Detecting the color chemicals in autumn leaves by using The Ambient Ionization Toolbox™

Summary

Some of the characteristic compounds that are responsible for the bright colors of autumn leaves are readily detected by using various ambient ionization methods with the AccuTOF-DART mass spectrometer system.

Introduction

Chlorophyll a is the dominant form of chlorophyll in plants that is responsible for the green color in leaves. Chlorophyll a has a chlorin ring (a porphyrin with partial hydrogenation) complexed to a magnesium atom. It has the elemental composition $C_{55}H_{72}MgN_4O_5$ and a monoisotopic molecular weight of 892.5353 u.

As chlorophyll production decreases in autumn, leaf color is dominated by other compounds such as carotenoids and anthocyanins[1]. These cause the bright yellows, oranges and reds that are associated with Fall color.

Lutein (yellow) and β-carotene (yellow-orange) are two of the principal carotenoids that are found in autumn leaves, remaining after chlorophyll is depleted. Anthocyanins, such as cyanidin and its glycosylated forms actually increase in some plants, resulting in bright red colors.

The structures of these compounds are shown in Figure 1. These include both polar and nonpolar compounds with very different chemical structures, so a single ionization method is not going to be ideal for detecting all components. Fortunately, the AccuTOF™-DART® Ambient Ionization Toolbox™ provides a variety of ambient ionization methods for detecting different compound types quickly and easily.

Experimental

Green and yellow leaves were collected from maple and oak trees in the area around JEOL USA, Inc. Red leaves were collected from a "Burning Bush" (*Euonymus alatus*) shrub in the vicinity. Leaves were cut up into small pieces and immersed in solvent solutions. Chlorophyll was extracted from the green leaf into methanol and a standard sample of chlorophyll a in methanol was measured for comparison. The carotenoids were extracted from the yellow leaf into hexane, and the anthocyanins were extracted from Burning Bush into water with sonication.

Chlorophyll a (standard and leaf extract) was detected by PaperSpray® by spotting a small amount of liquid onto a paper triangle positioned in front of the AccuTOF Orifice 1 using the JEOL PaperSpray accessory. The chlorophyll standard (1 mg in ether) had dried in the sealed ampule while stored in the freezer, so it was reconstituted in dichloromethane. One drop of the reconstituted standard was added to 1 mL of methanol for PaperSpray analysis.

The spray voltage was set to 2800V and the atmospheric pressure interface (API) Orifice 1 potential was set to 40V. The Ring Lens and Orifice 2 potentials were both 5V.

Chlorophyll a and cyanidins were also detected by Matrix Assisted Ionization Vacuum (MAIV)[2] by adding excess 3-nitrobenzonitrile (NBN) to the methanol or methanol/water solution and aspirating the solution and NBN crystals directly into Orifice 1 with the same API potentials.

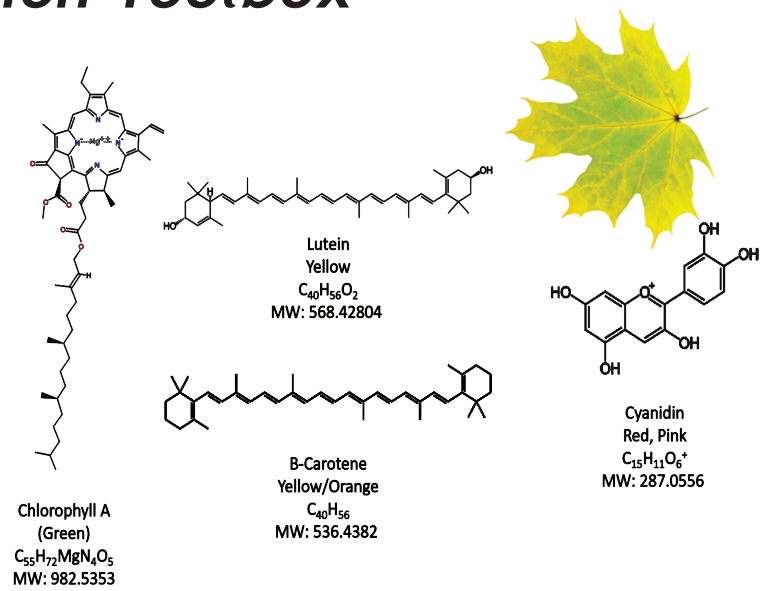

Figure 1. Structures, formulas, and monoisotopic molecular weights of some of the compounds responsible for leaf color.

Carotenoids were detected by positive-ion DART, using argon as the DART gas with chlorobenzene dopant. The measurement conditions have been described previously[3].

Jeffamine® M-600 and Ultramark® 1621 were measured with DART as reference standards for all measurements. There was no need to change hardware to switch between DART, PaperSpray®, and inlet ionization.

Results

Chlorophyll in a green leaf

The PaperSpray mass spectrum of a chlorophyll a standard is shown in Figure 2A. Chlorophyll a and a smaller amount of chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) is detected as the protonated and sodiated molecule. A peak at *m/z* 939.549 is determined from accurate mass measurements to have the composition $C_{56}H_{75}O_7N_4Mg$. A possible assignment is protonated 10-methoxylactone chlorophyll a[4]. This peak was not observed in the leaf extract, so it is likely a result of an in-vitro reaction occurring during long-term storage or reconstitution in new solvents.

The MAIV mass spectrum in Figure 2A also shows protonated chlorophyll a and chlorophyll b, but a major peak is loss of Mg^{2+} with the addition of 2 protons to give $C_{55}H_{75}O_5N_4^+$. This is only observed in the MAIV mass spectra, and must be an artifact from the matrix-assisted ionization.

PaperSpray of the leaf extract did not show chlorophyll, perhaps due to suppression by saccharides and other components in the extract (Figure not shown). On the other hand, the MAIV mass spectrum of the leaf extract (Figure 3) shows not only the chlorophyll, but also molecular ions for the carotenoids β-carotene and lutein. From this spectrum, we can see the deeply green chlorophyll is masking the yellow/orange colors from the carotenoids.

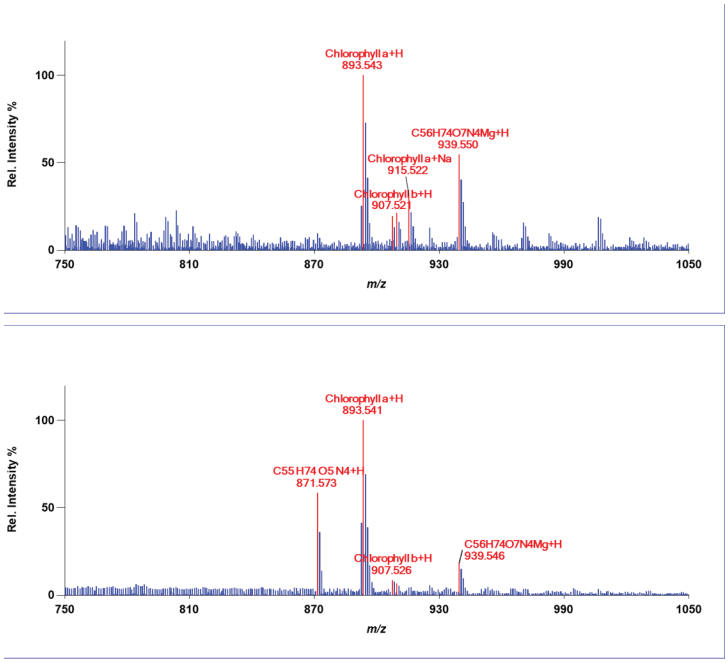

Figure 2. PaperSpray (A) and MAIV (B) mass spectra of a chlorophyll standard.

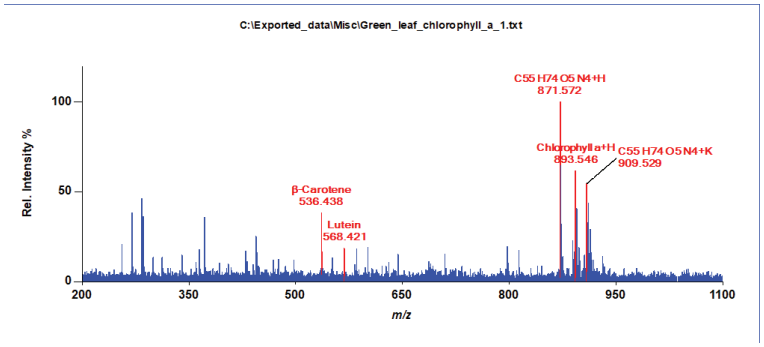

Figure 3. MAIV mass spectrum of a green leaf extract in methanol.

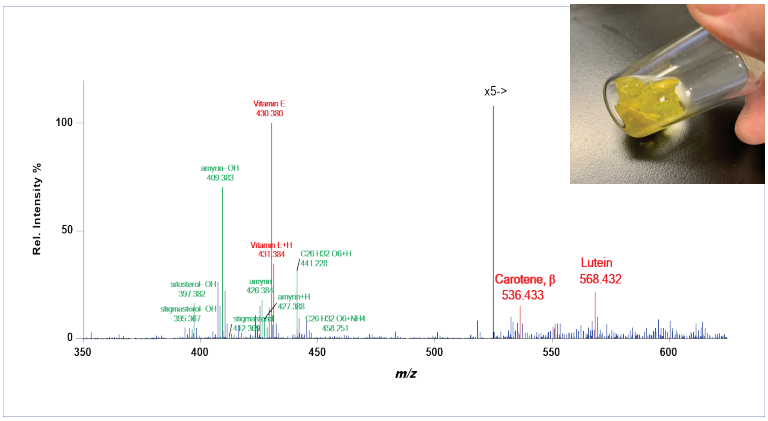

Figure 4. Argon DART mass spectrum (chlorabenzene dopant) of a hexane extract of a yellow leaf showing carotenoids as well as phytosterols (labeled in green) and α-tocopherol ("Vitamin E").

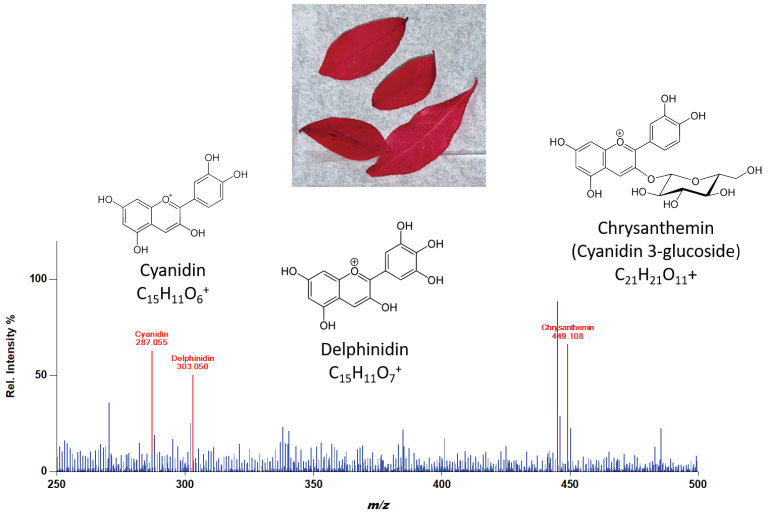

Figure 5. Cyanidin anthocyanins detected in a MAIV mass spectrum of a water extract of "Burning Bush" (Euonymus alatus) leaves.

Lutein and carotene in a yellow leaf

Helium DART gas is very versatile, but carotenoids such as β-carotene can be difficult to detect in the presence of abundant interferences (such as diglycerides) that are commonly found in the same *m/z* region. The carotenoids are highly conjugated molecules with low ionization energies, so they can be detected with high selectivity by using argon DART gas with chlorobenzene dopant. This will form molecular ions by charge exchange with the ionized chlorobenzene[3]. Figure 4 shows the lutein and β-carotene in the hexane extract of a yellow leaf. Abundant lipophilic molecules with low ionization energies (phytosterols and α-tocopherol or "Vitamin E") are also detected by this method.

Anthocyanins in a "burning bush" leaf

Anthocyanins are colorful cationic compounds that are readily soluble in water and which provide color to many botanical products (such as red wine). These polar compounds are not suitable for DART analysis, but they can be detected by electrospray ionization (ESI) PaperSpray[5] and MAIV. Figure 5 shows a MAIV mass spectrum of the cyanidin aglycoside as well as the glycosylated forms in the water extract of "burning bush" leaves.

Conclusion

The AccuTOF-DART Ambient Ionization Toolbox® provides multiple methods for chemical analysis with ambient ionization, without having to change ion sources. This makes it fast and easy to characterize chemical compounds with different polarities and chemical structures, such as the chemical compounds that produce the bright colors of autumn leaves.

For the leaf extracts, Argon DART with chlorobenzene dopant was highly selective for detecting conjugated nonpolar compounds such as phytosterols and carotenoids. PaperSpray and MAIV were effective for detecting charged and polar compounds such as chlorophyll and anthocyanins.

References

[1] The Chemicals Behind the Colours of Autumn Leaves. https://www.compoundchem.com/2014/09/11/autumnleaves/.

[2] E. D. Inutan, S. Trimpin. Matrix assisted ionization vacuum (MAIV), a new ionization method for biological materials analysis using mass spectrometry. Molecular & Cellular Proteomics, 2013, 12, 792.

[3] R. B. Cody , A. J. Dane. Dopant-Assisted Direct Analysis in Real Time Mass Spectrometry with Argon Gas Rapid Communications in Mass Spectrometry, 2016, submitted.

[4] D. Dolphin, The Porphyrins, Volume V. Physical Chemistry,