

Integrated introduction to sophomore undergraduate organic laboratory

Isolation and spectroscopic characterization of the flavone, hispidulin, from
Artemisia frigida”



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Abstract

- An integrated sophomore level organic chemistry laboratory curriculum will spark or enhance the appreciation of research while providing the sophomore with opportunities to develop various laboratory skills. Incorporating the isolation and characterization of a natural product in the curriculum provides for such an opportunity. Of the various groups of natural products, flavonoids are readily amenable to be included in a sophomore organic laboratory because of their widespread occurrence, and the relative simplicity of their spectral data. This report details the isolation and characterization of the flavonoid hispidulin, from *Artemisia frigida*. The experiment can be incorporated at various levels of the undergraduate curriculum depending on the complexity of the NMR spectroscopic data (1D or 2D NMR) that is desired.



Still valid after 300 years!

- Tell me, and I forget
 - Teach me, and I may remember
 - Involve me, and I learn



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Recent publications

- Nazri, M. M.; Samat, F. D.; Kavanagh, P. V.; Walsh, J. J. J. Chem. Educ., **2012**, 89, 138-140.
 - Nature's cholesterol lowering drug: Isolation and structure elucidation of lovastatin from red yeast rice containing dietary supplements
- Dintzner, M. R.; Kinzie, C. R.; Pulkrabek, K. A.; Arena, A. F. J. Chem. Educ., **2011**, 88, 1434-1436.
 - SIPCAn (Separation, Isolation, Purification, Characterization and Analysis): A one-term integrated project for the Undergraduate laboratory



Artemisia frigida (Prairie sagewort)

- Family: Asteraceae
- Perennial subshrub
- Foliage: Grey-green
- Habitat: almost all states
- Source: <http://plants.usda.gov/java/profile?symbol=arfr4>



Isolation

- Collect aerial parts, dry under shade and powder (collected in September 2008 from Fertile, MN). Dried powder (500 g)
- Soaked in methanol (1 L) for two days
- Filtered, and repeated 2 more times
- Rotavapped the combined methanol extract (8.2 g)
- Kupchan partition
 - Suspended the methanol extract in water-methanol (9:1, 100 mL) and extracted with hexane (100 mL). Repeated three times and rotavap the combined hexane extract.
 - Added 50 mL MeOH to the aqueous layer and extracted with DCM (3 X 150 mL). Rotavapped the DCM extract.

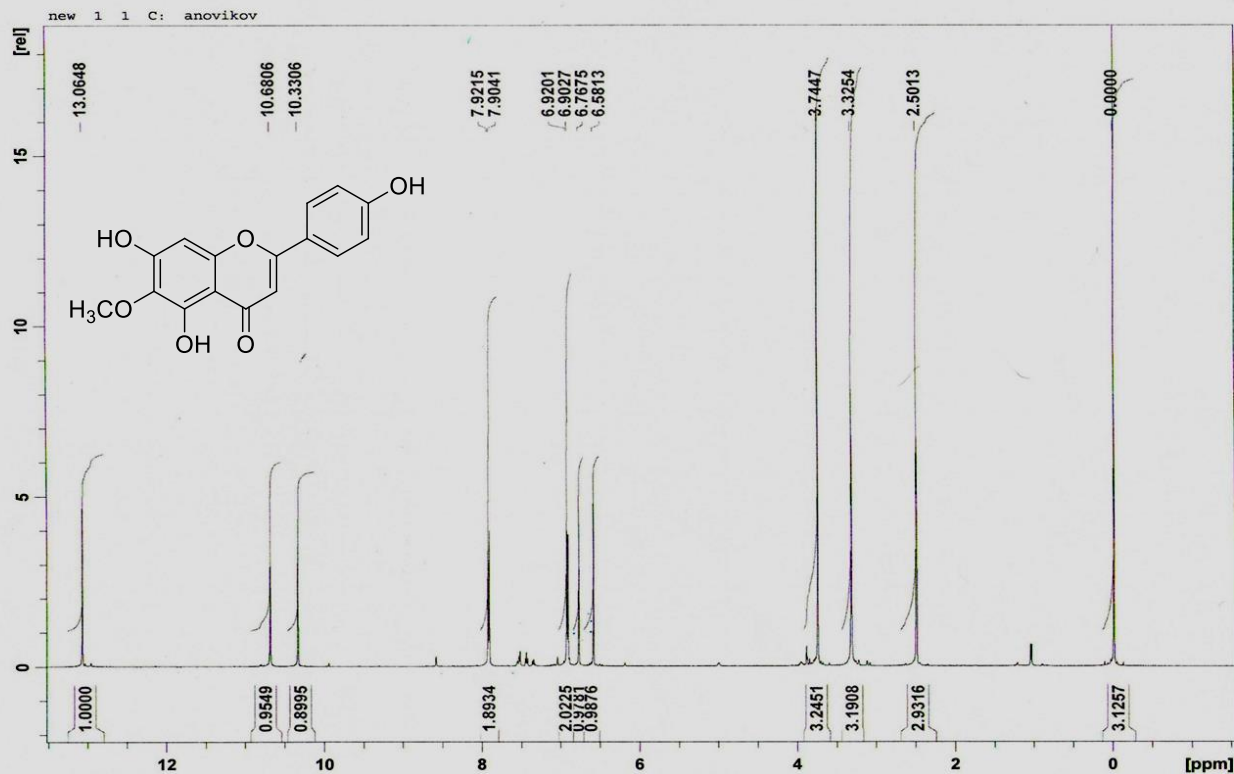


Isolation

- Separated the DCM fraction (2.3 g) on Sephadex LH-20.
 - Sephadex LH-20 from GE Healthcare. Mobile phase used MeOH.
- Controlled for hispidulin using Hexane-EtOAc (1:1) as the mobile phase on Si gel TLC plates.
 - Standard sample may be bought from Tocris Biosciences.
- Purified the fraction(s) of interest on Si gel to obtain hispidulin (5 mg) with greater than 95% purity.



^1H NMR of Hispidulin

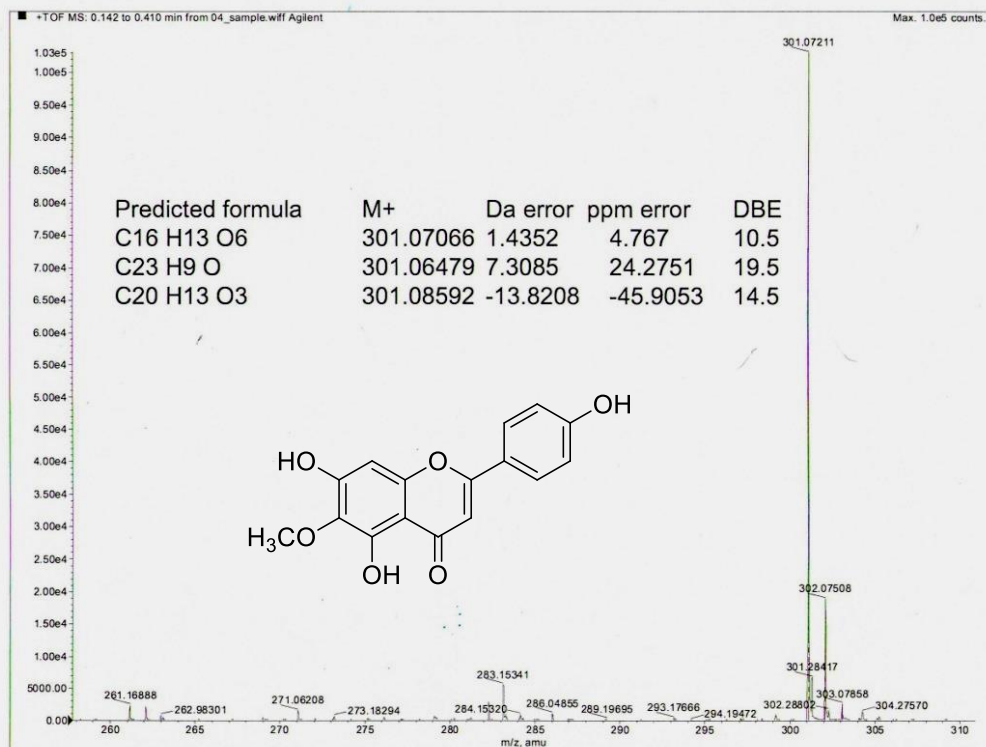


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Mass spectrum of hispidulin

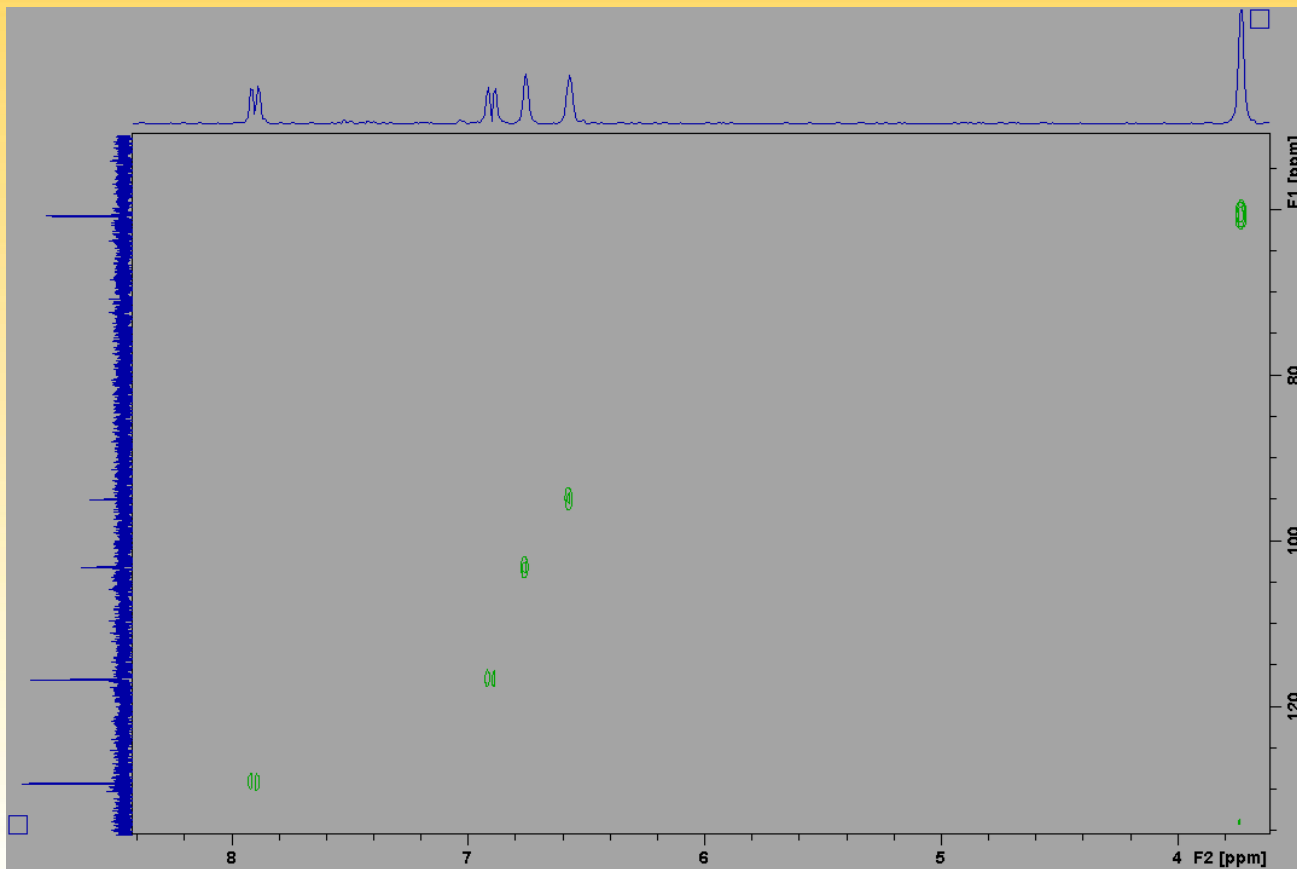
50% ACN 10 mM Ammonium acetate 10 μ L/min +ESI-TOF 4000V/ F150



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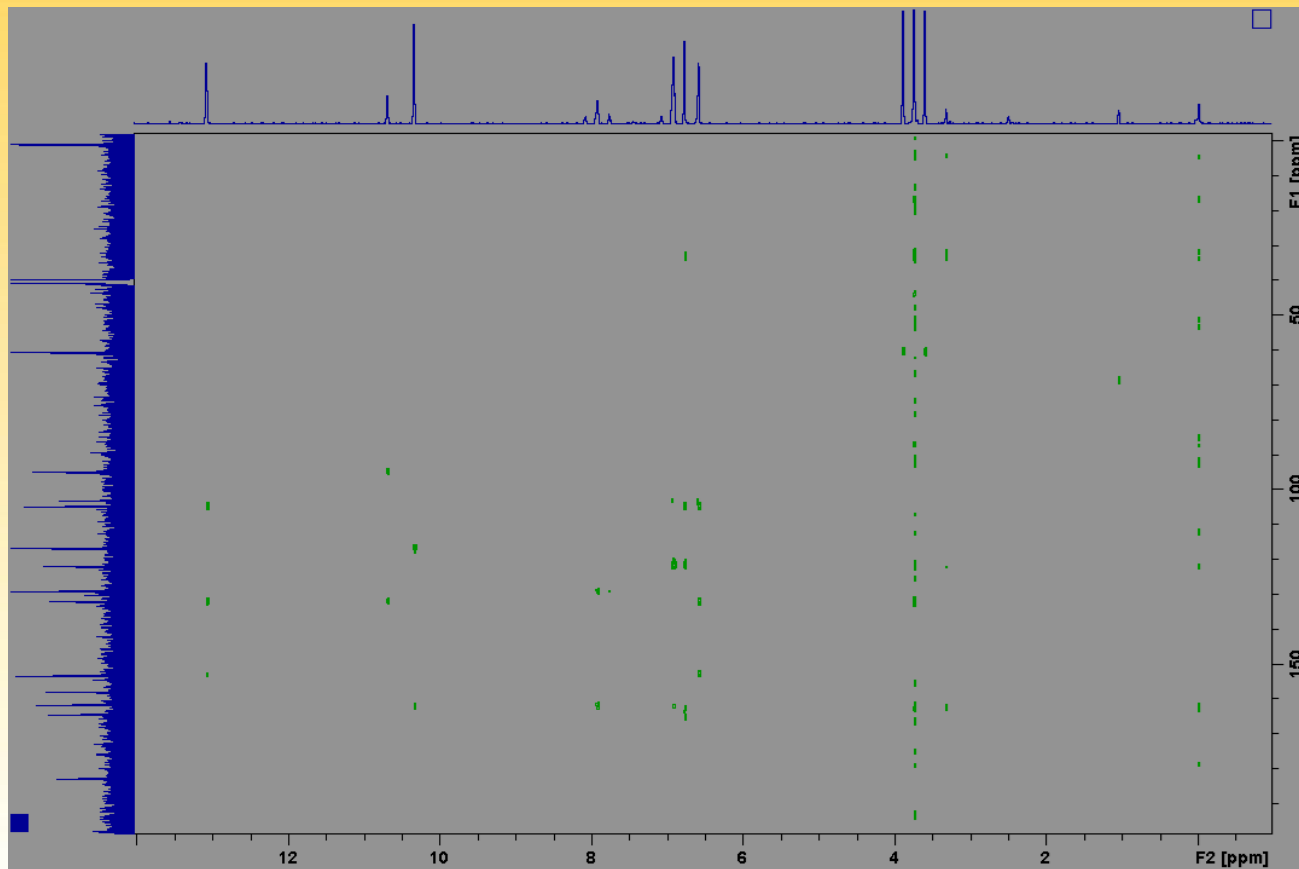
HMQC spectrum of hispidulin



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HMBC spectrum of hispidulin



UV spectra of flavonoids

- Band I (310-350 nm, cinnamoyl chromophore)
- Band II (250-280 nm, benzoyl chromophore)

- Exploiting UV to localize hydroxyl groups
 - 7-OH and 4'-OH lead to bathochromic shifts



What do students learn?

- Filtration
- Rotavapping
- Solvent-solvent extraction (distribution of polar vs non-polar compounds)
- Thin Layer Chromatography
- Column chromatography (size exclusion and adsorption)
- Interpretation of 'real' spectra
- Sharing resources, planning, writing project reports



Extrapolations

- Flavonoids could perhaps be separated from neutral compounds (a sub project)
- UV analysis
- Discussions regarding use of Size exclusion chromatography in biochemistry



Summary

- Sophomore organic chemistry I students will learn many of the techniques that are normally taught in a 'regular' organic laboratory.
- They will feel a sense of ownership of the project.
- Informal observations suggest that when they feel ownership, they learn.



Acknowledgements

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