BUFOBUTANOIC ACID AND BUFOPYRAMIDE, TWO NEW INDOLE ALKALOIDS FROM THE CHINESE TRADITIONAL DRUG CH'AN SU

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Abstract- Two new bioactive indole alkaloids, 5-hydroxytryptamine (serotonin) *N*-4-oxobutanoic acid and 5-hydroxytryptamine *N*-acetyl-*N*-pyrrolecarboxamide , named as bufobutanoic acid (**2**) and bufopyramide (**3**), respectively, were isolated from the Chinese traditional drug Ch'an Su in addition to known bufotenine (**1**). The structures were elucidated on the basis of spectroscopic data. Bufobutanoic acid (**2**) and bufopyramide (**3**) exhibited cytotoxicity against murine P388 lymphocytic leukemia cells with IC_{50} values of 22 μg/mL and 7.6 μg/mL, respectively.

The Chinese traditional drug Ch'an Su (蟾酥) was prepared from the skin secretions of the local toads such as *Bufo bufo gargarizans* Cantor or *Bufo melanostrictus* Schneider. Although the steroidal bufadienolides with an α-pyrone ring at C-17 position were the major biologically and pharmaceutically active components, the water-soluble basic components such as catecholamines and indolealkylamines were also responsible for the activity.[1] Until now, the twelve indolealkylamines, serotonin, bufotenine, bufotenidine, bufoviridine, bufothionine, *O*-methylbufotenine, dehydrobufotenine, bufoviridine-*N*-sulfate, *O*-methylbufotenine-*N*-sulfate, 5-hydroxy-*N*-methyltryptamine, 5-methyltryptamine and 5-methyl-*N*-methyltryptamine, have been isolated from many kinds of toads. However, from Ch'an Su, only bufotenine,[2,3] bufotenidine,[2,4~6] bufothionine[7] and dehydrobufotenine[5] were isolated. A consideration of the above facts and an interest in the water-soluble components of Ch'an Su have led to the present investigation. Here we describe the isolation and structural elucidation of two new indole alkaloids, 5-hydroxytryptamine *N*-4-oxobutanoic acid and 5-hydroxytryptamine *N*-acetyl-*N*-pyrrolecarboxamide, named as bufobutanoic acid (**2**) and bufopyramide (**3**) respectively, in addition to the isolation of known bufotenine (**1**)(Figure. 1).

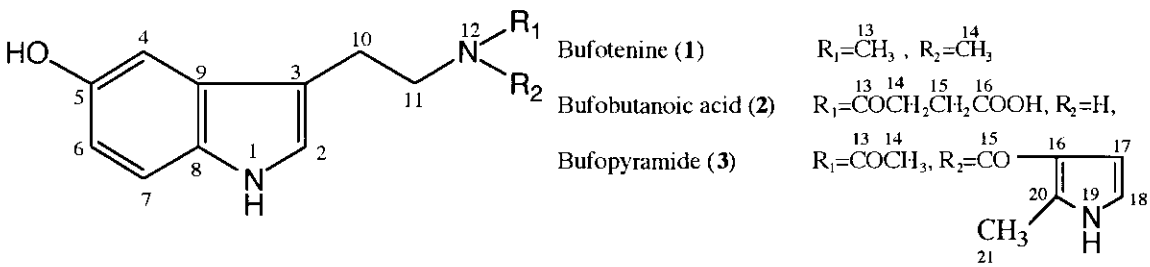


Figure 1

Table 1 ^{1}H and ^{13}C NMR Spectral Data of Indole Alkaloids (**1**～**3**).

	Bufotenine (1)		Bufobutanoic acid (2)		Bufopyramide (3)	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	10.70 (1H, s)		10.45 (1H, s)		11.52 (1H, s)	
2	7.14 (1H, d, J=2.2)	119.74	7.02 (1H, d, J=1.7)	122.99	7.46 (1H, d, J=2.2)	110.89
3		103.24		110.82		111.49
4	6.92 (1H, d, J=2.1)	98.19	6.82 (1H, d, J=2.2)	102.14	7.94 (1H, s)	104.23
5-OH	8.73 (1H, br s)	146.43	8.55 (1H, s)	150.10	8.73 (1H, s)	152.37
6	6.65 (1H, dd, J=8.6, 2.1)	107.65	6.58 (1H ,dd, J=8.6, 2.2)	111.18	7.50 (1H, d, J=8.6)	112.54
7	7.16 (1H, d, J=8.6)	107.81	7.11 (1H, d, J=8.6)	111.55	7.28 (1H, d, J=8.6)	112.97
8		126.75		130.75		132.43
9		123.36		127.83		129.44
10	3.07 (2H, m)	14.68	2.70 (2H, t, J=7.8)	25.29	3.37 (2H, t, J=7.9)	28.78
11	3.52 (2H, m)	61.22	3.26 (2H, t, J=7.0)	39.44	4.75 (2H, t, J=7.9)	50.86
12			7.92 (1H, t, J=5.4)			
13	3.17 (3H, s)	48.11		170.70		167.56
14	3.17 (3H, s)	48.11	2.43 (2H, t, J=6.7)	29.15	2.17 (3H, s)	23.41
15			2.32 (2H, t, J=6.7)	30.04		187.68
16				173.78		127.63
17					7.24 (1H, d, J=1.8)	124.40
18					7.79 (1H, d, J=1.3)	121.70
19					10.78 (1H, s)	
20						122.96
21					2.34 (3H, s)	27.13

The MeOH extract (49.7 g) of Ch'an Su (200 g) was separated on HP-20 column with aq MeOH solution. The crude material (5.3 g) eluted with H_2O-MeOH (60 : 40) was rechromatographed on ODS column by preparative HPLC with H_2O-MeCN (98 : 2) containing 0.05% trifluoroacetic acid (TFA) to give two compounds (**1**) (12.9 mg) and (**2**) (6.8 mg). ODS column chromatography of another crude material (4.53 g) was eluted with H_2O-MeOH (40 : 60), using H_2O-MeOH (42 : 58) afforded compound (**3**) (11.6 mg) after purification by preparative HPLC with H_2O-MeCN (85 : 15～87 : 17).

The ^1H and ^{13}C NMR spectral data of compounds (**1-3**) were indicated in Table 1. Based on the NMR spectral data and other spectral (UV, IR and MS. See Experimental for IR and MS.) data, compound (**1**) was found to be an indole alkaloid. The UV absorption of **1** indicating the presence of indole moiety appeared at λ_{max} 225, 274, and 300 nm. In the ^1H NMR spectra of **1**, the NH signal and *N,N*-dimethyl signal were observed at δ 10.70 (1H, s) and δ 3.17 (6H, s) respectively. The compound (**1**) was identified to be known bufotenine by direct comparison with the authentic sample[2,3].

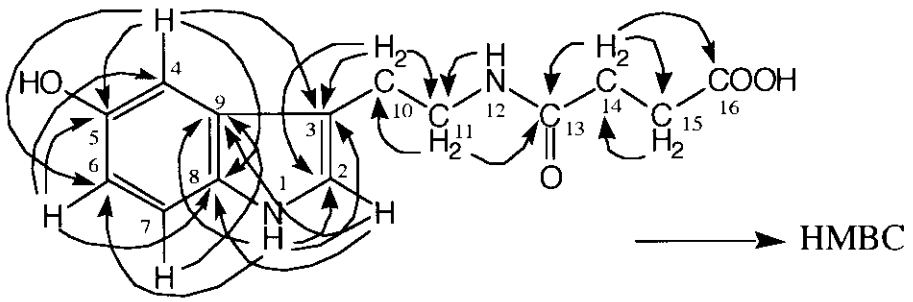
Compound (**2**) showed the following physical and spectral data: UV (MeOH) λ_{max} 225, 274 and 300 nm; IR (KBr) ν_{max} 3320, 1630 and 1185 cm^{-1} and EI-MS (m/z) M^+ 276. The molecular formula of **2** was *determined* as $C_{14}H_{16}N_2O_4$ by HRFAB-MS. From these and the NMR spectral data (Table 1), compound **2** was also suggested to be an indole alkaloid. In ^1H NMR spectra of compound (**2**), two signals at δ 10.45 (1H, s) and 7.92 (1H, t, J=5.4) were assigned to NH of indole moiety and NH of ethyl side chain respectively. In ^{13}C NMR spectra, two signals at δ 170.70 and δ 173.78 were assigned to CO of amide *group and CO of carboxylic acid respectively*. *The assignment* was *supported* from IR spectral data indicated above. Also, the other two $-CH_2CH_2-$ units were provided from the 2D ^1H-^1H and ^{13}C-^1H COSY experiments. The sequence on structure was obtained by HMBC analysis, as shown in Figure 2. Thus, compound (**2**) was found to be 4-{[2-(5-hydroxy-1*H*-3-indolyl)ethyl]amino}-4-oxobutanoic acid corresponding to the *N*-4-oxobutanoic acid of serotonin (5-hydroxytryptamine), *which was named as* bufobutanoic acid.

Compound (**3**) showed the following data: UV (MeOH) λ_{max} 208, 225 and 302 nm; IR (KBr) ν_{max} 3200, 1400 and 1190 cm^{-1}, EI-MS (m/z) M^+ 325. The molecular formula of **3** was determined as $C_{18}H_{19}N_3O_3$ by HRFAB-MS. From the ^1H and ^{13}C NMR spectral data (Table 1), the presence of indole moiety in the structure of compound (**3**) was suggested. In the ^1H NMR spectra, a new NH signal at δ 10.78 (1H, s) was observed, in addition to NH signal (δ 11.52, 1H, s) on indole ring. The four ^{13}C signals at δ 127.63, 124.40, 121.70 and 122.96 and the correlating two ^1H signals at δ 7.24 (1H, d, J=1.8) and 7.79 (1H, d, J=1.3) suggested the presence of another heterocyclic ring moiety, which was assigned to be a pyrrolecarboxamide group. As shown in Figure 3, the careful structural correlation by HMBC analysis illustrated a reasonable structure for compound (**3**). Thus, the structure of compound (**3**) was found to be *N*-3-acetyl-*N*-3-[2-(5-hydroxy-1*H*-3-indolyl)ethyl]-2-methyl-1*H*-3-pyrrolecarboxamide corresponding to the *N*-acetyl-*N*-pyrrolecarboxamide of serotonin (5-hydroxytryptamine), which was named as bufopyramide.

The occurrence of new indole alkaloids in Ch'an Su, bufobutanoic acid (**2**) and bufopyramide (**3**), indicates a possibility of further isolation from original or other toads. And, this result may arouse our interest in the biosynthetic aspect.

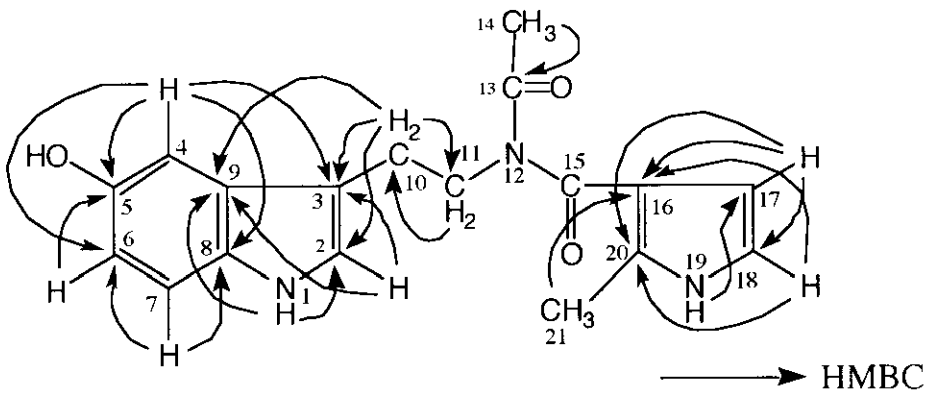
Interested in biological activities, we carried out various biological screenings for the two new alkaloids,

bufobutanoic acid (**2**) and bufopyramide (**3**). At this time, we report that a moderate activity (22 μg/mL for **2** and 7.6 μg/mL for **3** as IC_{50} value) against murine P388 lymphocytic leukemia cells has been recorded.



Bufobutanoic acid (**2**)

Figure 2



Bufopyramide (**3**)

Figure 3

EXPERIMENTAL

General Details-For chromatography and biological test, commercial solvents of analytical grade were used after redistillation. Ultraviolet spectra were measured with a SHIMAZU, UV 2500PC spectrophotometer, and IR spectra on a Jasco, FT/IR-300 spectrophotometer. HRFAB-MS and EI-MS spectra were taken with a JEOL JMS-AX505H mass spectrometer. For separation, HP-20 (DIAION, Mitsubishi Chemical Corp.) column and ODS (Chromatorex, Fuji Silysia Chemical LTD) column were used. HPLC was performed with an Inertsil PREP-ODS column (20 mm i.d.×250 mm, GL Science Inc.) packed with 10 μm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) or precoated DC-platten RP-18 F_{254} (Merck). The spots were detected by UV (UV GL-25, Mineralight lamp of Upland, CA, USA) and spraying with Dragendorff's reagent or anisaldehyde-sulfuric acid solution, and heating (hot plate). NMR spectra were recorded on a Brucker DRX-500 spectrometer. For

the homo- and hetero-nuclear NMR measurements, a solution of 5 mg of samples in 0.5 mL of DMSO-d_6 or pyridine-d_5 was used. The NMR coupling constants (J) are given in Hz.

Isolation- Ch'an Su (200 g) was extracted with MeOH (500 mL) three times at 45℃ for 3 h to give the extract (49.7 g), which was subjected to HP-20 column chromatography using H_2O and MeOH gradiently as solvents. Elution with H_2O-MeOH (60 : 40) afforded 5.3 g of crude materials, of which 540 mg was separated carefully by preparative HPLC using ODS column with H_2O-MeCN (98 : 2) solution containing 0.05% TFA at 220 nm to afford two compounds, bufotenine (**1**)(12.9 mg) and bufobutanoic acid (**2**) (6.8 mg). On the other hand, from elution with H_2O- MeOH (40 : 60), 4.53 g of another crude material was obtained. This was chromatographed on ODS column with H_2O-MeOH (42 : 58) to give the five fractions. The fourth fraction (700 mg) was purified by preparative HPLC using H_2O-MeCN (85 : 15～83 : 17) ODS column at 254 nm to give 11.6 mg of bufopyramide (**3**). The ^1H and ^{13}C NMR spectral data of **1**, **2**, and **3** were indicated in Table 1.

Bufotenine (**1**) : a colorless amorphous solid ; TLC: Rf 0.15 (3 : 7 $CHCl_3$/MeOH) and 0.28 (1 : 9 $CHCl_3$/MeOH) for normal phase, Rf 0.18 (4 : 6 MeOH/H_2O) and 0.37 (6 : 4 MeOH/H_2O) for reverse phase; Detection: UV (+), deep red (Dragendorff's reagent) and purple (anisaldehyde-sulfuric acid), EI-MS (m/z) M^+ 204; IR (KBr) ν_{max} 2300 and 800 cm^{-1}. The sample was identical to the authentic specimen.

Bufobutanoic acid (**2**) : an amorphous solid ; TLC: Rf 0.38 (3 : 7 $CHCl_3$/MeOH) and 0.65 (1 : 9 $CHCl_3$/MeOH) for normal phase, Rf 0.66 (4 : 6 MeOH/H_2O) and 0.86 (6 : 4 MeOH/H_2O) for reverse phase; Detection: UV (+), deep red (Dragendorff's reagent) and reddish purple (anisaldehyde-sulfuric acid), HRFAB-MS (m/z) $[M+Na]^+$ 299.1004 for $C_{14}H_{16}N_2O_4Na$; Δ -0.4 mmu.

Bufopyramide (**3**) : a colorless amorphous solid ; TLC: Rf 0.90 (3 : 7 $CHCl_3$/MeOH) and 0.93 (1 : 9 $CHCl_3$/MeOH) for normal phase, Rf 0.23 (4 : 6 MeOH/H_2O) and 0.46 (6 : 4 MeOH/H_2O) for reverse phase; Detection: UV (+), deep red (Dragendorff's reagent) and purple (anisaldehyde-sulfuric acid), HRFAB-MS (m/z) $[M+Na]^+$ 348.1311 for $C_{18}H_{19}N_3O_3Na$; Δ -1.3 mmu.

Bioassay-The murine P388 lymphocytic leukemia cells were maintained in tissue culture flasks and grown in 96 well microtiter plates for assay. Appropriate dilutions of the test compounds were added to the culture. After incubation at 37℃, 5% CO_2, for 72 h, the survival rate of cells in cultures was evaluated by the MTT method. The effect was shown as IC_{50}, which is the concentration of test compound (μg/mL) to give 50% inhibition of the growth of P388 cells.

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