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Alkaloids from the Traditional Chinese Medicine ChanSu: Synthesis-enabled structural reassignment of bufopyramide to bufoserotonin C†

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A synthesis of putative bufopyramide has shown the structure assigned to the natural product to be incorrect. The spectroscopic data for the natural product bufopyramide matches that obtained from a synthetic sample of bufoserotonin C, confirming that the two natural products are not distinct, but instead the same compound.

The Traditional Chinese Medicine ChanSu is prepared from the skin secretions of the toads *Bufo bufo gargarizans* Cantor and *Bufo melanostictus* Schneider.[1] ChanSu has been used for centuries as an anti-inflammatory, antiarrhythmic, anaesthetic and in chemotherapy regimens, for which several bufadienolides and indolealkylamines are responsible for the observed therapeutic effects.[2-6]

Our research group has an ongoing interest[7] in the total synthesis of structurally unique alkaloids present in ancient therapeutics and as such, were intrigued by a report that ChanSu contains the novel alkaloid bufopyramide (**1**),[8] a cytotoxic serotonin-pyrrole hybrid linked by an acyclic imide moiety (Figure 1).

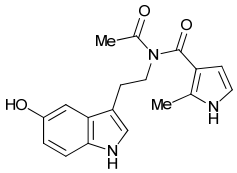
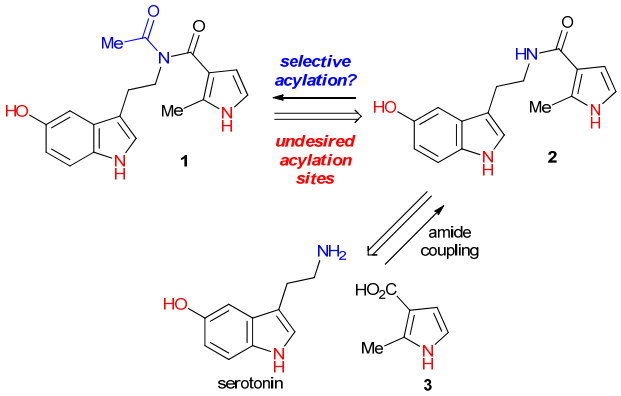


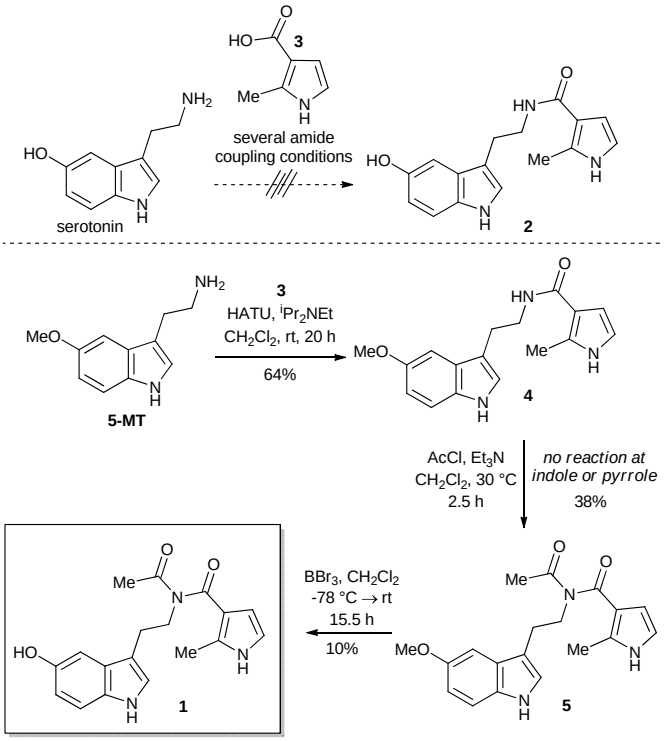
Figure 1. Bufopyramide (**1**)

A protecting group free-synthesis of bufopyramide (**1**) was envisaged, one that hinged on the selective acylation of the amide **2** (available from readily available serotonin and pyrrole **3**) in the presence of the unprotected indole and pyrrole heterocycles (Scheme 1). Due to the partially anionic character of the amide oxygen, the acylation of amides proceeds through an initial *O*-acylation and rearrangement of the resulting isoimide to the imide,[9] and it would be interesting to to see if this could be exploited in a protecting-group free synthesis of **1**.



Scheme 1. Proposed protecting group-free approach to bufopyramide (**1**).

Although serotonin has been successfully employed in amide couplings,[10] attempts to secure quantities of amide **2** by coupling serotonin and 2-methylpyrrole-3-carboxylic acid (**3**)[11] were met with failure, with the best yield obtained never exceeding 5% despite several coupling agents being trialled (HATU, HBTU, carbodiimides, etc). As such, our strategy was adjusted slightly, and 5-methoxytryptamine (5-MT) was coupled with pyrrole **3** to give **4** in good yield (Scheme 2). Although the amide **4** differs slightly to the substrate proposed in the initial retrosynthesis, the selective acylation would still be challenging in the presence of both heterocyclic N-H's.

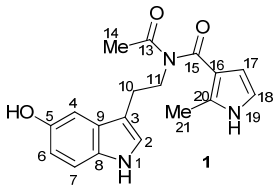


Scheme 2. Synthesis of putative bufopyramide (**1**)

With **4** in hand, its selective acylation was attempted. Although a detailed overview is not provided herein, extensive attempts at effecting the selective acetylation using both acetic anhydride and acetyl chloride (various temperatures and solvents, with and without DMAP) led to highly complex product mixtures arising from indiscriminate acylation of the amide, indole and pyrrole sites. In a satisfying breakthrough, one equivalent of acetyl chloride and triethylamine in dichloromethane effected selective acylation of the amide to give **5**, with no evidence of any other products by ^{1}H NMR or TLC. Although the subsequent demethylation proceeded in poor yield, a concise synthesis of bufopyramide (**1**) had been accomplished (Scheme 2).

Comparing the NMR data for synthetic **1** and that of the natural product bufopyramide[8] revealed several key differences (Table 1). Notably, the chemical shifts of the pyrrole C-H protons in synthetic **1** are observed significantly further upfield in both the ^{1}H (δ_H 6.88, 6.57 ppm) and ^{13}C NMR (δ_C 117.9, 110.9 ppm) compared to those in the natural product (δ_H 7.24, 7.79 ppm and δ_C 124.4, 121.7 ppm). This trend was also evident in the chemical shifts of the pyrrole methyl group (C21; δ_C 13.3 vs δ_C 27.1 ppm) and the pyrrole carboxamide (C15; δ_C 172.8 vs δ_C 187.7 ppm). A structural reassignment of bufopyramide was required.

Table 1. NMR data for authentic bufopyramide[8] and synthetic **1** (pyridine-d_5)



Atom	Bufopyramide ^{1}H (500 MHz)	Synthetic **1** ^{1}H (400 MHz)	Bufopyramide ^{13}C (125 MHz)	Synthetic **1** ^{13}C (100 MHz)
NH (1)	11.52 (s)	11.52 (br s)	-	-
CH (2)	7.46 (d, *J* 2.2)	7.28 – 7.25 (m)	110.9	124.7
C (3)	-	-	111.5	112.3
CH (4)	7.94 (s)	7.84 (d, *J* 2.2)	104.2	104.6
C(5)-OH	8.73 (s)	10.82 (br s)	152.4	152.7
CH (6)	7.50 (d, *J* 8.6)	7.28 – 7.25 (m)	112.5	113.3
CH (7)	7.28 (d, *J* 8.6)	7.50 (d, *J* 8.6)	113.0	113.0
C (8)	-	-	132.4	132.9
C (9)	-	-	129.4	130.0
CH_2 (10)	3.37 (t, *J* 7.9)	3.48-3.44 (m)	28.8	26.5
CH_2 (11)	4.75 (t, *J* 7.9)	4.53 – 4.49 (m)	50.9	48.0
CO (13)	-	-	167.6	172.1
COMe (14)	2.17 (s)	2.37 (s)	23.4	25.8
CO (15)	-	-	187.7	172.8
C (16)	-	-	127.6	117.1
CH (17)	7.24 (d, *J* 1.8)	6.88 (t, *J* 2.7)	124.4	117.9
CH (18)	7.79 (d, *J* 1.3)	6.57 (t, *J* 2.7)	121.7	110.9
NH (19)	10.78 (s)	12.48 (br s)	-	-
C (20)	-	-	123.0	137.5
Me (21)	2.34 (s)	2.59 (s)	27.1	13.3

In search of information that would help uncover the correct structure of bufopyramide, an inventory of all the known alkaloid constituents present in ChanSu was compiled. In particular, a 2007 paper reporting the isolation of bufoserotonin C (**6**, Figure 2) from ChanSu,[12] some eight years after the isolation of bufopyramide,[8] grabbed our attention. Like nominal bufopyramide (**1**), bufoserotonin C (**6**) is also an indole-pyrrole hybrid and critically, the two structures share the same molecular formula ($C_{18}H_{19}N_3O_3$). Our suspicions that the two natural products could be the same compound could not be confirmed immediately because: (1) the separate isolation reports recorded the NMR spectra in different solvents (**1** in pyridine-d_5 and **6** in DMSO-d_6)[8,12] and (2) correspondence with the authors of both isolation reports revealed neither had samples of the natural product remaining, precluding a full spectroscopic comparison in the same NMR solvent. The only

way to determine if bufopyramide and bufoserotonin C were identical was to synthesise the latter.

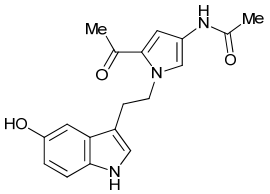
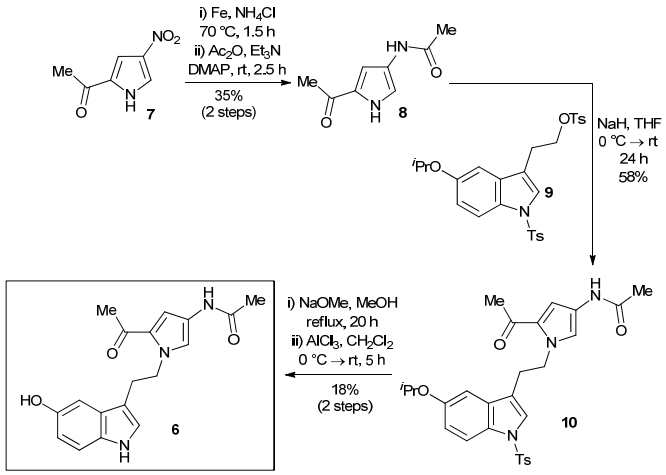


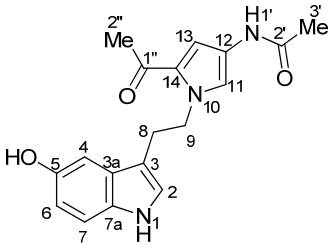
Figure 2. Bufoserotonin C (**6**)

The synthesis of bufoserotonin C is shown in Scheme 3. The reductive acetylation of pyrrole **7**[13] gave **8**, itself a natural product present in ChanSu.[14] Pyrrole **8** was *N*-alkylated with the ditosylate **9**[15] to give **10** in good yield. Detosylation and dealkylation then gave an synthetic sample of bufoserotonin C (**6**), the gross structure of which was confirmed by detailed spectroscopic analysis, which was identical in all aspects to that of the isolation report.[12,16] With the structure of the natural product bufoserotonin C confirmed, its NMR data was collected in the same solvent used in the characterisation of the natural product bufopyramide (pyridine-d_5, Table 2). While the NMR data for synthetic bufoserotonin C and authentic bufopyramide are remarkably consistent and point toward structural equivalency, some minor discrepancies were evident. We posit the difference between the chemical shifts of the C2-H in the ^{1}H NMR data (δ_H 7.27 vs δ_H 7.46 ppm) is due to a typographical error by the isolation chemists and should read δ_H 7.26 ppm. Secondly, a difference (1.7 ppm) between the chemical shift of the C12 carbon in synthetic bufoserotonin C (δ_C 124.7 ppm) versus bufopyramide (δ_C 123.0 ppm) requires comment. In the ^{13}C NMR spectrum of synthetic bufoserotonin C, two peaks in very close proximity at δ_C 124.7 ppm (C12) and δ_C 124.8 ppm (C2) were apparent.[16] It is likely that in the ^{13}C NMR of natural bufopyramide, these two peaks appeared as one (δ_C 124.4 ppm) and an impurity was subsequently assigned to the quaternary C12 (δ_C 123.0).[8] Finally, despite close analysis of the HMBC data,[16,17] the presence of the peak at δ_H 8.73 ppm assigned to the C5-OH in authentic bufopyramide could not be confirmed,[8] as the pyridine solvent peaks obscure this region of the ^{1}H NMR spectrum.



Scheme 3. Synthesis of bufoserotonin C (**6**)

Table 2. NMR data for synthetic bufoserotonin C (**6**) and authentic bufopyramide[8] (reassigned) (pyridine-d_5)



Atom (bufoserotonin C numbering)	Synthetic bufoserotonin C **^{1}H (400 MHz)**	Natural bufopyramide[a] **^{1}H (500 MHz)**	Synthetic bufoserotonin C **^{1}C (100 MHz)**	Natural bufopyramide[a] **^{13}C (125 MHz)**
NH (1)	11.55 (br s)	11.52 (s)		
CH (2)[b]	7.274 – 7.266 (m)	7.46 (d, *J* 2.2)	124.8	124.4
C (3)	-	-	111.8	111.5
C (3a)	-	-	129.8	129.4
CH (4)	7.95 (d, *J* 2.3)	7.94 (s)	104.6	104.2
C(5)-OH[b]	Not observed	8.73 (s)	152.8	152.4
CH (6)	7.28 (dd, *J* 8.8, 2.3)	7.28 (d, *J* 8.6)	113.4	113.0
CH (7)	7.51 (d, *J* 8.8)	7.50 (d, *J* 8.6)	112.9	112.5
C (7a)	-	-	132.8	132.4
CH_2 (8)	3.38 (t, *J* 7.9)	3.37 (t, *J* 7.9)	29.2	28.8
CH_2 (9)	4.76 (t, *J* 7.9)	4.75 (t, *J* 7.9)	51.3	50.9
CH (11)	7.80 (d, *J* 1.9)	7.79 (d, *J* 1.3)	122.1	121.7
C (12)[b]	-	-	124.7	123.0
CH (13)	7.24 (d, *J* 1.9)	7.24 (d, *J* 1.8)	111.2	110.9
C (14)	-	-	128.0	127.6
NH (1')	10.80 (br s)	10.78 (s)	-	-
CO (2')	-	-	167.9	167.6
Me (3')	2.17 (s)	2.17 (s)	23.8	23.4
CO (1")	-	-	188.1	187.7
Me (2")	2.35 (s)	2.34 (s)	27.5	27.1

[a] The NMR data for authentic bufopyramide[8] has been reassigned to the bufoserotonin C structure; [b] The discrepancies at C2-H, C5-OH and C12 are discussed in the main text.

A synthesis of putative bufopyramide has shown the structure assigned to the natural product to be incorrect. The spectroscopic data for authentic bufopyramide matches that obtained from a synthetic sample of bufoserotonin C. A synthesis-enabled reassignment of a natural product to a separate, structurally distinct natural product is not common.[18] Furthermore, clarifying the structural identity of a cytotoxic alkaloid consumed by thousands of people on a daily basis is of clear importance, one that may contribute to the medicinal properties displayed by the Traditional Chinese Medicine ChanSu.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental procedures and compound characterization data. See DOI: 10.1039/c000000x/

References

1. The State Pharmacopoeia Commission of PR China, "Pharmacopoeia of the People's Republic of China (2000 Edn.), Vol. 1," Chemical Industry Publishing House, Beijing, 2000, pp. 316–317.
2. Z. Hong, K. Chan, and H. W. Yeung, *J. Pharm. and Pharmacol.,* 1992, **44**, 1023–1026,
3. A. Enomoto, M. C. Rho, K. Komiyama, and M. Hayashi, *J. Nat. Prod.,* 2004, **67**, 2070–2072.
4. T. J. Qin, X. H. Zhao, J. Yun, L. X. Zhang, Z. P. Ruan, and B. R. Pan, *World J. Gastroenterol.*, 2008, **14**, 5210–5216,
5. (a) W. S. Ko, T. Y. Park, C. Park, Y. H. Kim, H. J. Yoon, S. Y. Lee, S. H. Hong, B. T. Choi, Y. T. Lee and Y. H. Choi, *Oncol. Rep*., 2005, **14**, 475–480; (b) C. Li, S. M. Hashimi, S. Cao, A. S. Mellick, W. Duan, D. Good and M. Q. Wei, *Evidence-Based Complementary and Alternative Medicine*, 2013, Article ID 849054, 11 pages, doi:10.1155/2013/849054
6. P. Zhang, Z. Cui, Y. Liu, D. Wang, N. Liu and M. Yoshikawa, *Chem. Pharm. Bull.,* 2005, **53**, 1582–1586.
7. M. B. Calvert and J. Sperry, *Chem. Commun*., 2015, **51**, 6202–6205.
8. Y. Komano, H. Morita, R. Takano, A. Kotake, T. Nogawa, H. Hashima, K. Takeya, H. Itokawa and G. R. Pettit, *Heterocycles,* 1999, **50**, 499–503.
9. D. Davidson and H. Skovronek, *J. Am. Chem. Soc.,* 1957, **80**, 376–379.
10. (a) K. Yamada, S. Teranishi, A. Miyashita, M. Ishikura and M. Somei, *Heterocycles,* 2011, **83**, 2546-2562; (b) G. Ortar, M. G. Cascio, L. De Petrocellis, E. Morera, F. Rossi, A. Schiano-Moriello, M. Nalli, V. de Novellis, D. F. Woodward, S. Maione and V. Di Marzo, *J. Med. Chem.,* 2007, **50**, 6554-6569; (c) L. Guo, F. Zhang, W. Hu, L. Li and Y. Jia, *Chem. Commun.,* 2014, **50**, 3299-3302.
11. Prepared in one step (76%) by hydrolysis of the commercially available ethyl ester, see ESI.
12. R.-H. Liu, H. Luo, Y.-L. Li, M. Yang, H.-L Li, Y.-H. Shen, C. Zhang, J. Su and W.-D. Zhang, *Helv. Chim. Acta.,* 2007, **90**, 2427–2431.
13. H. Oda, T. Hanami, T. Iwashita, M. Kojima, M. Itoh and Y. Hayashizaki, Y. *Tetrahedron*, 2007, **63**, 12747–12753.
14. R.-H. Liu, H. Luo, Y. L. Li, M. Yang, X.K. Xu, H. L. Li, Y. H. Shen, C. Zhang, J. Su, and W. D. Zhang, *Chem. Nat. Compd.,* 2009, **45**, 599–600.
15. Prepared by the ditosylation of 5-isopropoxytryptophol, see ESI.
16. See ESI for full details
17. The C5-OH is visible when the ^{1}H NMR of synthetic bufoserotonin C is run in d_6-DMSO. See ESI.
18. For selected examples of two distinct natural products that were subsequently shown to be the same compound, see: (a) J. S. Clark, L. Delion and L. J. Farrugia, *Chem. Eur. J.,* 2015, **21**, 4772-4780; (b) P. Sharma, B. Lygo, W. Lewis and J. E. Moses, *J. Am. Chem. Soc.,* 2009, **131**, 5966-5972.