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Direct analysis of camptothecin from *Nothapodytes nimmoniana* by desorption electrospray ionization mass spectrometry (DESI-MS)

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Desorption electrospray ionization was employed for fast and direct ambient detection of the anti-tumor drug, camptothecin, and its derivative, 9-methoxycamptothecin in *Nothapodytes nimmoniana*. Different parts of the plant such as leaves, stems and bark were examined. The ion intensities suggest that the concentration in bark is higher than that in the leaves and stems. The method does not require any sample preparation or preseparation. The identity of the alkaloids was further confirmed by tandem mass spectrometry.

Nothapodytes nimmoniana (Graham) (family Icacinaceae), also known as *Nothapodytes foetida* (Wight, Sleumer) or *Mappia foetida* (Miers), is a small spreading sub-canopy tree (Fig. 1A) distributed in the moist and dry deciduous forests and sometimes in evergreen rain forests of the Western Ghats region of India. Camptothecin (CPT, Fig. 1B) is a monoterpene indole alkaloid known for its anti-tumor activity.[1–4] CPT is extracted from the wood chips of *N. nimmoniana*[5,6] and the herbal extract carrying the trade name "Ghanera" is traded extensively. Irinotecan and topotecan, two water-soluble derivatives of CPT, have been approved by the Food and Drug Administration (FDA) for treating colorectal and ovarian cancer.[7–9] In fact, CPT is regarded as one of the most promising anticancer drugs of the twenty-first century.[10] The projected global demand for CPT in 2002 was valued at US$ 4 billion.[11] CPT is also produced by *Camptotheca acuminata* Decaisne (Nyssaceae);[12] *Merrilliodendron megacarpum*[13,14] and *Nothapodytes nimmoniana*[15] (family Icacinaceae); *Ophiorrhiza mungos*[15] and *O. pumila*[16] (family Rubiaceae); *Eravatamia heyneana* (family Apocynaceae) and *Mostuea brunonis* (family Loganiaceae).[17] Among these, the highest concentration of CPT (about 0.3% on a dry weight basis) has been reported from *N. nimmoniana*[6] which has a threat status of endangered/vulnerable.[18]

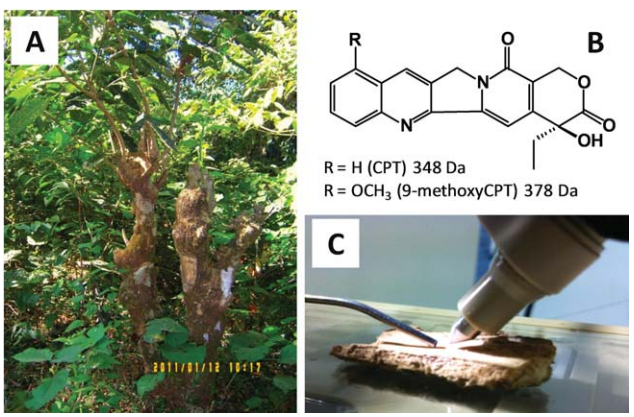

Fig. 1 (A) Photograph of *Nothapodytes nimmoniana* from Western Ghats, India. (B) Chemical structure of camptothecin and 9-methoxycamptothecin. (C) Photograph of the DESI-MS setup, with bark of the tree from which spectra are collected.

Chemical determination of CPT and related alkaloids from plants is usually performed by liquid chromatography coupled with mass spectrometry (MS) or fluorescence detection,[19,20] which requires significant sample preparation and makes the "*in situ*" analysis of the material prohibitive.

The introduction of two ionization techniques, desorption electrospray ionization (DESI) and direct analysis in real time (DART), in late 2004[21] and early 2005,[22] respectively, started a new family of ambient ionization methods.[23] In this family, the ionization of the sample occurs in the native environment, at atmospheric pressure, and without the requirement of sample preparation or pre-separation. In DESI, a spray of charged liquid droplets is directed to the sample creating a thin solvent film on the surface. Further droplets hit this film splashing secondary droplets containing the analytes into the mass spectrometer.[21]

Here, we demonstrate the application of DESI to the direct analysis of CPT in the leaves, stems and bark of *N. nimmoniana.* The specimen used was collected from the Dandeli Wildlife Sanctuary (Karnataka, India). The popular name in the local habitat is *Arbudhavinashini* (Sanskrit, literally means destroyer of cancer). However, names such as *Guwada* (Konkan-Maratha) or *Durvasane Mara* (Kannada) both meaning smelling human feces are also common.

All the mass spectra were acquired under the identical conditions of 2 μL min^{-1} solvent flow rate, 110 psi nebulizer gas (N_2) pressure, and 5 kV spray voltage on an ion trap LTQ XL mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a 2D moving stage (Prosolia, Indianapolis, IN). The solvent used here is an acetonitrile:

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water mixture in the v/v ratio of 7:3. Approximately 2 mm distance between the mass spectrometer inlet and the spray tip was maintained throughout the experiment and the solvent was sprayed at about a 60° angle to the surface. These two factors are very important for optimization of the signals in DESI-MS experiments. The spot size of the spray was not directly measured. However, it is known to be around 200 microns when using these experimental conditions.[24] Mass spectra were acquired in the positive ion mode and the subsequent tandem mass spectrometry was made using the same conditions to confirm the compound, CPT, and its methoxy derivative, namely 9-methoxyCPT.

The direct analysis of the leaves and stems did not show the presence of CPT or its derivatives since these compounds are not exposed on the surface and DESI is a soft ionization method which does not damage the surface. However, after creating a local incision at the surface of the leaves and stems using a razor blade, peaks corresponding to $[CPT + H]^+$ (*m/z* 349) and $[9\text{-methoxyCPT} + H]^+$ (*m/z* 379) were observed in the mass spectra (data not shown). Although quantitative experiments cannot be performed by DESI without the addition of internal standards,[25] the ion intensities in the mass spectra suggest that the concentration found in the bark (Fig. 2) is higher than those found in leaves and stems. This observation is also supported by previous reports which involved extraction and subsequent high-performance liquid chromatography (HPLC) analysis, showing that the amount of CPT and 9-methoxyCPT is higher in bark followed by stems and leaves.[26] The presence of CPT and 9-methoxyCPT was observed only in the internal surface of the bark. The external surface did not reveal any of the compounds. This observation could be made only by DESI which is highly surface sensitive. Other extractive methods process the bark as a whole losing the spatial information about the distribution of the molecules.

Experiments by MS/MS were performed in order to confirm the compounds CPT and 9-methoxyCPT. Selection and fragmentation of the $[CPT + H]^+$ precursor ion at *m/z* 349 showed a loss of 44 Da, forming the product ion at *m/z* 305 (Fig. 3, left column), presumably due to the loss of CO_2. Selection and fragmentation of the ion at *m/z* 305 showed consecutive losses of neutrals of 28 Da, forming the peaks at *m/z* 277 and *m/z* 249. The neutral losses can be assigned to ethylene and carbon monoxide, respectively. The selection and fragmentation of the ion at *m/z* 249 showed losses of 28 Da and 43 Da and are presumably due to rearrangements in the molecular skeleton. The methoxy derivative showed analogous fragmentation pathways. Selection and fragmentation of the 9-methoxyCPT precursor ion at *m/z* 379 showed a loss of 44 Da, forming the product ion at *m/z* 335 (Fig. 3, right column). Selection and fragmentation of the ion at *m/z* 335 showed consecutive losses of 28 Da, forming the peaks at *m/z* 307 and *m/z* 279. Again, the neutral losses can be assigned to ethylene and carbon monoxide, respectively. The selection and fragmentation of the ion at *m/z* 279 showed a loss of 28 Da and is due to rearrangement in the molecular skeleton. All fragments observed and neutral losses proposed are in agreement with the literature which confirms the presence of CPT and 9-methoxyCPT.[27]

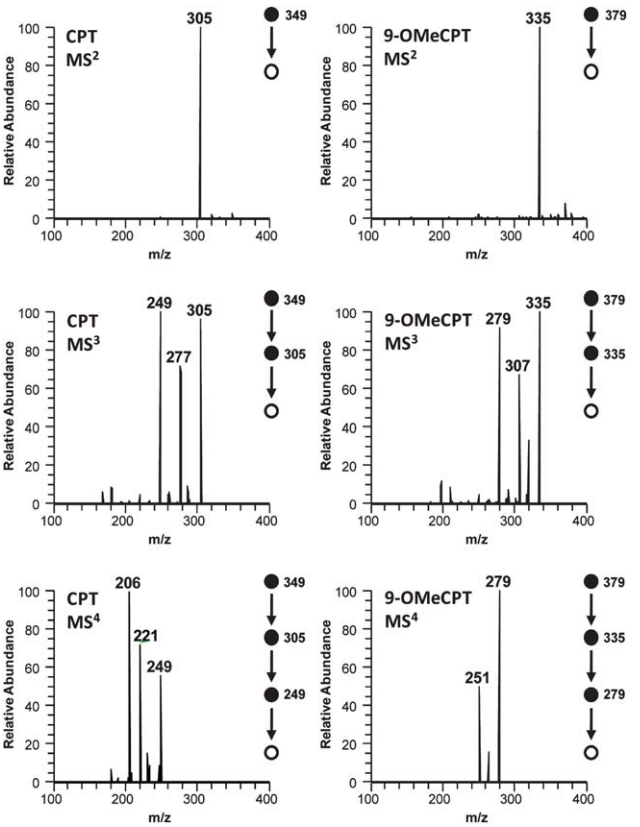

Fig. 3 Tandem mass spectra of camptothecin and 9-methoxycamptothecin.

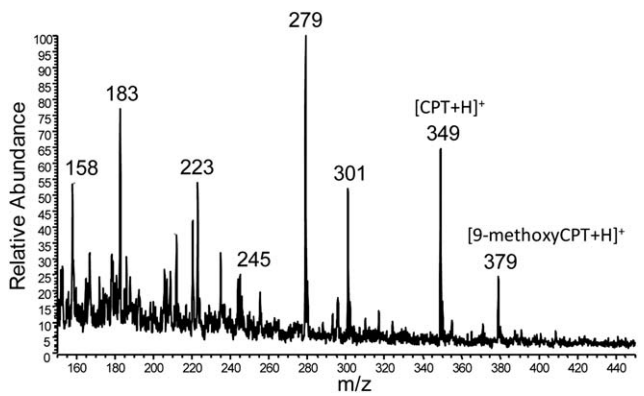

Fig. 2 Positive ion mode DESI-MS spectrum of *N. nimmoniana* bark chip.

The remarkable versatility of MS and its different ionization techniques (*e.g.* ESI, DART, MALDI, SIMS, DART and APCI) allow the accurate analysis of high molecular weight biopolymers;[28] the creation of chemical images of compounds distributed on surfaces;[29] and ionization of compounds in their native environment.[30] Evolving work on miniaturization of mass spectrometers may also allow chemical characterization *in situ* without the requirement of transporting samples to the laboratory.[31] Considering the biodiversity of the tropics, we believe that the use of DESI for direct analysis of natural products, as described here for the examination of CPT and its derivatives in leaves, stems and bark, will strongly facilitate the identification and discovery of molecules of value in biology and medicine.

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