

Identification of Medicinally Active Compounds in Prairie Plants by HPLC Coupled to Electron Impact-Mass Spectrometry

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Prairie plants of the Great Plains have historically been used as medicines by North American Indian tribes, settlers, and physicians since the early 19th century. Ethnobotanical studies have documented over 200 of these plants, which have been used for medicinal purposes. Two of these plants utilized for the treatment of multiple ailments are *Acorus calamus* L. and *Monarda fistulosa* L.

The leaves and rhizomes of *Acorus calamus* (Sweet Flag) contain an aromatic oil that has been used medicinally since ancient times and is cultivated in present-day Asia for this reason. The leaves and rhizomes are considered to possess antispasmodic, carminative, and antihelminthic properties and are also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers, and glandular and abdominal tumors. Additionally, the plant has been utilized for the treatment of kidney and liver troubles, rheumatism, sinusitis, and eczema.¹

Monarda fistulosa (Beebalm) was primarily used to treat ailments associated with the digestive system, such as relieving gas or alleviating the symptoms of nausea and vomiting. However, it was a common practice of Plains Indian tribes to prepare hot water extracts to treat colds, catarrh, headaches, and aching kidneys. Furthermore, the teas were employed to ease fevers, appease sore throats, induce sweating and urination, and as a stimulant. *Monarda fistulosa* leaves were also used externally to treat a wide variety of skin eruptions, including pimples and cuts. Native tribes used the leaves fresh or dried, achieving comparable results with both. Additionally, the dried leaves were often placed in food stores to function as an insect repellent.¹

In an attempt to uncover the relationship between the constituents and the purported medicinal properties, *Acorus calamus* and *Monarda fistulosa* leaves were analyzed by liquid chromatography-electron impact-mass spectrometry (LC-EI-MS). This method permits the rapid assessment of plant extracts for the presence of medicinally active compounds with a minimum of prepurification. Extracts are separated by reversed-phase LC prior to volatilization of the LC effluent and introduction into the electron impact ionization chamber. The advantage to acquiring EI fragmentation data lies within the subsequent ability to use existing deconvolution and search programs to match results with commercially available EI mass spectral databases.

Experimental

The *Acorus calamus* and *Monarda fistulosa* leaves were chopped (~1-cm pieces) and the organic constituents extracted first with methanol, then overnight with stirring with hexane:dichloromethane (1:1). The leaves were removed by suction filtra-

tion, a small amount of water was removed in a separatory funnel, the organic solvent was washed with saturated sodium chloride, and the solvent was rotary evaporated to obtain a viscous dark oil.² As an alternative preparation, the leaves were steam-distilled in order to obtain the essential oils. The distillate was extracted with diethylether (2 × 100 mL); the ether was washed with saturated sodium chloride; dried over sodium sulfate; and rotary evaporated, resulting in a golden-colored oil. The samples obtained by either preparative method were dissolved in acetonitrile for LC-EI-MS.

For LC-EI-MS, a particle beam (PB) interface was employed. The system included the following components. The liquid chromatograph used was an Agilent model 1100 modular system with quaternary pump, vacuum degasser, 100-vial autosampler, and variable wavelength detector (Agilent Technologies, Palo Alto, CA). The HPLC column used was a Zorbax SB-C18 (Agilent pn 830990-902), narrow bore 2.1 × 150 mm with 3.5- μ m particle size. The Genesis II particle beam interface (CSS Analytical Co., Shawnee, KS) was attached to an Agilent 5973 MSD (mass selective detector) so that samples could be analyzed by LC-MS with electron impact. The Genesis II is an improved particle beam interface that delivers a higher amount of analyte to the ion source compared to previous commercial interfaces. The mass spectrometer used was an unmodified Agilent 5973 MSD with turbomolecular pump. The benchtop quadrupole mass spectrometer has a mass range of 1.6–800 mass units, 10,000-V high-energy conversion dynode (HED), and is available with EI or EI-CI (chemical ionization) capabilities (see Figure 1).

Results and discussion

An HPLC chromatographic method was successfully developed using a reversed-phase column with UV detection at 254 nm that could resolve approx. 35 peaks from the *Acorus calamus* and 25 peaks from the *Monarda fistulosa* leaf extracts. When this separation was coupled with the PB-LC-EI-MS, the total ion chromatograms (TIC) shown in Figures 2 and 3 were obtained. 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). The deconvolution program found 189 ion signatures in the particle beam TIC for the organic extract of the *Acorus calamus* leaves. Similarly, the deconvolution program found 93 components in the particle beam



Figure 1 Genesis II particle beam interface.

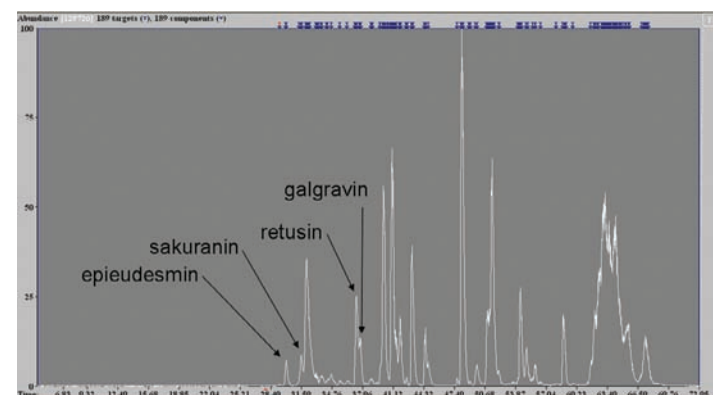


Figure 2 TIC from PB-LC-EI-MS for *Acorus calamus*.

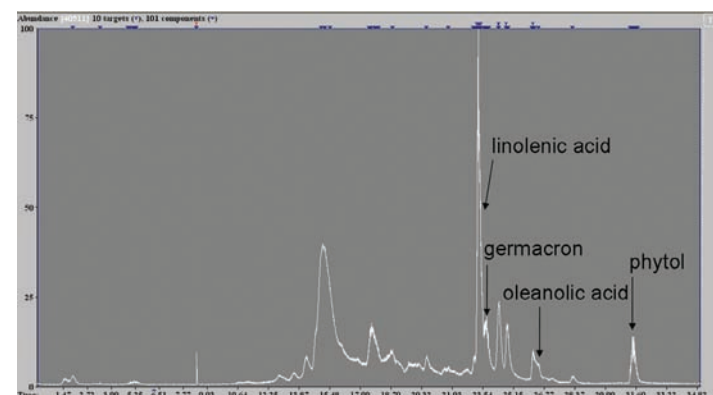


Figure 3 TIC from PB-LC-EI-MS for *Monarda fistulosa*.

TIC for the organic extract of the *Monarda fistulosa* leaves. Comparisons of unidentified constituents and AMDIS library matches are shown in Figure 4.

The deconvolution program isolates and identifies every component ion signature that it believes is unique, and frequently will match the same component several times to one peak in the TIC. This exhaustive analysis does produce some redundancies, but is critical to the discovery and investigation of coeluting component peaks. Our evaluation of the EI data yielded about 60 completely unique ion signatures in the analysis of the *Acorus calamus*

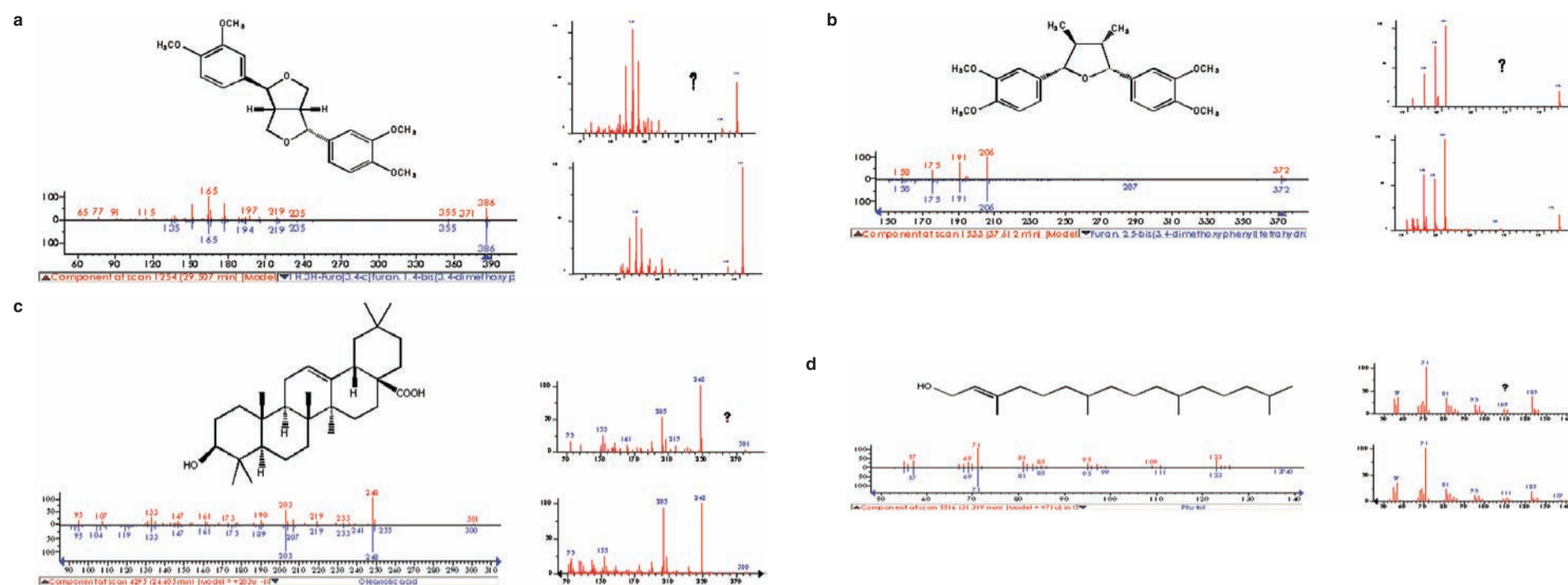


Figure 4 NIST library match for a) epieudesmin, b) galgravin, c) oleanolic acid, and d) phytol.

leaf constituents from the PB-LC-EI-MS, and the authors were able to identify 29 constituents by mass spectral matching. Using the PB-LC-EI-MS data collected from the *Monarda fistulosa* leaves, 17 constituents were identified conclusively. While the matching does postulate the presence of the constituent, it alone is not proof of the absolute identity. Several of these assignments were further supported by comparison to previously published accounts of the constituents.

Interesting results were obtained from both the LC-EI-MS analysis of the *Acorus calamus* and *Monarda fistulosa* leaf extract. One of the newly identified constituents is epieudesmin, which belongs to the class of plant molecules known as lignans. In addition to

the mass spectral match, the identity of epieudesmin was confirmed by comparison to an authentic synthesized standard provided by Dr. Brown's laboratory.³ Several of epieudesmin's confirmed pharmacological actions correlate to the purported herbal medicinal actions or uses of *Acorus calamus* leaves or rhizomes, and its presence in the leaves could implicate it as the medicinally active agent. Galgravin, also a lignan, was identified and is used for the treatment of malaria and rheumatism in addition to its neurotrophic effects.⁴ Another constituent, sakuranin, has been shown to have antihyperlipidemic activity, and plant extracts containing sakuranin have been used as an herbal treatment for diabetes. The flavanoid retusin was identified and has previously been shown to be an antitumor and psychoactive agent. Teas containing this compound function as antiinflammatory and analgesic preparations, and have been utilized as a purgative, laxative, and cathartic.

The discovery of two plant lignans, epieudesmin and galgravin, in the leaves of the plant potentially explains several of the purported activities attributed to *Acorus calamus*. Epieudesmin has been shown to have antineoplastic activity against the murine P388 lymphocytic leukemia cell line and several human cancer cell lines (BXPC-3, MCF-7, SF268, NCI-H460, KM20L2, and DU-145).⁵ Galgravin has demonstrated activity in preventing neuronal death and stimulating neurite growth. Structurally similar lignans have also shown neuroprotective activity in in vitro models for Alzheimer's and Parkinson's disease.⁴ Both epieudesmin and galgravin were identified in the methanolic extracts of *Acorus calamus* leaves.

Many of the previously unidentified constituents in *Monarda fistulosa* have been linked to medicinal properties. The medicinally active constituents were identified from the PB-LC-EI-MS data in Figure 3. Again, comparisons of unidentified constituents and AMDIS library matches are shown in Figure 4. Oleanolic acid is reported to be used as an antiinflammatory, while linolenic acid has been used to treat heart disease and rheumatism.^{6,7} The constituent phytol, used in making vitamins E and K, has been researched in connection with preventing various illnesses.⁸ The sesquiterpene germacron was identified and has been shown to work against bronchitis, and has also been known to benefit skin.

To this point only a fraction of the chemical constituents of the *Acorus calamus* and *Monarda fistulosa* leaves have been identified. While the authors were able to find several previously identified constituents, several constituents that have not previously been reported in the literature have been identified. All of the medicinally active compounds that were identified as novel components were discovered with the aid of the PB-LC-EI-MS. The PB-LC-EI-MS provided an analytical tool for exploring constituents of plant extracts not suitable for analysis by standard GC-MS or LC-ESI-MS techniques.

References

1. Kindscher, K. *Medicinal Wild Plants of the Prairie: An Ethnobotanical Guide*, University Press of Kansas: Lawrence, KS, 1992.
2. Mazza, G. J. *Chromatogr.* **1985**, *328*, 179–94.
3. Swain, N.A.; Brown, R.C.D.; Bruton, G. J. *Org. Chem.* **2004**, *69*, 122–29.
4. Pettit, G.; Meng, Y.; Gearing, R.; Herald, D.; Pettit, R.; Doubek, D.; Chapuis, J.; Tackett, L. J. *Nat. Prod.* **2004**, *67*, 214–20.
5. Fukuyama, Y.; Mitsumoto, Y.; Esumi, T.; Moriyama, M.; Inoue, T.; Zhai, H. *Biol. Pharm. Bull.* **2005**, *28*, 289–93.
6. Ismaili, H.; Milella, L.; Fkih-Tetouani, S.; Ildrissi, A.; Camporese, A.; Sosa, S.; Altinier, G.; Della Loggia, R.; Aquino, R. J. *Ethnopharm.* **2004**, *91*, 31–6.
7. Mozaffarian, D. *Altern. Ther.* **2005**, *11*, 24–30.
8. Rajab, M.; Cantrell, C.; Franzblau, S.; Fisher, N. *Planta Med.* **1998**, *64*, 2–4.

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