

Cite this: *Analyst*, 2012, **137**, 1082

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COMMUNICATION

Rapid detection of urushiol allergens of *Toxicodendron* genus using leaf spray mass spectrometry†Fatkhulla K. Tadjimukhamedov,^a Guangming Huang,^a Zheng Ouyang^b and R. Graham Cooks^{*a}

Received 8th November 2011, Accepted 26th December 2011

DOI: 10.1039/c2an16077c

A new ambient ionization method - leaf-spray mass spectrometry - is used to detect allergenic urushiols directly from poison ivy (*T. radicans*) leaves with no sample preparation. These simple measurements show all the urushiols previously reported using liquid chromatography mass spectrometry methods. Tandem mass spectrometry analysis of the leaf spray ions confirms the identifications. Enhanced detection of some urushiols was achieved in the negative mode with the addition of chloride anions to the spray solvent.

Introduction

In the chemical analysis of complex samples, such as plant tissue,^{1–7} liquid chromatography – mass spectrometry (LC-MS) is considered the gold standard due to its excellent quantitation, specificity, and sensitivity. The technique, however, relies heavily on laborious analyte extraction and purification steps prior to relatively lengthy LC separation processes. Given the need for instantaneous *in situ* detection of compounds by mass spectrometry in many applications, new ambient ionization methods have emerged during the past decade that allow direct analysis of compounds without sample preparation or purification.^{8–13} Paper-spray¹⁴ and the derived method of leaf-spray¹⁵ are amongst the most recent of the ambient mass spectrometry methods that allow immediate analysis of complex samples in their native form. Leaf-spray offers the simplest method yet to study various plants under native conditions. Chemicals unique to a given plant can be directly analyzed and identified¹⁵ although the scope of the methodology remains to be ascertained. This communication tests the method by attempting direct analysis of allergens in poison ivy.

Plants of *Toxicodendron* genus in North America, such as poison ivy (*T. radicans*) and western poison oak (*T. diversilobum*) release urushiols that cause an allergic reaction after contact with skin.¹⁶ Although the study of urushiols and their structural characterization dates back more than a century, the first mass spectrometric

investigation of urushiol extracts after gas chromatographic separation was reported in 1975 by Corbett and Billets.¹⁷

More recently, Draper and co-workers demonstrated a method to characterize urushiol extracts from western poison oak using LC followed by atmospheric pressure chemical ionization with tandem MS measurements, using collision-induced dissociation (CID) to obtain characteristic product ion mass spectra of urushiols.¹⁸ Kim *et al.*¹⁹ also sought to identify urushiols obtained from the sap of Korean lacquer trees, based on their tandem MS fragmentation profiles which bore strong resemblances to the spectra of Draper and co-workers. Each of these careful studies, however, employed meticulous extraction processes prior to LC separation and rapid identification and classification of poisonous species has not been demonstrated.

Here we document the direct detection and identification of urushiol homologs using leaf spray mass spectrometry. The main reason for choosing to study these compounds is that they are neither strongly basic or acidic so are not readily ionized and characterized by an ambient ionization method. Nevertheless, all six allergenic urushiols reported previously with LC/MS and GC-MS methods were detected within 30 s by the present method. The MS/MS identification of all detected urushiols is achieved and possible structures of the product ion species are discussed.

Results and discussion

Poison ivy is widely encountered in Indiana and specimens for the experiments were collected locally. Fresh leaf samples were cut into triangular shapes (base, ~7 mm; height, ~10 mm). A copper clip was attached to the base of the triangle while the apex was directed towards the mass spectrometer, for immediate measurements. The measurements were carried out using a Thermo Fisher Scientific LTQ instrument (San Jose, CA).

A diagram and a photograph of the experimental setup are shown in Supplementary Information.† Various spray solvents, such as methanol, acetonitrile, chloroform, dichloromethane as well as mixtures of solvents miscible with water in various ratios were investigated for the best signal intensity (solvent is necessary in this case to obtain stable signals). All solvents were purchased from Sigma-Aldrich, St. Louis, MO). The measurements demonstrated that amongst the solvent systems tested, 100% methanol showed the highest sensitivity in the positive mode of ionization, presumably since urushiols dissolve well in alcohols. Fig. 1 illustrates the positive

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2an16077c

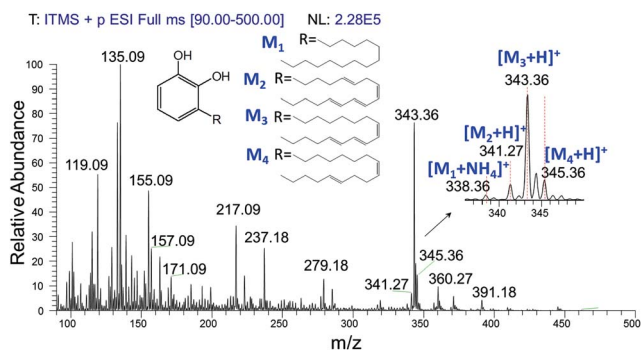


Fig. 1 Mass spectrum of poison ivy leaf sample obtained with leaf spray ionization in the positive ion mode using methanol as spray solvent. Inset shows expanded view of signals from four different urushiols observed between m/z 338 and 345.

ion mass spectrum recorded on a poison ivy leaf after applying of 15 μ L HPLC grade methanol to the leaf and supplying 4 kV DC potential *via* an attached copper clip. The cluster of peaks between m/z 341 and 345 (labelled M_2 through M_4) corresponds to protonated urushiol with C_{17} hydrocarbon chains, but with varying numbers of double bonds in the chain. Each ion upon CID yields product ions that are characteristic of the urushiols in general and of the particular hydrocarbon chain. These data are identical to those reported in the literature (Supplementary Information†).

In the negative ion mode, deprotonated non-polar urushiols could not be directly observed at high intensities in the mass spectrum. However, addition of chloride into the spray solution enhanced ionization and allowed detection of urushiols due to facile attachment of Cl^- ion. Fig. 2 shows the mass spectrum of a poison ivy sample

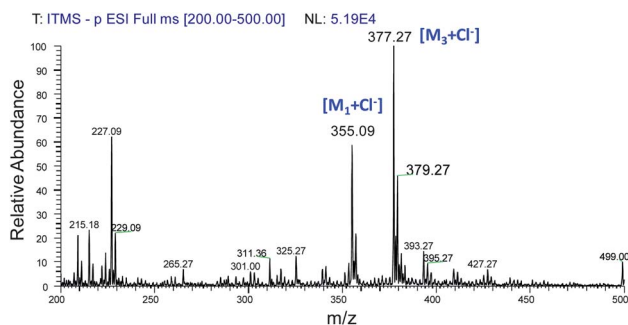


Fig. 2 Leaf spray mass spectrum of poison ivy in negative ionization mode with 9 : 1 methanol and water containing 0.01% HCl.

Table 1 Urushiols detected using leaf spray MS

m/z	Ion Detected	Hydrocarbon Side-chain	Double Bonds in Side-chain	Empirical Formula
<i>Positive mode</i>				
317	$[M + H]^+$	C_{15}	2	$C_{21}H_{32}O_2$
319	$[M + H]^+$	C_{15}	1	$C_{21}H_{34}O_2$
341	$[M + H]^+$	C_{17}	4	$C_{23}H_{32}O_2$
343	$[M + H]^+$	C_{17}	3	$C_{23}H_{34}O_2$
345	$[M + H]^+$	C_{17}	2	$C_{23}H_{36}O_2$
<i>Negative mode</i>				
355	$[M + Cl]^-$	C_{15}	0	$C_{21}H_{36}O_2$
341, 377	$[M - H]^-$, $[M + Cl]^-$	C_{17}	3	$C_{23}H_{34}O_2$
379	$[M + Cl]^-$	C_{17}	2	$C_{23}H_{36}O_2$

analysed using methanol and water (9 : 1) with 0.01% HCl used as spray solvent. The peak at m/z 355 is due to chloride anion attachment to the urushiol with MW = 320.27. This confirms the presence of this urushiol in the leaf sample confirming the above ammonium adduct data. The peaks at m/z 377 and 379 are due to the addition of Cl^- to an urushiol with a C_{17} side chain containing three double bonds and its ^{37}Cl isotope, respectively. The higher than expected relative abundance of the ^{37}Cl isotope peak ($\sim 45\%$) is due to a contribution from the C_{17} urushiol with two double bonds in the side chain (this urushiol appears at m/z 345 in Fig. 1). Enhancement in the efficiency of ionization with adding a dopant or reactant species to spray solutions have been successfully employed using other ambient ionization methods, specifically for desorption electrospray ionization method, where numerous reactant species have been shown to expand its applications.^{20,21} Here adding the chloride anion to the spray solution increased the intensity of the C_{15} urushiol (M_1) (seen at m/z 355 as the chloride adduct in Fig. 2) from 3.5% to 50% relative to the intensity of the C_{17} urushiol (M_2). The observation of facile attachment of adduct ions (ammonium cation in the positive and chloride in the negative ion modes) to urushiols containing saturated hydrocarbon side-chain seems to be favored compared to those with multiple double bonds in the side-chain. This can be attributed to the increased conformational mobility of the fully saturated hydrocarbon side-chain which allows access to the catechol hydroxyl groups by the adduct ions. A list of all urushiols detected in both the positive and negative ion modes is shown in Table 1. Among the six urushiols listed in Table 1 four were detected in the mass spectra at high intensity (Fig. 1 and 2).

Conclusions

Instantaneous detection of allergenic compounds from poison ivy leaves was achieved by leaf spray mass spectrometry. Measurements showed that all of the urushiols from poison ivy previously reported were detected using this simple ambient MS method. Coupling leaf spray to a portable mass spectrometer is underway to allow *in situ* chemical identifications of which the poison ivy case is a model example. Indeed, very recent studies have shown the capability of paper spray with a portable mass spectrometer for instantaneous *in situ* detection of agrochemicals from fruits with no sample preparation.²² These findings provide qualitative information on the presence of urushiol allergens based on their MS and tandem MS profiles. The technique is rapid by comparison with traditional LC-MS methods.

Acknowledgements

The authors acknowledge the National Science Foundation under grant numbers CHE-08-48650 for funding.

References

- 1 J. Boatright, F. Negre, X. L. Chen, C. M. Kish, B. Wood, G. Peel, I. Orlova, D. Gang, D. Rhodes and N. Dudareva, *Plant Physiol.*, 2004, **135**, 1993.
- 2 A. Goyer, T. L. Johnson, L. J. Olsen, E. Collakova, Y. Shachar-Hill, D. Rhodes and A. D. Hanson, *J. Biol. Chem.*, 2004, **279**, 16947.
- 3 W. J. Yang, P. J. Rich, J. D. Axtell, K. V. Wood, C. C. Bonham, G. Ejeta, M. V. Mickelbart and D. Rhodes, *Crop Sci.*, 2003, **43**, 162.
- 4 E. Baloglu and D. G. I. Kingston, *J. Nat. Prod.*, 1999, **62**, 1448.
- 5 K. V. Wood, C. C. Bonham, D. Miles, A. P. Rothwell, G. Peel, B. C. Wood and D. Rhodes, *Phytochemistry*, 2002, **59**, 759.
- 6 L. Z. Lin and J. M. Harnly, *J. Agric. Food Chem.*, 2007, **55**, 1084.
- 7 L. Z. Lin, S. Mukhopadhyay, R. J. Robbins and J. M. Harnly, *J. Food Compos. Anal.*, 2007, **20**, 361.
- 8 R. G. Cooks, Z. Ouyang, Z. Takats and J. M. Wiseman, *Science*, 2006, **311**, 1566.
- 9 R. Alberici, R. Simas, G. Sanvido, W. Romão, P. Lalli, M. Benassi, I. Cunha and M. Eberlin, *Anal. Bioanal. Chem.*, 2010, **398**, 265.
- 10 J. Laskin, A. Laskin, P. J. Roach, G. W. Slys, G. A. Anderson, S. A. Nizkorodov, D. L. Bones and L. Q. Nguyen, *Anal. Chem.*, 2010, **82**, 2048.
- 11 P. J. Roach, J. Laskin and A. Laskin, *Anal. Chem.*, 2010, **82**, 7979.
- 12 A. S. Galhena, G. A. Harris, M. Kwasnik and F. M. Fernandez, *Anal. Chem.*, 2010, **82**, 9159.
- 13 G. A. Harris, A. S. Galhena and F. M. Fernandez, *Anal. Chem.*, 2011, **83**, 4508.
- 14 H. Wang, J. J. Liu, R. G. Cooks and Z. Ouyang, *Angewandte Chemie, International Edition*, 2010, **49**, 877.
- 15 J. Liu, H. Wang, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2011, 7608.
- 16 D. G. Crosby, *The Poisoned Weed: Plants Toxic to Skin*; Oxford University Press: New York, 2004.
- 17 M. D. Corbett and S. Billets, *J. Pharm. Sci.*, 1975, **64**, 1715.
- 18 W. M. Draper, D. Wijekoon, M. McKinney, P. Behniwal, S. K. Perera and C. P. Flessel, *J. Agric. Food Chem.*, 2002, **50**, 1852.
- 19 D. H. Kim, J. O. Choi, J. S. Yang and D. W. Lee, *J. Liq. Chromatogr. Relat. Technol.*, 2003, **26**, 17.
- 20 H. Chen, I. Cotte-Rodriguez and R. G. Cooks, *Chem. Commun.*, 2006, 597.
- 21 I. Cotte-Rodriguez, C. C. Mulligan and G. Cooks, *Anal. Chem.*, 2007, **79**, 7069.
- 22 S. Soparawalla, F. K. Tadjimukhamedov, J. S. Wiley, Z. Ouyang and R. G. Cooks, *Analyst*, 2011, **136**, 4392.