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Differential Rapid Screening of Phytochemicals by Leaf Spray Mass Spectrometry†

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Ambient ionization can be achieved by generating an electrospray directly from plant tissue ("leaf spray"). The resulting mass spectra are characteristic of ionizable phytochemicals in the plant material. By subtracting the leaf spray spectra recorded from the petals of two hibiscus species *H. moscheutos* and *H. syriacus* one gains rapid access to the metabolites that differ most in the two petals. One such compound was identified as the sambubioside of quercitin (or delphinidin) while others are known flavones. Major interest centered on a $C_{19}H_{29}NO_5$ compound that occurs only in the large *H. moscheutos* bloom. Attempts were made to characterize this compound by mass spectrometry alone as a test of such an approach. This showed that the compound is an alkaloid, assigned to the polyhydroxylated pyrrolidine class, and bound *via* a C_3 hydrocarbon unit to a monoterpene.

Key Words : Ambient ionization, Alkaloids, Tandem mass spectrometry, Natural products

Introduction

The past four decades have seen the rapid development of mass spectrometry with many applications throughout the sciences. These applications ultimately rest on an understanding of the unimolecular chemistry of gas phase ions, a field to which M. S. Kim has made significant contributions through his studies of the dissociation kinetics of ions with known internal energy.[1] The topic of natural products chemistry has long benefitted from advances in mass spectrometry, including the development of exact mass measurements,[2] tandem mass spectrometry[3] and soft ionization methods.[4] Typically structural elucidation of natural products involves extensive use of chromatographic separation and use of other spectroscopic methods in concert with mass spectrometry.[5]

Ambient ionization[6] refers to the creation of ions for mass spectrometry by examination of native materials in the open environment. In the family of ambient ionization methods, samples – typically complex mixtures – are ionized without prior sample preparation and in their native environment. A typical example[7] is the identification of the anti-tumor drug camptothecin from the bark of the tree *Nothapodytes nimmoniana* using desorption electrospray ionization (DESI)[6] and tandem mass spectrometry. Another example is the determination of the structures and distributions of chlorophyll catabolites using DESI in the imaging mode.[8] A more recently introduced version of ambient ionization is paper spray,[9] in which samples are ionized from a wet porous material like paper, cut to a fine point and supplied with a high voltage. Micro-droplets are emitted from the point under the influence of the high electric field and they carry ionized forms of analyte into the mass spectrometer. It is possible to perform quantitative analysis using paper spray experiments and this has been pursued rigorously for biological fluids with the aim of utilization at the point-of-care.[10] Plant materials are conveniently examined by a variant on this method in which the plant material itself serves as the porous substrate, originally reported as leaf spray mass spectrometry.[11] Other plant parts can be examined too in both living plants and preserved samples.[11]

In this study we utilize a simple differential method in which ambient ionization mass spectra of two related plant samples are subtracted from each other in order to quickly access characteristic differences between the phytochemicals in the two samples. The primary purpose of this paper is to test the usefulness of this approach. A second objective is to explore the degree to which mass spectrometry alone can be used to determine the structure of an entirely new compound. This compound was observed by differential ambient ionization of the petals of two Hibiscus species, *H. moscheutos* and *H. syriacus*. (The family Malvaceae is widely known for cultural, medical, horticultural as well as phytochemical uses and *H. syriacus* also known as Rose of Sharon, is the national flower of South Korea.) Data were recorded at unit resolution and also at high resolution to obtain molecular formulae, in both the positive and negative ion modes. Notably, multiple stages of MS were used to acquire structural information.

Experimental

Instrumentation. Leaf spray and electrosonic spray ionization (ESSI) experiments were carried out using a Thermo LTQ mass spectrometer (Thermo-Fisher, San Jose, CA) as previously described.[11,12] In order to facilitate compound identification tandem mass spectrometry experiments were performed using the Thermo LTQ and exact mass measure-

†This paper is to commemorate Professor Myung Soo Kim's honourable retirement.

ments were performed using a Thermo Scientific Velos LTQ-Orbitrap using direct infusion ESI.

Leaf Spray Experiments. The freshly collected petals of *H. moscheutos* and *H. syriacus* (photographs in Supporting information, Fig. S1) were cut to a point (Fig. 1(a) and Supporting information, Fig. S2). A copper clip was used to hold the triangular plant tissue in front of the atmospheric inlet of the mass spectrometer at a distance of about 0.5 cm (Fig. 1(b)). The heated-capillary temperature was set to 150 °C, capillary voltage and tube-lens voltage were (+/–) 15.5 V and (+/–) 66.7 V, respectively. Full MS spectra were routinely recorded in the range of *m/z* 50 to *m/z* 1000. Tandem mass spectrometry data were recorded using an isolation window of 1.5-2 mass units and a collision energy of 20-25% (manufacturer's arbitrary units). As soon as a high voltage (3.5 kV, positive or negative) was applied to the tissue, the surface of the plant tissue was wetted with 10-20 μL of methanol. A stable spray (spray current in the range of 3 to 0.2 μA) occurred and lasted for approximately 1 min after addition of solvent. This was adequate time to record mass spectra in the positive and negative ion modes and to record MS/MS spectra. When stable signals were needed for longer times, a syringe pump at a flow rate of 7 μL/min was used to continuously pump methanol onto the center of the petal triangle (*via* a fused silica capillary 100 μm ID, 360 μm OD). This signal lasted until the petal triangle had to be exchanged (*ca.* 10-15 min). Within the first 10 min of leaf spray analysis only minor changes of the relative signal intensities could be observed. In particular the potassiated molecular ions of ethanolamine (*m/z* 104), betaine (*m/z* 156) as well as the unknown compound at *m/z* 352 showed slightly decreasing relative intensities, while the signal intensities of the potassiated molecular ions of glucose and sucrose (*m/z* 219 and *m/z* 381) increased with duration of the experiment. The absolute signal intensities were found to decrease by approximately 50% within the first 5 min of continuous leaf spray analysis, but were observed to be constant during the second half of the experiment (maybe due to the fact that in the beginning of the experiment a lot of material is dissolved/extracted from the edges of the petal triangle).

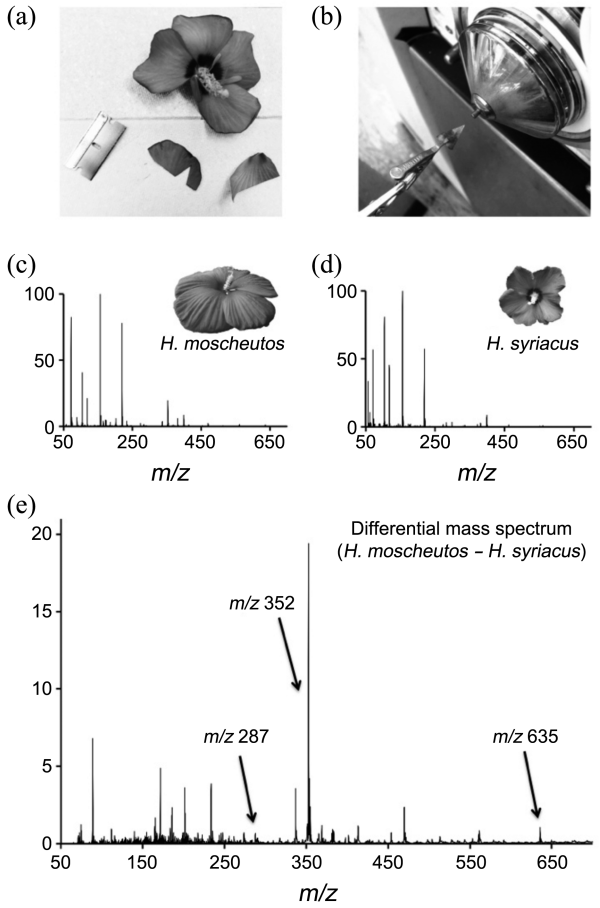


Figure 1. (a) *H. syriacus* petal cut to a point using a razor blade. (b) Tissue triangle held by a high voltage connector in front of the atmospheric inlet of a mass spectrometer. (c) and (d) positive ion mode spectra obtained by direct leaf spray analysis of *H. moscheutos* and *H. syriacus* petals. (e) Differential mass spectrum showing signals that appear exclusively in the *H. moscheutus* petal (for the result of simple spectral subtraction see Supporting Information, Fig. S4). The anthocyanin cyanidine at *m/z* 287, a highly abundant compound at *m/z* 352 which is the main topic of the study and the potassiated sambubioside of delphinidine or quercitin (isobaric aglycones) at *m/z* 635 are highlighted.

HR-MS and H/D Exchange Experiments. For high-resolution mass spectrometry 0.5 cm^2 of petal was extracted at room temperature using 500 μL methanol. Prior to the direct infusion ESI experiment (using 5 μL injections on the LTQ-Orbitrap Velos) 10 μL of the red colored, clear supernatant was diluted to 1 mL using methanol:water 3:1 (v/v). For H/D exchange experiments, 0.25 cm^2 of the petal was extracted using 100 μL CD_3OD. 10 μL of the red colored, clear supernatant was diluted to 500 μL using CD_3OD prior to the direct infusion ESSI on the Thermo Scientific LTQ instrument.

Results and Discussion

Leaf spray in the positive as well as negative ion-mode was used as a rapid method to analyze freshly collected leaves and petals of two different hibiscus species (*H. moscheutos* and *H. syriacus*) (Fig. 1(a), (b) and Supporting Information, Fig. S1 and S2). Stable spray conditions, particularly necessary for the performance of MS/MS experiments, were obtained by applying 10 μL portions of methanol onto the tissue triangles soon after the high voltage was applied. Low molecular weight compounds in the mass range below *m/z* 400 dominated the positive ion mode spectra of petals (Figure 1(c) and 1(d)) as well as leaves (Supporting Information, Fig. S3).

MS/MS experiments revealed these intense signals to be related inter alia to ethanolamine (*m/z* 104), the quaternary ammonium compound betaine, which is known to be a nontoxic osmolyte compensating saline in dry environments (*m/z* 118 and *m/z* 156 in case of the free carboxylic acid and its potassium salt, respectively),[13,14] and the potassiated molecular ions of glucose (*m/z* 219) and sucrose (*m/z* 381).

In order to compare the metabolic fingerprints of *H. moscheutos* and *H. syriacus*, the mass spectra from the leaf

spray analyses of the two species were subtracted after normalizing the individual spectra with respect to *m/z* 219 assuming that glucose is a "house-keeping" metabolite (see Supporting Information Fig. S4). Many abundant peaks (positive or negative relative intensity) were due to prominent compounds in both species whose signals do not exactly cancel when subtraction is performed. To enhance the quality of visualization, we also calculated the mass spectrum (Fig. 1(e)) of ions due to compounds which are exclusively present in the *H. moscheutus* petal but not in the petal of *H. syriacus* (not even at a very low abundance). This differential spectrum provides an immediate and distinct comparison of the two metabolic profiles.

Known anthocyanins and anthocyanidins[15-17] were identified from to their MS/MS fragmentation patterns, *e.g.* cyanidin occurs at *m/z* 287 (M^+) and the potassium adduct of a sambubiosylated compound at *m/z* 635 ($[M+K]^+$). While the aglycone of this ion can either be assigned to the flavone quercitin[16] or to the pH 5-6 form of the anthocyanin delphinidine (Fig. 2(a); loss of $C_{15}H_9O_7$). The glycone was identified as a sambubiose unit by the fragmentation of the deprotonated molecular ion at *m/z* 595 in the negative ion mode (Fig. 2(b)).

Significantly, the differential mass spectrum (Fig. 1(e)) showed a highly abundant signal at *m/z* 352 indicating the presence of a nitrogen-containing compound of molecular weight 351 Da. The compound was absent from the leaves of *H. Moscheutos* (Supporting Information, Fig. S3). Neither the addition of acetic acid or triethylamine to the spray solvent (up to 3% v/v) gave evidence for the presence of the corresponding potassiated or sodiated molecular ions, nor could the corresponding deprotonated ion $[M-H]^-$ be detected in the negative ion mode. Fragmentation of the isolated molecular ion at *m/z* 352 in the linear ion trap of the LTQ instrument showed a single, highly abundant fragment at *m/z* 334 due to the loss of water. All other fragment ions were of very low abundance. Although the sequential product ion MS^3 spectrum of the fragment *m/z* 334 (generated from *m/z* 352) gave a fragmentation pattern with good signal/noise (Fig. 3(a)), the compound could not be assigned to any known natural product listed in metabolomic databases.

In an attempt to explore the boundaries of structural characterization of an unknown natural product by mass spectrometry without the help of complementary methods like chromatography or optical spectroscopy, further MS analyses were performed. The molecular formula of the ion and those of its most important fragments were determined

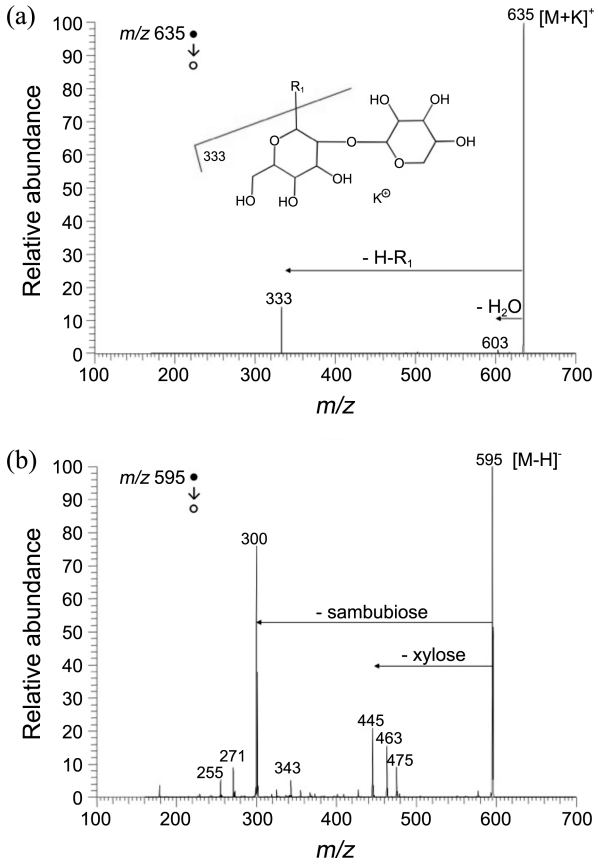


Figure 2. MS/MS spectra of the sambubioside of quercitin or the pH 5-6 form of delphinidine ($R_1 = C_{15}H_9O_7$). (a) MS/MS product ion spectrum of the potassiated molecular ion (*m/z* 635) showing the prominent loss of the aglycone. (b) Negative ion mode MS/MS of the deprotonated molecular ion (*m/z* 595) with fragmentations of the sugar moiety.

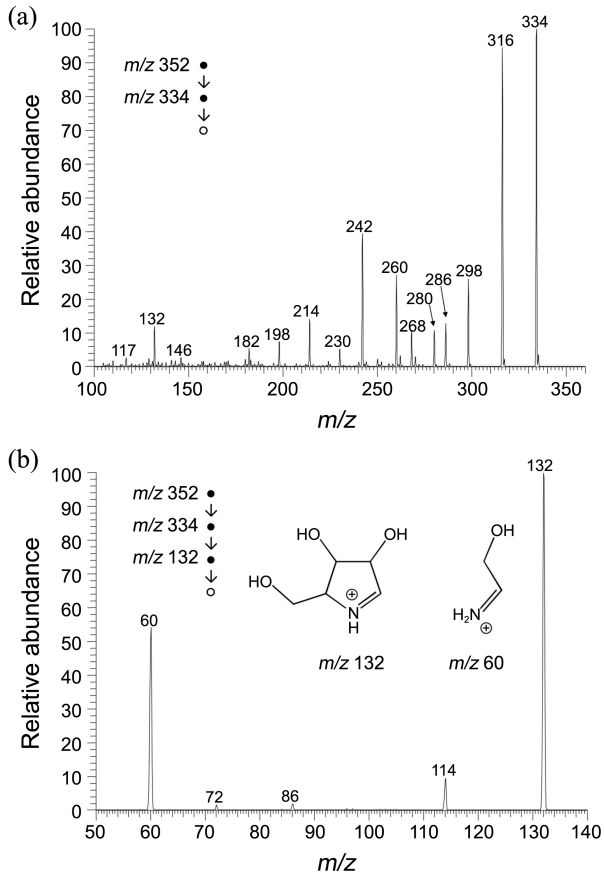


Figure 3. Selected MS^n spectra of the ion detected at *m/z* 352. (A) MS^3 of *m/z* 352 fragmenting *via m/z* 334 and (B) MS^4 of the fragment *m/z* 352 fragmenting in turn *via m/z* 334 and *m/z* 132; proposed structures of the fragment ions $C_5H_{10}NO_3^+$ at *m/z* 132 (iminium form) and $C_2H_6NO^+$ at *m/z* 60 are shown.

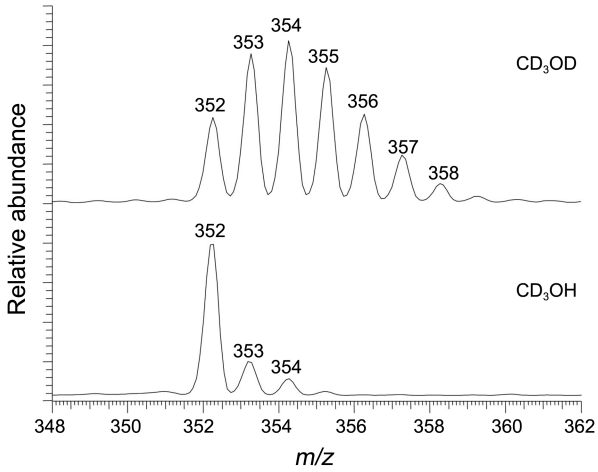
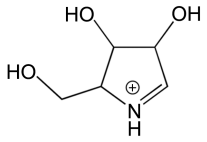


Figure 4. H/D exchange experiment. Molecular ions of the unknown compound from *H. moscheutos* at *m/z* 352 after the extraction using CH_3OH (bottom) and CD_3OD (top). For full spectra see Supporting Information, Fig. S5. A comparison of the MS/MS spectra of the deuterated and undeuterated compounds is made in Supporting Information, Fig. S6.

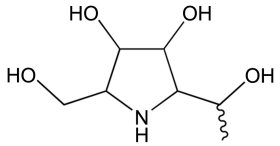
by high-resolution mass spectrometry using an Orbitrap mass spectrometer (for a comprehensive overview see Table 1). Although it was already known that an increase in the distance between the plant tissue and the atmospheric inlet of the mass spectrometer would reduce the amount of the sprayed material and help to avoid contamination of the highly sensitive instrument,[11] we preferred to use a different experimental approach. A methanolic extract of a small area of the plant tissue was diluted to an appropriate concentration and then analyzed using ESI (infusion) without any further sample preparation. Thus the elemental composition of the molecular ion of the unknown compound at *m/z* 352.2119 was determined as $C_{19}H_{30}N_1O_5^+$ (with a double bond equivalent of 6). Information about the number of exchangeable protons and their localization within the molecule was obtained by performing an H/D exchange experiment using CD_3OD as solvent for the quick extraction of the analyte from the plant tissue (Fig. 4).

The fragmentation behavior of the unknown compound molecular weight 351 ($[M+H]^+$ *m/z* 352) suggests that it is comprised of two units, of which one is polyhydroxylated while the other is lipophilic. Good evidence for multiple hydroxyl groups was given by the water solubility of the compound as well as H/D exchange data. Note that these data (Fig. 4) show that the number of exchangeable hydrogen atoms is five although not all of these are necessarily hydroxyl groups as acidic hydrogen atoms in the α-position of ketones and aldehydes could also undergo exchange. (Note too, that the fragmentation pattern of the deuterated compound (recorded with a wide isolation window to include all isotopomers, Fig. S6) was identical to that of the undeuterated compound, strongly indicating a single component at *m/z* 352.)

A prominent fragment ion at *m/z* 132 (elemental composition $C_5H_{10}NO_3^+$) turned out to be a key feature of the



Scheme 1. Tentative chemical formula of the fragment ion at *m/z* 132 with elemental composition $C_5H_{10}NO_3^+$.



Scheme 2. Chemical formula of the known hydroxylated pyrrolidine alkaloid DMDP with the proposed point of attachment of the remaining groups that constitute the unknown compound molecular weight 351 indicated by the wavy line.

MS/MS experiments. It apparently corresponded to a polyhydroxylated unit derived from pyrrolidine type alkaloids, which are well known to occur in hydroxylated versions.[18] Hence the fragment ion fragment ion *m/z* 132 is assigned to the tentative formula depicted in Scheme 1.

Further fragmentation of the ion *m/z* 132 gave a major product ion at *m/z* 60, which supports this assignment while not completely ruling out isomeric structures. On this basis, the full alkaloid unit is a derivative of the known compound $C_6H_{13}NO_4$ depicted in Scheme 2 (2,6-dihydroxy-3,4-dihydroxymethylpyrrolidine; DMDP).[19]

Support for this assignment is found in many other features of the MS^n data which are detailed in Table 1 and discussed extensively in the Supporting Information. The characteristic processes include the loss of the unit C_3H_4O after at least one dehydration step. This fact points to cross-ring cleavage in the partially unsaturated hydroxymethylpyrrolidine structure as the source of this particular neutral fragment.

The structural identification of the lipophilic unit of the structure turned out to be more puzzling. A number of mechanistic considerations (extensively described in the Supporting Information) helped to reduce the selection of possible structures which readily fulfill the criteria given by the fragmentation data. The lipophilic part corresponds to $C_{19}H_{29}NO_5 - C_6H_{12}NO_4$ that is to a $C_{13}H_{17}O$ unit. There is direct evidence for this unit in the fragmentation data (see Table 1 row E) in the form of the ion *m/z* 189 derived by four successive losses of water then C_6H_5N from the protonated molecule. It is also indicated by the loss of the neutral $C_{14}H_{20}O_4$ from the $[M+H-H_2O]^+$ ion, assuming cleavage on the other side of the 2-hydroxymethylene substituent of the pyrrolidine which gives the diagnostic ion *m/z* 132 depicted in Scheme 1.

In particular the loss of neutral $C_9H_{10}O$ provided important information for a basic structural identification of the second part of the molecule. $C_9H_{10}O$ is lost in three processes, after two, three and four eliminations of water (see Table 1, rows c-d). The $C_9H_{10}O$ unit therefore has to be a part of an exposed skeletal unit with a double bond equi-

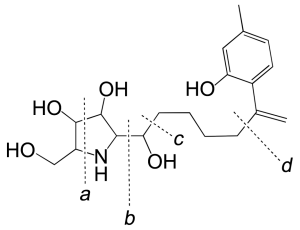
Table 1. Comprehensive overview of important MS^n fragmentations of the molecular ion, *m/z* 352[a]

(a)	**$C_{19}H_{30}NO_5^+$ (m/z 352.2119)**							**$[M+H]^+$**
(b)	H_2O	**$C_{19}H_{28}NO_4^+$ (m/z 334.2013)**						m/z 352 ● → ○
		$C_{14}H_{18}O$	**$C_5H_{10}NO_3^+$ (m/z 132.0650)**					m/z 334 ● → ○
			H_2O	$C_5H_8NO_2^+$ (m/z 114)				m/z 132 ● → ○
				CO	$C_4H_8NO^+$ (m/z 86)			m/z 114 ● → ○
					H_2O	$C_5H_6N^+$ (m/z 68)		m/z 86 ● → ○
			$C_2H_2/CO/H_2O$	$C_6H_6NO^+$ (m/z 60)				m/z 132 ● → ○
		CH_2O/H_2O	**$C_{18}H_{24}NO_2^+$ (m/z 286.1802)**					m/z 334 ● → ○
			H_2O	**$C_{18}H_{22}NO^+$ (m/z 268.1696)**				m/z 286 ● → ○
				H_2O	$C_{18}H_{20}N^+$ (m/z 250.18)			m/z 268 ● → ○
				CO	$C_{17}H_{22}N^+$ (m/z 240.18)			m/z 268 ● → ○
			CO	$C_{17}H_{24}NO^+$ (m/z 258.18)				m/z 286 ● → ○
				H_2O	$C_{18}H_{22}N^+$ (m/z 240.18)			m/z 258 ● → ○
				$C_9H_{15}NO_2$	**$C_9H_9^+$ (m/z 117.0693)**			m/z 334 ● → ○
(c)	H_2O	H_2O	**$C_{19}H_{26}NO_3^+$ (m/z 316.1907)**					m/z 334 ● → ○
			C_3H_4O	**$C_{16}H_{22}NO_2^+$ (m/z 260.1645)**				m/z 316 ● → ○
				H_2O	**$C_{16}H_{20}NO^+$ (m/z 242.1539)**			m/z 260 ● → ○
					H_2O	$C_{16}H_{18}N^+$ (m/z 224.18)		m/z 242 ● → ○
					CO	**$C_{15}H_{20}N^+$ (m/z 214.1590)**		m/z 242 ● → ○
					$C_5H_{11}N$	**$C_{10}H_9^+$ (m/z 129.0693)**		m/z 214 ● → ○
					C_8H_8	$C_7H_{12}N^+$ (m/z 110.09)		m/z 214 ● → ○
					$C_{11}H_{12}$	$C_4H_8N^+$ (m/z 70.09)		m/z 214 ● → ○
			C_9H_{10}	**$C_{10}H_{16}NO_3^+$ (m/z 198.1125)**				m/z 316 ● → ○
				H_2O	$C_{10}H_{14}NO_2^+$ (m/z 180.09)			m/z 198 ● → ○
				CO	$C_9H_{16}NO_2^+$ (m/z 170.09)			m/z 198 ● → ○
			$C_9H_{10}O$	**$C_{10}H_{16}NO_2^+$ (m/z 182.1176)**				m/z 316 ● → ○
				H_2O	$C_{10}H_{14}NO^+$ (m/z 164.09)			m/z 182 ● → ○
					H_2O	**$C_{10}H_{12}N^+$ (m/z 146.0957)**		m/z 164 ● → ○
(d)	H_2O	H_2O	H_2O	**$C_{19}H_{24}NO_2^+$ (m/z 298.1802)**				m/z 334 ● → ○
				C_3H_4O	**$C_{15}H_{20}NO^+$ (m/z 230.1539)**			m/z 298 ● → ○
					H_2O	$C_{15}H_{18}N^+$ (m/z 212.18)		m/z 230 ● → ○
					C_2H_5N	**$C_{13}H_{15}O^+$ (m/z 187.11174)**		m/z 230 ● → ○
				$C_9H_{10}O$	**$C_{10}H_{14}NO^+$ (m/z 164.10617)**			m/z 298 ● → ○
				$C_{12}H_{17}NO_2$	$C_7H_7^+$ (m/z 91.09)			m/z 298 ● → ○
(e)	H_2O	H_2O	H_2O	H_2O	**$C_{19}H_{22}NO^+$ (m/z 280.1686)**			m/z 334 ● → ○
					CO	$C_{18}H_{22}N^+$ (m/z 252.18)		m/z 280 ● → ○
					C_6H_5N	$C_{13}H_{17}O^+$ (m/z 189.09)		m/z 280 ● → ○
				C_9H_{10}	$C_{10}H_{12}NO^+$ (m/z 162.09)			m/z 189 ● → ○
				$C_9H_{10}O$	**$C_{10}H_{12}N^+$ (m/z 146.0957)**			m/z 189 ● → ○
(f)	H_2O	H_2O	H_2O	H_2O	H_2O	$C_{19}H_{20}N^+$ (m/z 262.18)		m/z 334 ● → ○
					C_6H_5N	$C_{13}H_{15}^+$ (m/z 171.09)		m/z 262 ● → ○

[a]Positive ion mode. Molecular ion ($[M+H]^+$) is in top row (a), fragment ions due to (consecutive) losses of water are in rows (b)-(f). High-resolution data from the Orbitrap are printed bold.

valent of 5. Furthermore, it contains an oxygen atom, which must not readily be lost as water. The highly unsaturated nature of the $C_9H_{10}O$ group seems to demand an aromatic ring. Evidence for an aromatic system is provided by three low abundance signals at *m/z* 129, *m/z* 117 and *m/z* 91. (Table 1, rows b, c and d) The elemental composition of two of these ions, *m/z* 117 and *m/z* 129, was found to be $C_9H_9^+$ and $C_{10}H_9^+$; these ions most likely arise from an aromatic system. The third signal at *m/z* 91 also appeared in several spectra (although in very low abundance) and might be a tropylium ion indicating the presence of an alkylated benzene unit.

Assuming that $C_9H_{10}O$ is the neutral cleavage product of a monoterpenoid (C_{10} unit), possible elemental compositions for the second skeletal unit of compound MW 351 could be $C_{10}H_{14}O$ or, equally well, by the alternative formula $C_{10}H_{12}O$ (for details of the arguments see the Supporting Information). Both are common elemental compositions of monoterpenoids, *e.g.* alcohols derived from *p*-cymene (4-isopropyltoluene) or the more unsaturated 8,9-dehydrothymol, respectively. Depending on the degree of saturation in the monoterpenoid unit, the missing linkage between the alkaloid and terpenoids has an elemental composition of (i) -C_3H_4- (diradical) on the basis of an $C_{10}H_{14}O$ terpenoid or (ii) -C_3H_6- (diradical) presuming a neutral $C_{10}H_{12}O$ terpenoid to be the origin of the lipophilic unit. While -C_3H_4- can hardly be other than allylic, -C_3H_6-



Scheme 3. Proposed chemical structure of the neutral compound mol. wt. 351 ($C_{19}H_{29}NO_5$). Diagnostic cleavage positions are marked with dashed lines: *a* = loss of C_3H_4O after a loss of H_2O; *b* = loss of the alkaloid unit as an immonium ion $C_5H_{10}NO_3^+$; *c* = loss of the alkaloid unit *via* four consecutives losses of H_2O and loss of C_6H_5N; *d* = loss of terpene moiety as $C_9H_{10}O$ with loss of two, three or four H_2O molecules.

corresponds to a saturated alkyl chain. In summary we propose the basic skeleton of the unknown compound 351 to be [pyrrolidinyl]-[alkyl/alkenyl]-[monoterpenyl]. Despite the large number of chemical structures that fit this pattern (see Supporting Information, Scheme S7), two molecules derived from the common terpenoids cymen-8-ol and 8,9- dehydrothymol best fulfill the structural prerequisites for the observed fragmentation behavior. Based on the observation in several fragmentation sequences of loss of CO, a known process in phenolic compounds, and in spite of the absence of the expected ted anion radical in the negative ion mass spectrum (Supporting Information) we hypothesize tentatively that the compound molecular weight 351 has the chemical constitution depicted in Scheme 3. Note, however, that several closely related but less likely alternatives exist, as shown in Supporting Information, Figure S7, especially the cymen-9-ol derivatives A and C.

Conclusion

An unknown compound, molecular weight 351, has been identified by differential ambient ionization through subtraction of the mass spectra recorded from the petals of two species, *Hibiscus moscheutos* and *Hibiscus syriacus*. A tentative structure has been assigned to this compound on the basis of mass spectrometry only, specifically using data acquired by multiple stages of mass spectrometry (MS^n) and exact mass measurements. The compound belongs to a well-known class of polyhydroxylated pyrrolidines. Although polyhydroxylated alkaloids with lipophilic moieties (hydroxyaryl or methoxyaryl substitutents) are known,[20,21] it is unusual for a lipophilic monoterpene to be bound *via* a linker.

We expect much future work to be done using differential leaf spray mass spectrometry and differential ambient ionization in general. The speed and convenience of the experiment should suit it to examination of genetic, environmental, and climatic effects on phytochemical production. This might include relative yields of desirable products such as fragrances and drugs, evaluation of growth conditions on productivity of genetically identical species.

Studies using individual plants as their own controls to examine the effects of stress, chemical transport and photosynthesis would be facilitated.

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