# New Lipophilic Alkaloids from *Mantella* frogs collected in Madagascar

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**Abstracts**

Total alkaloid skin extracts from eleven species *Mantella* frogs *M. aurantiaca*, *M. baroni, M. betsileo, M. crocea, M. laevigata, M. madagascariensis, M. milotympanum* var. red and green, *M. nigricans, M. cf nigricans, M. pulchra* and *M. viridis* have been examined. The latters have been collected in the North, North-East and Centre-East regions of Madagascar. All the above extracts were submitted to TLC, GC, GC/MS and GC/FTIR analysis. Extended studies of the MS/EI and FTIR data allowed to proposed structures for two new compounds dehydro-5,8-I **251P** and 5,6,8-I **267U.**

Similarly, the studies of total alkaloid skin extracts from six species *Mantella* frogs *M. baroni, M bernhardi, M. betsileo, M. cowani, M expectata* and *M. haraldmeieri* collected in the Centre, West, South-West and South-East regions of Madagascar have been performed. The alkaloid fraction of *M. betsileo*, collected in Kirindy forest, is characterized by the occurrence of new coccinelline tricyclic alkaloids, whose representatives are **193L, 261C** and **277C**.

Structure of **193L** was proposed, based on its MS and FTIR spectra.
The evidence that the tricyclic **261C** comes from coccinelline species was also given.
As a resume, over 50 new alkaloids were detected.

**Keywords**: *Mantella*, skin frogs, lipophilic alkaloids.

## Introduction

Madagascar is home to unique « Nature Biodiversity » and also an endemic genus frog *Mantella* that have captured the imagination of naturalist photographs and terrarium hobbists the world over. *Mantella* is the most prominent genus of Madagascar.

*Mantella* species demonstrate remarkable convergence in appearance, habits and coloration with the brightly colored neotropical "poison arrow frogs" of the family Dendrobatideae.
All display a wide array of eye-catched colour and pattern.

Thus, the unicolored *Mantella aurantiaca* is comparable to *Phyllobates terribilis*, a highly toxic frog from Amazonia. Both of them are known as "Golden frogs" (see figure 1: upper part). Some *Mantella* are convergent mainly with *Dendrobates* because the iridescant coloring is displayed as rounded markings and even limb bracelets. *Mantella cowani,* which resembles some populations of *Dendrobates histrionicus*, is the best example of a Dendrobates-like pattern, it is shown in figure 1 : lower part.

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In addition, *Mantella* exhibits a high level of convergence with *Dendrobates* in accumulating of skin alkaloids. A wide range of alkaloid representations, over twenty structural classes, have been characterized from skin extracts of mantellid frogs (*Mantella*) from Madagascar, dendrobatid frogs (*Epipedobates, Minyobates, Phyllobates*) from tropical Central and South America, bufonid toads (Melanophryniscus) from subtropical to temperate South Americas, myobatrachid frogs (Pseudophrynes) from Australia and salamanders (Salamandra) from Eurasia. (1,2,3).

In 1992, Homobatrachotoxin was reported to occur in the skins and feathers of three passerine bird species in the genus Pitohui (family Pachycephalideae) endemic to New Guinea and considered toxic by New Guineans. (4) More recently, Batrachotoxins, including many congeners, have been detected in feathers and skins of five New Guinean bird species of genus *Pitohui* as well as a species of a second toxic bird genus *Ifrita kowaldi* . (5)

**<u>Upper part:</u> File "Golden Twins 1"**

**<u>Lower Part:</u> File "Twins 2"**

Over the years [(*)], beside well known vasoactive analgetic and antibiotic peptides, biogenic amines, cardioactive bufadienolides, amphibian skin has produced a rich harvest of unique alkaloids.

That means amphibian skin is a remarkable source of biologically active arthropod alkaloids. Currently, over 800 lipophilic alkaloids have been discovered in amphibian skins, and with the exception of less than thirty, none have been detected elsewhere in Nature. The few that have been found elsewhere occur in ants, beetles and millipedes. (2,3,8,9).

Some of the latters had a major impact on biomedical research leading to the introduction or development of majority of all therapeutic agents. In particular, Epibatidine may be cited as an example of tremendous impact on research on nicotine receptors and function. Epibatidine was a trace alkaloid in the extracts at levels 1µg per frog from Ecuadorean frog, collected in 1974. It had proved to be 200-fold more potent than morphin as analgetic. This analgetic activity was due to agonist activity at nicotinic receptors. (3,10).

The pumiliotoxins/allopumiliotoxins were shown to have myotonic and cardiotonic activity due to positive modulation of sodium channels and to have stimulating effects, probably indirect, on the formation of inositol phosphates.

Such alkaloids occur in almost all alkaloid-containing species and populations from diverse habitats in South America, Australia, and in Madagascar as well. Many of the other classes of frog skin alkaloids, including decahydroquinolines, various izidines, piperidines, pyrrolidines and spirolizidines also have proven to be blockers of nicotine channels. (1,2,3). For this reason, since 1989 a part of our own natural products research has shifted to *Mantella* frogs and the biologically active alkaloids found in their skins. (1,2,8,9).

The present paper reports the occurrence of skin alkaloids in fifteen species of *Mantella,* collected during the fieldworks from July 2003 to April 2004, throughout diverse regions of Madagascar. They were supplied by FADES [(**)].

The occurrence among amphibian genera of some twenty classes of lipophilic alkaloids is presented in Table 1.

[(*)] For Daly *et al,* four decades have been devoted to amphibian skin alkaloids research

[(**)] FADES : Fonds d'Appui au Développement de l'Enseignement Supérieur, FADES SPO2v2_04 World Bank Program

**Table 1: Occurrence of various classes of lipophilic alkaloids in amphibian skins and other sources**

| Class of Alkaloids | Type of Alkaloids | Amphibians | | | | | | | | | Animal | | | | Plant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mantel linea | Dendrobatidae | | | | Bufo nidae | Myoba-tra-chidae | Rani-dae (6) | Sala-mander | Birds (4,5) | | Beetles | Ants (2,3) | Plant (7) |
| | | *Mantella* | *Dendrobates* | *Epipedobates* | *Minyobates* | *Phyllobates* | *Melanophrynicus* | *Pseudophrynes* | *Limnonectes kuhli* | *Salamandra* | *Pitohui* | *Ifrita* | *Beetles* | *Myrmicine Solenopsis* | *Anabasis Chenopodirceae* |
| Monocyclics | 2,5- disubstituted Pyrrolidines | **Traces** | **Traces** | | | | | | | | | | | + | |
| | Piperidines | | + | | | | | | | | | | | + | |
| Bicyclics | Pumiliotoxins | + | + | + | + | + | + | + | **Traces** | | | | | | |
| | Allopumiliotoxins | + | + | + | + | + | + | + | | | | | | | |
| | Homopumiliotoxins | + | + | | | | + | | **Traces** | | | | | | |
| | (pyrol-, indol-, quinol- ) Izidines | + | + | + | + | + | + | | | | | | | | |
| *Others classes* | Azabicyclo[5,3,0] decane (**275A**) | | + | | | | | | | | | | | | |
| | Decahydroquinolines | + | + | + | + | + | + | | | | | | | | |
| | Histrionicotoxins | | + | | | | | | | | | | | | |
| Tricyclics | Gephyrotoxins | | + | | | | | | | | | | | | |
| | Cyclopenta [*b*] quinolizidines | | | | + | | | | | | | | | | |
| | Coccinellines | + | + | + | + | + | | | | | | | + | | |
| | Spiropyrrolizidines | + | + | | | | + | + | | | | | | | |
| Stéroïdals | Samandarines | | | | | | | | | + | | | | | |
| | Batrachotoxins | | | | | + | | | | | + | + | | | |
| | Homobatrachotoxins | | | | | | | | | | + | | | | |
| Pyridines | Epibatidine | | | + | | | | | | | | | | | |
| | Norabasamine and anabasine | | | | | + | | | | | | | | | + |
| Indoles | Pseudophrynamines | | | | | | | + | | | | | | | |
| | Calycanthine/chimonanthine | | | | | + | | | | | | | | | |

**Results and Discussion**

*Sites of collection and habitats*

The number of recognized species of *Mantella* has about tripled in the last decade, due to discovery of new species concurrent with increased fieldwork in Madagascar.
Currently, the fifteen recognized species of *Mantella* are as follow :

*Mantella aurantiaca*
*Mantella baroni*
*Mantella bernhardi*
*Mantella betsileo*
*Mantella cowani*
*Mantella crocea*
*Mantella expectata*
*Mantella haraldmeieri*
*Mantella laevigata*
*Mantella madagascariensis*
*Mantella manery*
*Mantella milotympanum*
*(var. red) and (var. green)*
*Mantella nigricans*
*Mantella pulchra*
*Mantella viridis*

The fourteen *Mantella* species sampled are shown in figure 2. (2,9).

The joint expeditions to the localities sampled, purchased with the Biologists of DBAUA Department have been partitioned into two main regions :

- *Part I*, including the northern, north-eastern and centre-eastern subregions Madagascar, for the **A** group of researchers;
- *Part II,* including the central, western, south-western and south-eastern subregions Madagascar for the **B** group of researchers.

A total of 29 collection sites located in 7 subregions have been visited, three times, corresponding to the following biological prereproduction, reproduction and hibernation periods. In general, the frogs were very active in their habitat during the reproduction period.


Figure 1 : Convergence in colour patterns of *Mantella* and aposematic Dendrobatids

For each site, the local geographical data (humidity, temperature, pH of the surrounding streams) and the ecobiological data (vegetation and microsympatric animal species) as well, have been carefully recorded. The localities sampled are mapped in figure 3. The following tables, 2 and 3 respectively, summarize field observations. (11,12).

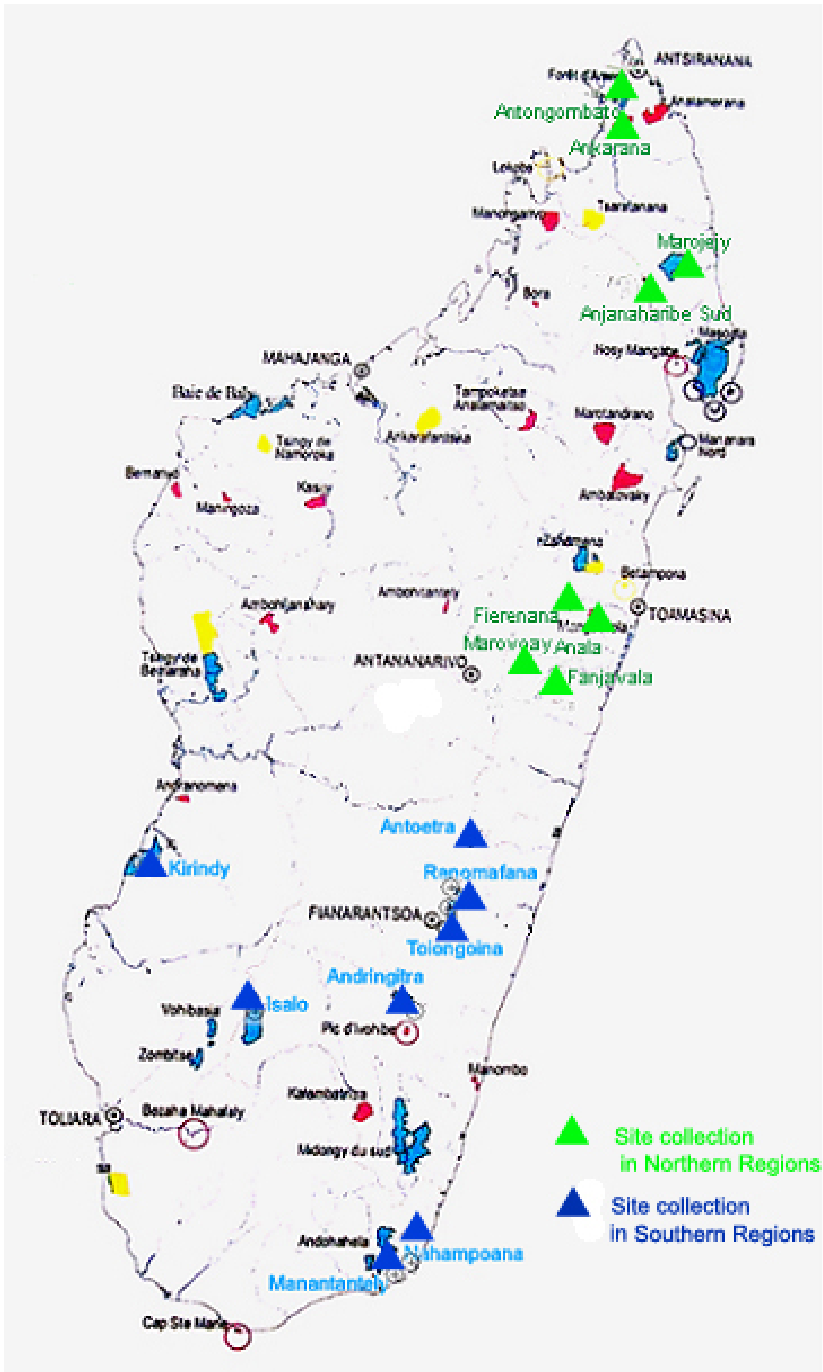


Table 2: Collection sites habitats and occurrence of species in the Northern and Centre-Eastern regions of Madagascar

| Région | Site | Habitat | **Occurrence of *Mantella* species** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *M. aurantiaca* | *M. baroni* | *M betsileo* | *M. crocea* | *M. laevigata* | *M. madagascariensis* | *M. mylotympanum (red)* | *M. mylotympanum (green)* | *M. nigricans* | *M pulchra* | *M. viridis* |
| I North | Antongombato | | | | | | | | | | | | |
| | Andranomantsina | A nearly dry streambed. Vegetation is weak and especially shrubby, except some mangoes tree Frogs were along the stream under fallen leaves and wet stones | | | | | | | | | | | + |
| | Antamotamo | Along the stream under fallen wet leaves and stones | | | | | | | | | | | + |
| | Andohantany | Along the stream under fallen lwet eaves and stones | | | | | | | | | | | + |
| | Ankarana | | | | | | | | | | | | |
| | Ankadirano | Along a streambed with some mangoes tree Frogs were found under wet stones | | | + | | | | | | | | |
| | Perte de Rivière | Dry rocky cliff | | | + | | | | | | | | |
| II North-East | Anjanaharibe Sud | | | | | | | | | | | | |
| | Andranomenabe | *Aframomum sp* vegetation with stones | | | | | | | | | + | | |
| | Marolakana | Along the streambed Vegetation consisted mainly of *Aframomum sp* | | | | | | | | | + | | |
| | Marojejy | | | | | | | | | | | | |
| | Camp Mantella | Bamboo forest Marojejy | | | | | + | | | | | | |
| | Camp Marojejy | Hillside forest with rupicolous vgetation. Samples were collected along the stream. | | | | | | | | | + | | |
| III Centre-East | An'Ala | | | | | | | | | | | | |
| | Andohan'i Sity 1 | Riparian forest | | | | | | | | | | + | |
| | Andohan'i Sity 2 | Meandering streams in forest exhibiting varying degrees of disturbance | | + | | | | | | | | | |
| | Antsahatsahana | | | | | | | | | | | + | |
| | Road to Anosibe an'Ala | | | | | | | | | | | | |
| | Andobo Andranomandry | Swamp forest | + | | | | | | | | | | |
| | Fanjavala | Undisturbed forest | | + | | | | + | | | | | |
| | Fierenana | | | | | | | | | | | | |
| | Sahamarolambo | Swamp secondary growth vegetation | | | | | | | + | | | | |
| | Sahavakoana | - | | | | | | | | + | | | |
| | Marovoay Gara | | | | | | | | | | | | |
| | Ankosy | Swamp secondary growth vegetation | | | + | | | | | | | | |
| | Marisiaka | Wet area with treeferns | | | + | | | | | | | | |
| | Ambodivoasary | Swamp secondary growth vegetation with ferns | | | + | | | | | | | | |

Table 3 : Collection sites habitats and occurrence of species in the Central, Western and Southern regions of Madagascar

| Region | Collection Site | Habitat | Occurrence of *Mantella* species | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *M. baroni* | *M. bernhardi* | *M. betsileo* | *M. cowani* | *M. expectata* | *M. harald-meieri* | beetles |
| IV Centre | Ranomafana | | | | | | | | |
| | Menavava River | Relatively undisturbed forest ; Vegetation *Aframomum sp*. | + | + | | | | | |
| | Vohiparara forest | Second growth forest along a relatively large stream | + | | | | | | |
| | Tolongoina | | | | | | | | |
| | Kirenabe Forest | Bordering of a disturbed forest along the tiny stream that led into the rice field. Vegetation consisted mainly of *Aframomum sp* (Zingiberacea). | | + | | | | | |
| | Antoetra | | | | | | | | |
| | Amparihimazava-Ampasimpotsy | Bordering of an undisturbed forest along a stream and in a cornfields in which remain the ashes after a forest fire | + | | | + | | | |
| V West | Kirindy | | | | | | | | |
| | Kirindy River | Typical West dry forest a nearly dry riverbed of Kirindy river | | | + | | | | + |
| VI South-west | Andringitra | | | | | | | | |
| | Korokoto | Undisturbed forest | + | | | | | | |
| | Isalo | | | | | | | | |
| | Oasis | Relatively dry area with standing pools of water with bush where tadpoles were observed. | | | | | + | | |
| | Andrehitogna | Vegetation along the streambed consisted mainly of shrubby vegetation and bush | | | + | | | | |
| VII South –east | Tolagnaro | | | | | | | | |
| | Nahampoana | Hill-side forest bordering a small boulder-strewn stream, Vegetation consisted mainly of small *Pandanus* | | | | | | + | |
| | Manantantely | Hill-side forest bordering a boulder-strewn stream. Vegetation, consisted mainly of *Pandanus,* is thick in comparison of Nahampoana | | | | | | + | |

*Mantella spp* occur in habitats, including a wide range of type localities :

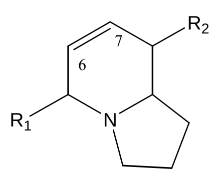
- upland swamp forest habitats, wich contained varying amounts of standing water, grassy hillocks, a variety of trees, Andasibe;
- semiarid streambed, massif Isalo;
- bamboo grove, Marojejy forest
- stream-side forest, Moramanga.

Some habitats are disturbed, while others are not. (11,12)

**Chemistry section**

Each skin amphibian alkaloid structure has a code identification based on use of the molecular weight and an identifying letter(s) in bold face. This code is usually preceded by the name of the structural class. (***)
A total of over 50 samples have been collected, and all have been investigated for skin alkaloids. Nonetheless, alkaloid extracts from 1 to 5 *Mantella* skins have not been available in quantities sufficient for allowing us to isolation of some of their components. Thus, tentative structures have been proposed based only on GC-MS and GC-FTIR data, obtained from containing as many as 10 to more than 70 alkaloids.
The following accounts provide general information on gas chromatographic profiles, and levels of alkaloid as well. They also deal with extended results for two selected species : *Mantella laevigata* collected in "Camp Mantella" Marojejy from the northern Madagascar, during the first expedition, and *M. betsileo* in Kirindy Morondava forest, from the south-western Madagascar.

Currently, a number of detailed analysis are in progress for further publications.
The levels of alkaloids varied considerably from one population to another for the same species, and for different species as well. The gas chromatography profiles also differed considerably in the same way. (11,12) These data reflect various habitats and diet. They are consonant with our previous results. (1,3,9)
*Mantella laevigata* alkaloid extract is characterized by the occurrence of a new dehydro-5,8-disubstituted-indolizidine structural class, which representative, one of the major compounds, is the 5,8-disubstituted azabicyclo[4,3,0]non-6,7-en **251P** [$C_{16}H_{29}NO$]. Its retention time is at 14,01 minutes. It displays an internal double bond, and its structure may be presented as $\Delta^{6,7}$-5,8-I **251P** follow:




$R_1 = C_7H_5O$
$R_2 = CH_3$

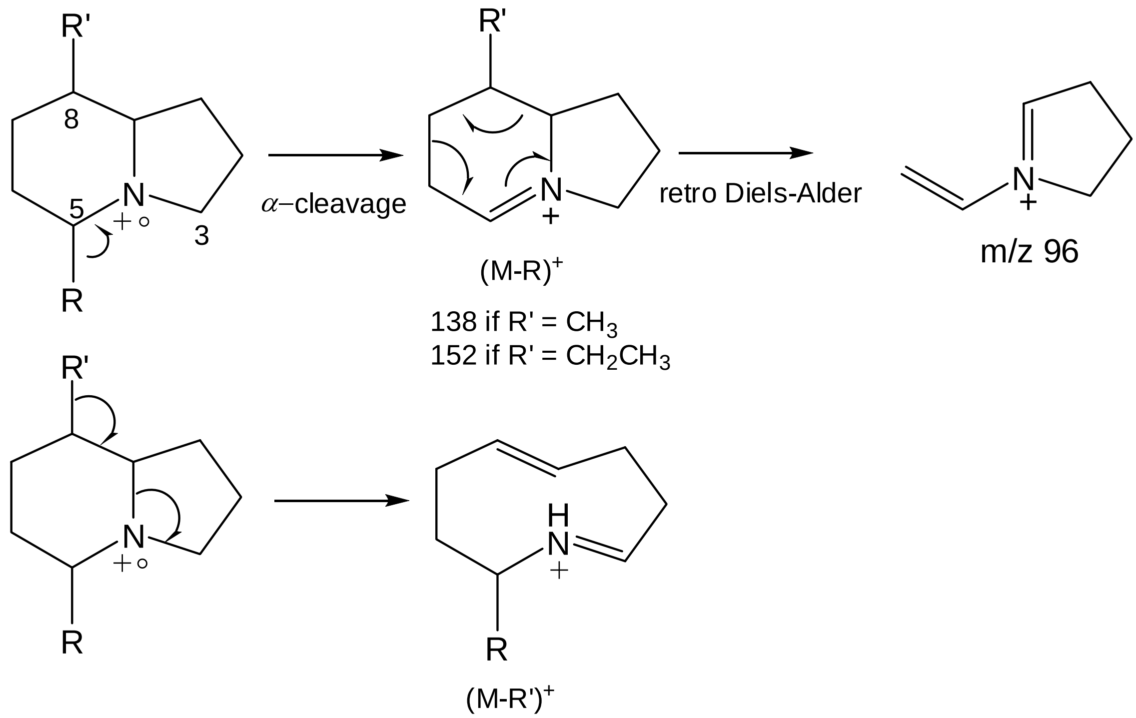
The tentative structure have been based on MS and vapor-phase FTIR spectra analysis. These are shown in scheme 1 and figure 4 respectively.
The comparison of mass fragmentation pathways for known disubstituted-5,8-indolizidines with proposed mass fragmentation of the new structural disubstituted-dehydro-5,8-I exhibits a major α-cleavage base peak [$M^+$-$C_7H_{10}O$] at m/z 136 (100) and a second daughter ion [$M^+$-$C_7H_{12}O$] at m/z 134 (43), obtained from the former by loss of 2H°. That is consonant with the

(***) Daly *et al,* Alkaloids from Amphibian skins, current revised version **2005**, unpublished occurrence of the double bond located at $C_6$-$C_7$. This fragment ion characterizes the new structural class dehydro-5,8-indolizidine.

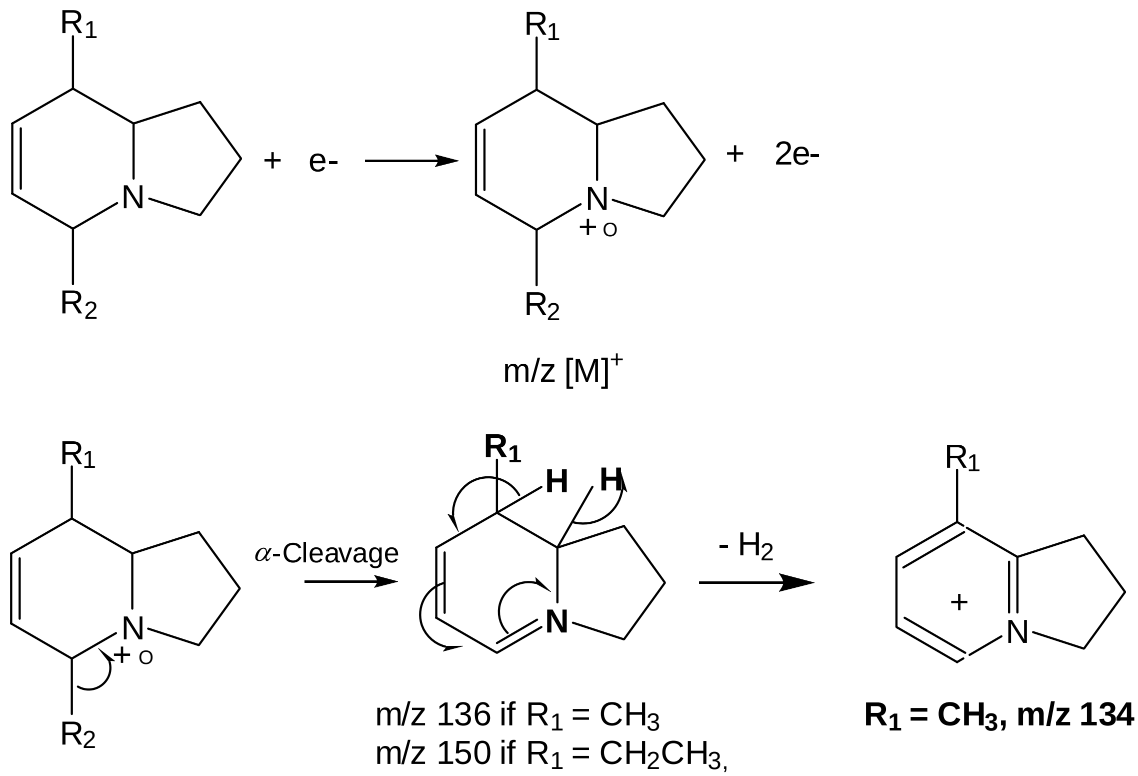
The absence of the base peak at m/z 96(100), diagnostic of saturated 5,8- disubstituted-indolizidines, is noteworthy.

The vapor-phase FTIR spectrum of **251P** exhibits absorption peaks at:

- 3660 $cm^{-1}$, typical for a non-hydrogen-bonded hydroxyl group on the side chain;
- 2969 $cm^{-1}$, indicating an internal double bond;
- 2788 $cm^{-1}$, weak Bohlmann band.




Scheme 1: Proposed EIMS fragmentation for 5,8-disubstituted Indolizidines




Scheme 2: Proposed EIMS fragmentation for Dehydro-5,8-disubstituted Indolizidines **251P** (5,8-disubstituted azabicyclo[4,3,0]non-6,7-en)

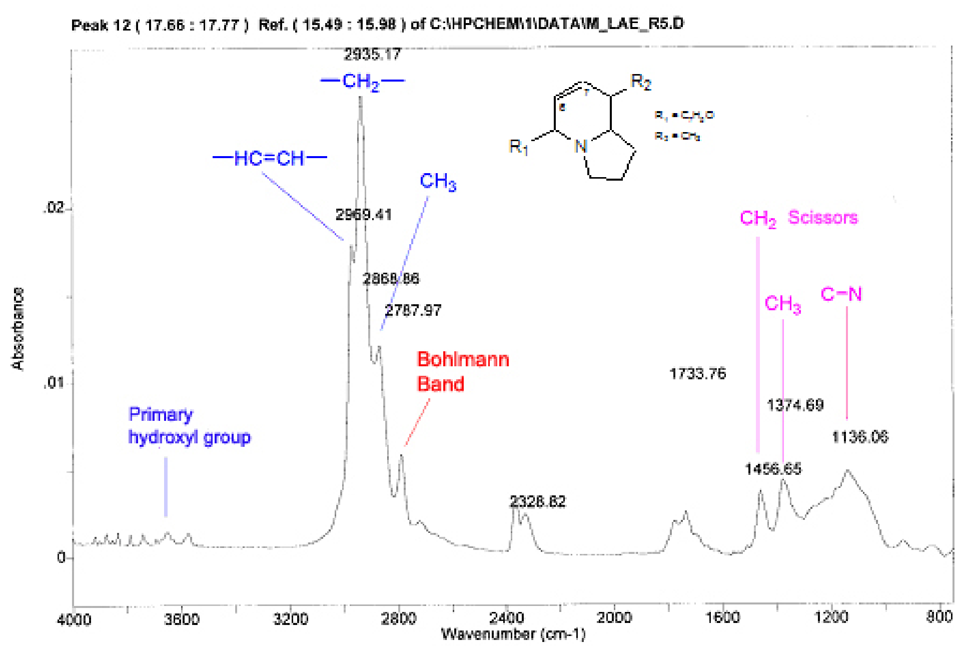
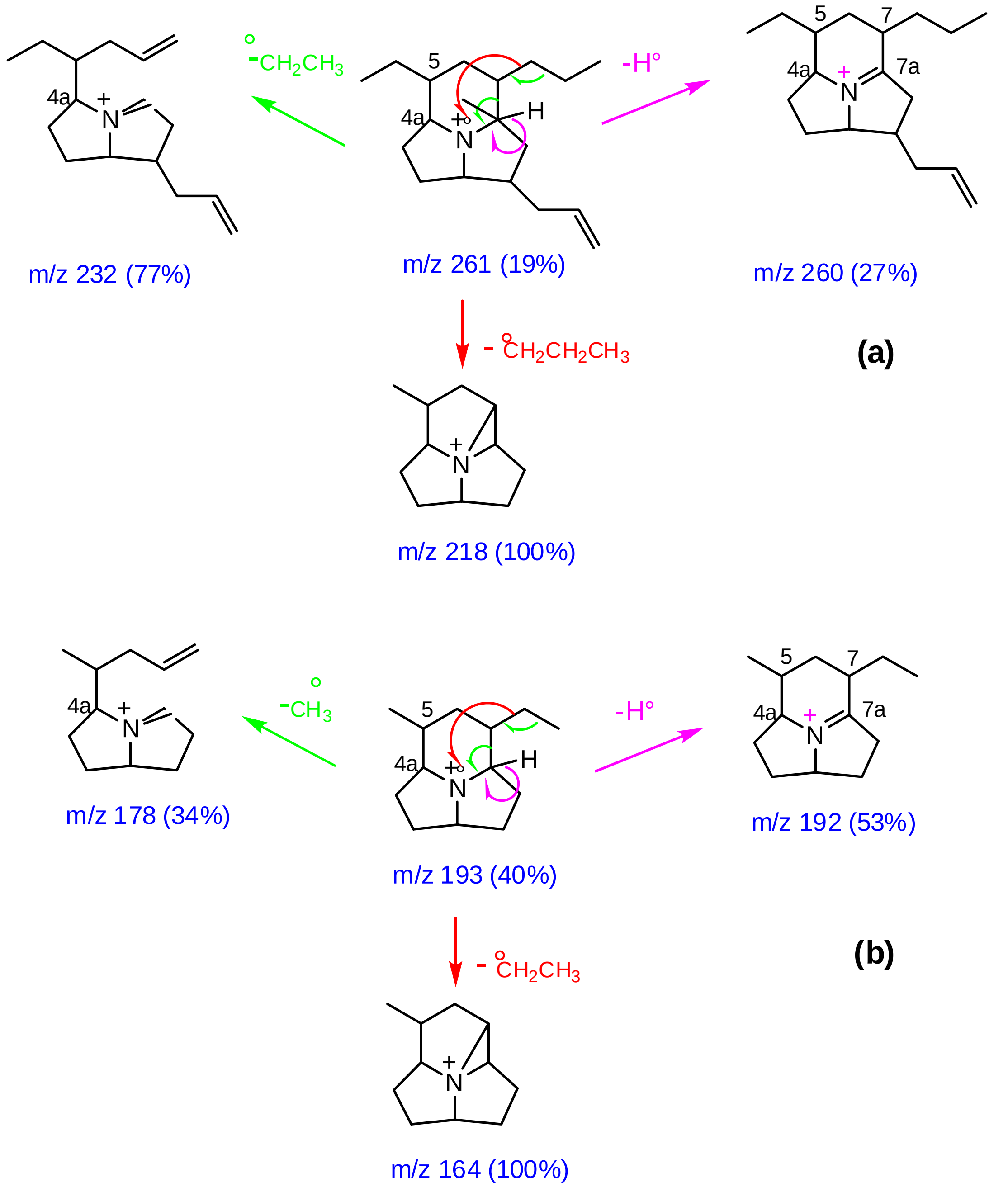



Figure 4: Vapor-phase FTIR spectrum of Dehydro-5,8-disubstituted Indolizidine **251P**

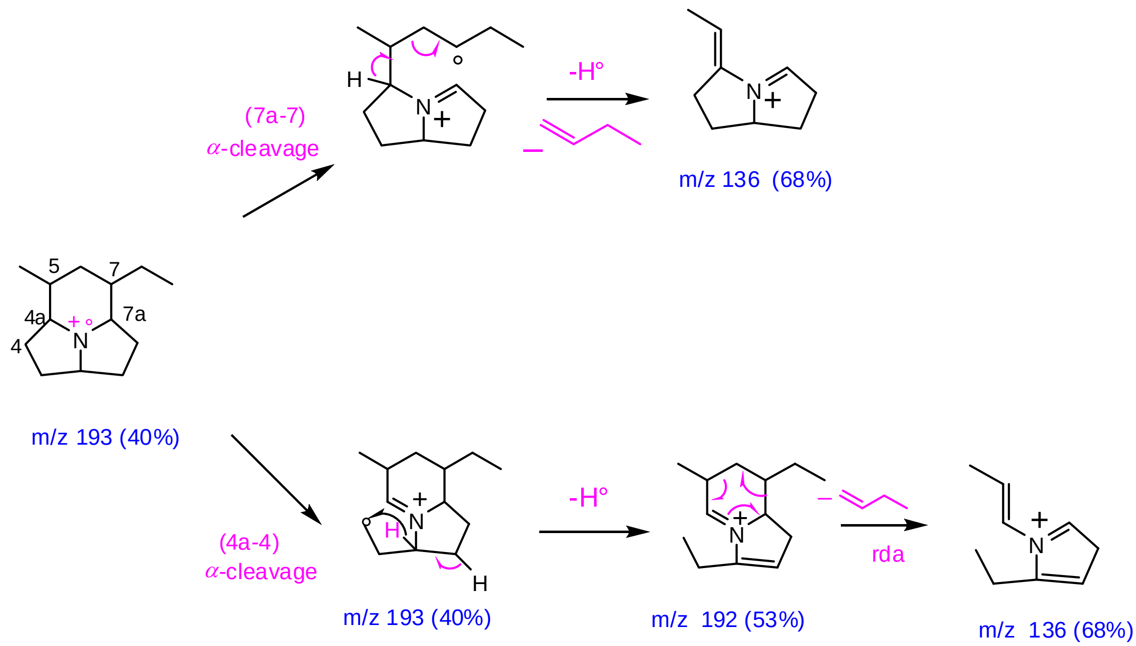
As a resume, in *Mantella laevigata* alkaloid fraction over 27 alkaloids, beside a number of alkaloids in trace levels, have been detected: 11 are of known structures and 16 belong to new or unknown structures. Two of the latters have been described the dehydro-5,8-I **251P** and a 5,6,8-trisubstituted-I **267U** (not presented herein). (12).

Similarly, the *Mantella betsileo* alkaloid extract have been analyzed. It is characterized by the occurrence of alkaloids of a new coccinelline tricyclic class with an unusual 6,5,5, instead 6,6,6 common structure. Three of them **193L**, **261C** and **277F,** alkaloids at moderate to high levels, were detected. The structure of the representative **261C** [$C_{18}H_{31}N$] of this class has been recently published (13).

The tentative structure of Tricyclic **193L** [$C_{13}H_{23}N$] **was** based on EIMS spectrum. In particular, a comparison of EIMS fragmentation pathways for some **261C** major daughter ions with EIMS fragmentation of **193 L** allowed to the conclusion the latter alkaloid was to be related in structure to **261C**. The alkaloid **193L** yielded a major α-cleavage base peak of [$M^{+\circ}$-$C_2H_5^{\circ}$] at m/z 164(100) and a significant ion at m/z 136(68), diagnostic of lack of propyl and allyl groups on the side chains. The mentioned EIMS fragmentation pathways are shown in schemes 3 and 4, respectively. The properties of dehydro-5,8-I **-251P** and Tricyclic **193L** are tabulated in the experimental section.




Scheme 3: Comparison of proposed EIMS fragmentation of tricyclics 261C (a) and 193L (b)

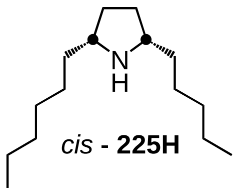



*Conclusion of the Chemistry section*

This preliminary survey of alkaloids in skin extracts of 15 species of *Mantella* reveals the presence of over 100 compounds. The structures of some of them are known, having been previously isolated and structurally defined from New World dendrobatid frogs. (1,2).

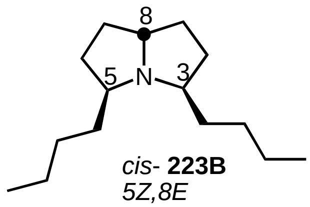
The pumiliotoxins and congeners are widely distributed in all mantellid frogs. Other well known structural classes, particularly izidines, pyperidines, decahydroquinolines have also been detected from some *Mantella* skin extracts.
Over 50 alkaloids belonging to new classes or unclassed structures occur in *Mantella*, including dehydro-5,8-indolizidine, coccinelline tricyclic alkaloid and 7,8-dehydro-8-desmethyllpumiliotoxin.

Very recently, the results for representatives of some of the new structural classes have been published, Tricyclic **261C** (13), and 7,8-dehydro-8-desmethylpumiliotoxin **235C** (*J. Nat. Prod.* **2005**, in press). Some of the major structural classes are shown in figure 5.



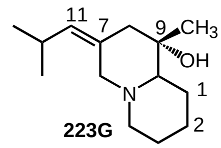
*cis* - **225H**

**2,5-disubstituted Pyrrolidines**



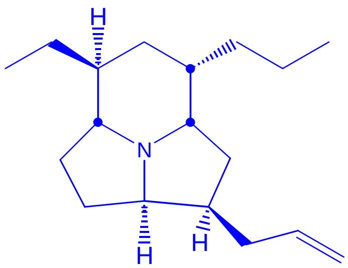
*cis*- **223B**
*5Z,8E*

**3,5-disubstituted Pyrrolizidine**



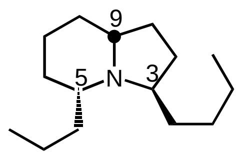
**223G**

**Homopumiliotoxin**



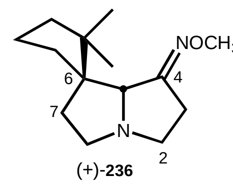
$C_{18}H_{31}N$

Tri **261C**, recently isolated from *M. betsileo*
Andrehitogna Isalo



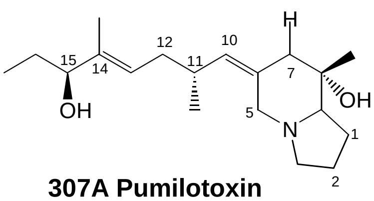
*cis*-**223AB**
*5E,9E*

**3,5-disubstituted Indolizidine**

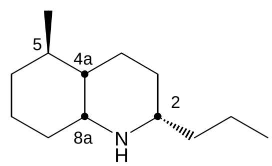


(+)-**236**

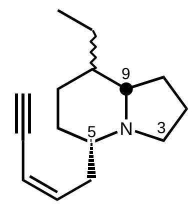
**(+)-spiropyrrolizidine 236**



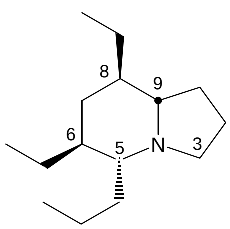
**307A Pumilotoxin**



**Décahydroquinoline 195A**

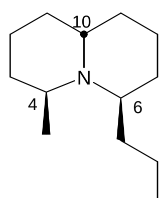


**217B**
**5,8 -disubstituted Indolizidine**



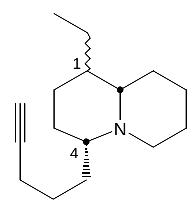
**223A**

**5,6,8-trisubstituted Indolizidine**



**195C**

**4,6-disubstituted Quinolizidine**



**231A**

**1,4-disubstituted Quinolizidine**

*Evidence for the dietary source of alkaloid 261C*

Sequestering of lipophilic alkaloids provided to a frog diet has been demonstrated for *Mantella.* (3,14,16) In addition, in our previous results we indicated skin tricyclic alkaloids, including or related to the coccinellines, undoubtly come from beetles, while the spiro pyrrolizidine oximes come from small millipedes. (3,15,16) It was obvious that in order to identify the dietary source, one would have to collect and analyse either arthropods or leaf-litter arthropods from a locale, where the frog had moderate or high levels of the particular alkaloid interest. That was what we purchased for Trycyclic 261C detected in the above described *M. betsileo* collected in Kirindy Morondava forest.

Coccinelline beetles as well as samples of *M. betsileo* were collected in the same site, and at the same time. The alkaloid fractions of both samples have been analyzed, using classical procedures, particularly GC-MS. As a results, the occurrence of the expected Tricyclic alkaloid **261C** was observed for each alkaloid fraction sample. (11)

Thus, the evidence of this alkaloid source has been proved. (****)

## Experimental Section

*General Experimental Procedures*

Thin Layer Chromatography (TLC) were performed with precoated silicagel plates DC-Alufolien Kieselgel 60 $F_{254}$ MERCK 1.05554.

A GC-14A SHIMADZU gas chromatograph with FID detector was used. The column was a 30 m x 0.25 mm, 1µm film BP1 (dimethylpolysiloxane) capillary column programmed from 100°C to 270°C at a rate of 5°C/min with a nitrogen as carrier gas. 2µl, equivalent to 2mg of weight skin, was injected.

A Finnigan GCQ instrument having a Phenomenex Zebron ZB-5 column (30 m x 0.25 mm i.d., 0.25 µm) was used to obtain EIMS or CIMS with either $NH_3$ or $ND_3$ as reagent gas.

GC-MS-FTIR spectra were obtained using a Hewlett-Packard model 5890 gas chromatograph fitted with a Phenomenex Zebron ZB-5 (5%- Phenyl 95% Diméthylpolysiloxane) fused silica bonded capillary column (30 m x 0.25 mm, 0.25 µm) with the same program as above for GC-MS analysis, and interfaced with a Hewlett-Packard model 5971 Mass Selective Detector and a Hewlett-Packard model 5965B IR instrument with a narrow-band (4000-750 $cm^{-1}$) detector and a Hewlett-Packard ChemStation (DOS-based) was used to generate the chromatograms, EIMS, and FTIR spectra.

High resolution mass spectrometry peaks (HRMS) were measured with a JEOL SX 102 instrument fitted with a 15 m × 0.20 mm HP-5 column.

*Biological Material and Extraction*

Alkaloid fractions were prepared in the usual manner (1) from MeOH extracts of 1 to 5 skins of the Madagascan frogs, collected in various sites through seven regions of Madagascar.

All Voucher specimens are in collections of the “Département de Biologie Animale, Université d’Antananarivo” (DBAUA).

For every collection site, precise location was given using GPS instrument and its physical (local temperature, pH of surrounding streams) and ecological data were carefully recorded.

Generally, every alkaloid fraction showed several alkaloids with various amounts, from trace to high level amounts. Each alkaloid fraction dissolved in MeOH was subjected to TLC, GC, GC-MS and Vapor Phase GC-FTIR analysis as described above.

(****) Poster communication presented at ICNPR – International Congress on Natural Products Research - in Phoenix Arizona USA July 31-August 4, 2004

The properties of some alkaloids are reported: molecular formulas were obtained by HRMS, EIMS with intensities relative to the base peak set equal to 100, vapor-phase FTIR absorptions with intensities relative to the maximum absorbance set equal to 100.
**Dehydro-5,8-I 251P**. . $C_{16}H_{29}NO$. EIMS: 251(5), 250(11), 236(4), 136(100), 134(38), 120(5). FTIR: 3660 (14), 2970 (70), Moderate Bohlmann band 2791 (30) $cm^{-1}$.
**Tricyclic 193L** (a). '$C_{13}H_{23}N$'. EIMS: 193(38), 192(54), 178(33), 164(100), 152(13), 150(29), 136(72), 122(22), 110(11), 84(26).

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## References

1. Garraffo, H. M.; Daly, J. W.; Spande, T. F.; Andriamaharavo, N. R.; Anndriantsiferana, M. A Alkaloids in Madagascan frogs (*Mantella)* : Pumiliotoxins and homopumiliotoxins, indolizidines, pyrrolizidines and quinolizidines. *J. Nat. Prod.* **1993**, *56*, 1016-1038
2. Daly, J. W.; Andriamaharavo, N. R.; Andriantsiferana, M.; Myers, C. W. Madagascan Poison Frogs. *Am. Mus. Novitates*. **1996,** *3177*, 1-34.
3. Daly, J. W. Ernest Guenther Award in Chemistry of Natural Products. Amphibian Skin: A remarkable source of biologically active arthropod alkaloids. *Journal of Medical Chemistry* **2003**, *46*, 445-452.
4. Dumbacher, J. P.; Beehler, B. M.; Garraffo, H. M.; Daly J. W. Homobatrachotoxin in the genus *Pitohui*: Chemical defense in birds? *Science* **1992**, *258*, 799-801.
5. Dumbacher, J. P.; Spande, T. F.; Daly J. W. Batrachotoxin alkaloids from passerine birds: A second toxic bird genus (*Ifrita kowaldi*) from New Guinea. *Proc. Natl. Acad. Sci. U.S.A.* **2000,** 12970-12975.
6. Daly, J. W.; Noimai, N.; Kongkathip, B.; Kongkathip, N.; Wilham, J. M.; Garraffo, H. M.; Kaneko, T.; Spande, T. F.; Nimit, Y.; Nabhitabhata, J.; Chan-Ard T. Biologically active substances from amphibians: preliminary studies on anurans from twenty-one genera of Thailand, *Toxicon* **2004**, *44*, 805-815.
7. Paris, R. R. ; Moyse, H. « Anabasine », in Précis de Matière Médicale, Tome III, Masson et Cie, Paris **1971**, 183.
8. Jain, P.; Garraffo, H. M.; Yeh, H. J. C.; Spande, T. F.; Daly, J. W.; Andriamaharavo, N. R.; Anndriantsiferana, M. A 1,4-disubstituted quinolizidine from a Madagascan Mantelline frog *(Mantella). J. Nat. Prod.* **1996**, *59*, 1174-1178.
9. Andriamaharavo, N. R. Studies of Biologically Active Alkaloids From The Skin of *Mantella* Frogs, endemic to Madagascar, *Thèse de Doctorat 3ème Cycle*, Chimie Organique "Produits Naturels", Université d'Antananarivo, **2003**, 157 pages.
10. Badio B. ; Daly J. W. Epibatidine, a potent analgetic and nicotinic agonist, *Molecular Pharmacology*. **1994**, *45*, 563-569.
11. Harisoa, C. H. S. Contribution à l'étude des grenouilles *Mantella* des Régions Centre, Ouest, Sud-Ouest et Sud-Est : Origine de l'alcaloïde tricyclique **261C**. *Mémoire de DEA,* Chimie Organique « Option Produits Naturels »,,Université d'Antananarivo, **2005**.

12. Razafindrabe Razafindrakoto, C. Contribution à l'étude des grenouilles *Mantella* des Régions Nord, Nord-Est et Centre-Est : Étude des Composés azabicyclo[4,3,0]non-6,7-ène 5,8-disubstitués, *Mémoire de DEA,* Chimie Organique « Option Produits Naturels », Université d'Antananarivo, **2005**
13. Kaneko, T.; Spande, T. F.; Garraffo, H. M.; Yeh, H. J. C.; Andriamaharavo N. R., Andriantsiferana M. A Structure For 261C, a novel Tricyclic Alkaloid From Madagascan Poison Frog, *Mantella betsileo. Heterocycles* **2003**, *58*, 745-757.
14. Daly, J. W.; Garraffo, H. M.; Hall, G. S. E.; Cover Jr, J. F. Absence of Skin Alkaloids in Captive Raised Madagascan Mantelline Frogs *(Mantella)* and Sequestration of Dietary Alkaloids. *Toxicon* **1997**, *35*, 1131-1135.
15. Daly, J. W.; Garraffo, H. M.; Jain, P.; Spande, T. F.; Snelling, R. R.; Jaramillo, C.; Rand A. S. Arthropod-Frog Connection: Decahydroquinoline and Pyrrolizidine Alkaloids Common to Microsympatric Myrmicine Ants and Dendrobatid Frogs. *J. Chem. Ecol.* **1994**, *26*, 73-85.16. Smith B. P., Tyler M. J., Kaneko T., Garraffo H. M., Spande T. F., Daly J. W., Evidence for biosynthesis of Pseudophrynamines alkaloids by an Australian Myobatrachid frogs (*Pseudophryne)* and for sequestration of dietary Pumiliotoxin, *J. Nat. Prod*., (**2002**), *65*, 439-447.