

Amphibian Alkaloid Identification in Brazilian Dendrobatidae frog *Dendrobates Galactonotus* by Eletrospray Ionization Tandem Mass Spectrometry

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Dendrobates galactonotus is a diurnal, bright black frog with a brilliant splash of color across its back. Nearly 500 alkaloids of 22 different structural classes have been detected in skin extracts from frogs of the family *Dendrobatidae*. All seem to have been sequestered unchanged from dietary arthropods. In this work, electrospray ionization tandem mass spectrometry (ESI-MS/MS) was used to investigate the occurrence of alkaloids in the *D. galactonotus* skin secretion. The alkaloids were first methanol extracted from the skin of 31 adult specimens of *D. galactonotus*, collected in the Amazon forest. The crude skin extract, after a semipurification process, was purified by reversed phase high performance liquid chromatography using a liquid chromatography system with a C₁₈ analytical column, an acetonitrile/TFA 0.1% gradient and an UV-VIS detection at 220 and 254 nm. The chromatographic fractions were analyzed using a ESI triple quadrupole mass spectrometer. Five classes of alkaloids (histrionicotoxins-HTX; gephyrotoxins-GTX; 8-desmethylpumiliotoxins-8-dmPTX; decahydroquinoline-DHQ; indolizidine-IZI) were identified in the skin secretion from *D. galactonotus* by the mass fragmentation pattern. The DHQ 231 was characterized by the peaks at m/z 91, 105, 117, 136 and a diagnostic peak at m/z 152, due to the loss of side methyl group at C-5. The IZI 219 was characterized by a pair of fragment ions at m/z 136/134 and a peak at m/z 120, which suggest an 6,7-dehydro-5,8-disubstituted indolizidine. The IZI 263 was characterized by the loss of the α -substituent at C-5, followed by a retro Diels-Alder reaction that provides a diagnostic ion at m/z 110 and by the ion at m/z 70 that, even when weak, suggests an indolizidine. These diagnostic peaks suggest an 5,6,8-trisubstituted indolizidine. The GTX 287 was characterized by a fragment ion at m/z 244, due to the α -cleavage of the CH₂CH₂OH substituent. The 8-dmPTX 317 was characterized by fragment ions at m/z 152 (C₉H₁₄NO) and 70 (C₄H₈N). The HTX 235 was characterized by a fragment ion at m/z 96, due to the α -cleavage of the R₁ side chain. The alkaloids structures, most of which are tentative, were established by mass spectroscopic analysis and published data correlation. The results demonstrate for the first time the occurrence of six different alkaloids in the *D. galactonotus* skin secretion.