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Note

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Bufospirostenin A and Bufogargarizin C, Steroids with Rearranged Skeletons from the Toad *Bufo bufo gargarizans*

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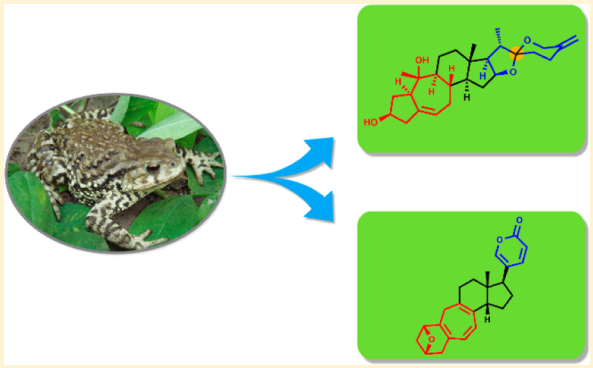
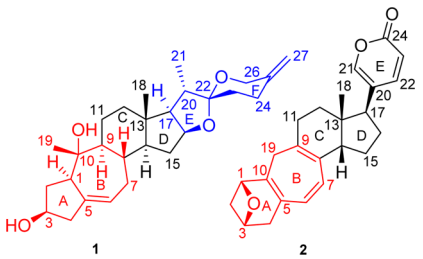
Supporting Information

ABSTRACT: Bufospirostenin A (**1**) and bufogargarizin C (**2**), two novel steroids with rearranged A/B rings, were isolated from the toad *Bufo bufo gargarizans*. Compound **1** represents the first spirostanol found in animals. Compound **2** is an unusual bufadienolide with a cycloheptatriene B ring. Their structures were elucidated by spectroscopic analysis, single crystal X-ray diffraction analysis, and computational calculations.



Toads are amphibians, and there are 34 genera and 410 species all over the world.[1,2] Toads have long been recorded as a valuable source of traditional Chinese medicine.[3] The medicinal parts of toads include toad skin, toad venom, toad liver, and toad bile.[3] The venom of toad, locally called ChanSu, is used to treat toothaches, heart failure, and other conditions.[4] HuaChanSu injections, prepared from the skin of the toad *Bufo bufo gargarizans* or *B. melanostictus*, have been shown to be a valuable anticancer drug for the treatment of many kinds of cancers in China.[5] Chemical components found in toads include bufadienolides, indole alkaloids, C_{23} steroids, peptides, and bile acids.[6−11] Among them, bufadienolides were reported to be the main active ingredients, which inhibit Na/K ATPase to regulate several important cellular processes and play potential therapeutic roles in various diseases.[12] In our previous studies, some new bufadienolides in the toad venom were found.[13,14] As a continuing investigation of the chemical constituents of *Bufo* species, bufospirostenin A (**1**), a spirostanol with an unprecedented 5/7/6/5/5/6 ring system, and bufogargarizin C (**2**), a bufadienolide with an unusual cycloheptatriene B ring, were isolated from the bile and venom of the toad *Bufo bufo gargarizans*. Herein, we report the isolation, structure elucidation, and Na/K ATPase (NKA) inhibitory activities of **1** and **2**.

The gallbladders and venom of toads were collected from Baoying Toad Breeding Base, Baoying country, Jiangsu province of China. The concentrated bile and dried venom were each extracted with 95% EtOH, respectively. Then the concentrated extract of the bile was suspended in H_2O and partitioned with cyclohexane, EtOAc, and *n*-BuOH, and that of the venom was dissolved in 20% EtOH and partitioned with CH_2Cl_2. Compound **1** was isolated from the EtOAc fraction of the bile, and **2** from the CH_2Cl_2 part of the venom extract.



1 **2**

Compound **1** was obtained as a colorless block. A protonated molecule at *m/z* 429.2999 $[M + H]^+$ in its HRESIMS spectrum indicated that the molecular formula of **1** was $C_{27}H_{40}O_4$, with eight degrees of unsaturation. The ^1H NMR spectrum showed the presence of three olefinic protons [δ_H 4.74 and 4.77 (1H each, br s, H_2-27); 5.65 (1H, m, H-6)], three methyl signals [δ_H 0.83 (3H, s, H_3-18), 1.09 (3H, s, H_3-19), and 0.95 (3H, d, J = 7.0 Hz, H_3-21)], and four oxygenated methine protons [δ_H 3.93 (1H, m, H-3); 4.43 (1H, dd, J = 13.6, 7.7 Hz, H-16); 3.82 and

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Table 1. ^{1}H and ^{13}C NMR Data for 1 and 2 (δ in ppm)[a]

	1[b]		2[c]			1[b]		2[c]	
No.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	No.	δ_C,	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	53.4, CH	2.81, t (9.5)	73.4, CH	5.23, d (5.9)	15α	33.8, CH_2	2.14, m	34.3, CH_2	1.60, ov
2α	38.3, CH_2	2.16, ov	29.2, CH_2	1.71, m	β		1.31, ov		2.33, m
β		1.75, ov		2.18, ov	16α	81.9, CH	4.43, dd (3.6,7.7)	29.2, CH_2	1.75, m
3	71.9, CH	3.93, m	73.7, CH	4.73, m	β				2.03, m
4α	44.3, CH_2	2.48, dd (14.3,6.5)	36.5, CH_2	2.44, d (16.5)	17	64.2, CH	1.77, ov	49.4, CH	2.60, dd (9.5,7.5)
β		2.17, ov		3.31, dd (16.5,5.5)	18	16.9, CH_3	0.83, s	23.4, CH_3	0.78, s
5	144.9, C		128.5, C		19α	15.9, CH_3	1.09, s	35.2, CH_2	1.94, d (15.9)
6	123.1, CH	5.65, m	127.2, CH	6.89, d (7.8)	β				2.18, ov
7a	33.7, CH_2	1.79, m	127.8, CH	6.97, d (7.8)	20	43.0, CH	1.94, m	120.4, C	
β		2.10, m			21	14.9, CH_3	0.95, d (7.0)	148.3, CH	7.30, br s
8	39.0, CH	1.48, ov	137.0, C		22	110.5, C		145.1, CH	7.34, dd (9.6,2.6)
9	61.2, CH	1.34, m	129.7, C		23α	34.0, CH_2	1.74, ov	115.8, CH	6.34, d (9.6)
10	75.0, C		138.6, C		b		1.70, ov		
11α	24.1, CH_2	1.48, ov	22.0, CH_2	2.49, dt (16.8,5.5)	24α	29.5, CH_2	2.25, m	161.9, C	
β		2.00, m		2.73, m	β		2.56, m		
12α	41.0, CH_2	1.73, ov	33.7, CH_2	1.67, m	25	145.2, C			
β		1.17, m		1.60, ov	26α	65.8, CH_2	3.82, d (11.9)		
13	41.8, C		42.1, C		β		4.27, d (11.9)		
14	57.1, CH	1.27, m	50.0, CH	2.77, m	27α	109.0, CH_2	4.74, br s		
					β		4.77, br s		

[a]ov: overlapped proton resonances. [b]Measured in CD_3OD, 300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR. [c]Measured in $CDCl_3$, 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR.

4.27 (1H each, d, J = 11.9 Hz, H_2-26)]. The ^{13}C NMR and DEPT spectra revealed the existence of 27 carbon signals. Based on the extensive analysis of COSY, HSQC, HMBC, and ROESY spectra, the ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in Table 1. Comparison of the NMR data of **1** with those of neoruscogenin[15,16] suggested that **1** had the same substructure as neoruscogein in rings C, D, E, and F. Further structural information on **1** came from the inspection of the COSY spectrum, which demonstrated the presence of three isolated spin systems as shown in Figure 1a. The HMBC correlations between H_2-4 and C-6, between H_3-19 and C-1, between H-2β and C-10, and between H-7β and C-5 indicated rings A and B were connected via a C-1 to C-5 bond, which established a 5/7 ring system. The detailed interpretation of the HMBC correlations (Figure 1a) allowed the establishment of the planar structure of **1**.

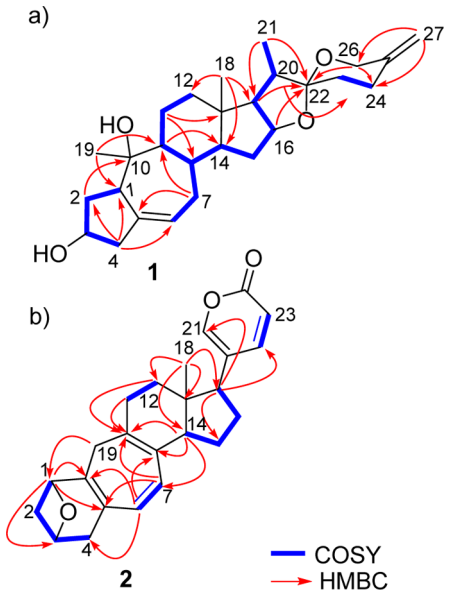

Figure 1. COSY and key HMBC correlations of **1** (a) and **2** (b).

In order to determine the relative configurations of **1**, the ROESY spectrum was extensively analyzed (Supporting Information, Figure S2). NOE correlations between H_3-19 and H-2β/H-4β/H-8, and between H_3-18 and H-8/H-20 suggested these protons were in the same orientation. Furthermore, NOE correlations between H-1 and H-3α/H-7α/H-9, between H-14 and H-16/H-7α, as well as between H-17 and H-16 indicated those protons had the same orientation. Crystals suitable for single-crystal X-ray diffraction analysis were obtained from a MeOH solution of **1**. Therefore, the relative configuration of **1** was fully established by X-ray crystallographic analysis (Figure 3). A relatively small Flack parameter 0.0 (3) from the final refinement enabled the determination of the absolute configurations of **1** to be 5Z, 1R, 3S, 8S, 9S, 10R, 13S, 14S, 16S, 17R, 20S, and 22R, which was also consistent with biosynthetic considerations.[17,18] Thus, the complete structure of compound **1** was established, which was named as bufospirostenin A.

Compound **2** was isolated as a white powder. A protonated molecule at m/z 363.1959 $[M + H]^+$ indicated that the molecular formula of **2** was $C_{24}H_{26}O_3$, with 12 degrees of unsaturation. The UV absorption maximum at 300 nm suggests the presence of a 2H-pyran-2-one moiety. In the ^{1}H NMR spectrum, characteristic signals for a 2H- pyran-2-one moiety [δ_H 6.34 (1H, d, J = 9.6 Hz) and 7.30 (1H, br s), 7.34 (1H, dd, J = 9.6, 2.6 Hz)], one pair of mutually coupled olefinic protons [δ_H 6.89 (1H, d, J = 7.8 Hz) and 6.97 (1H, d, J = 7.8 Hz)], two oxygenated protons [δ_H 4.73 (1H, m), 5.23 (1H, d, J = 5.9 Hz)], and one angular methyl [δ_H 0.78 (3H, s)] were observed. The analysis of the ^{13}C NMR and DEPT spectra permitted differentiation of 24 carbon resonances into one methyl, seven methylenes, nine methines, and seven nonprotonated carbons. This evidence suggested that **2** was a bufadienolide derivative.

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Via combined analysis of the COSY, HSQC, HMBC, and ROESY spectra of **2**, the ^{1}H and ^{13}C NMR signals were unambiguously assigned (Table 1).

In the COSY spectrum, five spin systems could be observed as shown in bold face in Figure 2b. In the HMBC spectrum, the

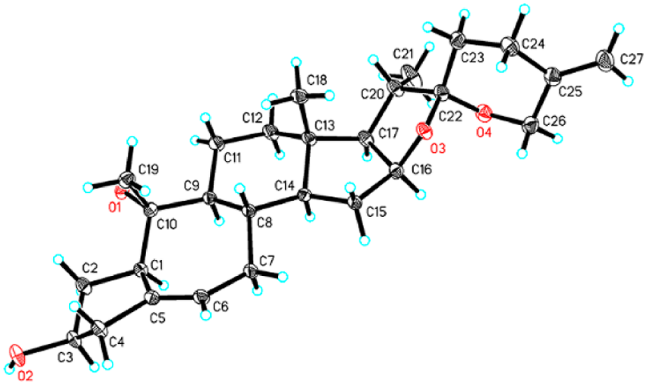

Figure 2. X-ray crystal structure of **1**.

correlations between H-17 and C-21/C-22 indicated that the 2H-pyran-2-one moiety was connected to C-17 as for typical bufadienolides. The HMBC correlations between H_3-18 and C-12/C-13/C-14/C-17 revealed the angular methyl was attached at C-13. This evidence suggested the presence of the rings C/D/E. Furthermore, HMBC correlations between H-1 and C-5/C-10, between H-6 and C-4/C-8/C-10, between H-7 and C-5, as well as between H-19α (δ_H 1.94) and C-1 supported the establishment of rearranged A/B rings, containing a rare cycloheptatriene ring B. Moreover, the connection of the C/B rings was accomplished by HMBC correlations between H-14 and C-7/C-8/C-9 and between H_2-11/H_2-12 and C-9. In addition, taking account of the molecular formula of **2**, as well as the deshielded chemical shifts of H-3 (δ_H 4.73) and C-3 (δ_C 73.7) compared with those of a common bufadienolide (δ_H 3.89 and δ_C 67.1, Supporting Information), the structure of **2** should possess one more degree of unsaturation corresponding to a four membered cyclic ether constructed by an oxygen bridge between C-1 and C-3. The above evidence allowed the establishment of the planar structure of **2**.

The relative configuration of **2** could be determined by a ROESY experiment (Figure 3a). The NOE correlations between H_3-18 and H-14/H-21 indicated that H-14 was in the same β-orientation as the C-18 angular methyl group and the C-17 lactone ring. According to a comparison with our previously reported bufadienolides[8,14] and on biosynthetic considerations, the absolute configurations of C-13, C-14, and C-17 were deduced to be 13*S*, 14*S*, and 17*S*. However, the configuration of the oxetane group in ring A was still unclear due to an uncertain conformation of rings A/B. In order to establish the full absolute configuration of **2**, a quantum chemical calculation was carried out. Aiming to get one reasonable configuration, two optimized stereoisomers, (1*R*, 3*R*, 13*S*, 14*S*, 17*S*)-**2** and (1*S*, 3*S*, 13*S*, 14*S*, 17*S*)-**2**, were built based on the X-ray single crystal structure of 8,9-didehydro-14-deoxybufalin (Supporting Information), which was a structurally modified bufadienolide derivative with the same C, D, and E rings as **2**.[20] Furthermore, their ECD spectra were calculated at the level of PBE0/6-311++G (2d, 2p) with the PCM solvent continuum model and MeOH as the solvent, and the stereoisomer with the 1*R*, 3*R* configurations (Figure 4)

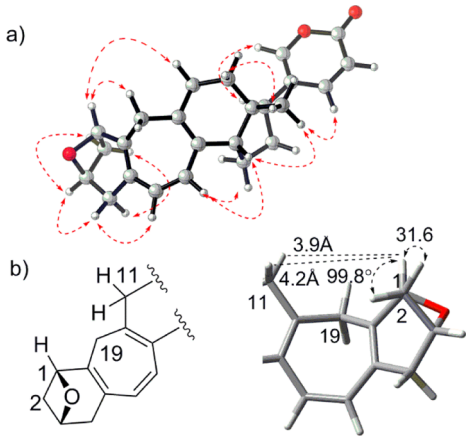

Figure 3. (a) Key NOE correlations of **2**; (b) conformational analysis of rings A/B in **2**: an optimized stereoisomer shows the dihedral angles between H-1 and H-2α/H-2β and the spatial distance between H-1 and H-11α/H-11β.[19]

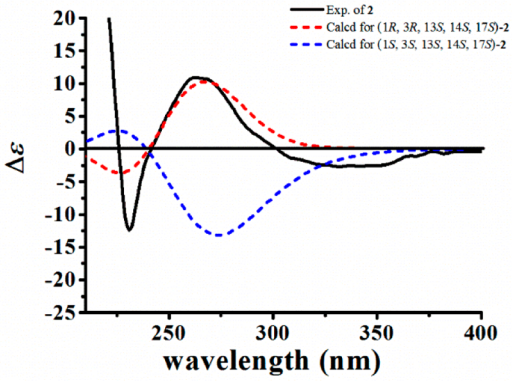

Figure 4. Experimental ECD spectrum of **2** in MeOH and the calculated ECD spectra of two optimized stereoisomers.

matched well with the experimental spectrum, which was strong evidence that it was the most reasonable absolute configuration.[21] It is quite interesting that the signal of H-1 displayed as a doublet with a coupling constant of 5.9 Hz in the ^{1}H NMR spectrum, though the vicinal C-2 bears two protons. Through a conformation analysis, it was found that the dihedral angle between H-1 and H-2α was 99.8°, corresponding to a coupling constant close to zero, while that between H-1 and H-2β was 31.6°, in line with a coupling constant of 5.9 Hz based on the Karplus equation (Figure 3).[22] Moreover, the analysis of the spatial distances between the protons illustrated well the NOE correlations observed in its ROESY spectrum (Figure 3b). The spatial distances between H-1 and H-11α/H-11β were 4.2 and 3.9 Å due to the arch-shape of the molecule, supporting the NOE correlations between those protons. Thus, the structure of **2**, named bufogargarizin C, was fully established.

Many traditional Chinese medicines containing bufadienolides or other steroid-like compounds have been reported to have a cardioactive effect and promote the blood circulation via inhibition of NKA.[23] Thus, compounds **1** and **2** together with bufalin (the main bufadienolide in *Bufo bufo gargarizans*) were evaluated for their inhibitory activities against NKA. At 12.5 and 25 μM, compound **1** showed 21% and 43% inhibition of NKA, respectively. At the same concentrations, bufalin showed complete inhibition of NKA, while compound **2** was inactive,

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which suggested that a bufadienolide with a rearranged A/B ring structure is not favorable for NKA inhibition.

In the present work we report two novel steroids from toad bile and venom, respectively. It is very intriguing for toads to synthesize such interesting A/B ring rearranged steroids. Compound **1** has an unusual 5/7/6/5/5/6 ring system, which is the first example of a spirostanol with alteration of rings A and B. Compound **2** is a steroid presumably biosynthesized from a normal bufadienolide, through a series of steps to form an unprecedented 6/7/6/5/5 ring system containing a cycloheptatriene in its backbone. The discovery of these compounds enriches the structural patterns of the steroid family. The biosynthetic pathways and the endogenous biological functions of these compounds in toads need further investigations.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting point was obtained with an X-5 melting point apparatus without correction. Optical rotations were measured in MeOH on a Jasco P-1020 polarimeter at room temperature. UV spectra were recorded in MeOH on a Jasco V-550 UV/vis spectrophotometer. ECD spectra were obtained on a Jasco J-810 spectropolarimeter. NMR spectra were acquired with Bruker AV-300, AV-400, and AV-600 spectrometers using TMS as an internal standard. HRESIMS spectra were measured on an Agilent 6210 ESI/TOF mass spectrometer. The X-ray diffraction study was carried out on an Agilent Gemini S Ultra with Cu Kα radiation. Column chromatographic methods were carried out on commercial silica gel (100−400 mesh, Qingdao Marine Chemical Plant). TLC analyses were carried out using precoated silica gel GF_{254} plates (Qingdao Marine Chemical Plant). Analytical high-performance liquid chromatography (HPLC) was carried out on an Agilent 1200 system chromatograph equipped with a Quat Pump and DAD detector. Semipreparative HPLC (semi-RP-HPLC) was performed on a WUFENG LC-100 system equipped with an UV detector using a COSMOSIL Packed $5C_{18}$-MS-II column (particle size 5 μm, 250 × 10 mm). All solvents used in column and HPLC were of analytical grade (Shanghai Chemical Plant) and chromatographic grade (Fisher Scientific), respectively.

Animal Materials. The gallbladders and venom of toads were collected from Baoying Toad Breeding Base in Jiangsu province of China, and authenticated as *Bufo bufo gargarizans* Cantor by Guang-Xiong Zhou (Jinan University, Guangzhou, P. R. China). They were sacrificed according to a procedure approved by the Institute Animal and Use Committee of Jinan University (No. 20130729001) and in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (seventh edition).

Extraction and Isolation. The collected gallbladders (240 g) and venom (1.5 kg) were extracted thoroughly with 95% EtOH under ultrasonic condition (40 min × 3, 40 °C). The combined EtOH extracts were filtered and concentrated under reduced pressure to afford 24.0 g of extract for the bile and 900.0 g for the venom. The concentrated residue of bile was then suspended in H_2O and partitioned with cyclohexane, EtOAc, and *n*-BuOH, and those of the venom were dissolved in 20% EtOH and partitioned with CH_2Cl_2 three times. The EtOAc fraction (1.6 g) of the bile extract was subjected to silica gel column chromatography (200−300 mesh) with a gradient elution of CH_2Cl_2/MeOH (from 50:1 to 2:1) to yield 15 subfractions (TBE-1 to 15). Compound **1** (1.9 mg, t_R 24.6 min) was separated from fraction TBE-2 by semipreparative HPLC eluting with 82% MeOH/H_2O. The CH_2Cl_2 portion of the venom extract was then chromatographed on silica gel (10 times, 200−300 mesh), eluting with cyclohexane-−cetone (5:1, 3:1, 1:1) to yield 15 fractions (Fr. 1 to 15). Fr. 8 was separated by reversed-phase C18 silica gel, eluting with MeOH/H_2O gradients (20:80−90:10) to give six subfractions (Fr. 8.1−Fr. 8.6). Fr. 8.6 was purified by preparative HPLC using MeOH/H_2O (72:28) as eluent to yield **2** (2.0 mg).

*Bufospirostenin A (**1**).* Colorless needle crystals; mp 187−189 °C; $[\alpha]_D^{27}$ + 54 (*c* 0.1, MeOH); UV (MeOH): λ_{max} (log ε) 208 (3.57) nm; IR (KBr): 3414, 2956, 2935, 1617, 1204 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and S1; HRESIMS: *m*/*z* 429.29985 $[M + H]^+$ (calcd for $C_{27}H_{41}O_4$, 429.29994).

*Bufogargarizin C (**2**).* White powder; $[\alpha]_D^{25}$ + 20 (*c* 0.1, MeOH); UV (MeOH): λ_{max} (log ε) 300 (3.80) nm; ECD (0.02 mg/mL, MeOH), λ_{max} ($\Delta\varepsilon$) 342 (−2.63), 263 (10.89), and 231 (−12.40) nm; IR (KBr): 2927, 2858, 1715, 1666 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, Tables 1, S2 and S3; ESI-MS: *m*/*z* 363 $[M + H]^+$, 385 $[M + Na]^+$ and 747 $[2M+Na]^+$; HRESIMS: *m*/*z* 363.1959 $[M + H]^+$ (calcd for $C_{24}H_{27}O_3$, 363.1955).

Biological Assay. Na/K-ATPase from pig kidney microsomal membranes was prepared by treatment with SDS and purified by differential centrifugation.[23] The specific activities of the Na/K-ATPase of various kidney preparations were in the range 900 to 1200 μmol Pi/mg/h, which was more than 95% of the total ATPase activity. The inhibitory activities of different concentrations of compounds **1**, **2**, and bufalin (purity over 98% by HPLC analysis) on 0.5 μg of Na/K-ATPase were determined by measuring the release of inorganic phosphate from ATP over a 15 min period at 37 °C in an assay medium containing 100 mM NaCl, 20 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 20 mM Tris-HCl, and 2 mM ATP-Mg^{2+} mixture, pH 7.4. The absorbance at 620 nm was measured with a microplate reader (SpectraMax 340PC384, Molecular Devices) after treatment using the Biomol Green Reagent.[24]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b01018.

Detailed NMR spectra; HR-ESI-MS, ESI-MS, UV spectra; and X-ray crystal data of **1** and 8,9-didehydro-14-deoxybufalin (PDF)

X-ray crystallography data (cif) for **1** (CCDC 1434619) (CIF)

X-ray crystallography data (cif) for 8,9-didehydro-14-deoxybufalin (CCDC 1471863) (CIF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Yang, Q.; Zhou, X. X.; Zhang, M.; Bi, L. L.; Miao, S.; Cao, W.; Xie, Y. H.; Sun, J. Y.; Tang, H. F.; Li, Y.; Miao, Q.; Wang, S. W. *Am. J. Transl. Res.* **2015**, *7*, 1−14.

(2) Wen, G.; Yang, W.; Fu, J. *Zool. Sci.* **2015**, *32*, 427−434.

(3) Editorial Committee of the Administration Bureau of Traditional Chinese Medicine. *Chinese Materia Medica (Zhonghua Bencao)*; Shanghai Science and Technology Press: Shanghai, 1999; Vol. *9*, pp 362−367.

(4) Gao, H. M.; Zehl, M.; Leitner, A.; Wu, X. Y.; Wang, Z. M.; Kopp, B. *J. Ethnopharmacol.* **2010**, *131*, 368−376.

(5) Yang, T.; Shi, R. L.; Chang, L.; Tang, K.; Chen, K.; Yu, G.; Tian, Y. F.; Guo, Y. L.; He, W.; Song, X. D.; Xu, H.; Ye, Z. Q. *J. Exp. Clin. Cancer Res.* **2015**, *34*, 21.

(6) Gao, H. M.; Popescu, R.; Kopp, B.; Wang, Z. M. *Nat. Prod. Rep.* **2011**, *28*, 953−969.

(7) Zhang, P.; Cui, Z.; Liu, Y. S.; Wang, D.; Liu, N.; Yoshikawa, M. *Chem. Pharm. Bull.* **2005**, *53*, 1582−1586.

(8) Tian, H. Y.; Wang, L.; Zhang, X. Q.; Zhang, D. M.; Wang, Y.; Liu, J. S.; Jiang, R. W.; Ye, W. C. *Steroids* **2010**, *75*, 884−890.

(9) Tian, H. Y.; Luo, S. L.; Liu, J. S.; Wang, L.; Wang, Y.; Zhang, D. M.; Zhang, X. Q.; Jiang, R. W.; Ye, W. C. *J. Nat. Prod.* **2013**, *76*, 1842−1847.

(10) Konig, E.; Zhou, M.; Wang, L.; Chen, T. B.; Bininda-Emonds, O. R. P.; Shaw, C. *Toxicon* **2012**, *60*, 967−981.

(11) Yoshii, M.; Une, M.; Kihira, K.; Kuramoto, T.; Akizawa, T.; Yoshioka, M.; Butler, V. P., Jr.; Hoshita, T. *J. Lipid Res.* **1994**, *35*, 1646−1651.

(12) Prassas, I.; Diamandis, E. P. *Nat. Rev. Drug Discovery* **2008**, *7*, 926−935.

(13) Tian, H. Y.; Zhang, P. W.; Liu, J. S.; Zhang, D. M.; Zhang, X. Q.; Jiang, R. W.; Ye, W. C. *Chin. Chem. Lett.* **2014**, *25*, 1104−1106.

(14) Tian, H. Y.; Wang, L.; Zhang, X. Q.; Wang, Y.; Zhang, D. M.; Jiang, R. W.; Liu, Z.; Liu, J. S.; Li, Y. L.; Ye, W. C. *Chem. - Eur. J.* **2010**, *16*, 10989−10993.

(15) Park, J. E.; Woo, K. W.; Choi, S. U.; Lee, J. H.; Lee, K. R. *Helv. Chim. Acta* **2014**, *97*, 56.

(16) Barbic, M.; Willer, E. A.; Rothenhofer, M.; Heilmann, J.; Furst, R.; Jurgenliemk, G. *Phytochemistry* **2013**, *90*, 106−113.

(17) Niu, C. S.; Ho, D. M.; Zask, A.; Ayral-Kaloustian, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1535.

(18) Brown, G. D. *Nat. Prod. Rep.* **1998**, *15*, 653−696.

(19) Legault, C. Y. *CYLview*, 1.0b; Université de Sherbrooke: 2009 (http://www.cylview.org).

(20) Jiang, R. W.; Yu, T.; Tian, H. Y.; Li, J.; Ye, W. C. CN. Patent 10,300,670, 2013.

(21) Li, X. C.; Ferreira, D.; Ding, Y. Q. *Curr. Org. Chem.* **2010**, *14*, 1678−1697.

(22) Burgueno-Tapia, E.; Hernandez, L. R.; Resendiz-Villalobos, A. Y.; Joseph-Nathan, P. *Magn. Reson. Chem.* **2004**, *42*, 887−892.

(23) Chen, R. J.; Jinn, T. R.; Chen, Y. C.; Chung, T. Y.; Yang, W. H.; Tzen, J. T. *Acta Pharmacol. Sin.* **2011**, *32*, 141−151.

(24) Klodos, I.; Esmann, M.; Post, R. L. *Kidney Int.* **2002**, *62*, 2097−2100.

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