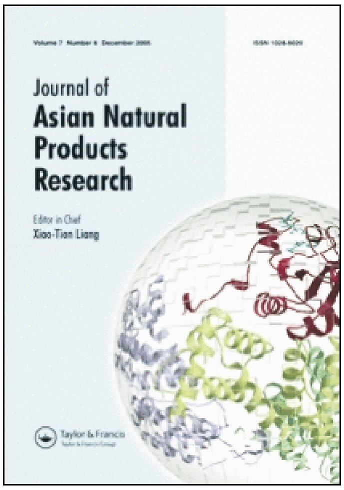

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One new bufadienolide from Chinese drug “Chan’Su”

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A new bufadienolide named 16β-acetoxy-bufarenogin (**1**), together with six known bufadienolides, namely, 11α,12β-dihydroxy-bufalin (**2**), bufotalin (**3**), hellebrigenin (**4**), desacetylbufotalin (**5**), gamabufotalin (**6**), and resibufagin (**7**) were isolated from Chan’Su. Of these, **2** was a new natural product. Their structures were elucidated by spectral methods. The cytotoxic activities *in vitro* of these compounds have been assayed against HeLa cell line. They all showed strong cytotoxic activities.

Keywords: Chan’Su; bufadienolides; 16β-acetoxy-bufarenogin; 11α, 12β-dihydroxy-bufalin; cytotoxic activity

1. Introduction

Bufadienolides represent a type of steroids with A/B *cis* and C/D *cis* structures and a β-2-pyrone ring at the 17-position. These compounds possess potent cardiotonic, blood pressure-stimulating, antiviral, and local anaesthetic activities. More than 300 bufadienolides have been isolated from natural sources including plants and animals, which have been reported to have significant anti-tumour activities.[1–4] In this paper, seven bufadienolides, namely, 16β-acetoxy-bufarenogin (**1**), 11α,12β-dihydroxy-bufalin (**2**), bufotalin (**3**), hellebrigenin (**4**), desacetylbufotalin (**5**), gamabufotalin (**6**), and resibufagin (**7**)[6–9] were isolated from Chan’Su. Amongst these, **1** was a new compound, and **2** was a new natural product. This report describes their isolation and characterisation.

2. Results and discussion

Compound **1**, with $[\alpha]_D^{20} + 23.0$ (c 0.3, CH_3OH), was obtained as colourless plate (CH_3OH), and positive to Liberman–Burchard reaction. The molecular formula was established as $C_{26}H_{34}O_8$ by HRFAB-MS at *m/z* 474.2303 $[M]^+$. The UV and IR absorption spectra suggested the presence of 2-pyrone ring (295 nm, 1635 cm^{-1}) and ketone group (1712 cm^{-1}).[5] In the ^1H NMR spectrum, H-21, H-22, H-23 signals (7.46, 8.10 and 6.22) were characteristic of the 2-pyrone ring of bufadienolide.[5] All the information above showed that compound **1** was a bufadienolide (Figure 1). In the HMBC spectrum, the correlations between the proton signal at δ 3.47 and C-20 (δ 116.8), C-21 (δ 151.8), and C-22 (δ 149.9) indicated that the proton at δ 3.47 was assigned to H-17. The methyl proton signal at δ 0.42 was assigned to H-18 according to its HMBC correlations with C-17 (δ 51.3), C-12 (δ 77.9), C-13 (δ 59.8), and C-14 (δ 81.5). In the HMBC spectrum the correlation between H-12 (δ 3.99) and C-11 (δ 210.2) indicated that C-11 was a carbonyl carbon. The signal at δ 73.5 was assigned to C-16 since its corresponding proton signal at δ 5.44 was coupled with H-17. The HMBC correlations between H-16 (δ 5.44) and C-1′ (δ 169.7), C-2′ (δ20.8) exhibited that the acetoxy group was linked to C-16. Another oxygen-bearing carbon signal at δ 64.5 was therefore assigned to C-3. The HMBC correlations from 12-OH proton (δ 4.83) to C-11 (δ 210.2) and C-12 (δ 77.9) were also shown in HMBC spectrum (Figure 2).

The relative stereochemistry of compound **1** was determined by the analysis of NOESY spectrum (Figure 2). The NOESY correlation of H-17/H-12 indicated that the 2-pyrone ring was β-oriented. The NOESY correlation of H-16/H-12 showed that H-16 was α-oriented, so the acetoxy was β-oriented. Furthermore, the chemical shift of C-18 was shifted upfield to δ 11.0 at the presence of a 12β-OH due to γ-gauche effect, which was very common in a serial of bufadienolides.[10] Thus, compound **1** was elucidated as 16β-acetoxy-bufarenogin.

Compound **2** was obtained as white powder, $[\alpha]_D^{20}$ -24.8 (c 0.1, CH_3OH). The molecular formula was established as $C_{24}H_{34}O_6$ by HRFAB-MS (*m/z* 418.2355 $[M]^+$). The ^{13}C NMR and ^1H NMR spectra of **2** were similar to compound **1**, which suggested that **2** was also a bufadienolide (Figure 1), showing the typical proton signals of the 2-pyrone ring at δ 7.82 (1H, dd, $J = 2.1$, 9.6 Hz), 7.43 (1H, d, $J = 2.1$ Hz), 6.29 (1H, d, $J = 9.6$ Hz). Comparing the NMR signals of the two compounds, the acetoxy in **1** was substituted by the

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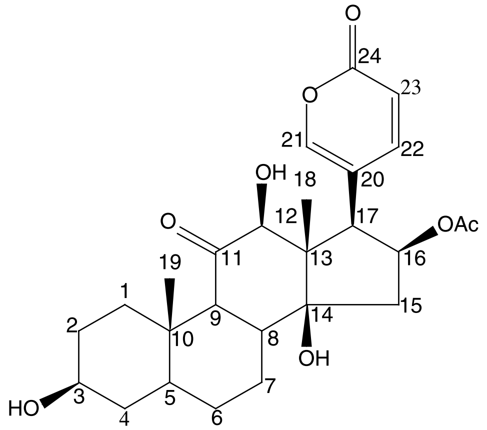
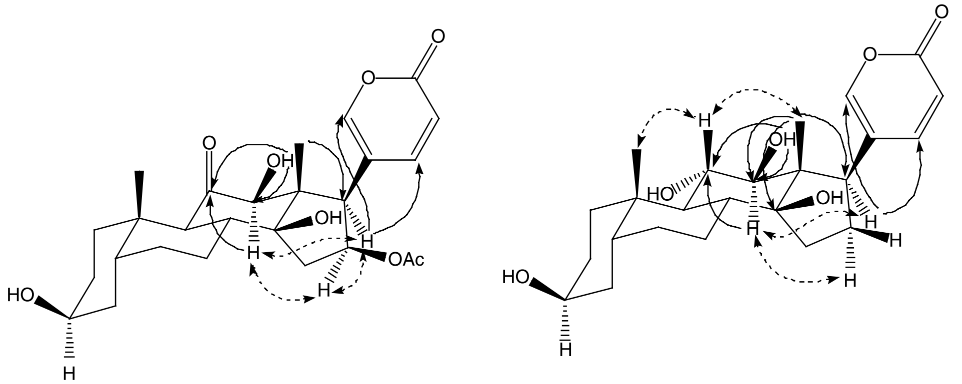


Figure 1. Structures of compounds **1** and **2.**



1

2

Figure 2. Key HMBC and NOESY correlations of compounds **1** and **2.** Dotted line, NOESY; solid line, HMBC.

methylene in **2**, due to the disappearance of the proton at δ 5.44 and its corresponding carbon at δ 73.5 and the appearance of the proton at δ 2.03 and the corresponding carbon at δ 28.6. Another difference between **1** and **2** was the carbonyl in **1** was replaced by a hydroxyl group in **2** for the absence of the carbon signal at δ 210.2 and the presence of a proton signal at δ 3.24 and its corresponding carbon at δ 71.2 in **2**. The β-configuration of H-11 was confirmed by the NOESY correlations of H-11/H-18 and H-11/H-19. Thus, compound **2** was elucidated as 11α,12β-dihydroxy-bufalin. Compound **2** was a new natural product and was reported in 1973 by Sigrid Spengel as a synthetic intermediate,[11] but unfortunately the author did not give any experimental data. In this paper, the spectral data of compound **2** were reported for the first time.

3. Experimental

3.1 *General experimental procedures*

Melting points were measured with a Yanako MS-S3 (Yanaco Co. Ltd, Kyoto, Japan) micro melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1601. IR spectra were measured on a Bruker IFS 55 spectrometer. All the NMR spectra were taken on a Bruker ARX-600 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz). HRFAB-MS spectra were measured on a VG Atospec spectrometer. Column chromatography was performed on silica gel G (200–300 mesh, Qingdao Haiyang Chemical Factory) and C-18 preparative HPLC (Shimadzu).

3.2 *Material*

The thin-plate Chan'Su was obtained in Anguo Folk-Medicinal Market, Hebei province, China, in March of 2005, and identified as dried secretion of *Bufo bufo gargarizans Cantor* by Prof. Qi-shiSun.

3.3 *Extraction and isolation*

Thin-plate Chan'Su (500 g) was ground into a rough powder and extracted with chloroform in a Soxhlet apparatus. The extract was concentrated under reduced

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of compounds **1** and **2** (in DMSO-d_6).

	1		**2**	
Position	δ_C	δ_H (J_{Hz})	δ_C	δ_H (J_{Hz})
1	29.1	1.88, 1.75	28.7	1.72, 1.75
2	25.9	1.74, 1.66	26.1	1.76, 1.62
3	64.5	3.85 (brs)	65.0	3.88 (brs)
4	33.0	1.76, 1.17	33.9	1.77, 1.18
5	36.5	1.59	37.7	1.69
6	27.8	1.51, 1.08	27.0	1.63, 0.91
7	21.9	1.33, 0.96	21.5	1.78, 1.15
8	40.1	1.90	32.5	1.82
9	43.1	1.89	37.6	1.64
10	34.5		36.4	
11	210.2		71.2	3.24 (q)
12	77.9	3.99 (d, 5.7)	79.3	2.95 (dd, 4.5, 9.6)
13	59.8		53.8	
14	81.5		83.1	
15	43.3	2.74 (d), 3.01 (m)	32.3	2.33
16	73.5	5.44 (t, 9.0)	28.6	2.03
17	51.3	3.47 (d, 4.5)	46.0	3.0 (m)
18	11.0	0.42 (s)	11.7	0.52 (s)
19	23.6	1.12 (s)	24.0	0.96 (s)
20	116.8		122.7	
21	151.8	7.46 (d, 2.1)	149.5	7.43 (d, 2.1)
22	149.9	8.10 (dd, 2.1, 9.6)	147.7	7.82 (dd, 2.1, 9.6)
23	112.1	6.22 (d, 9.6)	114.3	6.29 (d, 9.6)
24	161.1		161.5	
1′	169.7			
2′	20.8	1.86 (s)		
3-OH		4.22 (s)		4.12 (s)
11-OH				3.98 (s)
12-OH		4.83 (d, 5.7)		4.73 (d, 4.5)
14-OH				4.23 (s)

pressure, and the residue (150 g) was subjected to column chromatography on silica gel, eluting with petroleum ether/acetone gradient (increasing acetone 0–100%). Fifteen fractions (fractions 1–15) were obtained and fraction 10 (petroleum ether/acetone 100:30, 300 mg) was re-chromatographed on preparative HPLC. Compounds **1** (5 mg), **2** (7 mg) and **3** (4 mg) were obtained at methanol/water 46:54, and compounds **4** (5 mg), **5** (13 mg), **6** (7 mg) and **7** (8 mg) were obtained at methanol/water 39:61.

*3.3.1 Compound **1***

Colourless plate (MeOH); mp 192–194°C; $[\alpha]_D^{20}$ + 23.0 (c 0.3, CH_3OH); UV (MeOH) λ_{max} nm: 295, 203; IR (KBr) ν_{max}: 3400, 2953, 2869, 1712, 1635, 1243, 1148, 1031, 958 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data: see Table 1; HRFAB-MS *m/z*: 474.2303 $[M]^+$ (calcd for $C_{26}H_{34}O_8$, 474.2324).

*3.3.2 Compound **2***

White powder; mp 184–186°C; $[\alpha]_D^{20}$ −24.8 (c 0.1, CH_3OH); UV (MeOH) λ_{max} (nm): 294, 203; IR (KBr) ν_{max}: 3400, 2946, 1708, 1633, 1539, 1241, 1148, 1036, 829 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data: see Table 1; HRFAB-MS *m/z*: 418.2355 $[M]^+$ (calcd for $C_{24}H_{34}O_6$, 418.2364).

Cytotoxicity assay

The *in vitro* cytotoxic activities of **1–7** against the human tumour cell HeLa were tested by the MTT method.[12,13] Their IC_{50} values are given in Table 2.

Table 2. The IC_{50} values (μM) of **1–7** against HeLa cell line.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
HeLa	1.15	14.67	0.11	0.03	0.01	0.003	0.02

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