URL: http://www.iupac.org/symposia/proceedings/phuket97/daly.html

Biodiversity of Alkaloids in Amphibian Skin: A Dietary Arthropod Source

John W. Daly

Laboratory of Bioorganic Chemistry, NIH, Bethesda, MD 20892 USA

Abstract: Over four hundred alkaloids in amphibian skin have been detected and structures from twenty different classes have been elucidated. These include batrachotoxins, histrionicotoxins, pumiliotoxins, epibatidine, pyrrolidines, piperidines, decahydroquinolines, pyrrolizidines, indolizidines, quinolizidines and tricyclic gephyrotoxins, pyrrolizidine oximes, pseudophrynamines, coccinellines and cyclopentaquinolizidines. The alkaloids of amphibian skin are not synthesized by the amphibians, but instead are accumulated unchanged from dietary sources. Pyrrolidines, piperidines, decahydroquinolines, pyrrolizidines, indolizidines, and quinolizidines appear likely to be derived from ants, the coccinellines from beetles, and the pyrrolizidine oximes from millipedes. The origins of the batrachotoxins, histrionicotoxins, pumiliotoxins and epibatidine are of particular interest in view of their remarkable biological activity.

INTRODUCTION

Amphibian skin contains a remarkable spectrum of biologically active compounds, including biogenic amines, peptides, proteins, bufadienolides, tetrodotoxins and lipophilic alkaloids (ref.1). The lipophilic alkaloids include the samandarines and an incredible array of piperidine-based, pyrrolidine-based, and steroidal alkaloids. Such an array of over four hundred new alkaloids has been detected in skin extracts from four genera of dendrobatid frogs of New World tropics, the bufonid genus *Melanophryniscus* of Southeastern South America, the mantelline genus *Mantella* of Madagascar, and the myobatrachid genus *Pseudophryne* of Australia (refs. 2,3). Skin extracts from 71 other genera encompassing 11 anuran families have not contained alkaloids.

ALKALOIDS FROM ANURAN SKIN

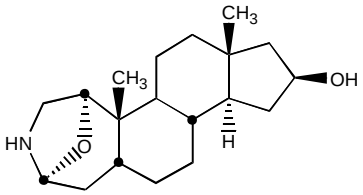
Over 20 major structural alkaloid classes, several of which may co-occur in a single frog, have been detected in anuran skin. Such alkaloids include the batrachotoxins (sodium channel activators), the histrionicotoxins (noncompetitive blockers of nicotinic channels), the pumiliotoxin, allopumiliotoxin, and homopumiliotoxin group (positive modulators of sodium

*Invited lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization, 23–27 November 1997, Phuket, Thailand. Other presentations are published in *Pure Appl. Chem.*, Vol. 70, No. 11, 1998.

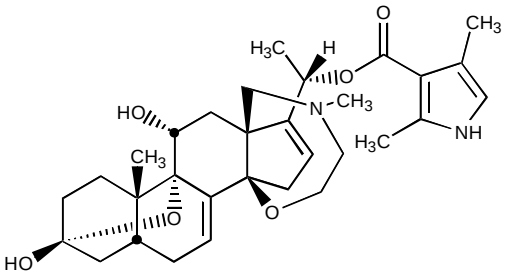
channels), decahydroquinolines, various izidines, epibatidine (a potent nicotinic agonist), the tricyclic coccinellines, the pseudophrynamines and spiropyrrolizidines (potent noncompetitive blockers of nicotinic channels). Structures of some alkaloids from amphibian skin are shown in Fig. 1.

Some of the anuran alkaloids are broadly distributed and others not. The batrachotoxins were known only from dendrobatid *Phyllobates* until homobatrachotoxin was detected in birds of the genus *Pitohui* from Papua New Guinea (ref. 4). The histrionicotoxins appear to be limited to three genera of the neotropical dendrobatids. All of the genera of anurans with lipophilic skin alkaloids contain compounds of the pumiliotoxin class, which comprises pumiliotoxins, allopumiliotoxins, homopumiliotoxins, and several desoxy, desmethyl and dehydro congeners. Epibatidine has been found in trace levels only in certain species of the South American dendrobatid genus *Epipedobates*. The pseudophrynamines are known only from the Australian myobatrachid genus *Pseudophryne*.

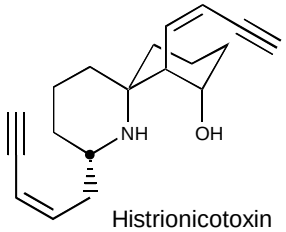
Virtually none of the alkaloids detected in anuran skin extracts were known to occur in plants or other animals and how they came to be in skin of anurans from certain genera in four amphibian families was a great mystery. It now appears that the frogs do not synthesize them, but instead sequester and accumulate alkaloids unchanged into skin glands from the diet (refs. 5,6).



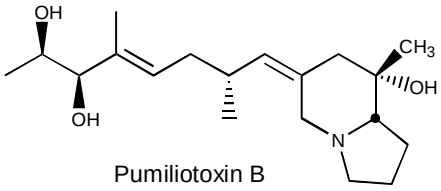
Samandarine



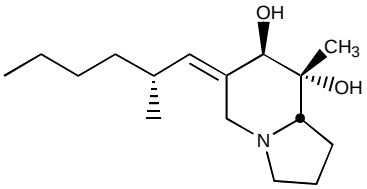
Batrachotoxin



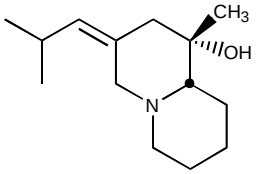
Histrionicotoxin



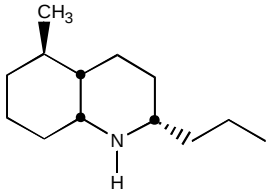
Pumiliotoxin B



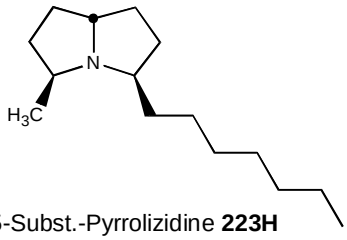
Allopumiliotoxin **267A**



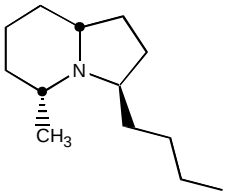
Homopumiliotoxin **223G**



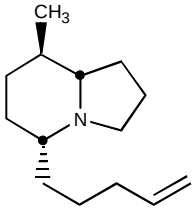
Decahydroquinoline **195A**



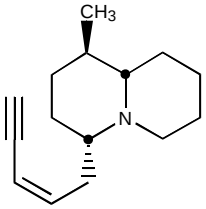
3,5-Subst.-Pyrrolizidine **223H**



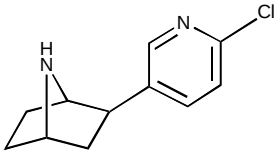
3,5-Subst.-Indolizidine **195B**



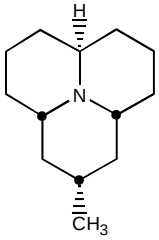
5,8-Subst.-Indolizidine **207A**



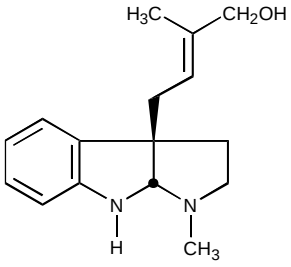
1,4-Subst.-Quinolizidine **217A**



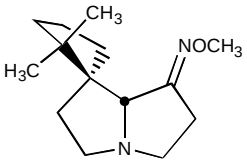
Epibatidine



Precoccinelline



Pseudophrynaminol



Pyrrolizidine oxime **236**

Fig.1: Representative Alkaloids of Amphibian Skin

The common evolutionary event appears to be the development or over-expression of an alkaloid-accumulating system. The decahydroquinolines, 3,5-disubstituted pyrrolizidines and indolizidines and 4,6-disubstituted quinolizidines likely come from myrmicine ants as may the pyrrolidines and piperidines. Two other classes of frog alkaloids with possible dietary sources are the spiropyrrolizidines, sequestered from very small millipedes, and the coccinelline-type tricycles, sequestered from small beetles. However, the majority of alkaloids from anuran skin lack known counterparts elsewhere in nature. These include batrachotoxins, pumiliotoxins, histrionicotoxins, epibatidine, 5,8-disubstituted indolizidines, 5,6,8-trisubstituted indolizidines, 1,4-disubstituted quinolizidines, and gephyrotoxins. All such alkaloids can be presumed to serve in chemical defense. The origins of the batrachotoxins, pumiliotoxins, histrionicotoxins, epibatidine and the pseudophrynamines are of particular interest because of their remarkable biological activity.

Batrachotoxins

The batrachotoxins are selective agents for stabilizing the open form of sodium channels (ref. 7) and had sufficiently high affinity so as to allow development of a radioligand to characterize such sites on sodium channels (ref. 8). Batrachotoxins have been widely used in research on sodium channels, and interact allosterically with other toxins and with local anesthetics, anticonvulsants and antiarrhythmics.

Pumiliotoxins

The pumiliotoxins, in particular pumiliotoxin B have marked cardiotonic and myotonic effects, apparently due to positive modulation of sodium channels (refs. 9,10). Activity is strongly dependent on structure, and appears to require at least three hydroxyl groups for optimal activity (ref. 11).

Histrionicotoxins

The histrionicotoxins are potent noncompetitive blockers of nicotinic channels (ref. 12). Activity is influenced by the length and nature of the two side-chains. A radiolabelled perhydrohistrionicotoxin (ref. 13) has proved useful in studying the interaction of many compounds, including phencyclidine, quinacrine, chlorpromazine and local anesthetics, with nicotinic channels (see ref. 2).

Epibatidine

Both enantiomers of epibatidine, proved to be incredibly potent nicotinic agonists with selectivity towards neuronal and ganglionic receptor subtypes (ref. 14). Agonist activity at central nicotinic receptors was the basis for the analgetic activity of epibatidine, which was about 200-fold more potent than morphine. Epibatidine now represents a powerful research tool for the study of nicotinic receptors and function, and analogs are being developed for possible therapeutic use as analgetics and cognitive enhancers.

PSEUDOPHRYNAMINES

Pseudophrynaminol is a very potent, noncompetitive blocker of nicotinic channels, showing no selectivity between ganglionic and neuromuscular subtypes (ref. 10).

SUMMARY

Anurans from four families have yielded a rich array of previously unknown alkaloids, some of which have become valuable research tools. If all of the frog alkaloids are sequestered from dietary sources, then small, even tiny arthropods may be a virtually untapped source of new pharmacologically active agents from the world's rain forests.

REFERENCES

1. J.W. Daly, C.W. Myers and N. Whittaker. *Toxicon*. **25**, 1023 (1987).
2. J.W. Daly, H.M. Garraffo and C.W. Myers. *Pharmaceut. News*. **4**, 9 (1997).
3. J.W. Daly, H.M. Garraffo and T.F. Spande. In *The Alkaloids*. (G.A. Cordell, ed.), pp.185-288. Academic Press, San Diego (1993).
4. J.P. Dumbacher, B.M. Beehler, T.F. Spande, H.M. Garraffo and J.W. Daly. *Science*. **258**, 799 (1992).
5. J.W. Daly, H.M. Garraffo, T.F. Spande, C. Jaramillo and A.S. Rand. *J. Chem. Ecol*. **20**, 943 (1994).
6. J.W. Daly, S.I. Secunda, H.M. Garraffo, T.F. Spande, A. Wisnieski and J.F. Cover Jr. *Toxicon* **32**, 657 (1994).
7. E.X. Albuquerque, J.W. Daly and B. Witkop. *Science*. **172**, 995 (1971).
8. W.A. Catterall, C.S. Morrow, J.W. Daly and G.B. Brown. *J. Biol. Chem*. **256**, 8922 (1981).
9. F. Gusovsky, D.P. Rossignol, E.T. McNeal and J.W. Daly. *Proc. Natl. Acad. Sci. USA*. **85**, 1272 (1988).
10. F. Gusovsky, W.L. Padgett, C.R. Creveling and J.W. Daly. *Mol. Pharmacol*. **42**, 1104 (1992).
11. J.W. Daly, F. Gusovsky, E.T. McNeal, S. Secunda, M. Bell, C.R. Creveling, Y. Nishisawa, L.E. Overman, M.J. Sharp and D.P. Rossignol. *Biochem. Pharmacol*. **40**, 315 (1990).
12. C.E. Spivak, M.A. Maleque, A.C. Oliviera, L.M. Masukawa, T. Tokuyama, J.W. Daly and E.X. Albuquerque. *Mol. Pharmacol*. **21**, 351 (1982).
13. A.T. Eldefrawi, M.E. Eldefrawi, E.X. Albuquerque, A.C. Oliveira, N. Mansour, M. Adler, J.W. Daly, G.B. Brown, W. Burgermeister and B. Witkop. *Proc. Natl. Acad. Sci. USA*. **74**, 2172 (1977).
14. B. Badio and J.W. Daly. *Mol. Pharmacol*. **45**, 563 (1994).
15. B. Badio, H.M. Garraffo, C.V. Plummer, W.L. Padgett and J.W. Daly. *Biochem. Pharmacol.* **53**, 671 (1997).

