New and Uncommon Indole- and Imidazole-Alkylamines in Skins of Amphibians from Australia and Papua New Guinea

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Indolealkylamines, Imidazolealkylamines, Amphibian Skin

Extracts of the skin of some amphibians from Australia and Papua New Guinea contained, in addition to the usual 5-hydroxyindolealkylamines and histamine: a. two new, hitherto unknown indolealkylamines, *i. e.* O-sulphate of bufotenidine and 2-(3-indolyl)ethyltrimethylammonium, a quaternary ammonium base of tryptamine. The rare O-sulphate of bufotenine was also present, the occurrence of which had previously been demonstrated only in the skin of some South American toads; b. a series of uncommon imidazolealkylamines, such as N′-acetylhistamine, N′-methylhistamine, N′,N′-dimethylhistamine, spinaceamine and 6-methylspinaceamine. It appears evident that amphibian skin continues to be an exceptionally rich source of aromatic amines.

Introduction

During the past decade the skins of approximately one hundred species of amphibians from Australia and Papua New Guinea have been submitted to chemical and pharmacological screening. In this communication the occurrence in this material of some new or uncommon indole- and imidazole-alkylamines will be briefly described. The suggested structures of the new compounds, inferred from indirect evidence, have been fully confirmed by synthesis.

Materials and Methods

A complete list of the amphibian material examined, together with a description of the simple procedure used to prepare the skin extracts will be published elsewhere [1].

Methods for the preparation and elution of alumina columns, for both paper and thin-layer chromatography involving silica gel and various solvent systems, for high voltage electrophoresis, as well as the colour reactions used to locate and identify the various indole- and imidazole-alkylamine spots have already been described in detail [2–4].

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The following synthetic compounds were available for comparison: 5-hydroxytryptamine and creatinine sulphate, bufotenine base, bufotenine O-sulphate, bufotenidine (bufotenine methiodide), bufotenidine O-sulphate, N′,N′-dimethyltryptamine methiodide, histamine. 2 HCl, N′-methylhistamine. 2 HCl, N′,N′-dimethylhistamine. 2 HCl, spinaceamine. 2 HCl, 6-methylspinaceamine. 2 HCl.

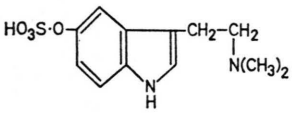
Indolealkylamines, with the exception of 5-HT, were synthesized by Dr. Temperilli at the Farmitalia Research Laboratories, Milan, and the N′-methylhistamines and the spinaceamines were synthesized by Prof. T. Vitali, Institute of Pharmaceutical Chemistry, University of Parma, Italy.

Results

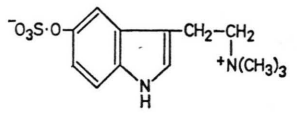
O-Sulphate of bufotenine

This compound was first tentatively identified in extracts of the skin of some South American toads *(e. g. Bufo spinulosus chilensis, B. marmoreus, B. perplexus, B. bocourti)* [5]. The availability of synthetic O-sulphate of bufotenine has now permitted to confirm the preceding identification and to demonstrate that the compound is also present in

the skin of the Australian leptodactylid frog *Litoria pearsoniana*.



Bufotenine O-sulphate



Bufotenidine O-sulphate

The natural compound considered to be the O-sulphate of bufotenine showed the following characteristics:

Alumina column: elution by 80% ethanol.

Paper chromatography: R_F 0.26 – 0.30 in *n*-butanol : acetic acid : water (40 : 10 : 50), 0.61 – 0.68 in *n*-butanol : 35% methylamine (80 : 30), 0.68 in 20% KCl, 0.76 – 0.78 in distilled water, 0.42 – 0.44 in 1-pentanol : pyridine : methylamine : water (40 : 40 : 1 : 10), and 0.61 – 0.65 in methylethylketone : pyridine : methylamine : water (65 : 15 : 0.5 : 10); yellow or orange-yellow colour with the NNCD reagent, violet turning to blue with *p*-dimethylaminobenzaldehyde; Gibbs and Pauly reactions negative.

Thin-layer chromatography on silica gel: R_F 0.2 in butanol : acetic acid : water (40 : 10 : 50), 0.43 in butanol : 35% methylamine (80 : 30), 0.03 in benzene : ethanol : methylamine (22 : 7 : 8).

Paper electrophoresis $E_{1.2} = 0.14$ bufotenine, $E_{5.8} = 0.25$ bufotenine.

After treatment with glacial acetic acid in a boiling water bath for 60 min followed by evaporation of the solvent, two substances, *i. e.* bufotenine and sulphuric acid, could be visualized on paper chromatograms.

The synthetic O-sulphate of bufotenine was indistinguishable from the natural compound.

The concentration of the O-sulphate of bufotenine in the different batches of *Litoria pearsoniana* skin varied between 40 and 600 μg per g dry skin.

O-Sulphate of bufotenidine

This hitherto unknown indole derivative was detected for the first time in extracts of the skin of *Nictimystes tympanocryptis*. Its characteristics are as follows:

Alumina column: elution by 99 – 90% ethanol, together with bufotenidine.

Paper chromatography: R_F 0.24 – 0.29 in butanol : acetic acid : water, 0.13 – 0.16 in butanol : 35% methylamine, 0.67 – 0.70 in 20% KCl, 0.69 – 0.72 in distilled water, 0.29 – 0.34 in pentanol : pyridine : methylamine : water, 0.24 in methylethylketone : pyridine : methylamine : water; colour reactions were exactly the same as those described for the O-sulphate of bufotenine.

Thin-layer chromatography: R_F 0.18 in butanol: acetic acid : water, 0.17 in butanol : methylamine, and 0 in benzene : ethanol : methylamine.

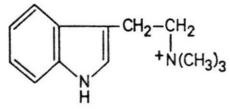
Paper electrophoresis: $E_{1.2} = 0.13$ bufotenidine, $R_{5.4} = 0.25$ bufotenidine.

After treatment with glacial acetic acid in a boiling water bath for 60 min followed by evaporation of the solvent, two substances *i. e.* bufotenidine and sulphuric acid, could be visualized on paper chromatograms.

The synthetic O-sulphate of bufotenidine was indistinguishable from the natural compound. The amount of compound occurring in different batches of *N. tympanocryptis* skins varied between 200 and 300 μg per g of dry skin.

Tryptamine trimethylammonium [2-(3-indolyl)ethyltrimethylammonium]

This new compound was found for the first time in extracts of the skin of *Litoria moorei*.



Tryptamine trimethylammonium

It showed the following characteristics:

Alumina column: elution by 95% ethanol.

Paper chromatography: R_F 0.67 – 0.72 in butanol : acetic acid : water, 0.32 – 0.40 in butanol : 35% methylamine, 0.61 – 0.63 in 20% KCl, 0.1 – 0.12 in distilled water, 0.61 – 0.64 in pentanol : pyridine : methylamine : water, 0.52 – 0.59 in methylethylketone : pyridine : methylamine : water; colour reactions were essentially the same as those described for the O-sulphates.

Thin-layer chromatography: R_F 0.27 in butanol : acetic acid : water, 0.09 in butanol : methylamine, and 0.03 in benzene : ethanol : methylamine.

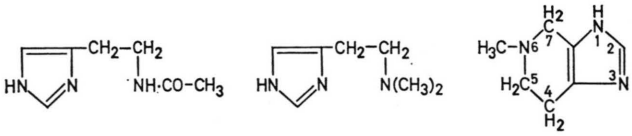
Paper electrophoresis: $E_{1.2} = 0.95 – 1.1$ tryptamine, $E_{5.8} = 0.9$ tryptamine.

Synthetic N′,N′-dimethyltryptamine methiodide was indistinguishable from the natural compound.

The amounts of tryptamine trimethylammonium occurring in the skin extracts of *L. moorei* were of the order of 30 – 50 μg per g dry skin.

Imidazolealkylamines

All the uncommon imidazolealkylamines occurring in the skins of amphibians from Australia and Papua New Guinea have already been described and fully characterized in previous studies on South American leptodactylid frogs [2, 3]. The structural formulae of three of them are as follows:



N′-Acetyl-histamine | N′,N′-Dimethyl-histamine | 6-Methyl-spinaceamine

N′-acetylhistamine may be considered a regular constituent of the skin whenever histamine is present in large amounts *(e. g. Litoria caerulea, L. aurea, L. moorei)*. Its concentration varied between 4 and 30 μg per g dried skin.

N′-methylhistamine and N′,N′-dimethylhistamine have so far been detected only in *Nictimystes disrupta* (N′-methylhistamine 100 – 150 μg/g; N′,N′-dimethylhistamine 5 μg/g) and *Litoria glandulosa* (N′-methylhistamine 15 μg/g), and similarly cyclized histamines only in *Nictimystes disrupta* (spinaceamine 7 μg/g, 6-methylspinaceamine 30 – 35 μg/g) and *Litoria moorei* (spinaceamine 30 μg/g).

Discussion

Results described in this paper show that as screening of skin extracts of additional species of amphibians progresses new indole- and imidazolealkylamines are added to the already substantial list of known compounds, thereby elucidating new metabolic pathways. For example, while the complete series of N′-methylated derivatives of 5-HT has been known for a long time, the discovery of tryptamine trimethylammonium demonstrates that a parallel series of N′-methylated derivatives of tryptamine may exist in animal tissues. Likewise, the discoveries of the O-sulphates of bufotenine and bufotenidine show that O-sulphoconjugation may occur for every 5-hydroxyindolealkylamine known.

As far as concerns imidazolealkylamines, N′-acetylation, N′-methylation and cyclization processes have been confirmed, thus demonstrating the striking similarity between the imidazolealkylamine spectrum of leptodactylid frogs of South America and that of leptodactylid frogs of Australia.

A comprehensive map of identified and unidentified amines occurring in the amphibian skin will be presented after completing our studies on more than 500 species collected almost all over the world.

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[1] M. Roseghini, V. Erspamer, and R. Endean, Comp. Biochem. Physiol., in press.
[2] V. Erspamer, M. Roseghini, and J. M. Cei, Biochem. Pharmacol. **13**, 1083 [1964].
[3] V. Erspamer, T. Vitali, and M. Roseghini, Arch. Biochem. Biophys. **105**, 620 [1964].
[4] V. Erspamer, T. Vitali, M. Roseghini, and J. M. Cei, Biochem. Pharmacol. **16**, 1149 [1967].
[5] J. M. Cei, V. Erspamer, and M. Roseghini, Evolution of the Genus Bufo (ed. W. F. Blair), p. 233, University of Texas Press, Austin and London 1972.