The inherent matrix properties of lichen metabolites in matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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The inherent matrix properties of lichen metabolites in MALDI-TOF MS

Pierre Le Pogam[1], Joël Boustie[2], Pascal Richomme[3], Antoine Denis[1] and Andreas Schinkovitz[3*]

[1] Institut d'Électronique et de Télécommunications de Rennes, Université de Rennes 1, UMR CNRS 6164, 263 Avenue du Général Leclerc, 35042 Rennes Cedex, France.

[2] Institut des Sciences Chimiques de Rennes, Université de Rennes 1, UMR CNRS 6226, 2 Avenue du Professeur Léon Bernard, 35043 Rennes Cedex, France.

[3] Université d'Angers, Campus du Végétal, SFR 4207 QUASAV, EA921 SONAS, 42 Rue Georges Morel, 49070 Beaucouzé Cedex, France.

* Corresponding author: andreas.schinkovitz@univ-angers.fr

Abstract

RATIONALE: Light-absorbing secondary metabolites from lichens were recently reported to exhibit promising Laser Desorption Ionization (LDI) properties, enabling their direct detection from crude lichen extracts. In addition, many of them display close structural homologies to commercial Matrix Assisted Laser Desorption Ionization (MALDI) matrices, which is incentive for the evaluation of their matrical properties. The current study systematically evaluated the matrix effects of several structural classes of lichen metabolites: monoaromatic compounds, quinone derivatives, dibenzofuran-related molecules and the shikimate-derived vulpinic acid. Their matrical properties were tested against a wide range of structurally diverse analytes including alkaloids, coumarins, flavonoids and peptides.

METHODS: Triplicate automatic positive-ion mode MALDI analyses were carried out and ionization efficiencies were compared with those of structurally related reference matrices (*i.e* DHB, HCCA, dithranol and usnic acid) in terms of (i) analyte absolute intensities and (ii) Matrix Suppressing Effect (MSE) scores.

RESULTS: Monoaromatic lichen metabolites revealed matrical properties similar to those of DHB under similar experimental conditions. Likewise, anthraquinone metabolites triggered ionization of tested analytes in a similar way to the structurally related dithranol. Finally, dibenzofuran derivatives displayed a broad ionization profile, reminiscent of that of (+)-usnic acid.

CONCLUSIONS: Lichen metabolites exhibit interesting matrix properties, especially for MALDI of medium and low molecular weight analytes. For many of the tested molecules, matrix ion formation was very limited. This proof-of-concept study paves the way for follow-up investigations to assess the matrix properties of lichen metabolites against a wider array of analytes as well as adapting experimental settings to individually optimize the performance of successfully tested candidates.

INTRODUCTION

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) is a greatly-expanding soft ionization technique.[1] As MALDI-MS requires the cocrystallization of analytes with a large excess of matrix, the choice of suitable MALDI matrices is of significant importance for analyte detection.[2] In general, MALDI matrices are small organic molecules exhibiting UV absorption in the range of the excitation wavelength of the laser as well as a high vacuum stability.[3] However, most conventional MALDI matrices produce strong cluster ions in the low mass region that often interfere with signals from small molecules.[4] For that reason, MALDI-MS is mainly used for the analysis of macromolecules (*i.e* proteins, peptides, polymers, lipids…).[5] Strategies to bypass this problem include replacing the matrix by textured silicon surfaces as known from Surface Assisted Laser Desorption Ionization (SALDI).[6] Alternatively, as each matrix forms a specific cluster of matrix ions, samples can be analyzed by two matrices in order to check for signal superpositions. In the specific field of natural products dereplication, MALDI-MS might open up new perspectives by substantially improving the throughput compared with benchmark LC/MS approaches.[7] However, since the development of MALDI-MS, only few matrices such as 2,5-dihydroxybenzoic acid (DHB) or *p*-hydroxy-α-cyanocinnamic acid (HCCA) have found broad use for various analyte series.[8] Therefore, t research for new MALDI matrices has gained considerable interest during the last decade.[9–11] Despite these notable efforts, it is still quite difficult to predict whether a compound will exhibit matrical properties or not; so the discovery of new matrices remains mostly empirical.[12] In this context, lichen metabolites may represent attractive candidates in the search for new matrix molecules.[13,14]

Lichens are self-supporting symbiotic consortia comprising a fungus and a photobiont partner (most often green algae that can either be replaced or accompanied by cyanobacteria).[15] As world-widespread partnerships, lichens display an extreme resistance to UV irradiance that is partly based on the biosynthesis of photoprotective polyphenolics, a majority of which is unique to the lichenized condition.[16] Most of them display UV chromophores that are structurally closely related to those of MALDI matrices. Moreover, a previous study has demonstrated that a wide range of chemically very diverse lichen metabolites can be easily ionized and detected by matrix-free Laser Desorption Ionization (LDI)-MS.[13] An interesting question arising from these findings is whether these compounds also exhibit matrix properties, as previously reported for the dibenzofuran-derived metabolite, usnic acid.[14]

However, LDI effects are not necessarily linked to matrix effect. Some lichen metabolites display satisfying LDI properties while not exhibiting matrix effects.[14]

With this in mind, the current study systematically evaluated the matrix properties of a wide range of lichen metabolites. Among them, three chemical groups appeared to be of special interest and will be discussed separately: monoaromatic metabolites and compounds similar to DHB such as orsellinic acid, β-orcinol methylcarboxylate (MOC), atranol and chloroatranol (**I**), quinones, and compounds similar to dithranol such as chrysophanol, citreorosein, haemoventosin, parietin and solorinic acid (**II**), as well as dibenzofurans and compounds similar to usnic acid such as pannaric acid, placodiolic acid, porphyrilic acid and schizopeltic acid (**III**). Moreover, the shikimate-derived vulpinic acid was also included in the study (**IV**). All lichen metabolites were evaluated on different test compounds belonging to various chemical families including a flavonoid (rutin), a coumarin (E-notopterol), alkaloids (brassinin, inosine and yohimbin) and peptides (substance P, a mixture of gramicidin A-D, Angiotensin II, Gly-Tyr, Leu enkephalin, Met enkephalin and Val-Tyr-Val). Eventually, each group was compared with a commercial reference matrix of the same structural class as well as with HCCA.

EXPERIMENTAL

Materials

Matrix reagent dithranol was purchased from Avocado Research Chemicals Ltd (Morecombe, UK). HCCA, DHB, Gramicidin A-D, substance P and the HPLC peptide mix (containing 0.5 mg of the following compounds: Angiotensin II, Gly-Tyr, Leu enkephalin, Met enkephalin and Val-Tyr-Val) were all purchased from Sigma-Aldrich (Steinheim, Germany). Small organic analytes were obtained from former phytochemical research projects or generously provided by collaborators. Likewise, lichen metabolites considered in the study were isolated during previous phytochemical investigations. Placodiolic acid and didymic acid were kindly provided by Dr M. Millot (Université de Limoges, Limoges, France). Citreorosein, porphyrilic acid and schizopeltic acid were generous gifts from Dr H. J. Sipman (Huneck's compounds library - National History Museum – Berlin, Germany). Purity evaluation of the lichen metabolites was based on ^{1}H NMR experiments. Each compound exhibited a degree of purity of at least 95%.

Stock solutions and sample preparation

Matrix solutions were prepared at a concentration of 29 mM in methanol (MeOH), dichloromethane (DCM) or a 9:1 (*v/v*) mixture of DCM and pyridine, depending on sample solubility. Sample stock solutions were prepared at a concentration of 6 mM in MeOH, DCM or water depending on the solubility of the test compounds. The Sigma HPLC mix was dissolved in 0.5 mL water. Working solutions were prepared by mixing one equivalent of sample stock solutions with two equivalents of matrix solutions. Eventually, 0.5 µL of the working solutions were deposited on a MALDI steel plate and air-dried.[17]

Mass spectrometry settings

All MALDI experiments were carried out on a Bruker Biflex III Time Of Flight (TOF) mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with a 337 nm pulsed nitrogen laser (model VSL-337i, Laser Sciences, Inc., Boston, MA, USA) emitting a laser beam with a diameter of approximately 100 µm. Mass spectra were acquired in the positive reflectron ionization mode within a mass range of *m/z* 20 to 2000. The acceleration voltage was 19 kV, the pulse ion extraction time was 200 ns and the laser frequency was 200 MHz.

Automatic mode data acquisition

Each sample was deposited in triplicate on a MALDI MTP 384 ground steel plate (Bruker Daltonik) with deposition area spots of 3 mm diameter. For each deposition area, 30 irradiation spots were randomly selected and analyzed 15 times, yielding an overall of 450 single acquisitions for each sample spot. Based on results from a first manual screening, automatic mode experiments were performed at a laser energy of 70% (71.9 µJ). Dithranol, HCCA, DHB and usnic acid were used as reference matrices. As not all samples could be analyzed within one day, the results of the reference matrices HCCA and DHB (Tables 1 and 2) are expressed as average of two different days.

Data analysis and MSE score calculations

Raw data were processed using Flex Analysis 2.0 software (Bruker Daltonik). Matrix Suppression Effect (MSE) scores were obtained by dividing the summed analyte signals by the sum of all observed signals (analyte + matrix) as previously outlined.[18] Elevated scores indicate strong analyte signals and weak matrix noise, while MSE scores close to 0 indicate intense matrix noise and comparatively weak analyte signals. Despite this, mass spectra exhibiting low MSE scores might still be analyzed as long as the matrix signals did not

overlap those of analytes (*i.e* no isobaric matrix ions). Signals having a signal-to-noise ratio of less than 50 were not considered. Likewise, compounds exhibiting MSE scores lower than 0.03 were eliminated.

RESULTS AND DISCUSSION

The present study evaluated the ionization properties of secondary lichen metabolites as MALDI matrices on a wide range of chemically very different analytes. Mass spectrometric signals retained for the test compounds along with consistent literature data are provided in Table S1 (supporting information). This strategy served two purposes. On one hand, classical MALDI approaches targeting the ionization of proteins and peptides were addressed. On the other hand, small molecules such as alkaloids, flavonoids and coumarins, known for exhibiting significant bioactivities, were also considered. As these low-molecular weight analytes display photoabsorbing properties, a first pertinent step was to exclude LDI effects under the given experimental conditions. The Positive-Ionization mode-LDI (PI-LDI) mass spectra obtained for these metabolites (brassinin, inosine, E-notopterol, yohimbin and rutin) are shown in Figure S1 (supporting information). Except for yohimbin, none of the tested compounds (brassinin, inosine, E-notopterol and rutin) exhibited LDI effects. For yohimbin, a weak $[M\text{-}H]^+$ ion signal was detected which may seem unusual at first sight, but this has been reported for the MALDI ionization process [3] and was also observed for the compound itself in LDI.[19] However, when adding a working matrix, a strong increase in the signal intensity was detected (Table 1).

A first assessment of matrix properties was performed on various chemically diverse lichen metabolites including depsides (perlatolic acid and erythrin), depsidones (physodic acid and variolaric acid), dibenzofurans (pannaric acid and placodiolic acid), monoaromatic metabolites (orsellinic acid and atranol), a diphenyl ether (buellin), anthraquinones (parietin and chrysophanol), paraconic and aliphatic acid derivatives (lichesterinic acid and roccellic acid) and a pulvinic acid-derived metabolite (vulpinic acid) using both positive and negative-ionization modes. It was observed that, in general, candidates exhibiting structural homologies to commercial MALDI matrices also showed notable ionization of test compounds. Among these candidates, best results were obtained for: monoaromatic metabolites (I), anthraquinones (II) dibenzofurans (III) and vulpinic acid (IV), which will be discussed separately in sections of the article. Each group was further compared with a

commercial reference of the same family (*i.e* DHB, dithranol or (+)-usnic acid). Consistent with our previous reports, all matrix candidates were sucessfully ionized in negative mode, mainly affording abundant deprotonated molecules.[13] However, no ionization of the test compounds was observed under these conditions. Consequently, all experiments evaluating the matrix properties of lichen metabolites were performed in the positive ionization mode. At this point, it should be mentioned that depsides and depsidones such as *m*-scrobiculic acid and stictic acid did not show matrix properties, which is in line with previously reported results.[14] The chemical structures of all the tested matrix candidates and of the low-molecular weight test compunds are outlined in Figures 1 and 2, respectively.

FIGURE 1

FIGURE 2

As MALDI-MS is known for its limitations in terms of reproducibility,[20,21] special precautions were taken to allow a most unbiased evaluation of matrix candidates. All samples were deposited in triplicate and randomly analyzed in automatic mode to avoid the selection of the so-called 'sweet spots' by a potentially biased operator.[22] However, automatic mode acquisitions required the use of a fixed level of ionization energy, which could not be modified during the experiment. The laser energy was set at 70% (71.9 µJ) as this value facilitated the sufficient ionization of any of the tested matrix compounds. Accordingly, a general comparison of ionization properties of all matrix molecules could be performed.

Matrices were also compared in terms of observed analyte signal intensities as well as their MSE scores. The latter were used to estimate the quality of the obtained spectra relating analyte to matrix signal intensities.[18]

A direct comparison of analytes signal intensities obtained with all matrix compounds is outlined in Table 1, and the MSE scores are compared in Table 2.

TABLE 1

TABLE 2

A compilation of mass spectra obtained using lichen metabolites as matrices is displayed in Figure 3. Further mass spectra, associating matrices of structural classes **I-IV**, are available in the supporting information.

FIGURE 3

Matrical effects of monoaromatic metabolites

Each of the tested monoaromatic lichen compounds revealed similar ionization trends, which were globally similar to those of DHB, in terms of signal intensity and MSE scores. These matrices efficiently assisted the ionization of small metabolites but failed to ionize peptides (*i.e* gramicidin, substance P and peptides of the commercial mixture). Only atranol could induce a weak ionization of gramicidin under the given experimental conditions. Some representative mass spectra obtained using lichen monoaromatic metabolites are shown in Figure S2 (supporting information).

With regard to analyte ion yields, HCCA exhibited the best ionization properties of all the matrices. PI-MALDI mass spectra obtained using this reference matrix are given in Figure S3 (supporting information). However, most monoaromatic lichen derivatives exhibited little matrix noise resulting in excellent MSE scores. While HCCA is among the most popular MALDI matrices, it is also known to produce intense matrix cluster ions potentially superposing analyte ions.[23] This effect may complicate the correct interpretation of spectra, which was also observed in the present study. The second reference matrix, DHB, is reported to display reduced matrix cluster signals but also decreased analyte ionization resulting in reduced sentivity,[24,25] (see also the mass spectra shown in Figure S4, supporting information). Overall, the results obtained for the monoaromatic lichen metabolite as matrices were quite close to those of DHB. It is worth mentioning that DHB is a reported to be a suitable matrix for the ionization of peptides,[26] but it failed to be so in the current experiments. However, as previously mentioned, due to the large number of tested matrices, the experimental conditions had to be very general and could not be specifically adapted to DHB. Using optimized instrument settings and sample preparation procedures similar to those previously reported will certainly solve this problem.[27–32] This also applies to any of the other tested matrices but exceeds the scope of the current manuscript.

Matrical effects of quinones derivatives

As previously observed for monoaromatic compounds, quinone-derived lichen metabolites showed similar ionization trends to their commercial reference dithranol. Representative mass spectra obtained with this structural class are displayed in Figure S5 (supporting information). Some small differences whithin the groups were nevertheless observed.

Dithranol triggered the ionization of small non-peptidic compounds and substance P, while gramicidin and the peptide mix could not be ionized under the given experimental conditions.

Mass spectra obtained using dithranol as a matrix are displayed in Figure S6 (supporting information). Haemoventosin was less efficient than dithranol, and could ionize neither peptides nor E-notopterol. Likewise, the intensity of ionized test compounds was generally lower than when using dithranol as matrix. Chrysophanol, parietin and solorinic acid could ionize the same compounds as dithranol, exhibiting comparable or seemingly higher ion yields (Tables 1 and 2). Within this group, substance P was best ionized by parietin. Citreorosein also facilitated the ionization of the HPLC peptide mixture (except for angiotensin II). Moreover, an excellent ion yield and MSE score were observed for gramicidins A-D (Figure 3F).

Despite showing higher signal intensities than monoaromatic lichen compounds, the MSE scores for quinones were mostly lower than those of these former. However, this had no negative impact on spectra quality. As the tested quinones barely fragmented, their mass spectra mostly comprised $[M+H]^+$ ions, limiting the risk of signal overlap with analyte signals.

Matrical properties of dibenzofurans

A previous report on (+)-usnic acid has shown that this compound exhibits a broad ionization profile towards many chemically diverse test compounds,[14] and the present study found similar results for some of the tested dibenzofurans. A selection of some representative mass spectra obtained for this group is shown in Figure S7 (supporting information).

Any of the dibenzofurans ionized all the single test compounds (except for placodiolic acid, which failed to ionize brassinin and inosine). While the signal intensities were generally in the same range as those of usnic acid, some dibenzofurans, *i.e* pannaric acid, porphyrilic acid and schizopeltic acid, exhibited higher MSE scores (Table 2). All the tested dibenzofurans facilitated the ionization of at least some of the components of the commercial peptide mixture. Pannaric and placodiolic acids ionized angiotensin II but failed to do so for the rest of the mixture. Conversely, schizopeltic acid solely ionized small peptides (*i.e* Gly-Tyr dipeptide, Val-Tyr-Val tripeptide and enkephalin derivatives) with notable signal intensities. Didymic and porphyrilic acids facilitated the ionization of all the components of the commercial peptide mixture, as was also observed for usnic acid (see Figure S8, supporting information). It is noteworthy that lower matrix noise was observed with these newly tested dibenzofuran matrices than with (+)-usnic acid.

Matrical properties of vulpinic acid

Among the pulvinic acid derivatives, only vulpinic acid was available in sufficient amount to allow the evaluation of its matrical properties. Some representative MALDI mass spectra are shown in Figure S9 (supporting information).

Vulpinic acid ionized most test compounds (including peptides and proteins) but failed to do so for E-notopterol and substance P. Despite quite intense analyte signals, the MSE scores were rather low (Table 2). However, a more tailored adaption of the ionization energy to the specific sample requirements may improve the MSE scores and this is part of ongoing research. Nevertheless, the present results suggest the evaluation of further vulpinic acid derivatives as an interesting class of MALDI matrices.

CONCLUSIONS

The close structural similarity of many natural products to commercial MALDI matrices makes them an interesting subject of research. In particular, secondary metabolites from lichen fulfill many requirements of MALDI matrices such as light-absorbing moieties. This is mainly due to their physiological fuction in the living organism. As lichen species are often exposed to high-light irradiance, their specialized metabolites often serve as efficient UV-protectants, which is essential for the survival of these organisms in harsh conditions.[16,33] The present results have shown that monoaromatic metabolites (*i.e* orsellinic acid, MOC, atranol and chloroatranol) and quinones (in particular, citreorosein, parietin and solorinic acid) may provide useful tools for the MALDI detection of small molecules. For both absolute signal intensities and MSE scores, these compounds showed similar ionization trends to DHB and dithranol.

Most of the studied dibenzofurans revealed a quite broad ionization profile covering both small organic metabolites and peptides. Their ion yields were comparable with those previously observed for (+)-usnic acid. In addition, some of them (*i.e* pannaric acid, porphyrilic acid and schizopeltic acid) showed promising MSE scores.

As outlined previously, the current experimental settings used a fixed laser energy, which allowed a good estimation of the general ionization properties of all the investigated matrix candidates. However, it should be noted that any matrix displays very specific ionization thresholds and pulse energies so that an optimization of these parameters might enhance their

performance. Likewise, alternative approaches of matrix deposition (*e.g* acoustic spraying[34] or sublimation[35]) may further increase the matrical performances of lichen metabolites. These aspects exceed the scope of the current manuscript but will be thoroughly studied in follow-up projects.

Nevertheless, the presented results underscore that lichen secondary metabolites represent an interesting group of new matrix candidates that deserves further attention.

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Table 1. Direct comparison of analytes signal intensities using lichen metabolites as matrices at a laser energy of 70% (71.9 µJ) (along with commercial matrices). For illustration purposes, absolute signal intensities were divided by 1250. The last five rows refer to components of the Sigma peptide mixture. Abbreviations: BRA Brassinin, INO inosine, NOT E-notopterol, YOH yohimbin, RUT rutin, GRAM gramicidin A-D, SUB. P substance P, G-T Gly-Tyr dipeptide, V-T-V Val-Tyr-Val tripeptide, LeuEnk Leucine Enkephalin, MetEnk Methionine Enkephalin and Ang. II Angiotensin II.

								HPLC peptide mixture				
Compound	**BRA**	**INO**	**NOT**	**YOH**	**RUT**	**GRAM**	**SUB. P**	**G-T**	**V-T-V**	**LeuEnk**	**MetEnk**	**Ang. II**
Orsellinic acid	0.57±0.05	0.82±0.22	15.61±1.49	10.53±2.91	7.91±6.32	0	0	0	0	0	0	0
MOC	5.82±4.52	1.60±0.62	56.47±11.76	1.90±0.11	3.57±2.54	0	0	0	0	0	0	0
Atranol	3.82±3.87	1.49±0.38	16.96±8.29	23.88±2.89	4.06±3.30	0.52±0.24	0	0	0	0	0	0
Chloroatranol	17.27±22.53	3.22±0.26	3.37±1.04	6.38±2.39	2.13±0.54	0	0	0	0	0	0	0
DHB[a]	2.09±1.95	7.74±6.00	53.01±20.73	12.52±7.54	5.60±4.05	0	0	0	0	0	0	0
HCCA[a]	24.28±9.82	30.65±8.69	177.65±17.26	118.20±20.08	72.94±29.76	1.03±2.02	17.21±10.84	2.94±0.68	3.34±1.24	2.84±0.47	2.74±0.40	2.11±0.07
Chrysophanol	14.13±8.23	8.68±1.21	50.62±9.41	132.39±11.92	25.71±5.86	0	0.76±0.41	0	0	0	0	0
Citreorosein	2.29±0.67	12.34±3.49	13.45±6.37	40.91±16.93	3.17±0.53	3.10±0.65	1.04±0.32	1.08±0.18	1.95±0.58	0.76±0.31	0.36±0.06	0
Haemoventosin	3.18±2.35	3.09±1.42	0	33.19±6.44	4.69±1.11	0	0	0	0	0	0	0
Parietin	27.19±10.23	18.96±5.76	30.32±6.70	153.77±25.47	14.58±7.70	0	1.80±0.47	0	0	0	0	0
Solorinic acid	14.03±0.46	6.51±1.25	14.25±5.04	78.10±17.45	18.27±7.44	0	0.79±0.30	0	0	0	0	0
Dithranol	11.92±1.35	5.77±3.91	31.28±26.29	51.77±3.42	15.15±4.96	0	0.25±0.13	0	0	0	0	0
Didymic acid	4.96±3.77	13.05±3.21	4.94±0.09	67.05±10.05	25.04±7.25	8.27±1.51	3.47±1.01	2.19±0.96	2.19±1.98	2.01±0.88	1.80±0.80	1.72±0.29
Pannaric acid	22.04±3.67	18.58±8.48	10.54±2.56	94.89±16.17	13.52±7.72	0.45±0.28	2.37±0.51	0	0	0	0	0.40±0.15
Placodiolic acid	0	0	0.74±0.10	1.94±0.36	7.89±6.31	0.63±0.33	4.31±2.35	0	0	0	0	1.33±0.68
Porphyrilic acid	10.15±6.30	4.36±1.73	3.05±0.69	44.94±9.61	5.23±2.67	1.92±0.05	5.04±0.19	0.84±0.05	1.94±0.65	2.01±0.80	1.50±0.53	1.39±0.66
Schizopeltic acid	14.14±0.08	8.48±2.26	4.57±1.18	49.66±14.49	9.49±2.59	2.46±0.29	2.60±0.82	6.41±2.43	8.45±2.15	3.33±0.19	2.62±0.21	0
Usnic acid	40.48±17.70	6.63±0.98	20.48±7.19	26.16±16.27	11.90±2.15	1.49±0.57	10.54±1.65	0	0.20±0.03	1.42±0.17	0.96±0.10	2.39±0.72
Vulpinic acid	53.61±17.27	63.62±16.63	0	269.45±20.89	19.00±2.00	12.82±5.59	0	2.47±1.44	3.70±0.84	4.00±1.30	2.52±0.59	1.90±1.25

[a] *As experiments were performed on two different days, signal intensities obtained by reference matrices DHB and HCCA were averaged.*

Table 2. MSE scores for lichen metabolites as matrices at a laser energy of 70% (71.9 µJ) (along with commercial matrices). For illustration purposes, absolute signal intensities were divided by 1250. The last five rows refer to components of the Sigma peptide mixture. Abbreviations: BRA Brassinin, INO inosine, NOT E-notopterol, YOH yohimbin, RUT rutin, GRAM gramicidin A-D, SUB. P substance P, G-T Gly-Tyr dipeptide, V-T-V Val-Tyr-Val tripeptide, LeuEnk Leucine Enkephalin, MetEnk Methionine Enkephalin and Ang. II Angiotensin II.

								HPLC peptide mixture				
Compound	**BRA**	**INO**	**NOT**	**YOH**	**RUT**	**GRAM**	**SUB. P**	**G-T**	**V-T-V**	**LeuEnk**	**MetEnk**	**Ang. II**
Orsellinic acid	0.78±0.07	0.80±0.02	0.87±0.06	0.83±0.02	0.85±0.03	0	0	0	0	0	0	0
MOC	0.86±0.12	0.90±0.03	0.98±0.02	0.98±0.02	0.94±0.02	0	0	0	0	0	0	0
Atranol	0.79±0.24	0.99±0.01	0.98±0.01	0.99±0.01	0.72±0.25	0.42±0.22	0	0	0	0	0	0
Chloroatranol	0.87±0.07	0.97±0.01	0.93±0.05	0.99±0.01	0.95±0.02	0	0	0	0	0	0	0
DHB[a]	0.76±0.05	0.46±0.05	0.97±0.01	0.98±0.016	0.88±0.10	0	0	0	0	0	0	0
HCCA[a]	0.38±0.05	0.66±0.21	0.66±0.08	0.85±0.03	0.46±0.07	0.16±0.22	0.44±0.20	0.13±0.03	0.15±0.06	0.15±0.02	0.14±0.02	0.11±0.03
Chrysophanol	0.60±0.13	0.27±0.08	0.53±0.03	0.95±0.01	0.57±0.11	0	0.05±0.01	0	0	0	0	0
Citreorosein	0.52±0.19	0.94±0.02	0.51±0.20	0.98±0.01	0.29±0.09	0.88±0.02	0.49±0.02	0.25±0.07	0.45±0.06	0.19±0.06	0.09±0.05	0
Haemoventosin	0.66±0.07	0.63±0.06	0	0.92±0.03	0.63±0.06	0	0	0	0	0	0	0
Parietin	0.63±0.20	0.56±0.10	0.60±0.04	0.98±0.01	0.47±0.15	0	0.15±0.03	0	0	0	0	0
Solorinic acid	0.25±0.10	0.32±0.04	0.13±0.02	0.93±0.05	0.23±0.08	0	0.03±0.01	0	0	0	0	0
Dithranol	0.54±0.05	0.23±0.26	0.94±0.01	0.96±0.05	0.61±0.15	0	0.06±0.02	0	0	0	0	0
Didymic acid	0.37±0.16	0.29±0.04	0.06±0.01	0.96±0.02	0.35±0.03	0.31±0.02	0.11±0.03	0.11±0.01	0.10±0.01	0.09±0.01	0.09±0.04	0.08±0.01
Pannaric acid	0.68±0.06	0.82±0.06	0.30±0.03	0.98±0.01	0.55±0.05	0.26±0.09	0.68±0.05	0	0	0	0	0.43±0.09
Placodiolic acid	0	0	0.16±0.02	0.96±0.02	0.48±0.18	0.09±0.05	0.34±0.15	0	0	0	0	0.76±0.04
Porphyrilic acid	0.94±0.03	0.64±0.10	0.68±0.07	0.98±0.01	0.89±0.12	0.58±0.03	0.69±0.01	0.06±0.02	0.15±0.02	0.12±0.01	0.10±0.01	0.09±0.02
Schizopeltic acid	0.47±0.01	0.39±0.11	0.44±0.02	0.98±0.01	0.64±0.01	0.44±0.02	0.34±0.01	0.27±0.07	0.35±0.05	0.14±0.03	0.11±0.03	0
Usnic acid	0.35±0.09	0.09±0.03	0.17±0.01	0.77±0.03	0.11±0.02	0.04±0.01	0.25±0.02	0	0.03±0.02	0.03±0.00	0.03±0.01	0.05±0.01
Vulpinic acid	0.28±0.07	0.33±0.05	0	0.76±0.01	0.20±0.00	0.71±0.03	0	0.09±0.03	0.14±0.04	0.17±0.10	0.11±0.01	0.11±0.01

[a] *As experiments were performed on two different days, signal intensities obtained by reference matrices DHB and HCCA were averaged.*

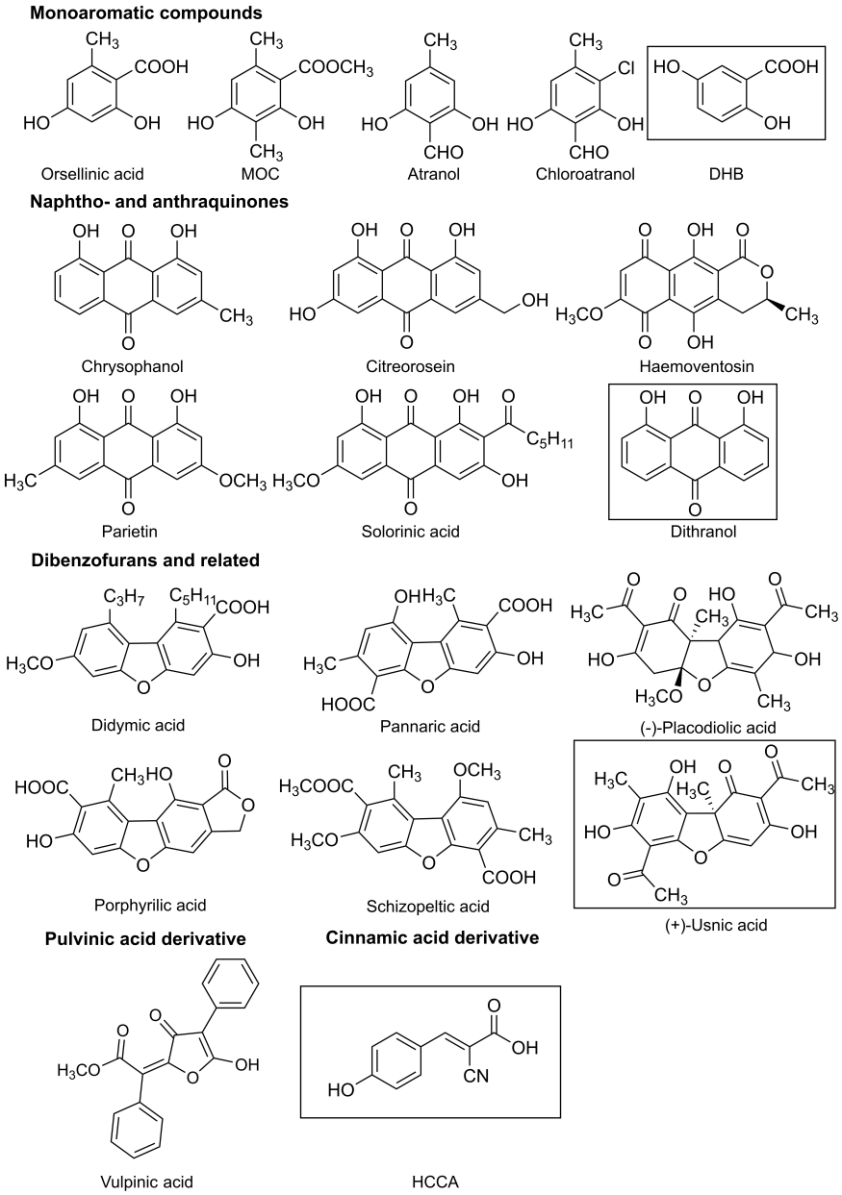

Figure 1. Structures of lichen metabolites that were evaluated for their matrical properties. Molecules in rectangular boxes refer to commercial MALDI matrices.

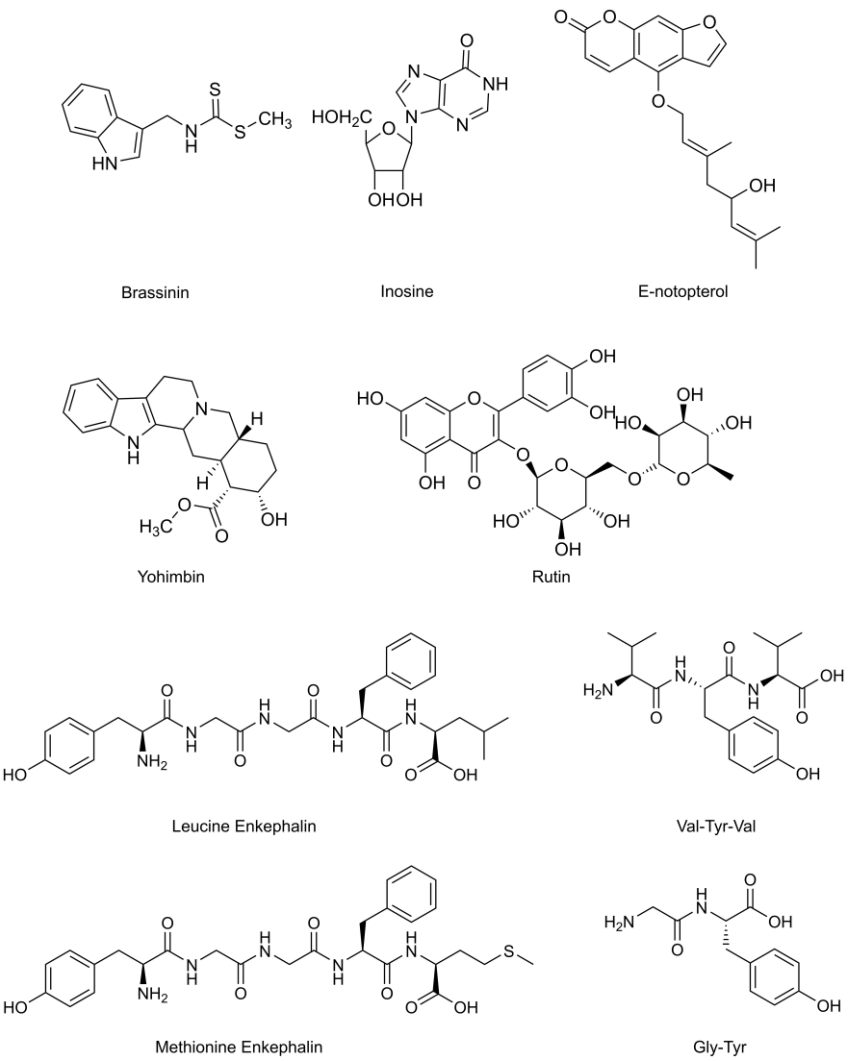

Figure 2. Structures of low molecular weight analytes considered in this study. Due to space limitations, structures of substance P, angiotensin II and gramicidins A-D are not shown.

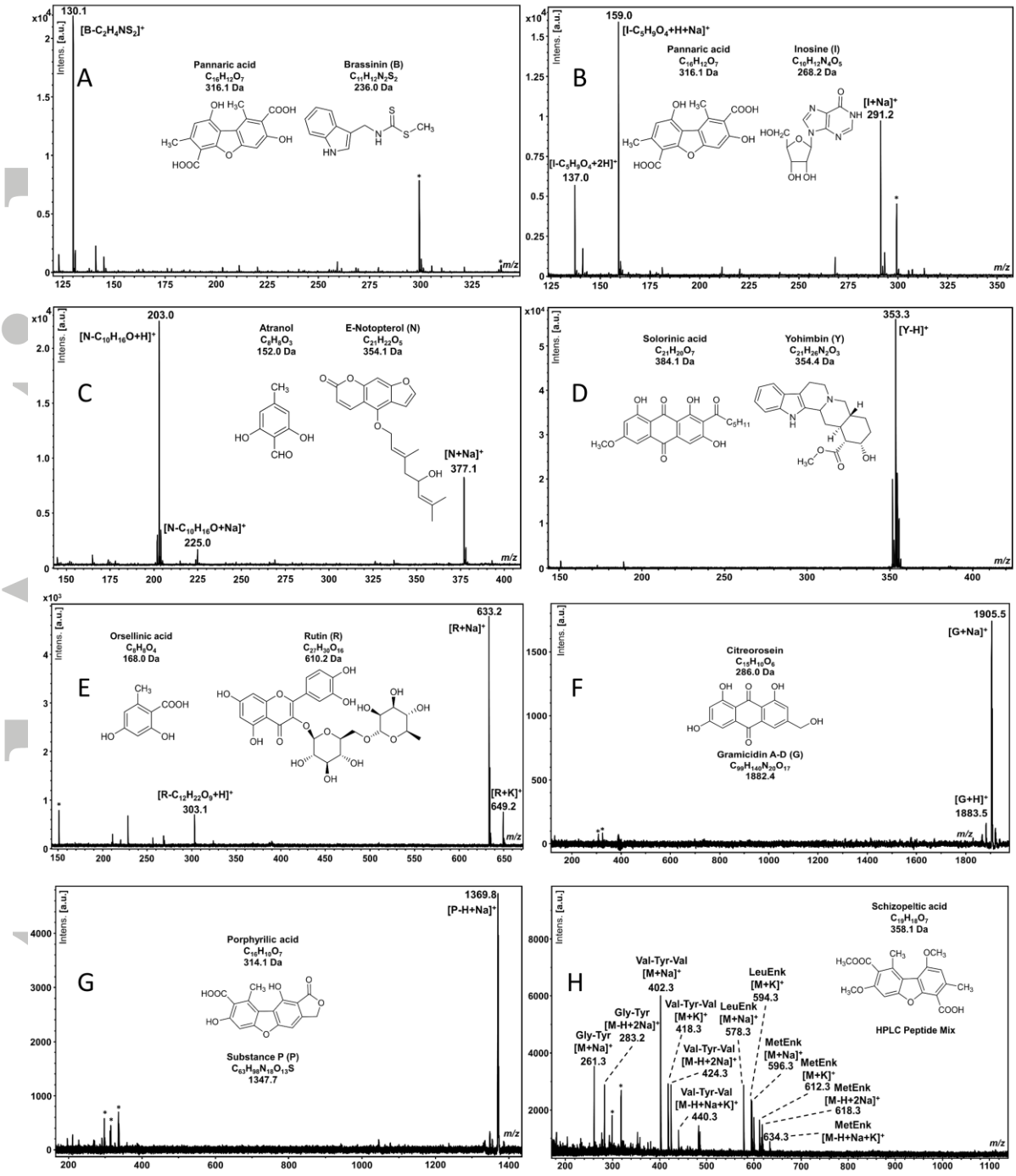

Figure 3. Compilation of some representative PI-MALDI mass spectra obtained using various lichen metabolites as matrices (matrix/analyte) A: pannaric acid/brassinin, B: pannaric acid/inosine, C: atranol/E-notopterol, D: solorinic acid/yohimbin, E: orsellinic acid/rutin, F: citreorosein/gramicidin A-D, G: porphyrilic acid/substance P and H: schizopeltic acid/HPLC peptide mixture. *Matrix signals.