Direct analysis of *Peucedanum palustre* samples by desorption atmospheric pressure photoionization-mass spectrometry

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Abstract

Desorption atmospheric pressure photoionization (DAPPI) is an ambient mass spectrometry (MS) technique that can be used for the analysis of polar and nonpolar compounds directly from surfaces. Here, the feasibility of DAPPI-MS in the screening of plant metabolites from dried *Peucedanum palustre* leaves and umbels was studied. DAPPI-MS requires no prior sample preparation or chromatographic separation, and the analysis can therefore be performed directly from the untreated plant material. *P. palustre* contains several linear and angular furanocoumarins, some of which are specific for the species. The DAPPI mass spectra of both leaf and umbel samples showed distinct ions at *m/z* 445 and 443 in positive and negative ion modes, respectively. MS^2 analyses of these ions confirmed that the ions were the protonated and deprotonated molecules, respectively, of peulustrin and its isomers, which have only been identified from *P. palustre*. The direct analysis of dried plant material by DAPPI-MS was shown to provide a fast and reliable means to confirm the identity of plant materials, to study the metabolite profiles of plants, and to screen biologically relevant compounds from plant surfaces.

Keywords

Desorption atmospheric pressure photoionization-mass spectrometry, *Peucedanum palustre*, milk parsley, furanocoumarin, peulustrin

1. Introduction

Since 1950s, the analysis of primary and secondary metabolites from plants has relied on various chromatographic separation methods coupled to spectroscopic detection (Marston and Hostettmann, 2009). Especially, the analysis of secondary metabolites, defence and signalling compounds of plant kingdom, has been in the centre of attention due to their many interesting pharmacological properties. However, the complex nature of plant extracts has posed a major challenge in the analytical work and is the main reason for the need to combine one or several separation steps before the detection technique. These steps add complexity and slow down the analysis procedure, and risk alteration of the sample.

In recent years, direct mass spectroscopic methods have gained increasing attention in natural product chemistry (Wolfender et al., 2015). These methods include ambient mass spectrometry (MS), and techniques such as desorption electrospray ionisation (DESI) (Takáts et al., 2004) and direct analysis in real time (DART) (Cody et al., 2005). In DESI, a spray of charged micro-droplets is directed to the surface under study, where analyte molecules are picked-up by the droplets and subsequently ionized. In DART, a hot gas stream activated by plasma discharge is used to desorb and ionize analytes from the sample surface. The main advantages of these methods are the lack of sample preparation and the short analysis time. Some reports on the use of these techniques in the analysis of plant samples have been published so far: DESI has been used for analysis of alkaloids from poison hemlock (*Conium maculatum*), jimsonweed (*Datura stramonium*) and deadly nightshade (*Atropa belladonna*) (Talaty et al., 2005), monitoring of the fermentation of tea leaves (Fraser et al., 2013) and analysis of camptothecin from *Nothapydetes nimmonia* (Srimany et al., 2011), while DART has been used for the analysis of volatile organic compounds in *Piper betle*, eucalyptus and *Bergenia crassifolia* (Bajpai et al., 2012; Chernetsova et al., 2012; Maleknia et al., 2009).

Desorption atmospheric pressure photoionization (DAPPI) is an ambient mass spectrometric technique that is efficient in detection of both polar and nonpolar compounds from surfaces (Haapala et al., 2007). DAPPI uses a microchip heated nebulizer, which provides a confined jet of vaporized dopant to desorb and vaporize analytes from the sample surface. Photons emitted by a krypton discharge lamp then initiate the ionization of analytes in the gas-phase similarly as in atmospheric pressure photoionization (Kauppila et al., 2002; Luosujärvi et al., 2008). In comparisons with DESI and DART, DAPPI has been shown to be more sensitive towards low polarity compounds (Haapala et al. 2007; Suni et al. 2012; Räsänen et al. 2014), less susceptible towards matrix effects than DESI (Suni et al., 2011) and have lower background noise than DART (Räsänen et al., 2014). In plant analysis, DAPPI has an advantage towards e.g. DESI and DART, since many of the natural compounds present in plant material are of low polarity. DAPPI can therefore detect a wider range of plant metabolites than other ambient MS methods. For the analysis of plant material by DAPPI, the sample is fixed on a glass microscope slide using double-sided tape, and analyzed as such (Fig. 1). In the analysis of natural compounds from plant surface, DAPPI-MS has been shown to be suitable for the analysis of cannabinoids from *Cannabis sativa* blooms (Kauppila et al., 2008, 2013) and cathinone and cathine from *Catha edulis* (khat) leaves (Kauppila et al., 2011). DAPPI has also been used for the direct analysis of pesticides from plant material (Vaikkinen et al., 2015).

Milk parsley, *Peucedanum palustre* (L.) Moench, is an umbelliferous plant growing in wetlands and damp environments in most parts of Europe (Meredith and Grubb, 1993). It contains a wide array of linear and angular furanocoumarins, as well as some simple coumarins (Eeva et al., 2004; Harborne, 1982; Yrjönen et al., 2016). These coumarins are thought to play a role in the defence of the plant against various environmental stress factors (Hadaček et al., 1994; Ojala et al., 1999; Siskos et al., 2008) and some of them also possess various pharmacological effects (Lim et al., 2014; Pae et al.,

2002; Sancho et al., 2004). It has been shown that some of these coumarin compounds are characteristic for a certain *Peucedanum* species, or their presence is limited to only a couple of species (Harborne, 1982). The coumarin composition of *Peucedanum* species can therefore be used to confirm the identity of a particular species or to aid the chemotaxonomic classification of the genus.

The aim of this study was to evaluate the feasibility of the DAPPI technique to the screening and identification of the main coumarins from dried *P. palustre* plant material.

2. Results and discussion

Dried leaf and umbel samples of *P. palustre* were analysed by DAPPI-MS without any pre-treatment steps. Both positive and negative ion modes with either acetone or toluene as the dopant were used. In positive ion mode a prominent ion was observed at *m/z* 445 with both dopants (Fig 2). Note that as the double-sided tape used in the study did not give any background ions to the DAPPI spectrum, it was not necessary to subtract the background. This ion is likely to be the protonated molecule ($[M+H]^+$) of peulustrin and its isomers. Peulustrin (Fig. 3) and its isomers are furanocoumarins that are characteristic to *P. palustre*, and have not been found in any other plants in the genus or family. Furthermore, these isomers are the main components in Finnish *P. palustre* specimens constituting approximately 50% of the total coumarin concentration in the aerial plant parts (Yrjönen et al., 2016).

With acetone dopant, the base peak was observed at *m/z* 229; in addition, notable ions were observed at *m/z* 329, 367, 205 and 163 (Fig. 2). The ion at *m/z* 329 is likely the $[M+H]^+$ ion of columbianadin and its isomer. Columbianadin (Fig. 3) is an angular furanocoumarin, which has been found in *Peucedanum* and *Angelica* genus plants (Chen et al., 1995; Harborne, 1982; Skalicka-

Woźniak et al., 2009). The ion at *m/z* 229 is likely to be a fragment of peulustrin or columbianadin, as it was also observed in the product ion spectra of the corresponding precursors (Table 1). The ions at *m/z* 367, 205 and 163 were especially high in umbels, and they were thought to be the $[M+H]^+$ and fragments of umbelliprenin. Umbelliprenin (Fig. 3) is a prenylated simple coumarin umbelliprenin, which is one of the major coumarin components in the leaves and umbels of *P. palustre* (Yrjönen et al., 2016). It has been isolated also from *P. arenarium* Waldst. & Kit., *P. oreoselinu*m (L.) Moench (Harborne, 1982) and *P. zenkeri* Engl. (Ngwendson et al., 2003). Although DAPPI cannot be used for absolute quantitation of the analytes, the hights of the ions in the spectrum can be taken as indicators of the relative proportions of the compounds within the sample.

When using toluene as the dopant, a minor molecular ion ($M^{+\cdot}$**)** of peulustrin at *m/z* 444 was detected in addition to the $[M+H]^+$ ion at *m/z* 445, and instead of the fragment ion at *m/z* 229, a fragment at *m/z* 228 was observed. In previous DAPPI work it has been shown that toluene as the dopant promotes the ionization of analytes through charge exchange, and therefore the formation of $M^{+\cdot}$ type ions (and their fragments) is more likely with toluene than with acetone (Haapala et al., 2007; Kauppila et al., 2008, 2013). Also, compounds of low polarity are typically more efficiently ionized with toluene than with acetone. The product ion at *m/z* 228 is suggested to have formed from the $M^{+\cdot}$ ion, since protonated molecules and molecular ions formed in DAPPI have previously been shown to fragment through different mechanisms (Kauppila et al., 2013). This can sometimes be utilized in structural identification of the analyte. The $[M+H]^+$ ion of columbianadin and its isomer at *m/z* 329, or the ions due to umbelliprenin, were not detected with toluene. Instead, intense ions at *m/z* 401, 383 and 365, probably originating from dihydroxylated sesquiterpene coumarins, were observed (Yrjönen et al., 2016).

DAPPI-MS^2 product ion spectra of precursor ions at *m/z* 329, 367, 401, and 445 in positive ion mode are presented in Table 1. The MS^2 analysis of precursor ion at *m/z* 329 yielded a product ion at *m/z* 229, corresponding to the cleavage of angelic acid or its isomer to form a columbianetin core structure. The same product ion at *m/*z 229 due to columbianetin was also observed in the MS^2 spectrum of the precursor ion *m/*z 445. MS^2 analysis of the precursor ion at *m/z* 367 yielded main product ions at *m/z* 205 and 163. For the precursor ion at *m/z* 401, intense product ions were observed at *m/z* 383, 365, 221, 203 and 163. The identification of columbianadin and umbelliprenin was confirmed by comparing their MS^2 product ion spectra to those of the corresponding standard compounds (Figure S1 in Supplementary material). Unfortunately, peulustrin standard was not available.The identification of columbianadin, umbelliprenin, and peulustrin and its isomers is in good agreement with previous HPLC-ESI-MS^2 experiments performed using the same plant material (Yrjönen et al., 2016). Also in the HPLC-ESI-MS^2 measurements, the peulustrin isomers were shown to be the main compounds in *P. palustre* leaves and umbels. However, the unknown ion at *m/z* 401 was only observed in the DAPPI measurements.

In negative ion mode the main ions were detected at *m/z* 215 and 443 (Fig. 2). In addition, minor ions were detected at *m/z* 161, 367, 451 and 476; and with toluene also at *m/z* 297 (spectrum not shown). The ion at *m/z* 443 is suggested to be the deprotonated molecule ($[M-H]^-$) of peulustrin isomers, but the identities of the other ions are unknown (Table 1). In general, the negative ion DAPPI-MS of umbels yielded a much higher number of ions compared to the positive ion mode. The detected ions are likely due to acidic compounds, which are readily ionized in negative ion APPI (Kauppila et al., 2004), such as phenolic acids, flavonoids and organic acids, all abundant in *Peucedanum* species (Sarkhail, 2014). The DAPPI-MS^2 product ions of the ion at *m/z* 443 in negative ion mode are shown in Table 1. To our knowledge, this is the first report of the negative ion mode MS^2 analysis of peulustrin and its isomers, and the proposed fragmentation pathway is

described in Fig. 4. The peulustrin isomers appear to lose CO_2 from the 2-pyrone ring of the coumarin core structure, and the side chain of the resulting molecule undergoes similar fragmentation as the intact deprotonated molecule. The cleavage of angelic, senecioic or tiglic acid moiety, depending on the isomer in question, results in the formation of fragment ions at *m/z* 361, 343, 317 and 299. The fragment ion at *m/z* 227 is suggested to be due to the columbianetin core structure.

DAPPI-MS was shown to provide a fast and efficient means for the identification of the main coumarins in *P. palustre* leaf and umbel samples. In positive ion mode, the diagnostic ion at *m/z* 445 belonging to peulustrin isomers was easily detected regardless of the dopant used. The ion at *m/z* 329 originating from columbianadin was only detected using acetone as the dopant, and this would support the use of acetone in DAPPI-MS of *P. palustre* samples. This choice of dopant is further substantiated by the more intense ions at *m/z* 367, 205 and 163 resulting from umbelliprenin, when using acetone instead of toluene. However, because of the differences in the ionization mechanisms with these two dopants, a suitable dopant should be chosen case-specifically. In negative ion mode, the diagnostic ion of peulustrin isomers at *m/z* 443 was also clearly detected with both solvents facilitating the identification of the plant material. In general, mass spectra in negative ion mode with acetone and toluene dopants resembled each other more closely than in positive ion mode.

Peulustrin and its isomers are ideal marker compounds for the plant material, as they have only been identified from *P. palustre* and no other plant species, and since in our earlier study, peulustrin isomers were detected in all Finnish *P. palustre* samples in moderate to high concentrations (Yrjönen et al., 2016). Columbianadin was also detected in almost all plant samples, but in lower concentrations. DAPPI-MS could provide an efficient tool for the fast screening of natural

compounds from plant material, or identification of plants based on characteristic compounds detected. The identification of compounds and separation of isobaric and isomeric compounds in DAPPI would be further facilitated by the use of high resolution mass spectrometry and/or ion mobility spectrometry.

3. Experimental

3.1. Plant Material

The same plant material that was previously used in the profiling of coumarins from *P. palustre* (Yrjönen et al., 2016) was used also in this study. Briefly, whole healthy flowering plants of *P. palustre* were collected from 43 sites in southern and central Finland in late July- early August 1988. The plants were identified by Ph. D. Pertti Uotila (Department of Botany, University of Helsinki, Finland) and different plant organs were then separated, dried, and stored at room temperature at the Division of Pharmaceutical Biosciences, University of Helsinki, Finland.

3.2. Sample Preparation

Dried umbels and leaves were fixed on a glass microscope slide using Fissaforte double-sided PE foam tape (Sicad group, Uboldo, Italy) and analysed as such using a custom-made DAPPI source (Fig. 1).

3.3. DAPPI-MS Analysis

In DAPPI, hot solvent vapour and nebulizer gas are delivered towards the solid sample using a heated nebulizer microchip, causing the thermal desorption of the analytes from the sample surface. Nitrogen was used as the nebulizer gas, and toluene and acetone as dopants. A krypton discharge VUV lamp was used to initiate the ionizing reactions. Agilent 6330 ion trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) in positive and negative ion modes was used to

measure the spectra. Nitrogen nebulizer gas flow rate was 180 mL/min and dopant flow rate 10 μL/min. The DAPPI set-up and instrumentation have been described in detail by Haapala et al. (Haapala et al., 2007).

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Table 1. DAPPI-MS^2 data of the most intensive ions in the *Peucedanum palustre* samples.

Detected ion	MS^2 product ions	Suggested identity
Positive ion mode		
329	229	$[M+H]^+$ of columbianadin
367	215, 205, 189, 163, 149, 135, 123, 109	$[M+H]^+$ of umbelliprenin
401[a]	383, 365, 221, 203, 163, 161, 147, 133, 119	Unidentified
445	229	$[M+H]^+$ of peulustrin
Negative ion mode		
215	133, 99	Unidentified
297[a]	223, 197, 123	Unidentified
367	365, 349, 311	Unidentified
443	399, 361, 343, 317, 299, 271, 227	$[M-H]^-$ of peulustrin

[a] detected only with toluene

Figure captions

Fig. 1. Schematic of the DAPPI source with a *Peucedanum palustre* leaf sample fixed on a glass microscope slide and placed on the sampling mount

Fig. 2. Analysis of an umbel sample by DAPPI-MS in positive ion mode using a) acetone, and b) toluene as the dopant, and in c) negative ion mode using acetone as the dopant

Fig. 3. Chemical structures and molecular weights of columbianadin, peulustrin and umbelliprenin

Fig. 4. Tentative fragmentation pattern for the $[M-H]^-$ ion of peulustrin in negative ion DAPPI-MS^2

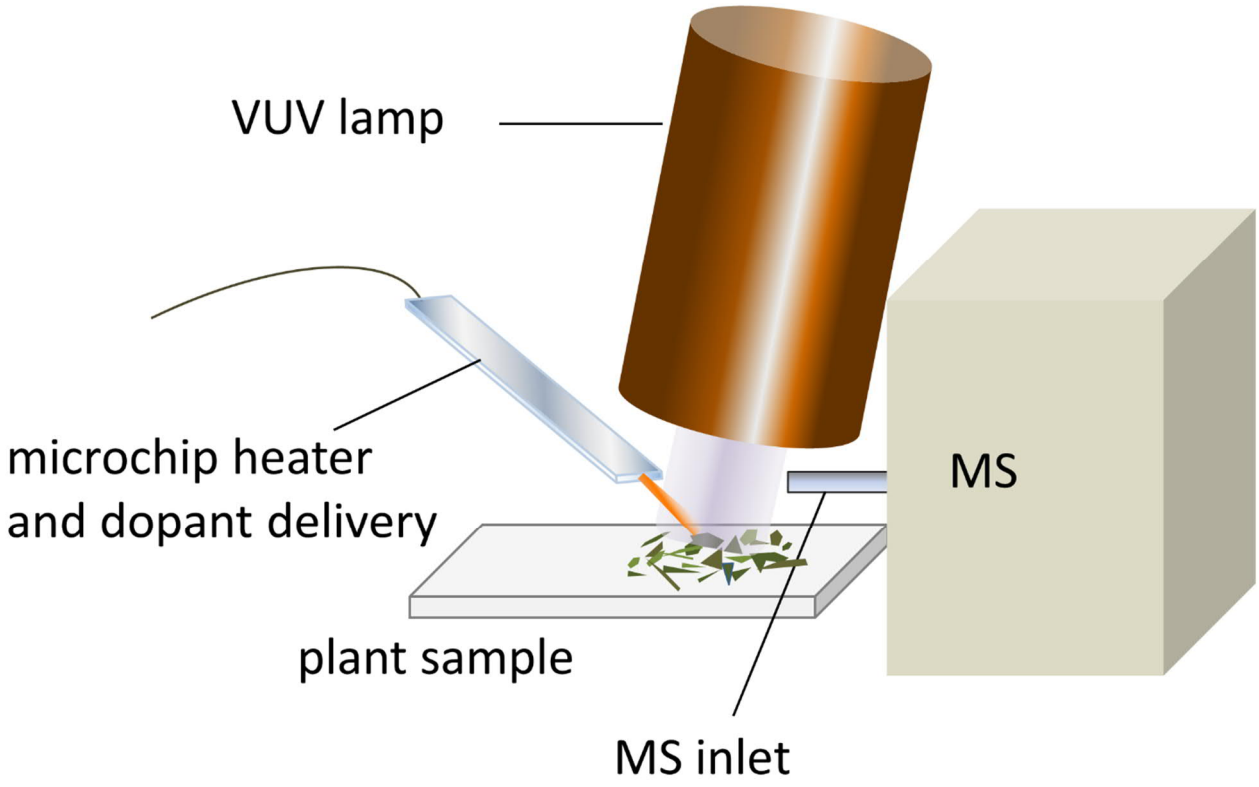
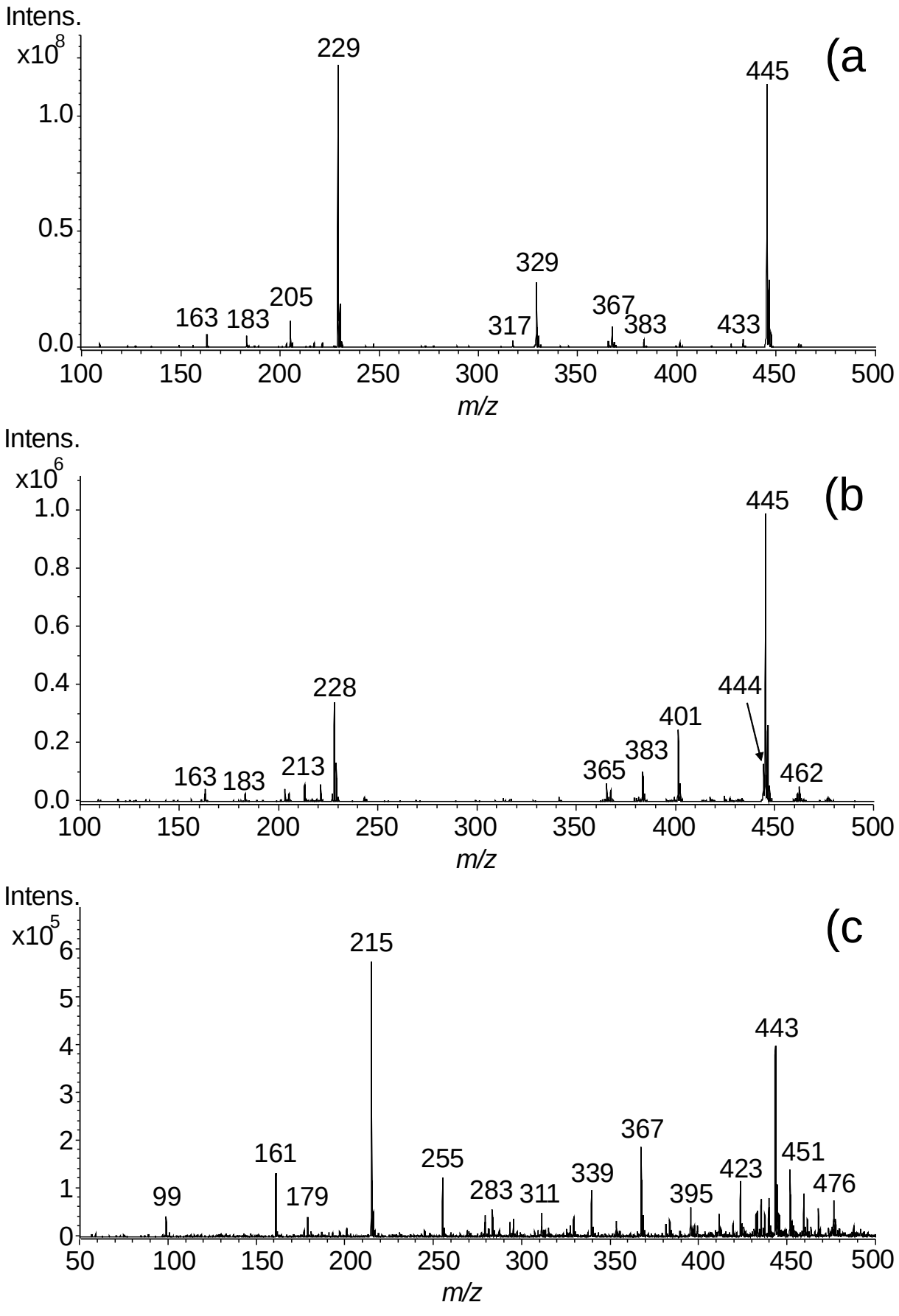


Fig. 1. (color online)


Intens.
x10^8
1.0
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229
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329
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m/z
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Intens.
x10^6
1.0
0.8
0.6
0.4
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250
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400
450
500
m/z
(b
Intens.
x10^5
6
5
4
3
2
1
0
215
443
367
161
255
339
451
423
476
99
179
283
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Fig. 2.

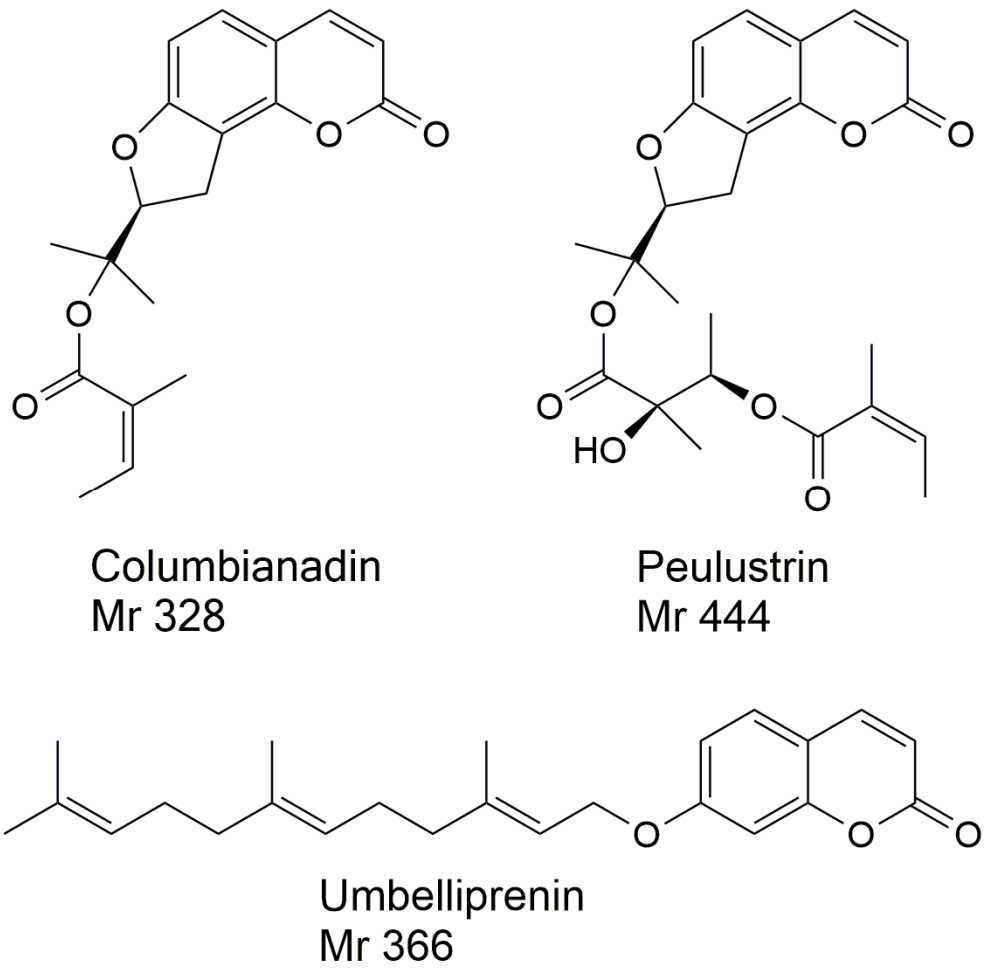
Columbianadin
Mr 328
Peulustrin
Mr 444
Umbelliprenin
Mr 366

Fig. 3.

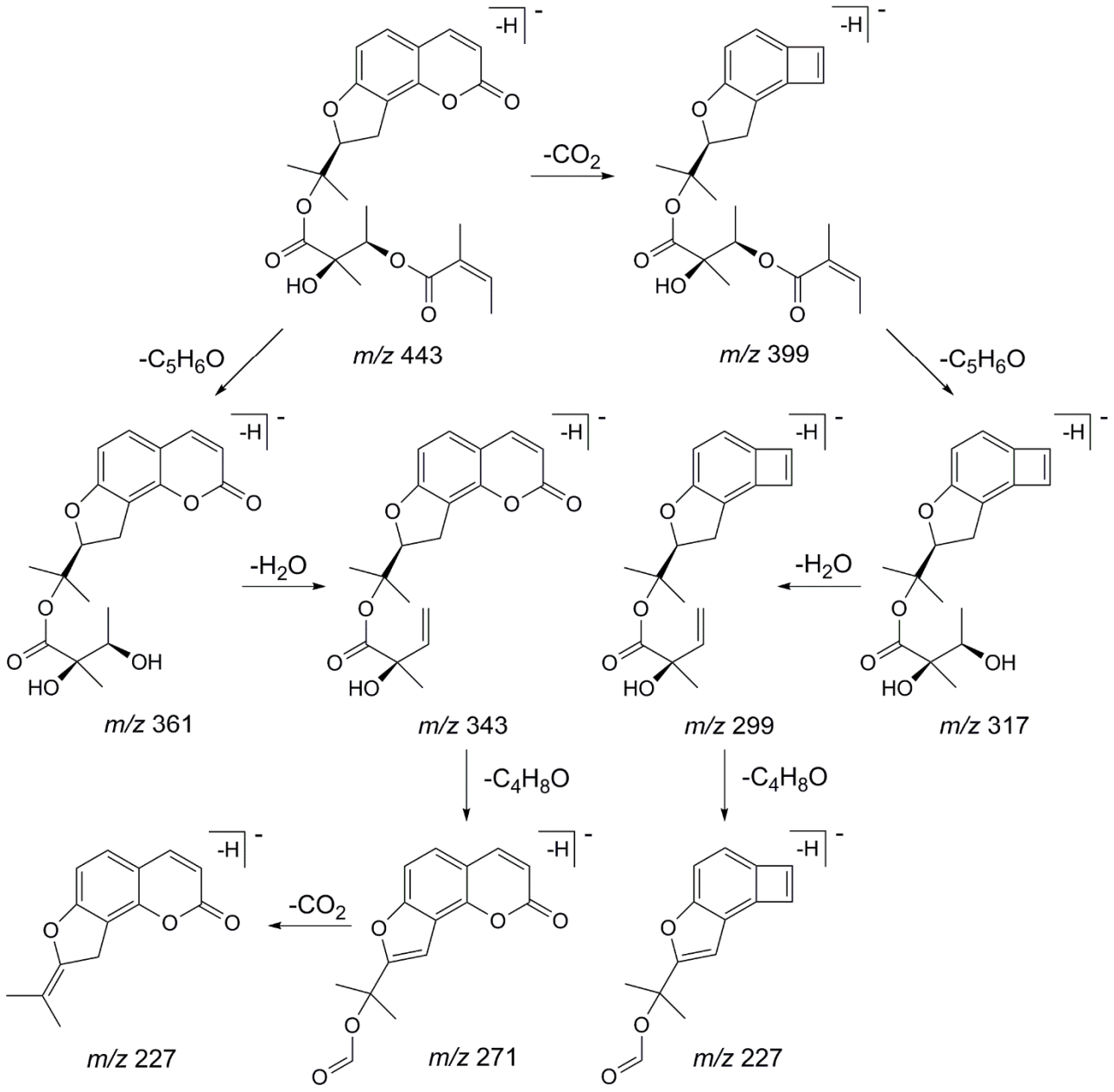
-H
-CO2
-C5H6O
m/z 443
m/z 399
-C5H6O
-H2O
-H2O
m/z 361
m/z 343
m/z 299
m/z 317
-C4H8O
-C4H8O
-CO2
m/z 227
m/z 271
m/z 227

Fig. 4.