Supporting Information

Differential Rapid Screening of Phytochemicals by Leaf Spray Mass Spectrometry†

Thomas Müller* **and R. Graham Cooks†,***

*Institute of Organic Chemistry, University of Innsbruck, 6020 Innsbruck (Austria). *E-mail: thomas.mueller@uibk.ac.at*
*†Department of Chemistry, Purdue University, West Lafayette, IN 47907 (USA). *E-mail: cooks@purdue.edu*
Received October 20, 2013, Accepted November 4 2014

Supporting information includes illustrations of plant material, differential mass spectra and H/D exchange data as well as details of fragmentation behavior and its rationalization in terms of the structures shown in main text.

Figure S1. *Hibiscus moscheutos* flower (top) and *Hibiscus syçacus* flower (bottom), West Lafayette, IN, USA, August 2013. Flower diameters are *ca.* 25 cm and 10 cm, respectively.

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

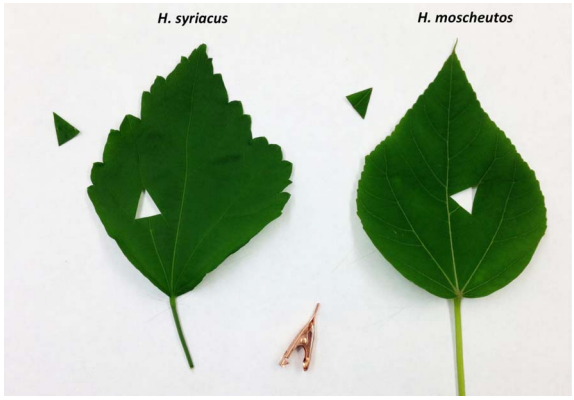


Figure S2. *Hibiscus syriacus* leaf (left) and *Hibiscus moscheutos* leaf (right), West Lafayette, IN, USA, August 2013. Unlike the flowers, the leaves are of similar size.

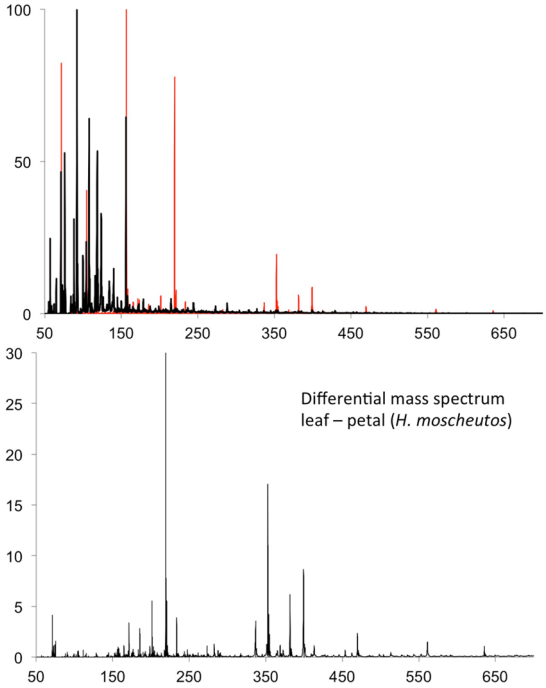


Figure S3. Top: Overlay of the leaf spray mass spectra obtained from a *H. moscheutos* petal (red) and *H. moscheutos* leaf (black). Bottom: Differential mass spectrum showing the set of difference ions detected in a *H. moscheutos* petal and a *H. moscheutos* leaf showing only positive excursions. The nitrogen-containing compound at *m/z* 352 is only present in the petals of *H. moscheutos*.

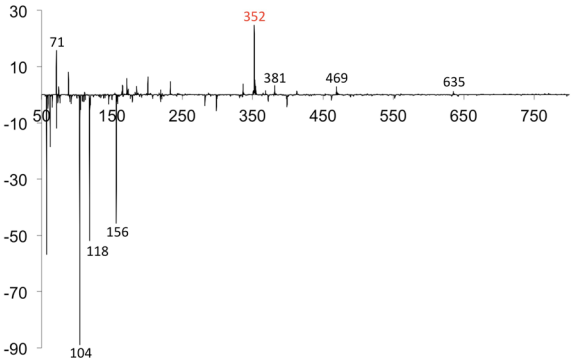


Figure S4. Difference mass spectrum obtained by subtraction of relative signal intensities of two different leaf spray mass spectra (both spectra were normalized with respect to the potassiated glucose at *m/z* 219 assuming glucose to be a "house-keeping" metabolite): rel. int. = rel. int (*H. moscheutos*) – rel. int. (*H. syriacus*). Positive signals are related to compounds that were only present (or appeared at higher relative intensities) in the petals of *H. moscheutos*, negative signal intensities represent compounds that were only present (or appeared at higher relative intensities) in *H. syriacus*. For the identification of signals that appear exclusively in the *H. moscheutos* petals see the differential mass spectrum depicted in main text Figure 1E.

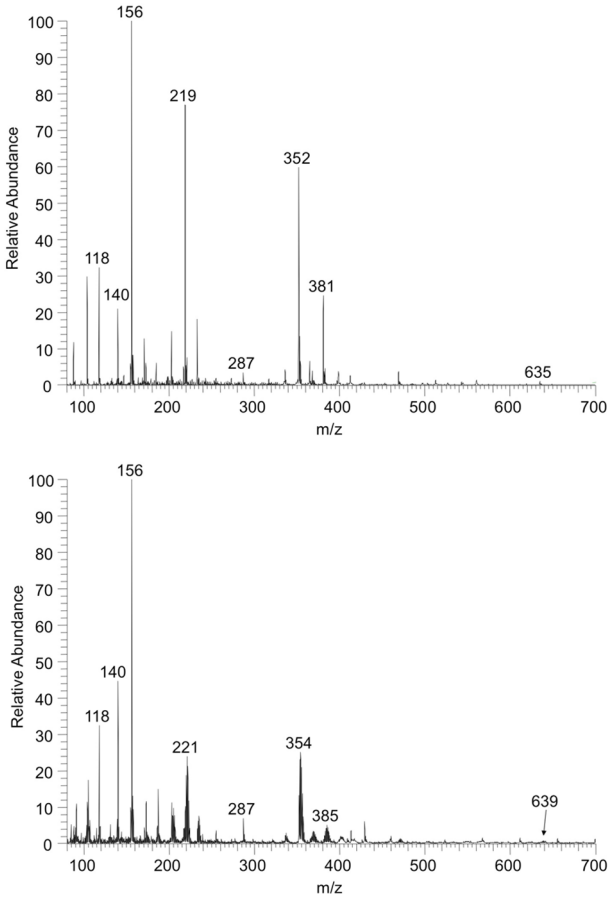


Figure S5. H/D exchange experiment. Top: Mass spectrum obtained from ESSI of the methanolic extract of a *H. moscheutos* petal. Bottom: Mass spectrum obtained from the ESSI analysis of the CD_3OD extract of a *H. moscheutos* petal.

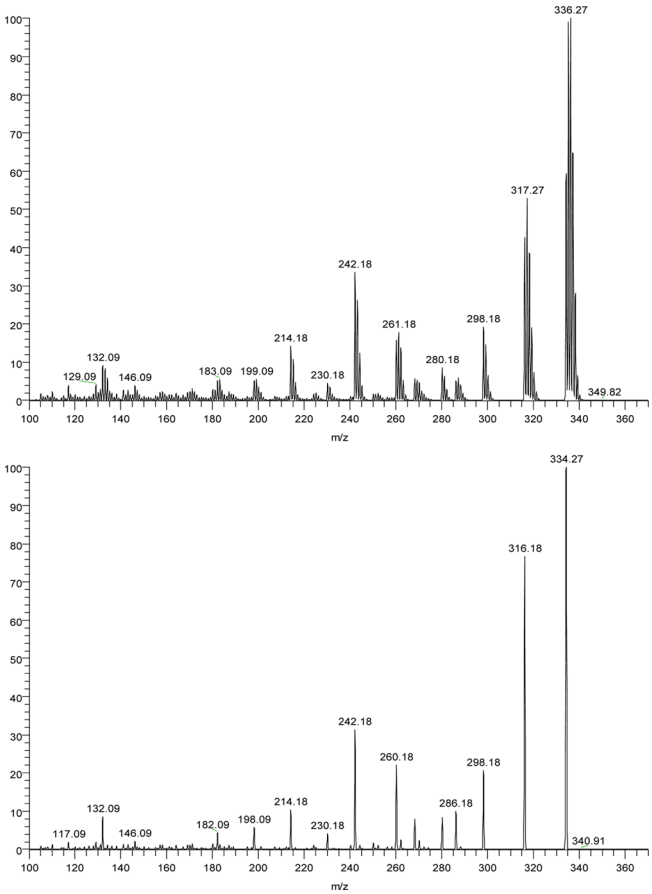


Figure S6. Top: MS/MS/MS sequential product ion spectra of H/D exchange product from *H. moscheutos* petal, recorded using an isolation window of 8 mass units centered on *m/z* 354 and monitoring the transitions *m/z* 354 → *m/z* 336 → fragments, compared, Bottom, to the MS/MS/MS product ion spectrum of the undeuterated compound, *m/z* 352 → *m/z* 334 → fragments.

Details of Fragmentation Mechanisms

$[M+H]^+$ Loss Water. An extremely ready loss of water occurs and has to be considered when proposing the chemical structure of the alkaloid (M) (*e.g.* the presence of a tertiary alcohol functionality or the formation of a conjugated product upon dehydration fragmentation). One expects this loss to occur either in the pyrrolidine unit, although its extent is a surprise, or in another exposed skeletal unit of the molecule.

$[M+H]^+$ Loss Water Followed by Loss of $C_{14}H_{18}O$. If water were lost from the alkaloid unit with its four hydroxyl groups it should stabilize and not facilitate the subsequent cleavage of the C_5 pyrrolidine. Hence it is the 5th oxygen that is lost most readily as water in a process that is distant from the pyrrolidine. The product ion must be the $C_5H_{10}NO_3^+$ immonium ion shown in Figure 3B and Scheme 1 of the main text (pyrrolidine substitutents 2-H, 3-OH, 4-OH, 5-CH_2OH). The product neutral is the primary alcohol $C_{14}H_{18}O$.

$[M+H]^+$ Loss of Water Twice, Three and Four Times, Each Followed by Loss of $C_9H_{10}O$. The neutral $C_9H_{10}O$ is lost in three separate fragmentations, after two, three and

four water eliminations (see fragmentations listed in Table 1 of the main text).

This unit has to be derived from an exposed skeletal unit and its oxygen must not readily be lost as water. Hence, the losses of water prior to the cleavage of $C_9H_{10}O$ are not from same site as in the process described section 2. The $C_9H_{10}O$ unit is lipophilic, its elements include 5 rings and double bonds as well as one oxygen atom. The highly unsaturated nature of the $C_9H_{10}O$ group seems to demand an aromatic ring, which is taken up in section 6 below.

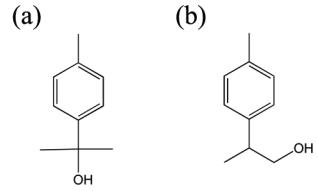
$[M+H]^+$ Loss of Two or Three Water Molecules Followed by Loss of C_3H_4O. This relatively unusual and highly unsaturated neutral loss is an important characteristic. It must be a branch point or at the terminus to be a first skeletal loss. The group –CH(OH)-CH=CH- (or the corresponding epoxide) seems most likely. We assume the C_3H_4O unit to be generated by water loss(es) from the pyrrolidine unit depicted in Scheme 1 of the main text. This is strongly indicated by the fact that ring cleavage after two water losses to give a pyrrole readily explains this fragmentation.

$[M+H]^+$ Loss C_6H_5N Loss. This last characteristic (and unusual) loss occurs after four and five successive water losses. So like C_3H_4O and $C_9H_{10}O$, the loss of C_6H_5N is from an exposed skeletal group. However, it requires four water losses before it is observed suggesting that these hydroxyls are on this unit, which is consistent with the proposed structure of the C_5 pyrrolidine (Scheme 2 of the main text). The final oxygen is elsewhere in the molecule and can be lost but need not be lost in the elimination of C_6H_5N. The process is consistent with and gives more evidence for the proposed C_5 pyrrolidine unit.

Evidence for Aromatic (Phenolic) Units. Evidence for an aromatic unit was provided by three low abundance signals at *m/z* 129, *m/z* 117 and *m/z* 91. Two of them, *m/z* 117 and *m/z* 129 appear in several MS^n spectra. From high-resolution data, their elemental compositions are $C_9H_9^+$ and $C_{10}H_9^+$. While $C_9H_9^+$ corresponds to the cleavage of *e.g.* methylstyrene or an isomer, $C_{10}H_9^+$ provides evidence for a terpenoid unit with a higher degree of unsaturation (see also section 7). The third signal at *m/z* 91 also appeared in several spectra although at very low abundance. It might be related to a tropylium structure indicating the presence of methylated aromatic unit. Furthermore, losses of CO occur at several points in the fragmentation scheme and are good evidence of phenol and not expected for hydroxyl substituents.

Constituent Units. The arguments in the paragraphs above reduce the number of units to three. There are two exposed skeletal units and a linking part which consists of the residual atoms (radicals or diradicals, as indicated by the dashes): (i) a C_6 pyrrolidinyl radical, $-C_6H_{12}NO_4$, for which a reasonable structure is available [Pyrr], (ii) a radical comprising a neutral $C_9H_{10}O$, which is highly unsaturated and may be terpenoid in origin [Ter], and (iii) a linking diradical $-C_xH_y-$.

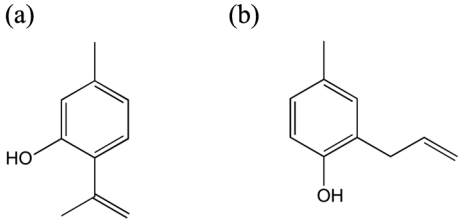
Terpenoid Unit. Assuming that [Ter] belongs to the monoterpenoid class (C_{10} unit), an assembly of [Ter] and a $-CH_2-$ together constitute the diradical group $-C_9H_{10}O-CH_2-$ *i.e.* a $-C_{10}H_{12}O-$ diradical corresponding to a neutral molecule $C_{10}H_{14}O$, which is a common monoterpenoid. There are many reasonable possibilities for the terpene including alcohols derived from *p*-cymene (4-isopropyltoluene) where the OH could be on the methyl or the isopropyl group or on the ring



Scheme S1. Monoterpenoids with elemental composition of $C_{10}H_{14}O$: Cymen-8-ol (a) and Cymen-9-ol (b).

(see Scheme S1).

Alternatively, a $-C_9H_8O-$ diradical can be presumed as the origin of the $C_9H_{10}O$ neutral; $-C_9H_8O-$ assembled with $-CH_2-$ then constitutes the diradical $-C_{10}H_{10}O-$ corresponding to a neutral molecule $C_{10}H_{12}O$, which is also a common elemental composition for monoterpenoids (see Scheme S2).



Scheme S2. Common monoterpenoids with an elemental composition of $C_{10}H_{12}O$: 8,9-Dehydrothymol (a) and 2-allyl-4-methylphenol (b).

Assembly of the Constituent Units. The basic structure of the unknown compound 351 can be proposed by assembling the constituent units [Pyrr]-[C_3H_x]-[Ter]. In case [Ter] corresponds to the terpenoid $C_{10}H_{14}O$, which has a higher degree of saturation, the remainder of the molecule would be [$C_{19}H_{29}NO_5$] – [pyrrolidinyl radical $-C_6H_{12}NO_4$] – [terpenoidyl radical $-C_{10}H_{13}O$] giving $-C_3H_4-$, which can hardly be other than an allylic diradical. If [Ter] is related to $C_{10}H_{12}O$, the remaining linker would be [$C_{19}H_{29}NO_5$] – [pyrrolidinyl radical $-C_6H_{12}NO_4$] – [terpenoidyl radical $-C_{10}H_{11}O$] giving $-C_3H_6-$, which corresponds to a saturated alkyl chain. Possible chemical structures for the unknown compound mol. wt. 351 are depicted in Figure S8. Structures A, B, C, D and F allow the loss of water in the terpenoid to give rise to a stabilized (conjugated) product.

Negative Ion Spectra. The negative ion data is of very poor quality. There is no $[M-H]^-$ as would be expected for a phenol but the main ion at *m/z* 133 corresponds to $C_9H_9O^-$ which parallels the loss of C_9H_9OH ($C_9H_{10}O$) seen in the positive ion data.

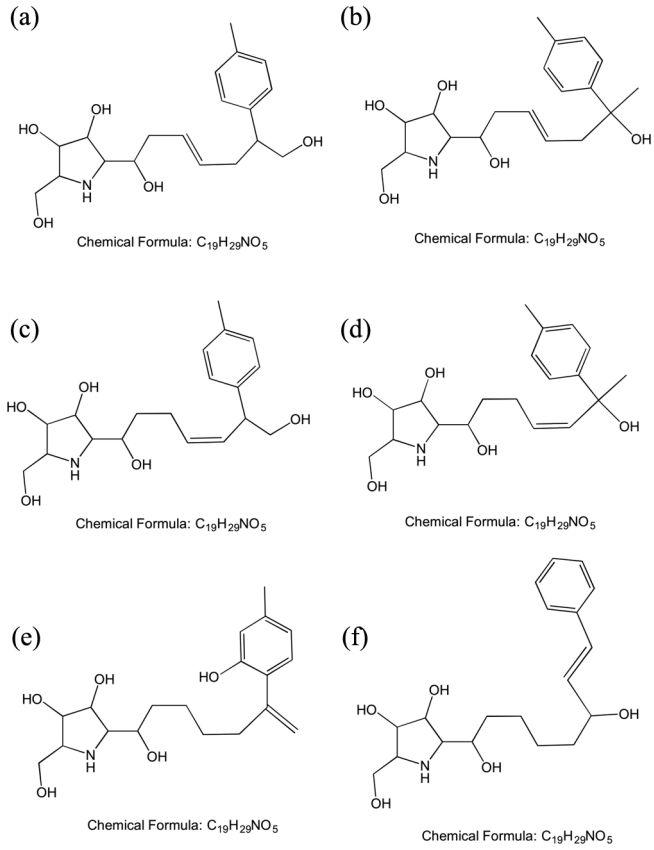


Figure S7. Possible chemical structures for the unknown compound 351 showing different constitutions of linker and terpenoid unit. The depicted structures are based on the terpenoid cymen-9-ol (a and c), cymen-8-ol (b and d), 8,9-dehydrothymol (e; a structure comprising the isomer 2-allyl-4-methylphenol is not shown), and others like cinnamic acid derivatives (f).