CONSTRUCTION OF AN ELECTRON IMPACT MASS SPECTRAL LIBRARY FOR THE AUTOMATED ANALYSIS OF POISON FROG ALKALOIDS

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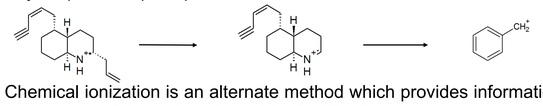
Background & Significance

Poison dart frogs are characterized by their vibrantly colored, yet toxic skin.[1] The toxicity of these poison frogs varies by the type and amount of alkaloids found on their skin. Alkaloids are naturally occurring, nitrogen containing, cyclic compounds that are found in the arthropods the frogs eat within their diet. These alkaloids are studied within our lab along with our collaborators to research their ecological and pharmacological possibilities. Through the use of gas/ liquid chromatography and mass spectrometry, we identify structural information and quantify the alkaloids present within the frog skin extracts provided by our collaborators.

Gas Chromatography - Mass Spectrometry

GC-MS is commonly used for the analysis of poison dart frog alkaloids, as most are volatile. We use a Thermo Trace GC ultra capillary gas chromatograph for separations.[2] This is interfaced to an iTQ1100 ion trap MS, which is capable of both, electron impact ionization (EI) and chemical ionization (CI).

Electron impact is an ionization method which provides structural information through fragmentation of a molecule. The electrons used for ionization are produced by passing a current through a filament. An electric field then accelerates the electrons across the source to produce a high energy (70eV, ~1600kcal/mol) beam. When an eluting analyte molecule passes through this beam, a valence shell electron is removed to produce a free electron and a radical cation. The kinetic energy given to the ion from this process is very high and causes fragmentation of the molecular ions as shown below for alkaloid **trans-243A**, a representative decahydroquinoline (DHQ).



Chemical ionization is an alternate method which provides information for molecular weight and assists with identification of co-eluting species when their masses differ. This information is obtained when NH_3 is used as the reagent gas. NH_3 is present in high concentrations relative to the eluting analyte molecules. The ammonium ion is significantly less acidic, thus tends to selectively protonate more basic species, being alkaloids.[4,5] Identifying exchangeable protons from NH, OH, and similar functional groups can be done by switching the reagent gas to ND_3.

Mass Spectral Library

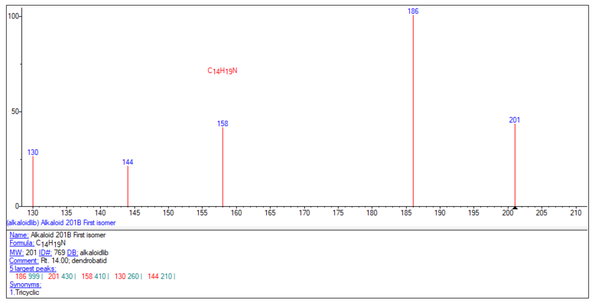
We are in the process of building a searchable mass spectral library of poison frog alkaloids that will be used for automatic identification through the NIST AMDIS program.[6] This process begins by acquiring alkaloid spectra from our GC-MS, which is then searching and comparing against known spectra that have been obtained through a similar process. Each library entry will include vital information about the alkaloid such as the spectrum, molecule name, structure, retention time, and data file that it was originally found within. When comparing this to the current manual method, the electronic library will allow for a much more simplified, efficient and accurate process. Manually analyzing the numerous spectra to a large range of alkaloids is a taxing procedure. In previous years, we were required to compare the manually found spectrum to formerly collected and classified spectra by the Daly group.

Existing Tabulated Library

The current electronic library that is used in our lab was created several years ago. It is mainly compiled of MS data presented in a review of over 800 alkaloids called the supporting information which was published by the Daly group in 2005.[1] Below is an example entry.

243A. DHQ. $C_{17}H_{25}N$. MS: 243(2), 242(1), 202(100). *Cis*-**243A**. Rt 12.32. FTIR[11]: No Bohlmann band. *Trans*-**243A**. Rt 12.68. 5-*Epi*-*trans*-**243A**: Rt 12.74. FTIRs[11]: Both weak Bohlmann bands 2805 cm^{-1}. NMRs.[12,17,46] 1D. H_8. *N*-Acetyl derivative. Dendrobatid, Bufonid.

This tabulated data was transcribed into the NIST MS Search software to produce searchable spectra as shown below for DHQ **243A**.

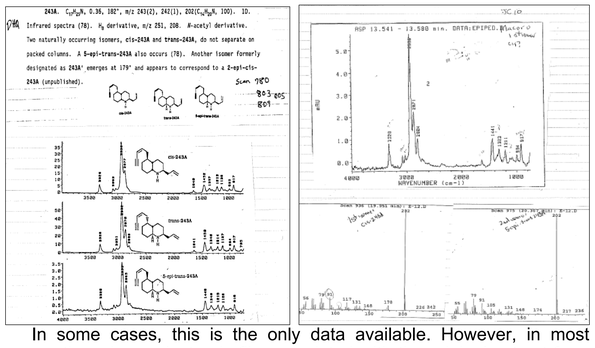


Using this library, we can sift through our alkaloid database of spectra and compare a sample spectrum to find a correct match in a semi-automatic fashion. This search produces an absolute list of possible matches that ranges from highest to least accurate comparison. However, this library does not include less abundant ion peaks as the tabulation focused more on larger fragments. These less abundant peaks can be crucial when distinguishing between similar alkaloids or isomers of pre-existing alkaloids. Numerous alkaloids of the same class have common fragmentation patterns and ratios, so comparing low abundance ions can be important when providing the proper identification.

Locating Archival Spectra

John W. Daly, a chemist and pharmacologist at the US National Institute of Health, dedicated nearly 50 years of his life to poison dart frog research. He worked to define and explain these naturally occurring, nitrogen containing, cyclic compounds' structures, as well as their effects and potential uses as medicines. We are grateful to have been granted custodianship of his collection of data and samples.

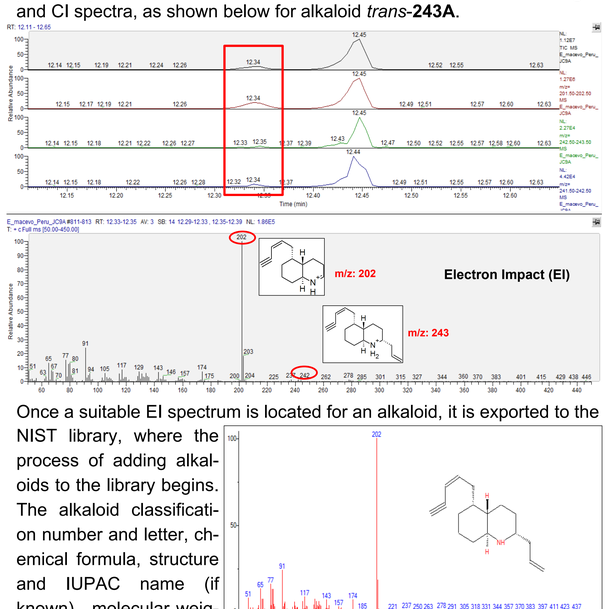
Magnetic sector, quadrupole, and ion-trap mass spectrometers were used to analyze the alkaloids.[1,3] For gas chromatographic separation, fused-silica-bonded capillary columns were primarily used. Much of this data was recorded in notebooks as shown.



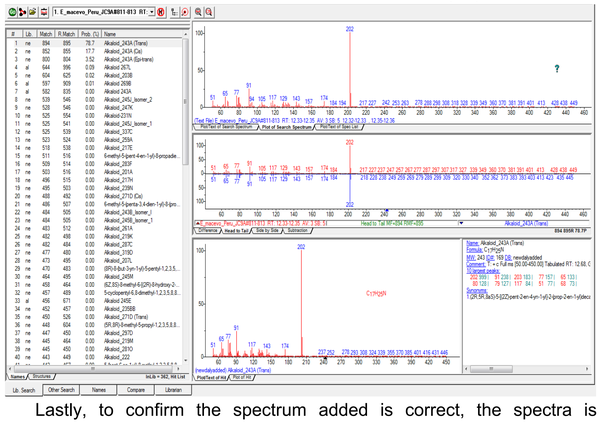
In some cases, this is the only data available. However, in most cases, there is an electronic file associated with the original extract from which the compound was identified or a closely related extract which can be matched to it. Where possible, the original dataset is used to extract spectra for incorporation into the library.

New Library Construction

The first step to confirming the identification of an alkaloid is to locate the original file where it once was found or a similar file in which it could potentially be found. The final assignment of the alkaloid is made using EI and CI spectra, as shown below for alkaloid *trans*-**243A**.



Once a suitable EI spectrum is located for an alkaloid, it is exported to the NIST library, where the process of adding alkaloids to the library begins. The alkaloid classification number and letter, chemical formula, structure and IUPAC name (if known), molecular weight, and retention time from both tabulated supporting information and from the GCQ file the alkaloid spectrum was found in with the name of the file, are all added to the library, along with the EI spectra. All of this information is added for validation purposes.



Lastly, to confirm the spectrum added is correct, the spectra is searched against the library spectrum list to assure that the correct spectra appears to be correctly matched with an accuracy percentage of at least 95%. In the case of *trans*-***243A*** the cis configuration is also very similar so the library results are portrayed as less accurate. This is a common occurrence when comparing isomers as the peaks are almost identical, however the retention times differ. The library allows for higher confidence identification of alkaloids when searched in automation.

Conclusions

At present, there are a total of 1,052 alkaloids to be added to the library from the 2005 tabulation and 612 spectra have been entered, of which, 139 were entered during the SURE 2019 program. Once complete, our goal is to make this library publicly available through NIST as well as in free public databases such as the Global Natural Product social Molecular Networking project (GNPS).[7] This way, researchers in this field will have access to high quality, fully curated spectral and chromatographic datasets from which to identify alkaloids in their own extracts. In particular, this should be of substantial value to ecologists who often lack access to reference compounds and high quality datasets.

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References

1.Daly, J. W.; Garraffo, H. M.; Spande, T. F. Alkaloids from Amphibian Skin: A Tabulation of Over Eight-Hundred Compounds. *J. Nat. Prod.*. **2005,** *68*. 1556-1575.
2.Chasteen, T. G. Split/Splitless Gas Chromatography Injection. http://www.shsu.edu/chm_tgc/primers/pdf/GC.pdf
3.de Hoffmann, E.; Stroobant, V. Mass Spectrometry: Principles and Applications, 2007, Wiley and Sons, New York, Ch 1, pp. 15-79; Ch 7, pp. 273-298.
4.Garraffo, H.M.; Spande, T.F.; Jones, T.H.; Daly, J.W., Ammonia chemical ionization tandem mass spectrometry in structure determination of alkaloids. I. Pyrrolidines, piperidines, decahydroquinolines, pyrrolizidines, indolizidines, quinolizidines and an azabicyclo [5.3.0] decane. *Rapid Commun Mass Spectrom* **1999**, *13*: 1553-1563.
5.Garraffo, H.M.; Spande, T.F.; Jain, P.; Kaneko, T.; Jones, T.H.; Blum, M. S.; All, T. M. M.; Snelling, R. R.; Isbell, L. A.; Robertson, H. G.; Daly, J.W., Ammonia chemical ionization tandem mass spectrometry in structural determination of alkaloids. II. Tetraponerines from pseudomyrmecine ants. *Rapid Commun Mass Spectrom* **2001**, *15*, 1409-1415.
6.AMDIS is freely available from NIST at https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis. NIST MS Search v2.0 is also available at https://chemdata.nist.gov/mass-spc/ms-search/docs/MSSearch_20_upgrade.html. NOTE, the NIST/EPA mass spectral database is not free and must be purchased from any of a number of vendors.
7.Wang, M.; Carver, J. J.; Phelan, V. V.; Sanchez, L. M.; Garg, N.; Peng, Y.; Nguyen, D. D.; et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnol.* **2016**, *34*, 828-837.

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