

Separation and Identification of cis-Monotetrahydrofuran Acetogenins by Chiral Chromatography Coupled to LC-EI-MS

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NOVEL ASPECT

Identification of structural and stereoisomers of acetogenins by chiral chromatography with normal phase (isopropanol:hexane) elution and effluent introduction into EI-MS.

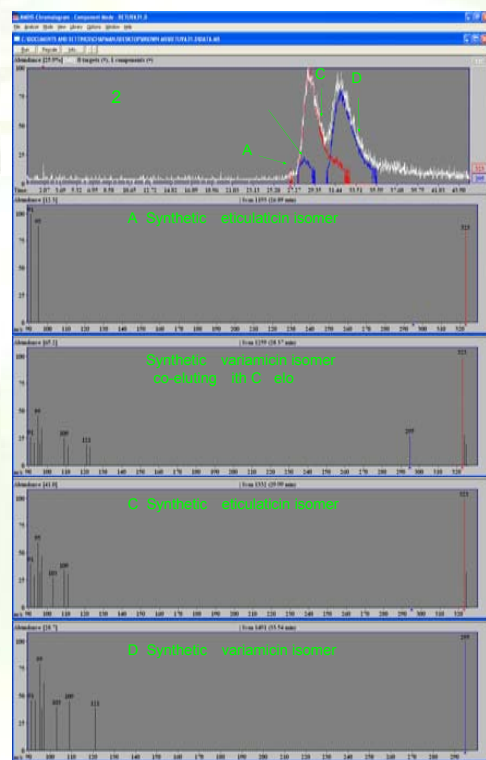
INTRODUCTION

Annonaceous acetogenins belong to a class of compounds that have been isolated from plants in the Annonaceae family. These acetogenins exhibit a variety of significant bioactivities including pesticidal, antiparasitic, and cytotoxic anti-tumor. Acetogenins are C 35 -C 39 compounds that typically contain two long hydrocarbon chains, one of which connects a terminal 2,4-disubstituted-γ-lactone to a variable number of tetrahydrofuran (THF) rings. The hydrocarbon chains possess a number of oxygenated functional groups at various positions. These groups can be hydroxyls, acetoxylys and/or ketones. The acetogenins also contain multiple stereocenters and this coupled with the existence of both structural and stereoisomers in the same plant source can make them very difficult to isolate.

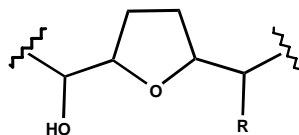
INSTRUMENTATION AND SEPARATION METHOD

The liquid chromatograph system included the following components: an Agilent 1100 modular system with quaternary pump, vacuum degasser, 100-vial autosampler, and variable wavelength detector. The HPLC column used was a Chiral CD-Ph, 4.6 × 250 mm. Chromatographic conditions were 0.200 ml/min flow rate, isocratic isopropanol:hexane (30:70), detection wavelength 220nm, and run time 45 minutes. For LC-EI-MS a Genesis II particle beam (PB) interface was employed. The particle beam interface was attached to an Agilent 5973 MSD so that samples could be analyzed by LC-MS with electron impact. The collected data for the particle beam LC-EI-MS were submitted for deconvolution and extracted ion analysis using the AMDIS program (Automated Mass Spectral Deconvolution and Identification System, version 2.1, DTRA/NIST, 2002).

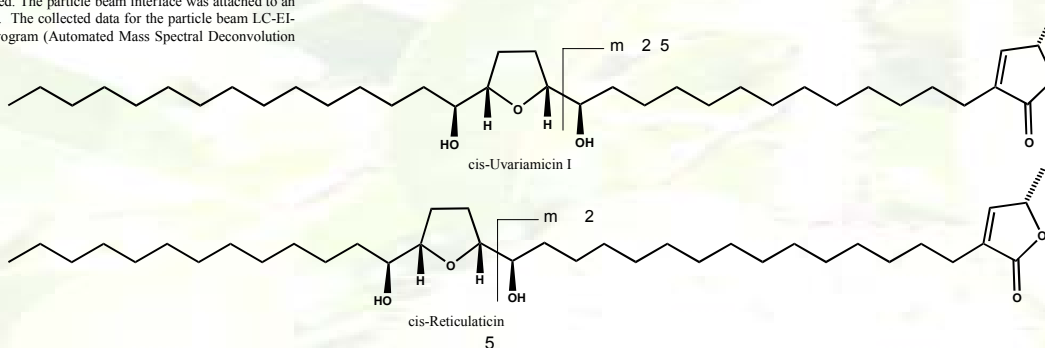
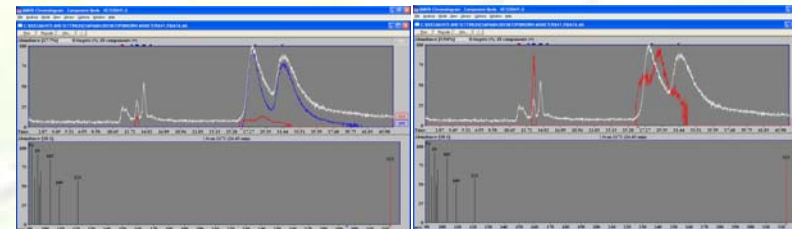
MASS SPECTRAL DATA



MONO-THF ACETOGENINS



MASS SPECTRAL DATA FOR NATURAL ISOLATE



DATA ANALYSIS

Figure 2 is the TIC and EICs of the synthetic mixture of reticulatin (2 isomers) and uvariamicin (2 isomers) illustrating that even though the peaks overlap and are not separable for UV analysis the EI-MS system is able to discern the difference in the four compounds. Figure 3 is the TIC and EICs for the natural isolate and illustrates that the natural isolate is a mixture of diastereomers and structural isomers.

CONCLUSIONS

Synthetically prepared standards of cis-uvariamicin I (a mixture of two stereoisomers) and cis-reticulatin (a mixture of two stereoisomers) were subjected to chiral chromatography on CD-Ph. The structural isomers cis-uvariamicin I and cis-reticulatin possess the same molecular mass and the same total number of methylene units (25). However, the two isomers differ in the number of methylene units that make up the two hydrocarbon chains attached to the tetrahydrofuran ring (13+12 for cis-uvariamicin I and 11+14 for cis-reticulatin). As a result, the fragmentation in the EI-MS yields unique ions that enable the two compounds to be easily differentiated. The difficulty arises not with characterizing the known standards, but with differentiating stereoisomers and structural isomers in extracts from the plants in the Annonaceae family. We have been able to chromatograph a natural isolate from *Annona muricata* and discovered the presence of two stereoisomers of cis-uvariamicin I and two stereoisomers of cis-reticulatin. Our method permits the chromatographic resolution of the structural and stereoisomers by Chiral CD-Ph, the introduction of the column effluent (isopropanol:hexane) into the EI-MS through the particle-beam interface, and subsequent identification of the compounds by comparison to synthetic standards.

ACKNOWLEDGEMENTS

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FUTURE WORK

The method utilizing analytical chiral chromatography columns at low flow rates and normal phase solvents for elution of the structural and stereoisomers of the natural product isolates promises to be useful for several other analytical applications.

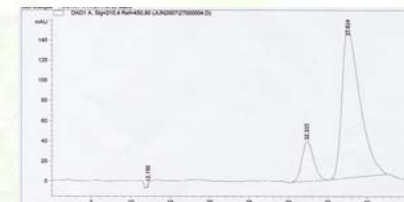
REFERENCES

Gleye, C., Duret, P., Laurens, A., Hocquemiller, R., and Cave, A. 1998. cis-Monotetrahydrofuran acetogenins from the roots of *Annona muricata*. Systematic Botany 13(2): 173-183.

6 Synthetic cis-reticulatin mixture of two isomers



Synthetic cis-uvariamicin mixture of two isomers



8 Synthetic cis-reticulatin and cis-uvariamicin

