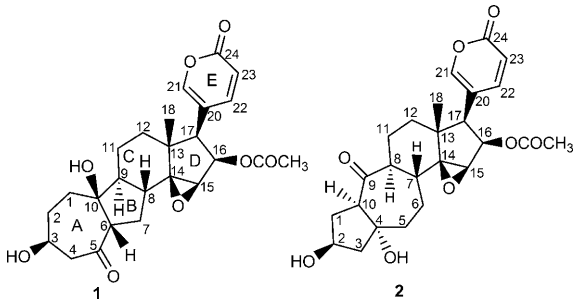
DOI: 10.1002/chem.201000847

Bufogargarizins A and B: Two Novel 19-Norbufadienolides with Unprecedented Skeletons from the Venom of *Bufo bufo gargarizans*

Hai-Yan Tian,[a, c] Lei Wang,[a, b] Xiao-Qi Zhang,[a, b] Ying Wang,[a, b] Dong-Mei Zhang,[a, b] Ren-Wang Jiang,[a, b] Zhong Liu,[a] Jun-Shan Liu,[a] Yao-Lan Li,[a, b] and Wen-Cai Ye*[a, b, c]

Toad venom, secreted from the postauricular and skin glands of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider, is useful as a chemical weapon against the natural enemies of toads. The dried toad venom has also been widely used as a traditional Chinese medicine in the treatment of superficial infection, odontalgia and skin cancer, although this secretion is known for its toxicity.[1] Bufadienolides bearing a δ-lactone ring at the C-17 in the steroidal skeleton had been proved to be active ingredients of toad venom, since these compounds showed significant cardiotonic, anesthetic, and antitumor activities.[2–3] To date, more than forty bufadienolides have been isolated from the venom of toads.[4–6] Bufalin, a main active component from toad venom, had been reported to induce apoptosis in various human cancer cell lines such as human leukemia HL60 and U937 cells, and the action mechanism was involved to influence the expression of apoptosis-related genes as *bcl*-2, *c-myc*, and *Tiam* 1.[7–8] Furthermore, bufadienolides were known to be specific inhibitors of Na^+/K^+ ATPase, which was reported as a potential target for anticancer drugs referring to the recent discovery of its signaling pathways.[9–10] The above pharmacological properties implied the promising therapeutic use of those compounds in, for example, cancer treatment. In our research for structurally unique and biologically interesting bufadienolides, two novel 19-norbufadienolides, bufogargarizins A (**1**) and B (**2**) (Scheme 1), with two unprecedented carbon skeletons, together with three presumably biosynthetic related intermediates **3**,[11] **4**, and **6** were isolated from the venom of *Bufo bufo gargarizans*. Herein, we describe the isolation and structure elucidation with the absolute configurations of **1** and **2**. In addition, a plausible biogenetic pathway of bufogargarizins A (**1**) and B (**2**) was also proposed.



Scheme 1. Chemical structures of **1** and **2** (the carbon atoms were numbered from rings A to E; No. 19 wasn't marked since the Me-19 was lost in **1** and **2**).

The roughly powdered toad venom was extracted with 95% ethanol under ultrasonic irradiation. The EtOH extract was filtered and concentrated under reduced pressure to afford a residue, which was then dissolved in 20% ethanol and partitioned with CH_2Cl_2. The extract, which showed significant cytotoxic activity, was then purified by chromatography on silica gel, reversed-phase C_{18} silica gel, and preparative HPLC columns to yield compounds **1**–**4**, and **6**.

Compound **1** was obtained as a colorless block. The quasi-molecular ion at m/z 459.2023 $[M+H]^+$ in its HR-ESI-MS indicated that the molecular formula of **1** was $C_{25}H_{30}O_8$. The UV absorption maximum at 295 nm and IR band at

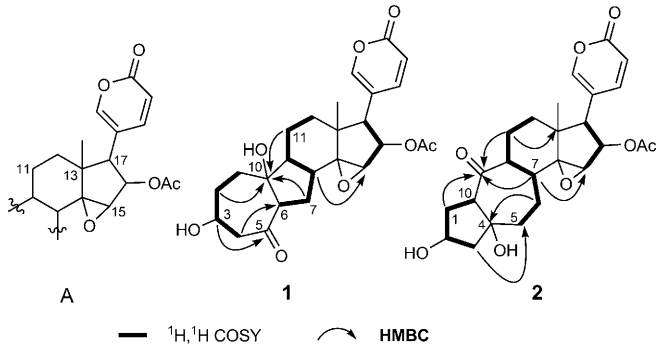
[a] H.-Y. Tian, Dr. L. Wang, Dr. X.-Q. Zhang, Dr. Y. Wang, Dr. D.-M. Zhang, Prof. Dr. R.-W. Jiang, Dr. Z. Liu, J.-S. Liu, Prof. Dr. Y.-L. Li, Prof. Dr. W.-C. Ye
Institute of Traditional Chinese Medicine and Natural Products
Jinan University, Guangzhou 510632 (P. R. China)
Fax: (+86) 20-8522-1559
E-mail: chywc@yahoo.com.cn

[b] Dr. L. Wang, Dr. X.-Q. Zhang, Dr. Y. Wang, Dr. D.-M. Zhang, Prof. Dr. R.-W. Jiang, Prof. Dr. Y.-L. Li, Prof. Dr. W.-C. Ye
Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University
Guangzhou 510632 (P.R. China)

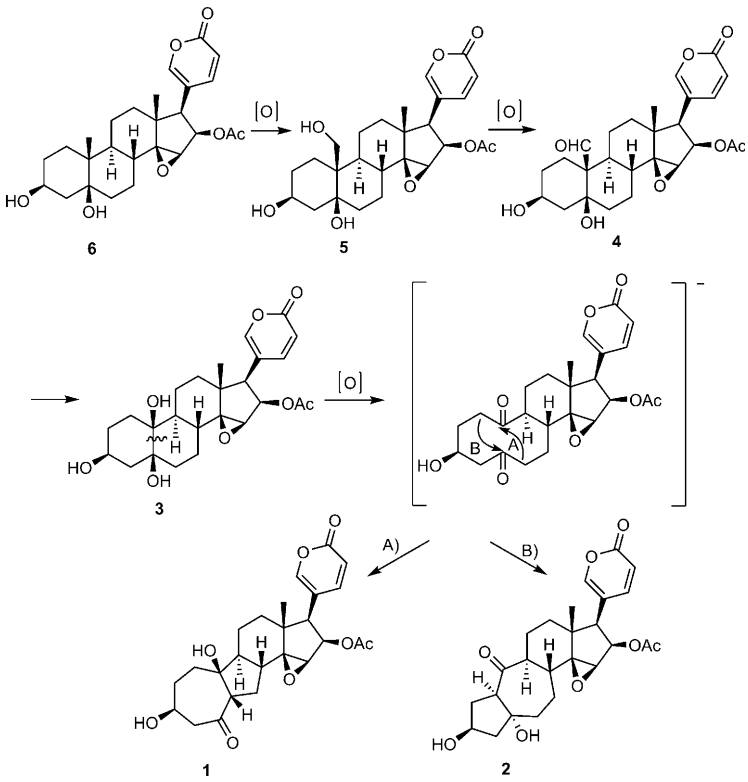
[c] H.-Y. Tian, Prof. Dr. W.-C. Ye
Department of Phytochemistry, China Pharmaceutical University
Nanjing 210009 (P. R. China)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201000847.

1725 cm^{-1} implied the presence of δ-lactone ring. The analysis of NMR spectra revealed that **1** possessed twenty-five carbons, including a methyl (δ_H 0.84, s), an acetyl group [δ_H 1.85 (1H, s); δ_C 171.6, 20.3], and a δ-lactone ring [δ_H 6.23 (d, J=8.0 Hz, 1H), 7.36 (d, J=1.0 Hz, 1H), 8.01 (dd, J=8.0, 1.0 Hz, 1H); δ_C 114.1, 118.2, 150.8, 153.6, and 163.9]. The ^1H and ^{13}C NMR signals assigned to fragment A (Scheme 2) were very similar to that of cinobufotalin (**6**)[6a] (Scheme 3),



Scheme 2. Key ^1H–^1H COSY and HMBC correlations of **1** and **2**.



Scheme 3. Plausible biogenetic pathway for **1** and **2**.

indicating that **1** had the same substructure as **6**, with an epoxy at C-14 and C-15, as well as an acetyl group at C-16. The $^1H,^1H$ COSY spectrum revealed the presence of the spin systems in bold as shown in Scheme 2. The HMBC correlations between H-3 (δ_H 3.62, m) and C-5 (δ_C 209.9), between H-4β (δ_H 2.85, dd, J=10.0, 10.0 Hz, 1H) and C-6 (δ_C 64.5), and between H-2β (δ_H 2.10, m, 1H) and C-10 (δ_C 81.4), as well as between H_2-7 (δ_H 1.58, m, 2H) and C-10 indicated that rings A and B were connected via C-6 and C-10 bond. The detailed interpretation of HMBC correlations allowed the establishment of the planar structure of **1**, which was a novel 19-norbufadienolide with 7/5/6/5 carbon rings.

In order to determine the relative configuration of **1**, the ROESY spectrum was extensively analyzed (for details see Supporting Information). However, the uncertainty of the conformation of the seven-membered ring made it difficult to determine the orientation of hydroxyl group at C-10 by NOE correlations. Fortunately, crystals suitable for single-crystal X-ray diffraction were grown from methanol solution. The relative configuration of **1** was unequivocally deduced by the result of X-ray diffraction analysis (Figure 1).[12] Meanwhile, some useful conformational information could be obtained from the crystal structure: the plane of δ-lactone ring was nearly perpendicular to the ring D, making H-21 (δ_H 7.36, d, J=1.0 Hz) and H-17 (δ_H 2.98, d, J=8.6 Hz) into the same orientation, which well accounted for the NOE correlation between H-21 and H-17; the seven-membered ring A and six-membered ring C were both in chair conformation; the hydroxyl group at C-10 was β-oriented, and **1** remained the A/B *cis*, B/C *trans*, as well as C/D *cis* ring junctions.

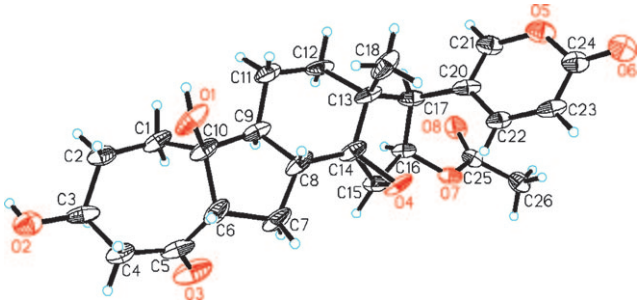
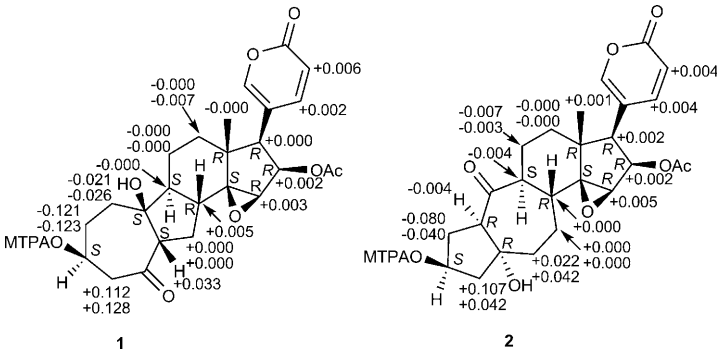


Figure 1. X-ray crystal structure of **1**.

Subsequently, the modified Mosher's method was applied to determine the absolute configuration of **1**.[13] Comparison of the ^1H NMR chemical shifts between (*S*)- and (*R*)-MTPA esters of **1** led to the assignment of *S* configuration of C-3 (Scheme 4). Therefore, the structure of **1** was fully established and deduced to possess the 3*S*, 6*S*, 8*R*, 9*S*, 10*S*, 13*R*, 14*S*, 15*R*, 16*R*, and 17*R* configurations.

Compound **2** was isolated as an amorphous powder and was shown to have the same molecular formula as **1** by its HR-ESI-MS. Comparison of the NMR data of **2** (Table 1) with those of **1** indicated that **2** also had fragment A in its structure (Scheme 2). The $^1H,^1H$ COSY spectrum revealed the presence of the partial units in bold as shown in Scheme 2. The HMBC correlations between H-1 (δ_H 2.04, 2H) and C-9 (δ_C 212.7), between H-3β (δ_H 1.41, dd, J=12.0, 10.0 Hz, 1H) and C-5 (δ_C 39.7), as well as between H-6 [δ_H 1.58 (1H, H-6α); 1.67 (1H, H-6β)] and C-4 (δ_C 79.9) re-



Scheme 4. $\Delta\delta$ values ($\delta_S-\delta_R$) for the MTPA esters of **1** and **2**.

vealed the presence of 5/7 consecutive carbocycles via C-4 and C-10 bond (Scheme 2). Furthermore, the HMBC correlations between H-11α (δ_H 1.97, 1H) and C-9 (δ_C 212.7), and between H-7 (δ_H 1.74, 1H) and C-15 (δ_C 61.0) implied that rings B and C were linked through C-7 and C-8 bond. Consequently, **2** was inferred to be an unprecedented 19-norbufadienolide with a 5/7/6/5 carbocycle system.

The ROESY spectrum (recorded in [D$_6$]DMSO) revealed correlations (Figure 2) between Me-18 (δ_H 0.74, s, 3H) and H-7 (δ_H 1.64, ddd, J=11.3, 11.3, 3.5 Hz), between H-7 and H-5β (δ_H 1.00, dd, J=14.1, 11.2 Hz), between H-5β and H-3β (δ_H 1.17, dd, J=12.0, 10.0 Hz), as well as between H-3β and 2-OH (δ_H 4.58, d, J=5.2 Hz), indicating that those protons were β-oriented. In contrast, the correlations between H-10 (δ_H 2.97, dd, J=8.7, 8.7 Hz) and H-8 (δ_H 2.69, ddd, J= 11.9, 11.3, 3.9 Hz), between H-10 and 4-OH (δ_H 4.74, s), between 4-OH and H-2 (δ_H 4.20, m), between H-17 (δ_H 2.88, d, J=9.3 Hz) and H-16 (δ_H 5.48, dd, J=9.3, 1.2 Hz), as well as between H-16 and H-15 (δ_H 3.74, d, J=1.2 Hz) revealed these protons were α-oriented. These findings suggested **2** also possessed A/B *cis*, B/C *trans*, and C/D *cis* ring junctions. Nevertheless, in contrast to the β-oriented OH and H at the junction positions of rings A and B in **1**, the corresponding OH and H were found to be α-oriented in **2**. To confirm the most reasonable conformation of **2**, a combination of computational analysis based on SYBYL software and interpretation of NMR data was applied.[14] Coincidentally, the seven-membered ring B in **2** also had the chair conformation as **1** when it was in a state of low energy (for details, see Experimental Section). Thus, the relative configuration of **2** was established and shown in Figure 2. As **1**, the result of the modified Mosher's method (Scheme 4) suggested that the absolute configuration of C-2 was *S*. Therefore, the structure of **2** was elucidated and assigned to have 2*S*, 4*R*, 7*R*, 8*S*, 10*R*, 13*R*, 14*S*, 15*R*, 16*R*, and 17*R* configurations.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1–2** (CD$_3$OD, J in Hz).[a,b]

No.	**1**		**2**		**2**[c]		No.	**1**		**2**		**2**[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α	1.05	34.0	2.04	35.6	1.84	34.6	11α	1.51	21.4	1.97	24.3	1.80	22.8
β	1.95 (m)		2.04		1.84		β	1.51		1.48		1.41 (dd, 14.1, 10.0)	
2α	1.85 (m)	32.3	4.38 (m)	71.7	4.20 (m)	69.4	12α	1.53	41.7	1.59	39.4	1.53	37.5
β	2.10 (m)		–		–		β	1.87		1.79		1.70 (ddd, 13.5, 3.0, 3.0)	
3α	3.62 (m)	70.8	1.94	51.9	1.80	51.0	13	–	47.0	–	46.5	–	44.7
β	–		1.41 (dd, 12.0, 10.0)		1.17 (dd, 12.0, 10.0)		14	–	73.5	–	72.3	–	70.9
4α	2.68 (dd, 10.0, 4.6)	54.9	–	79.9	–	77.3	15	3.80 (d, 1.3)	60.8	3.76 (brs)	61.0	3.74 (d, 1.2)	59.6
β	2.85 (dd, 10.0, 10.0)		–		–		16	5.46 (dd, 8.6, 1.3)	76.7	5.49 (d, 8.6)	76.4	5.48 (dd, 9.3, 1.2)	74.4
5α	–	209.9	1.95	39.7	1.82	38.3	17	2.98 (d, 8.6)	50.9	2.94 (d, 8.6)	51.2	2.88 (d, 9.3)	48.8
β	–		1.20 (dd, 14.1, 11.2)		1.00 (dd, 14.1, 11.2)		18	0.84 (s)	17.8	0.86 (s)	17.5	0.74 (s)	16.9
6α	–	64.5	1.58	22.4	1.50	20.8	20	–	118.2	–	118.1	–	115.8
β	3.17 (dd, 8.4, 8.4)		1.67		1.50		21	7.36 (d, 1.0)	153.6	7.38 (d, 1.0)	153.7	7.47 (d, 1.0)	152.3
7α	1.58	26.8	–	39.9	–	37.7	22	8.01 (dd, 8.0, 1.0)	150.8	8.00 (dd, 8.0, 1.0)	150.7	7.82 (dd, 8.0, 1.0)	148.3
β	1.58		1.74		1.64 (ddd, 11.3, 11.3, 3.5)		23	6.23 (d, 8.0)	114.1	6.24 (d, 8.0)	114.1	6.24 (d, 8.0)	112.9
8α	–	37.8	2.73 (ddd, 11.9, 11.3, 3.7)	58.1	2.69 (ddd, 11.9, 11.3, 3.7)	55.6	24	–	163.9	–	164.0	–	160.7
β	2.34 (m)		–		–		*C*OCH$_3$	–	171.6	–	171.6	–	169.3
9	1.09	58.0	–	212.7	–	210.7	CO*C*H$_3$	1.85 (s)	20.3	1.86 (s)	20.3	1.82 (s)	20.2
10	–	81.4	3.10 (dd, 8.2, 8.2)	62.8	2.97 (dd, 8.2, 8.2)	61.3	2-OH	–		–		4.58 (d, 5.2)	
							4-OH	–		–		4.74 (s)	

[a] Assignments were established by COSY, HSQC and HMBC spectra. [b] Overlapped signals were reported without designating multiplicity. [c] ^{1}H and ^{13}C NMR data were recorded in [D$_6$]DMSO.

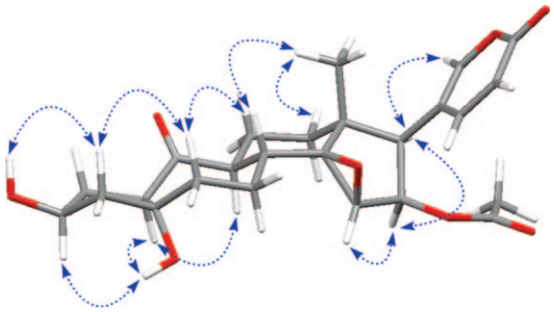


Figure 2. Key ROESY correlations of **2**.

Compounds **1** and **2** had unusual 7/5/6/5 and 5/7/6/5 ring systems, respectively, instead of the 6/6/6/5 skeleton presented in common bufadienolides, which interested us to presume the biogenetic pathway among these compounds. Cinobufotalin (**6**), 19-hydroxylcinobufotalin (**5**) and 19-oxocinobufotalin (**4**) were three known ingredients of toad venom.[15] The aldehyde group at C-10 in **4** should be formed by oxidation of the corresponding hydroxymethyl group in **5**, which was an oxide of **6**, as the formation of resibufagin and hellebrigenin.[5,6a,16] Then **4** might be changed into **3** through a Baeyer–Villiger reaction.[17] After undergoing an oxidation procedure of **3**, an presumably intermediate with diketone unit was formed.[18] Finally, **1** and **2** should be yielded through two intramolecular aldol condensation procedures of the intermediate (Scheme 3).[19]

Of all the tested bufadienolides, **6** and bufalin (as a positive control) showed potent antiproliferative effects on HeLa and HepG 2 cell lines, with IC_{50} values ranging from 0.1 to 3 μM. Compound **4** also exhibited cytotoxic activities with IC_{50} values of 4.01 ± 0.51 μM on HeLa cells and 7.84 ± 0.13 μM on HepG 2 cells, indicating that the presence of 10β-aldehyde group made no significant difference in cytotoxicity on the two cell lines. Compound **3**, a 19-nor derivative of **6**, showed weak antiproliferative effect on HeLa cells with IC_{50} value of 35.56 ± 4.19 μM, and no apparent effect on HepG 2 cells, suggesting 10β-hydroxyl substitution decreased the activities on these cancer cell lines. However, the antiproliferative effects of **1** and **2** were notably dropped, implying that the changes of rings A and B could greatly decrease the cytotoxic activity of bufadienolides on the two cancer cells (for details, see Supporting Information).

Bufogargarizins A (**1**) and B (**2**) were the first examples of bufadienolides with unusual alterations of rings A and B. The isolation and structure elucidation including absolute configurations of these compounds has added to a diverse and complex array of bufadienolide family. The plausible biogenetic pathway for **1** and **2** was reasonable and interesting. Bioassay result further confirmed that the essential steroidal skeleton is necessary for cytotoxic activities of these bufadienolides. Further chemistry and biological studies for such interesting compounds and other bufadienolides from the venom are currently ongoing.

Experimental Section

Extraction and isolation: The dried venom (1.5 kg) was ground into rough powder and then extracted with 95% ethanol under ultrasonic irradiation. The EtOH extract was filtered and concentrated under reduced pressure to afford a residue (900 g), which was then dissolved in 20% ethanol and partitioned with CH_2Cl_2. The CH_2Cl_2 solution was combined and concentrated to afford a residue (321 g). The residue was purified by chromatography on silica gel (200–300 mesh), eluted with cyclohexane/acetone 5:1, 3:1, and 1:1 to yield 15 fractions (fractions 1–15). Compound **6** (1.5 g) was obtained by recrystallization of fraction 7. Fraction 10 was separated using silica gel eluted with chloroform/ethanol 100:2, 95:5, and 90:10 to afford 11 subfractions (fractions 10a–10k). Fraction 10c was further separated by preparative HPLC eluted with acetonitrile/water/TFA 23:77:0.05 to yield **1** (8.0 mg), **2** (5.5 mg), and **3** (36.0 mg). **4** (80.0 mg) was obtained from fraction 10g using the same chromatography method.

Computational methods: The structure of **2** was calculated by using molecular modeling software package SYBYL 7.0 (Tripos, St Louis, MO, USA). All hydrogen atoms were added and overlaid with key correlations observed in the ROESY spectrum. The energy was minimized for 1000 steps using the Tripos force field and Powell method and the termination setting was 0.001 kcal $mol^{-1} \times A^{-1}$. Then a grid search was carried out with an interval of 5° for each rotatable bond to obtain the lowest energy conformation.

Bufogargarizin A (1): Colorless block crystals; m.p. 164–165 °C; $[\alpha]_D^{24} = -24.5°$ ($c = 0.2$, in CH_3OH); ^1H and ^{13}C NMR data, see Table 1. IR (KBr): $\nu_{max} = 3418, 1725, 1635, 1536, 1379, 1244, 1128, 1050, 954, 883, 839, 792, 754, 662\ cm^{-1}$; UV ($CH_3OH$): λ_{max} ($\log\varepsilon$) = 203 (3.1), 295 nm (2.7); HR-ESI-MS: m/z: calcd for $C_{25}H_{31}O_8$: 459.2013; found: 459.2020 $[M+H]^+$.

Bufogargarizin B (2): amorphous powder; $[\alpha]_D^{26} = +4.2°$ ($c = 0.1$, in CH_3OH); ^1H and ^{13}C NMR data, see Table 1; IR (KBr): $\nu_{max} = 3441, 1706, 1633, 1537, 1456, 1374, 1245, 1058, 966, 889, 841, 809, 782, 666\ cm^{-1}$; UV ($CH_3OH$): λ_{max} ($\log\varepsilon$) = 203 (2.9), 295 nm (2.7); HR-ESI-MS: m/z: calcd for $C_{25}H_{31}O_8$: 459.2013; found: 459.2017 $[M+H]^+$.

Acknowledgements

This work was supported by the National Natural Science Foundation of China for Outstanding Young Scientists (No. 30625039), Program for Changjiang Scholars (to W.C.Y.), National Natural Science Foundation of China (No. 90913020), Natural Science Foundation of Guangdong Province (No. 9451063201002969), New Century Excellent Talents Scheme (NCET-08-0612), and China Postdoctoral Science Foundation (No. 20090460786).

Keywords: biogenetic pathway · bufadienolide · cytotoxicity · natural products · structure elucidation

[1] Editorial Committee of the Administration Bureau of Traditional Chinese Medicine in *Chinese Materia Medica (ZhonghuaBencao), Vol. 9*, Shanghai Science and Technology Press, Shanghai, **1999**, pp. 362–367.
[2] P. S. Steyn, F. R. Van Heerdeen, *Nat. Prod. Rep.* **1998**, *15*, 397–413.
[3] W. Schoner, *Eur. J. Biochem.* **2002**, *269*, 2440–2448.
[4] L. Krenn, B. Kopp, *Phytochemistry* **1998**, *48*, 1–29.
[5] M. Ye, D. A. Guo, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1881–1892.
[6] a) T. Nogawa, Y. Kamano, A. Yamashita, G. R. Pettit, *J. Nat. Prod.* **2001**, *64*, 1148–1152; b) Y. Kamano, T. Nogawa, A. Yamashita, M. Hayashi, M. Inoue, P. Draar, G. R. Pettit, *J. Nat. Prod.* **2002**, *65*,

1001–1005; c) X. L. Xin, R. Lan, J. Huang, X. J. Wang, J. M. Jia, *Chin. Chem. Lett.* **2008**, *19*, 1445–1446.
[7] Y. Masuda, N. Kawazoe, S. Nakajo, T. Yoshida, Y. Kuroiwa, K. Nakaya, *Leuk. Res.* **1995**, *19*, 549–556.
[8] K. Nobuko, W. Masahiko, M. Yutaka, N. Shigeo, N. Kazuyasu, *Oncogene* **1999**, *18*, 2413–2421.
[9] I. Prassas, E. P. Diamandis, *Nat. Rev. Drug Discovery* **2008**, *7*, 926–935.
[10] T. Mijatovic, L. Ingrassia, V. Facchini, R. Kiss, *Expert Opin. Ther. Targets* **2008**, *12*, 1403–1417.
[11] Compound **3** was a new 19-norbufadienolide, and its spectral data was presented in the Supporting Information.
[12] X-ray analysis: colorless blocks, asymmetric unit 2 ($C_{25}H_{30}O_8$)·2.5 H_2O, monoclinic, $P2_1$, $a=12.8228(2)$, $b=9.8014(2)$, $c=18.9778(5)$ Å, $\beta=97.542(2)°$, $V=2364.7(3)$ Å^3, $Z=2$, $d_x=1.344$ Mg m^{-3}, $\mu(Cu_{K\alpha})=0.859$ mm^{-1}, $F(000)=1016$. Data collection was performed on a SMART CCD by using graphite monochromated radiation ($\lambda=1.54184$ Å) under low temperature (nitrogen gas); 7123 unique reflections were collected to $\theta_{max}=62.53°$, in which 5485 reflections were observed [$F^2>4\sigma(F^2)$]. The structures were solved by direct methods (SHELXTL version 5.1) and refined by full-matrix least-squares on F^2. In the structure refinements, non-hydrogen atoms were refined anisotropically. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the "ride on" method. Hydrogen atoms bonded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. The final $R=0.0959$, $Rw=0.1105$ and $S=1.026$. CCDC-743886 (**1**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[13] a) J. A. Dale, H. S. Mosher, *J. Am. Chem. Soc.* **1973**, *95*, 512–519; b) L. Liu, S. C. Liu, X. L. Chen, L. D. Guo, Y. S. Che, *Bioorg. Med. Chem.* **2009**, *17*, 606–613.
[14] P. Ciminiello, B. Catalanotti, C. Dell′Aversano, C. Fattorusso, E. Fattorusso, M. Forino, L. Grauso, A. Leo, L. Tartaglione, *Org. Biomol. Chem.* **2009**, *7*, 3674–3681.
[15] N. Horiger, D. Zivanov, H. H. Linde, K. Meyer, *Helv. Chim. Acta* **1972**, *55*, 2549–2562.
[16] P. M. Dewick in *Medicinal Natural Products (A Biosynthetic Approach)*, Wiley, New York, **1997**, pp. 241–246.
[17] a) E. Alvarez-Manzaneda, R. Chahboun, F. Bentaleb, E. Alvarez, M. A. Escobar, S. Sad-Diki, M. J. Cano, I. Messouri, *Tetrahedron* **2007**, *63*, 11204–11212; b) A. F. Barrero, E. J. Alvarez-Manzaneda, R. Alvarez-Manzaneda, R. Chahboun, R. Meneses, M. Aparicio, *Synlett* **1999**, 713–716; c) Y. Wu, G. F. Dai, J. H. Yang, Y. X. Zhang, Y. Zhu, J. C. Tao, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1818–1821.
[18] a) H. O. House, J. H. C. Lee, D. Van Derveer, J. E. Wissinger, *J. Org. Chem.* **1983**, *48*, 5285–5288; b) E. P. Balskus, J. Me'ndez-Andino, R. M. Arbit, L. A. Paquette, *J. Org. Chem.* **2001**, *66*, 6695–6704.
[19] C. L. Chandler, B. List, *J. Am. Chem. Soc.* **2008**, *130*, 6737–6739.

Received: April 5, 2010
Published online: August 16, 2010

