Three New Alkaloids from the Traditional Chinese Medicine ChanSu

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Three new alkaloids, named bufoserotonins A – C (**1** – **3**), were isolated from the traditional Chinese medicine ChanSu. Their structures were elucidated on the basis of spectroscopic analysis, especially of 2D-NMR data.

Introduction. – ChanSu, also called toad venom or toad poison, is an important traditional Chinese medicine (TCM) prepared from the skin secretions of giant toads, including *Bufo bufo gargarizans* CANTOR and *B. melanostictus* SCHNEIDER. It is one of the major components of many well-known patent TCM drugs like Liu-Shen-Wan, She-Xiang-Bao-Xin-Wan, and Chan-Su injection, and is frequently used in clinics to treat heart failure, sores, pains, and various cancers [1]. Chemical studies of toad venom showed that bofotoxins, bufogenins, and indole alkaloids were the main and characteristic constituents [2]. Within the scope of our continuous interest in traditional Chinese medicine, we examined the BuOH-soluble fraction of ChanSu, leading to the isolation of three new indole alkaloids: bufoserotonins A – C (**1** – **3**). This paper describes the isolation and structure elucidation of the new indole alkaloids.

Results and Discussion. – Bufoserotonin A[2]) (**1**) was obtained as colorless amorphous solid. It gave a positive *Dragendorff* reaction for alkaloids. The HR-ESI-MS determined its empirical molecular formula $C_{11}H_{13}N_3O_2$ (from $[M+H]^+$ at m/z 220.1091). The UV (MeOH) spectrum (221, 277, and 300 nm) showed the presence of an indole chromophore [3]. By means of ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), ^{1}H,^{1}H-COSY, HMQC, and HMBC (*Fig. 1*) data, bufoserotonin A[2]) was structurally elucidated to be *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]urea (**1**).

The ^{1}H-NMR spectrum of **1** displayed signals of protons due to an *ABX*-type aromatic ring (δ(H) 7.11 (*d*, $J = 8.6$ Hz); 6.83 (*d*, $J = 2.1$ Hz); 6.65 (*dd*, $J = 8.6, 2.1$ Hz)) and an olefinic-proton at δ(H) 7.02 (*d*, $J = 1.8$ Hz), suggesting that the indole unit was disubstituted. Four CH_2 protons at δ 3.23 (*q*, $J = 7.0$ Hz, 2 H) and 2.60 (*t*, $J = 7.0$ Hz, 2 H) showed the presence of a CH_2CH_2 unit, connected to the indole ring at

[1]) The first two authors contribute equally to the work of the paper.
[2]) Arbitrary numbering; for systematic names, see *Exper. Part.*

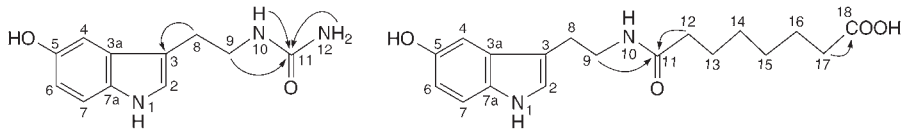


Fig. 1. *Structures and key HMBC correlations* (H → C) *of* **1** *and* **2**

Table 1. *^{1}H-NMR Data* (500 MHz, (D_6)DMSO) *of Compounds* **1**–**3**[2]). δ in ppm, J in Hz.

	1	**2**	**3**
H–N(1)	10.51 (*s*)	10.67 (*s*)	10.51 (*s*)
H–C(2)	7.02 (*d*, $J=1.8$)	7.01 (*d*, $J=1.8$)	6.97 (*d*, $J=2.3$)
H–C(4)	6.83 (*d*, $J=2.1$)	6.82 (*d*, $J=2.0$)	6.98 (*d*, $J=2.2$)
H–C(6)	6.65 (*dd*, $J=8.6, 2.1$)	6.58 (*dd*, $J=8.5, 2.0$)	6.59 (*dd*, $J=8.6, 2.2$)
H–C(7)	7.11 (*d*, $J=8.6$)	7.11 (*d*, $J=8.5$)	7.10 (*d*, $J=8.6$)
CH_2(8)	2.69 (*t*, $J=7.0$)	2.70 (*t*, $J=7.0$)	2.90 (*t*, $J=7.6$)
CH_2(9)	3.23 (*q*, $J=7.0$)	3.28 (*q*, $J=7.0$)	4.41 (*t*, $J=7.6$)
H–N(10)	6.06 (*t*, $J=5.6$)	7.84 (*t*, $J=5.0$)	
NH_2(12)	5.45 (*s*)		
H–C(11)			7.29 (*d*, $J=1.7$)
CH_2(12)		2.05 (*t*, $J=7.0$)	
CH_2(13) or H–C(13)		1.46–1.50 (*m*)	6.89 (*d*, $J=1.7$)
CH_2(14)		1.22–1.26 (*m*)	
CH_2(15)		1.22–1.26 (*m*)	
CH_2(16)		1.46–1.50 (*m*)	
CH_2(17)		2.15 (*t*, $J=7.0$)	
H–N(1′)			9.93 (*s*)
Me(3′)			1.95 (*s*)
Me(2″)			2.38 (*s*)

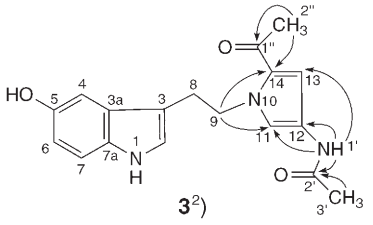
C(3), as established by the analysis of the ^{1}H,^{1}H-COSY and HMQC data. The ^{13}C-NMR spectrum showed 11 signals (2 CH_2 (sp^3), 4 CH (sp^2), and 5 C (sp^2)) (see *Table 2*). A *s* at δ 158.6 was assigned to a C=O group. The HMBC correlations (*Fig. 1*) from δ(H) 3.23 (*q*, CH_2(9)), 5.45 (*s*, NH_2(12)), and 6.06 (*t*, H–N(10)) to the C=O signal at δ(C) 158.6 (C(11)) revealed that **1** was a derivate of urea.

Compound **2** exhibited a quasimolecular-ion peak at *m*/*z* 333.1819 ($[M+H]^+$) in the HR-MS, which corresponded to the molecular formula $C_{18}H_{24}N_2O_4$. It also gave a positive *Dragendorff* reaction for alkaloids. Comparing with **1**, the UV data and NMR spectra (*Tables 1* and *2*) of **2** also showed the presence of a 3,5-disubstituted indole unit, and comparison with bufobutanoic acid [4] unambiguously determined **2** to be 8-{[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]amino}-8-oxooctanoic acid, named bufoserotonin B[2]).

The ^{1}H-NMR data of **2** were similar to those of bufobutanoic acid (=4-{[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]amino}-4-oxobutanoic acid) [4], except for four additional CH_2 groups (δ(H) 1.46–1.50 (*m*, 4 H); 1.22–1.26 (*m*, 4 H)) in **2**, which were bound to each other as established by the analysis of ^{1}H,^{1}H-COSY, NOESY, and HMBC data. The HMBC long-range correlations (*Fig. 1*) from protons due to two of the four CH_2 groups at δ(H) 1.46–1.50 (*m*, CH_2(13) and CH_2(16)) to the two C=O groups at δ(C)

Table 2. *^{13}C-NMR Data* (125 MHz, (D_6)DMSO) *of Compounds* **1**–**3**[2]). δ in ppm.

	1	**2**	**3**
C(2)	123.0	123.0	123.5
C(3)	110.9	110.9	109.8
C(3a)	127.8	127.9	127.8
C(4)	102.2	102.2	102.4
C(5)	150.1	150.1	150.2
C(6)	111.2	111.2	111.3
C(7)	111.5	111.6	111.6
C(7a)	130.7	130.7	130.7
C(8)	26.1	25.4	27.5
C(9)	39.8	39.4	49.4
C(11)	158.6	171.9	120.8
C(12)		35.5	122.9
C(13)		25.2	110.2
C(14)		28.5	126.4
C(15)		25.2	
C(16)		24.8	
C(17)		34.8	
C(18)		172.3	
C(2′)			166.7
C(3′)			23.0
C(1″)			187.3
C(2″)			27.1



3[2])

Fig. 2. *Structure and key HMBC correlations* (H → C) *of* **3**

171.9 (C(11)), and 172.3 (C(18)) indicated that the succinyl moiety of bufobutanoic acid was replaced by a suberoyl (= octanedioyl) moiety in **2**.

Compound **3** was obtained as brown amorphous solid, and showed a positive *Dragendorff* reaction for alkaloids. The ion at *m*/*z* 348.2 ($[M+Na]^+$) in the ESI-MS was in agreement with the formula $C_{18}H_{19}N_3O_3$, which was confirmed by the HR-ESI-MS ($[M+Na]^+$ ion at *m*/*z* 348.13352). Comparing the UV data and NMR spectra (*Tables 1* and *2*) of **3** with those of **1** and **2**, **3** was also identified as a derivative of a 3-ethyl-substituted indole. Further data (*Fig. 2*) established the structure of **3** as *N*-{5-acetyl-1-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]-1*H*-pyrrol-3-yl}acetamide, named bufoserotonin C[2]).

The ^{1}H-NMR resonances of **3** at δ(H) 7.29 (*d*, *J* = 1.7 Hz, H–C(11)), 6.89 (*d*, *J* = 1.7 Hz, H–C(13)), 2.38 (*s*, Me(2″)), and 1.95 (*s*, Me(3′)) showed HMQC cross-peaks with δ(C) 120.8 (C(11)), 110.2 (C(13)),

27.1 (C(13)), and 23.0 (C(3′)), respectively. The ^{13}C-NMR spectrum showed additional four signals in the downfield region at δ(C) 187.3 (C(1″)), 166.7 (C(2′)), 126.4 (C(14)), and 122.9 (C(12)). The HMBC long-range correlations (*Fig. 2*) δ(H) 7.29/δ(C) 122.9 (C(12)), 110.2 (C(13)), and 126.4 (C(14)), and δ(H) 6.89/δ(C) 120.8 (C(11)) and 126.4 (C(14)) indicated that there was a pyrrole ring in **3** (see *Exper. Part*). The HMBC correlations δ(H) 9.93 (H–N(1′))/δ(C) 166.7 (C(2′)), 110.2 (C(13)), 120.8 (C(11)), and 122.9 (C(12)), δ(H) 1.95/δ(C) 166.7 (C(2′)), and δ(H) 2.38/δ(C) 187.3 (C(1″)) and 126.4 (C(14)) suggested the presence of an acetyl and acetamide moiety, bound to C(14) and C(12) of the pyrrole ring, respectively. The location of the ethyl substituent at the N-atom of the pyrrole moiety was confirmed by the HMBC cross-peaks δ(H) 4.41 (CH_2(9))/δ(C) 120.8 (C(11)) and 126.4 (C(14)).

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Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA), *ODS* (25–40 μ; *Merck*), *MCI* gel *CHP-20P* (75–150 μm, *Mitsubishi Chemical Industries*, Tokyo, Japan), or silica gel (200–300 mesh; *Qingdao Marine Chemical Plant,* China). HPLC: *Shimadzu 2010* apparatus; semi-prep. column (*Zorbax SB-C18*, 9.4 × 250 mm; *Agilent*, USA). M.p.: *RY-2* apparatus (*Analytical Instruments Co.*, Tianjin, China); uncorrected. UV Spectra: *Shimadzu UV-210A*; λ_{max} in nm. NMR Spectra: *Bruker* NMR spectrometer *DRX-500*; 500 (^{1}H) and 125 MHz (^{13}C)); in (D_6)DMSO; δ in ppm rel. to $SiMe_4$, *J* in Hz. HR-ESI-MS: *Q-TOF-Micro-Mass* spectrometer.

Material. Thin-plate ChanSu was purchased from *Nantong Jianqiao* pharmaceuticals company in Jiangsu Province, China, in November 2004, and authenticated by Prof. *Han-Ming Zhang* of the Department of Pharmacognosy of this college. It appears as a dark brown rectangular thin plate (23 cm × 10 cm × 0.1 cm). A voucher specimen (20041125) has been deposited at the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried and powdered ChanSu (7.5 kg) was extracted with 90% EtOH (30 l). After evaporation of EtOH, the remaining aq. soln. (3 l) was partitioned successively with $CHCl_3$ (3 × 3 l), AcOEt (3 × 3 l), and BuOH (3 × 3 l). The BuOH extract (160 g) was subjected to CC (silica gel, gradient $CHCl_3$/MeOH and AcOEt/MeOH/H_2O): *Fractions A1–A5*. *Fr. A2* (15.24 g) was subjected to CC (*ODS*, MeOH/H_2O 20:80) to afford 5.56 g of crude material which was purified by prep. HPLC (10% MeCN/H_2O, detection at 227 nm): 5 mg of **1**. *Fr. A3* (9.34 g) was subjected to CC (*ODS*, MeOH/H_2O 15:85) to afford 3.25 g of crude material which was purified by prep. HPLC (8% MeCN/H_2O, detection at 230 nm): **2** (5 mg) and **3** (15 mg).

N-*[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]urea* (= *Bufoserotonin A*; **1**): Colorless, amorphous powder. M.p. 156–158° (MeOH). UV (MeOH): 221, 277, 300. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HMBC (500 MHz, (D_6)DMSO): H–N(1)/C(2), C(3), C(3a), C(7a); H–C(2)/C(3), C(3a), C(7a); H–C(4)/C(3), C(5), C(7a); H–C(6)/C(4), C(5), C(7a); H–C(7)/C(3a), C(5); CH_2(8)/C(9), C(2), C(3), C(3a); CH_2(9)/C(8), C(3), C(11). ESI-MS: 220.1 ([M + H]$^+$). HR-ESI-TOF-MS: 220.1091 ([M + H]$^+$, $C_{11}H_{14}N_3O_2^+$; calc. 220.1085).

*8-{[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]amino}-8-oxooctanoic Acid* (= *Bufoserotonin B*; **2**): Colorless, amorphous powder. UV (MeOH): 236, 309. ^{1}H- and ^{13}C- NMR: *Tables 1* and *2*. HMBC (500 MHz, (D_6)DMSO): H–N(1)/C(2), C(3), C(3a), C(7a); H–C(2)/C(3), C(3a), C(7a); H–C(4)/C(5), C(6), C(7a); H–C(6)/C(4), C(5), C(7a); H–C(7)/C(3a), C(5); H–C(8)/C(9), C(2), C(3), C(3a); H–C(9)/C(3), C(8), C(11); H–C(12)/C(11), C(13), C(14); H–C(13)/C(11), C(12), C(14); H–C(14)/C(13); H–C(15)/C(14); H–C(16)/C(17), C(18); H–C(17)/C(16), C(18). ESI-MS: 333.2 ([M + H]$^+$). HR-ESI-TOF-MS: 333.1819 ([M + H]$^+$, $C_{18}H_{25}N_2O_4^+$; calc. 333.1813).

N-*{5-Acetyl-1-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]-1*H*-pyrrol-3-yl}acetamide* (= *Bufoserotonin C*; **3**): Brown amorphous powder. M.p. 182–185° (MeOH). UV (MeOH): 221, 292, 302. IR (KBr): 3000–3500 (OH, NH), 1706, 1626, 1596, 1579, 1538, 1497, 1468, 1436, 1349, 1280, 1245, 1227, 1194, 1159, 1127, 948,

840. ^{1}H- and ^{13}C- NMR: *Tables 1* and *2*. HMBC (500 MHz, (D_6)DMSO): H–N(1)/C(2), C(3), C(3a), C(7a); H–C(2)/C(3), C(3a), C(7a); H–C(4)/C(3), C(3a), C(5), C(6), C(7a); H–C(6)/C(4), C(5), C(7a); H–C(7)/C(3a), C(5); H–C(8)/C(9), C(2), C(3), C(3a); H–C(9)/C(3), C(8), C(11), C(14); H–C(11)/C(9), C(13), C(12), C(14), C(1″); H–C(13)/C(11), C(14), C(1″); H–N(1′)/C(11), C(12), C(13), C(21); H–C(1′)/C(14), H–C(3′)/C(2′); H–C(2″)/C(14), C(1″). ESI-MS: 326.3 ($[M+H]^+$), 348.2 ($[M+Na]^+$). HR-ESI-TOF-MS: 348.13352 ($[M+Na]^+$, $C_{18}H_{19}N_3O_3Na^+$; calc. 348.13349).

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