PROCEEDINGS OF THE TWENTY-EIGTH CONFERENCE OF THE NORTH AMERICAN THERMAL ANALYSIS SOCIETY, OCTOBER 4-6, 2000, ORLANDO, FLORIDA

LOCALIZED EVOLVED GAS ANALYSIS USING A SCANNING THERMAL MICROSCOPE

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ABSTRACT

Micro-thermal analysis employs a scanning probe microscope fitted with a miniature resistive heater/thermometer to obtain images of the surface of materials and then perform localized thermoanalytical measurements. We have demonstrated that it is possible to use the same configuration to pyrolyze selected areas of the specimen by rapidly heating the probe to 600-800°C. This generates a plume of evolved gases which can be trapped using a sampling tube containing a suitable sorbent placed close to the probe. Thermal desorption-gas chromatography/mass spectrometry can then be used to separate and identify the evolved gases. This capability expands the normal visualization and characterization by micro-thermal analysis to include the possibility of localized chemical analysis of the sample (or a domain, feature or contaminant). The isolation and identification of natural products from a plant leaf are presented as an example to illustrate this approach.

INTRODUCTION

The herb feverfew [*Tanacetum parthenium* (L.) Schultz Bip. syn. *Chrysanthemum parthenium, Pyrethrum parthenium*] (figure 1) has attracted considerable interest for the treatment of migraine and arthritis *(1-3)*. This has been attributed to the presence of the sesquiterpene lactone, parthenolide, which is reported to be the active principle *(4,5)*. Smith and Burford reported studies on the selective extraction of this material from dried leaves of the plant using supercritical carbon dioxide *(6)*. Scanning electron microscopy of the leaf surface before and after extraction (figure 2) suggested that the parthenolide is present in oil cells which are ruptured during the extraction process *(7)*. Capillary gas chromatography of the extract also detected the monoterpene camphor as well as chrysanthenenol acetate and dihydroparthenolide.

In order to confirm the location of the parthenolide and its associated essential oils, a study of feverfew leaves was undertaken using micro-thermal analysis. This is a technique with combines the imaging capabilities of atomic force microscopy (AFM) with the ability to use the AFM tip to carry out localized physical characterization by thermal analysis (currently consisting of forms of localized calorimetry and localized thermomechanical measurements) *(8,9)*. Measurements of the melting of waxy coatings on the surface of plant leaves have

been reported elsewhere *(10)*. We have also developed a means of using the same instrument to pyrolyze specific regions of the sample, trapping evolved gases and subsequently analyzing them by thermal desorption-gas chromatography-mass spectrometry (GC/MS) *(11,12)*. This affords a means of localized chemical analysis, which complements the imaging and physical testing described above.

EXPERIMENTAL

Samples of feverfew were obtained from plants grown locally from seed. The fresh leaves were mounted on metal stubs using double-sided adhesive tape and allowed to dry out slightly in air overnight before analysis. Measurements were carried out on a TA Instruments 2990 Micro-Thermal Analyzer. Imaging and localized thermal analysis can be carried out in the usual way. For pyrolysis experiments the probe is placed in contact with the region of interest and rapidly heated to 600-800°C. At these temperatures the tip of the probe emits sufficient visible light so that its temperature can be estimated by optical pyrometry. The evolved gases were trapped in a specially designed tube packed with a suitable sorbent in (this case a mixture of Tenax and Carbopak). This ended in a short section of hypodermic tubing, the open end of which is placed immediately adjacent to the heated thermal probe using a micro-manipulator. As the tip was heated, a pump was used to draw gas through the tube which was then placed in a thermal desorption unit (TA Instruments Evolved Gas Collector) for analysis of the trapped volatiles by GC/MS (Hewlett-Packard 6890 Gas Chromatograph with HP5973 Mass Selective Detector). The GC was fitted with a HP-5 MS capillary column (30 m x 0.25 mm i.d. x 1.0 μm d.f.). The oven program consisted of a 5 minute hold at 40°C following desorption and then a ramp to 250°C at 15°C min^{-1} followed by a 10 minute hold at this temperature. Mass spectra (m/z 45-350) were acquired every 0.5 s. A blank desorption run was carried out before and after each experiment to confirm the cleanliness of the detection system.

RESULTS AND DISCUSSION

Contact mode atomic force microscopy (AFM) images of the leaf surface before and after the pyrolysis experiment are shown in figures 3a and 3b. In the center of figure 3a an oil cell can be observed. The thermal probe was placed on this region and a pyrolysis-GC/MS measurement carried out. The resulting "crater" in the sample is visible in figure 3b. The damage caused by the pyrolysis measurement is quite large and can be controlled by the proximity of the probe to the surface and the time and temperature of the heat pulse. In this case the leaf surface is quite delicate and a large damage area results under the mildest conditions required to obtain a sufficient yield of evolved gases.

The GC/MS chromatogram showing total ion count *vs.* retention time is shown in figure 4a. The mass spectrum of the peak at a retention time of 13 minutes is shown in figure 4b. A search of the library of mass spectra identified camphor as the most likely (>95%) match for this peak. The library spectrum and structure of this compound are shown in figure 5. Parthenolide was not detected under the chromatographic conditions used (it would probably elute beyond 30 minutes). Furthermore, parthenolide is thermally unstable *(4)* and it is probable

that any material present at this location would be degraded during the pyrolysis experiment. Measurements on other areas of the leaf did not detect camphor; thus it is probably concentrated in the oil cells.

CONCLUSIONS

This example illustrates the use of micro-thermal analysis to carry out localized chemical analysis of biological specimens in order to determine the distribution of natural products in plant leaves. In this instance, the target material (parthenolide) was not detected – probably due to the choice of experimental conditions and its thermal instability. Camphor was detected however, and from this work we were able to we were able to conclude that it is localized within oil cells on the leaf surface.

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Figure 1. Feverfew (*Tanacetum parthenium*)

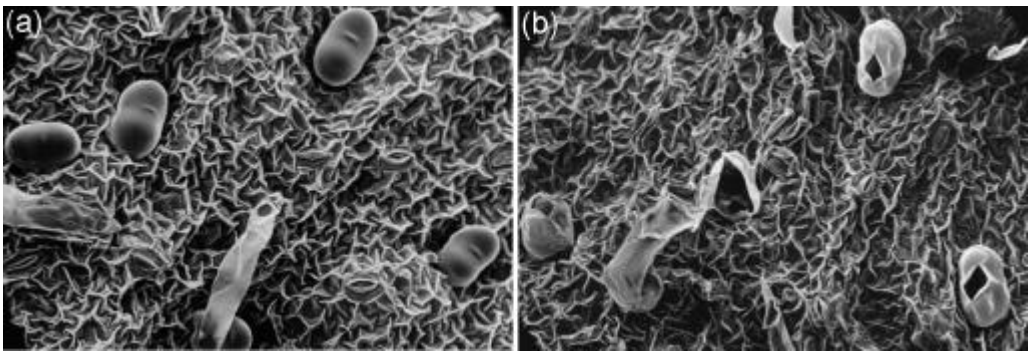

Figure 2. SEM images (225x 150 µm) of a feverfew leaf before (a) and after (b) extraction with supercritical CO_2

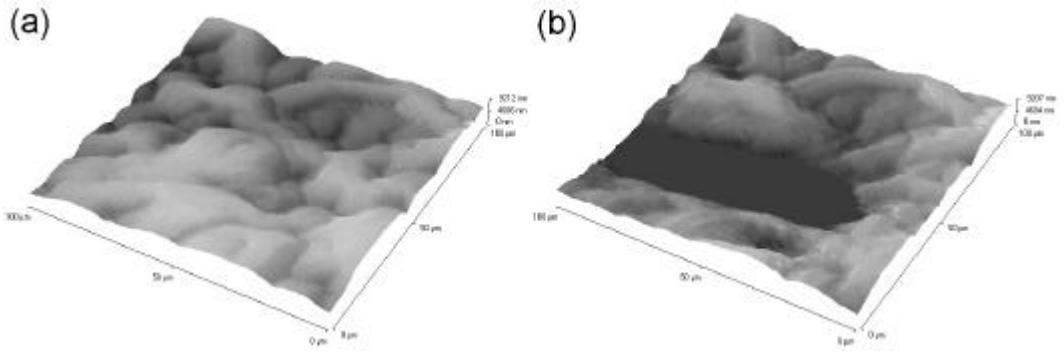

Figure 3. Topography (100 x 100 µm) of feverfew leaf before (a) and after (b) localized pyrolysis

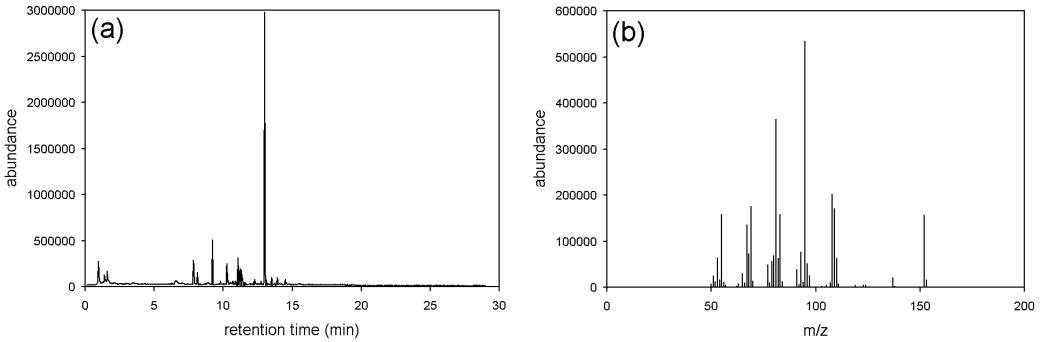

Figure 4. (a) GC/MS total ion chromatogram of gases from the pyrolysis of an oil cell. (b) Mass spectrum of peak at retention time of 13 minutes

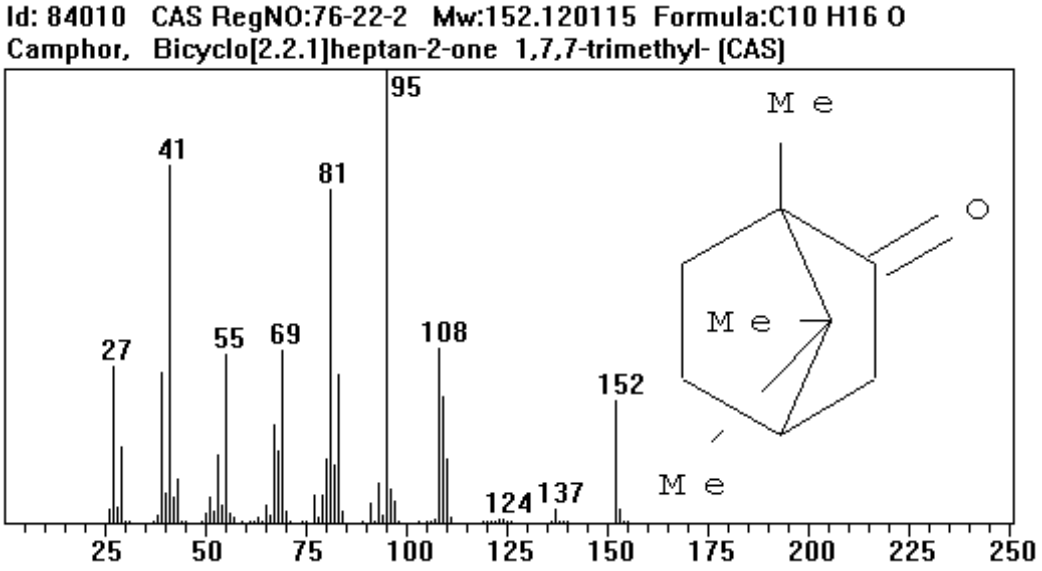

Figure 5. Library spectrum of camphor *c.f.* figure 4(b)