Mass spectrometry-based metabolomics unravel the extensive transfer of bioactive compounds between rye and neighboring plants

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May 23, 2021

Abstract

Translocation of metabolites between different plant species provides important hints in understanding the fate of bioactive root exudates. In the present study, targeted and untargeted mass spectrometry-based metabolomics was applied to elucidate the transfer of bioactive compounds between rye and several crops and weed species. Our results demonstrated that benzoxinoids (BXs) synthesized by rye were taken up by roots of neighboring plant species and translocated into their shoots. Furthermore, we showed roots of the rye plant took up compounds originating from neighboring plants. Among the compounds taken up by rye roots, wogonin was detected in the rye shoot, which indicates the root-to-shoot translocation of this compound. Elucidating the transfer of bioactive compounds between plants is essential for understanding plant-plant interactions, developing natural pesticides and understanding their modes of action.

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Abstract

Translocation of metabolites between different plant species provides important hints in understanding the fate of bioactive root exudates. In the present study, targeted and untargeted mass spectrometry-based metabolomics was applied to elucidate the transfer of bioactive compounds between rye and several crops and weed species. Our results demonstrated that benzoxinoids (BXs) synthesized by rye were taken up by roots of neighboring plant species and translocated into their shoots. Furthermore, we showed roots of the rye plant took up compounds originating from neighboring plants. Among the compounds taken up by rye roots, wogonin was detected in the rye shoot, which indicates the root-to-shoot translocation of this compound. Elucidating the transfer of bioactive compounds between plants is essential for understanding plant-plant interactions, developing natural pesticides and understanding their modes of action.

Keywords: Keywords: Metabolomics, Mass spectrometry, Plant-plant interactions, Secondary metabolites, Bioactive compounds

Introduction

Plant roots exude a complex mixture of organic compounds, including secondary metabolites, which are essential in mediating plant interaction with other organisms (Bais et al., 2006). Root-exuded secondary metabolites can act as defense compounds against microbial pathogens and herbivores (Zhang et al., 2020, Hu et al., 2018) or shape the beneficial microbiome (Sikder et al., 2021, Kudjordjie et al., 2019). Moreover, root exudates containing allelochemicals can directly inhibit or reduce the germination or growth of other plant species (Weir et al., 2004, Hazrati et al., 2021). Other plants must absorb root-exuded metabolites or their breakdown products (Etzerodt et al., 2006, Etzerodt et al., 2008) before acting as growth inhibitors (Chiapusio et al., 2004). However, studies on the transfer of bioactive compounds between plants are scarce, specifically on root uptake from the soil and translocation into the shoots. Elucidation of the transfer of bioactive compounds between plant species is vital for developing commercial natural pesticides (Davies and Caseley, 1999).

Benzoxinoids (BXs) are tryptophan-derived heteroaromatic metabolites that act as natural pesticides and are mainly present in the Poaceae roots, including rye, maize, and wheat (Hazrati et al., 2019, Frey et al., 2009). They are essential in plant interactions with microorganisms (Cotton et al., 2019), herbivores (Wouters et al., 2016), and other plant species (Hazrati et al., 2020). The growth-inhibitory ability of BXs has been confirmed on several plant species (Schulz et al., 2013). Just recently, Hazrati et al. (2020) demonstrated that root exuded BXs can be taken up by hairy vetch plants and subsequently translocate into their shoots. Studying whether root uptake and translocation of BXs occur in the same manner in other plant species is of particular interest from an agronomic perspective because it may promote the development of selective natural herbicides. Here we are comparing the root uptake and translocation of BXs in several crop and weed species.

Metabolomics represents a field of research, which provides us a better understanding of complex biological systems (Cevallos-Cevallos et al., 2009). There are two distinct metabolomics approaches: targeted and untargeted. Targeted metabolomics refers to identifying and quantifying selected groups of metabolites or validation of biomarkers identified using non-targeted metabolomics (Lu et al., 2008, Roberts et al., 2012). Targeted metabolomics deals with the known metabolites of interest and, therefore, the coverage of detected metabolites is limited (Vrhovsek et al., 2012). Untargeted metabolomics aims to globally profile the metabolome and gather as much information on metabolites as possible. Untargeted metabolomics is often applied to generate hypotheses and discover biomarkers (Vinayavekhin and Saghatelian, 2010). High-resolution mass spectrometers (HRMS) are routinely used for untargeted metabolomics studies due to their high sensitivity and selectivity, which maximize metabolic coverage (Theodoridis et al., 2012). Ultra-high-performance liquid chromatography (UHPLC) hyphenated with an HRMS is currently the dominant technique for global metabolite profiling of complex biological samples, e.g. from plants, due to its versatility in separation and detection of compounds with a wide range of polarities, as well as its robustness (Pezzatti et al., 2019). Metabolite annotation and identification from the vast amount of generated features is the most significant bottleneck of untargeted mass spectrometry-based metabolomics (Misra and van der Hooft, 2016, Dunn et al., 2013). Therefore, in most cases, mass spectrometry-based metabolic profiling aims to identify known/unknown putative metabolites, which could be confirmed by MS experiments or by comparing with authentic standards (Lee et al., 2010, Krauss et al., 2010). There are several studies, which used targeted approach to show the root uptake and translocation into shoot of particular metabolites or a specific class of metabilites (Hazrati et al., 2020, Chiapusio et al., 2004, Lewerenz et al., 2020). Up to date, no studies have revealed two-way transfer of bioactive compounds between plant species using an untargeted metabolomics approach. In the present study , a combination of targeted and untargeted mass spectrometry-based metabolomics was applied to elucidate the extensive transfer of bioactive compounds between rye and several crops and weeds species.

Material and Methods

Chemicals

2-Benzoxazolinone (BOA) and 6-methoxy-benzoxazolin-2-one (MBOA) were purchased from Sigma-Aldrich. The following eight BXs standards were obtained as part of an on-going patenting process in our lab: 2-hydroxy-1,4-benzoxazin-3-one (HBOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA), 2-β-d-glucopyranosyloxy-1,4-benzoxazin-3-one (HBOA-glc), 2-β-d-glucopyranosyloxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA-glc), 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), 2-β-d-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-glc), double hexose derivative of DIBOA (DIBOA-glc-hex) (structure not fully elucidated), and double hexose derivative of HBOA (HBOA-glc-hex) (structure not fully elucidated). 2-β-d-Glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-glc) was obtained as described in a previous study (Pedersen et al., 2017). High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were purchased from Rathburn (Walkerburn, Scotland); MS-grade methanol, acetonitrile, and isopropanol from Fischer Scientific (Loughborough, UK); and acetic acid from Baker (Griesheim, Germany). HPLC-grade water was obtained from a Milli-Q system (Millipore, Billerica, MA) for the analysis using LC-MS/MS. HPLC-MS grade water was purchased from Fisher Scientific for analysis in UHPLC-QTOF-MS.

Pot experiment

One-liter pots filled with sandy loam field soil (2.8% organic matter, 11.5% clay, 28.4% silt, and 57.2% sand) were used as the growth medium. Plants were grown under controlled conditions in a greenhouse at a 16/8 h photoperiod with a temperature of 22/18 °C (day/night) and were watered through a sub-irrigation system. Six rye seeds were sown in the center of the pot as target species, and 12 seeds of a neighboring crop/weed species were sown in a ring around the rye plants. After germination of the seeds, the number of rye and neighboring seedlings were thinned to 4 and 8 plants per pot. Neighboring plant species included in the study was seven crop species: rye, Alexandrian clover (*Trifolium alexandrinum*), hairy vetch (*Vicia villosa*), fodder radish (*Raphanus sativus*), oat (*Avena sativa*), subterranean clover (*Trifolium subterraneum*), Austrian pea (*Pisum sativum*) and two weed species: Lolium (*Lolium multiflorum*) and Sinapis *Sinapis arvensis* . Eight replicates were used for each treatment. Plants were harvested five weeks after sowing. At harvest, rye and neighboring plants were carefully separated from each other. Plant roots were slightly shaken to remove attached soil, washed with distilled water, and separated from their shoot. Harvested plant material was immediately transferred into the liquid nitrogen before transferring to a freezer at -80 @C. Samples were freeze-dried, and the lyophilized root and shoot material was grounded to a fine powder using a mechanical disrupter Genogrinder 2010 from Spex (Metuchen, NJ) at a speed of 1500 rpm for 90 s (repeated twice).

Metabolite Extraction from Plant Material

Homogenized ground plant material (20 mg) was transferred into an Eppendorf tube, and 1 ml of 80% methanol containing 1% acetic acid was added. Samples were ultrasonicated for 45 min and centrifuged (Sigma 1–14 K micro-centrifuge, Buch and Holm, Herlev, Denmark) at 4500 g and 21°C for 10 min. Subsequently, the supernatant (˜ 0.9 ml) was collected in a new Eppendorf tube. One ml of extraction solvent was added to the pellet, and ultrasonication, centrifugation, and supernatant collection were repeated. Finally, ˜ 1.8 ml of supernate was collected and stored at -20°C. Extracts were filtered through a 0.22 μ m PTFE syringe filter and transferred into glass vials to quantify BXs by HPLC-QqQ-MS. For qualitative analysis by UHPLC-QTOF-MS, 1ml of extraction was transferred into Eppendorf tubes, and the solvent was evaporated until dryness in a SpeedVac (SPD121P, Thermo Scientific, USA) for six hours. Then 0.2 ml of 80% MeOH/H_2O was added to the Eppendorf tube, and it was centrifuged for 120 seconds. Extracts were transferred to vials for further qualitative analysis.

Quantification of BXs in Plant Material by LC-QqQ-MS

The plant extracts were analyzed by LC-MS/MS using an Agilent 1100 HPLC system coupled with a 3200 QTRAP mass spectrometer (AB SCIEX, Foster City, CA). The extracts were diluted to fit the signals from the analytes into the range of the standard curve. Negative electrospray ionization (ESI-) was used, and the mass spectrometry was operated in the multiple reaction monitoring mode (MRM). Analyst Software (version

1.6.2) was used for instrument control, data acquisition, and subsequent quantifications. The chromatography was performed using a 250 mm × 2 mm id 4 μ m Synergi Polar RP-80Å column (Phenomenex, Macclesfield, U.K.) with a flow rate of 300 μ L/min and an injection volume of 25 μ L. The temperatures of the column oven and autosampler were set at 30 and 10 °C, respectively. Two mobile phases (A: 7% acetonitrile in water and B: 78% acetonitrile in water, each containing 20 mM acetic acid) were used in a linear gradient system. The binary gradient was as following: 100 % A at 0-1 min, 92 % A at 1-3 min, 90 % A at 3-13 min, 30 % A at 13-14 min, 10 % A at 14-17 min, 100 % A at 17-25 min for equilibration. Standard compounds were used to optimize the compound-dependent parameters (Table 1). The most intense mass transition was considered as a quantifier and the second mass transition as a qualifier. Quantifications were done based on standard curves prepared in the range of 0.39-400 ng/mL. Data points of the standard curves were weighted according to x^{-1}.

Untargeted metabolomic profiling of plant samples using UHPLC-QTOF-MS

The metabolite profiling of roots and shoots was performed using an Agilent Infinity 1290 UHPLC system coupled to Agilent 6545 quadrupole time of flight (QTOF) mass spectrometer equipped with an Agilent dual-jet stream electrospray ion source. The whole system was controlled by Masshunter software. An HSS T3 (C18) 1.8 μ m, 2.1 mm × 150 mm was used for chromatographic separation of non-targeted compounds with a flow rate of 0.5 mL/min and a sample volume of 10 μ L was injected for each run. The temperature of the column oven was set at 40 °C. The mobile phases were (A): 100% LC-MS grade water with 0.02 M formic and (B): acetonitrile with 0.02 M formic acid. The gradient was as follows: 0 min, 0% B; 1 min, 0% B; 3 min, 10% B; 10 min, 70% B; 15 min, 100% B; 17.5 min, 100% B; 18 min, 0% B; 21 min, 0% B. Spectra were acquired in full scan MS1 and data-dependent MS2. Data were collected in ESI($\pm$) modes with a mass range of m/z 75–1500 Da. QTOF-MS parameters were set as follows: Fragmentor voltage at 120 V, capillary voltage at 4000 V, skimmer voltage at 65 V, collision energy at 30 eV, drying gas temperature at 325 °C (8 L/min), nebulising gas pressure at 40 psi, sheath gas temperature and flow at 300 °C and 12 L/min, respectively.

Data processing, statistics, and visualization

To compare the biomass and quantification of BXs in the plants, a one-way ANOVA with post-hoc Tukey's test was applied to compare each group with the other groups. Comparisons were performed using GraphPad Prism version 8.4.2 (La Jolla California, USA). Acquired raw metabolomics data were converted into ABF files using the freely available converter (*https://www.reifycs.com/AbfConverter/index.html*). Spectra deconvolution, peak alignment, gap filling, and metabolite annotation were performed by MS-DIAL software (ver. 4.38). NIST17 (*https://chemdata.nist.gov*) and MoNA (*https://mona.fiehnlab.ucdavis.edu*) were used for mass spectral library searches. MSP file containing MS/MS spectra, MS^1 isotopic spectrum, metabolite name, adducts, reverse-dot score, and tentative formula were exported from MS-DIAL for further data processing (Tsugawa et al., 2015). Detailed parameters of MS-DIAL can be found in the supplementary materials (Table S1). MS-FLO was utilized to improve the feature list's quality by curating the features for duplicates and adducts (DeFelice et al., 2017). The data file was filtered by removing duplicate reported metabolite before submitted to statistical analysis. The untargeted metabolomics data were submitted to the log-transformation and Pareto method. Principle component analysis (PCA) was performed for all the annotated compounds with SIMCA version 17.0 (Umea, Sweden). The hierarchical clustering heatmaps of normalized metabolites (peak intensities were log-transformed and pareto scaled) were made by MetaboAnalyst version 5.0 (Chong et al., 2018). ClassyFire was used to classify the annotated compounds based on their reported InChIKey from MS-DIAL (Djoumbou Feunang et al., 2016). All the raw data from untargeted metabolomics analysis can be found in the supplementary materials.

Results

Quantification of BXs in roots and shoots of neighboring plants

Six aglycone BXs (DIBOA, DIMBOA, BOA, HBOA, HMBOA and MBOA) and six glycoside BXs (DIBOA-glc, DIMBOA-glc, HMBOA-glc, HBOA-glc, HBOA-glc-hex and DIBOA-glc-hex) were identified and quan-

tified in the roots of eight neighboring plants species (Figure 1). DIBOA, DIBOA-glc, DIMBOA and DIMBOA-glc were found at the highest concentrations in neighboring plants. The concentration of BXs was significantly lower in Sinapis and subterranean clover compared to oat. Among the 12 BXs absorbed by the roots of neighboring plants, four (DIBOA, DIBOA-glc, DIBOA-glc-hex and DIMBOA-glc) were also present in the shoots. DIBOA-glc was the dominant BX present in the shoots of neighboring plants. The concentrations BXs in the shoots of Lolium were significantly higher than other plant species. Figure S1 shows the different concentrations of each BX in the shoot of the neighboring plant.

Metabolite profiling of rye samples

Untargeted LC-MS analysis detected 11670 ion features, of which 369 were annotated by spectrum matching with the reference mass spectra in the public and licensed libraries. The PCA score plot of the raw data acquired by HHPLC-QTOF-MS showed the accuracy and robustness of the instrument based on the grouping of QC and Blank samples (Figure S2).

Metabolites present in roots of rye plants growing alone were removed from the feature list, and only 58 metabolites originating from neighboring plants were kept. Figure 2 shows the classification of absorbed metabolites at superclass and class levels. Nearly 60% of the metabolites belonged to the phenylpropanoids and polyketides superclass, and flavonoids were the most abundant compounds taken up by rye root at the class level.

The PCA score plots (Figure 3) revealed the grouping of the samples based on the taxonomic relatedness of the neighboring species. Rye root samples growing with Alexandrian clover and subterranean clover that belongs to the same genus (Trifolium) were grouped. Root samples from rye plants growing together with fodder radish and Sinapis, two species belonging to the Brassicaceae family, were also grouped. Root samples of rye plants growing with oat, pea and hairy vetch were separated and did not group with other samples. The roots of the rye plant growing with Lolium did not have a distinct separation from rye plants growing alone. This observation suggests that Lolium and rye have similar metabolomes, and many of the metabolites exudated by Lolium plants and absorbed by rye were already present in rye roots and, hence, excluded in the data filtering process.

Figure 4 shows the hierarchical clustering heatmap of the metabolites absorbed by rye roots from plants growing alone or together with the eight other plant species. The intensity of all annotated metabolites in rye roots from plants growing alone was nearly zero. Therefore, we assumed that all the annotated metabolites were taken up from neighboring plants. Like the PCA analysis, clustering of heatmap indicated that rye root samples from plants grown with two clover species (Alexandrian and subterranean) clustered together and, similarly, samples from rye plants growing with fodder radish and Sinapis were clustered together. Malonylglycitin, wogonoside, and sissotrin were the annotated metabolites with the highest peak intensity in rye plant roots growing with legumes.

4-Methoxyglucobrassicin was the dominant annotated metabolite in roots of rye plants growing together with fodder radish and Sinapis (Figure S3).

Untargeted LC-MS analysis of rye shoot samples detected 5637 ion features, of which 176 features were annotated by spectrum matching with the reference mass spectra in the public and licensed libraries. The PCA score plot of 176 annotated metabolites in rye shoot showed no clear grouping of samples (Figure S4). Among all the annotated metabolites in the shoot, wogonin was the secondary plant metabolite found in the shoots of rye plants growing with hairy vetch, Alexandrian clover, and subterranean clover. Figure 5 shows the intensity of wogonin in rye root (A) and rye shoot (B). Wogonin was present in the roots of rye plants growing with Austrian pea, but it was absent in the shoots.

Discussion

A previous study confirmed that root-exuded BXs could be taken up by hairy vetch (*Vicia vilosa*) roots and translocated to the shoots (Hazrati et al., 2020). Nevertheless, it was unclear if the uptake of root-exuded BXs varied among plant species. This study is the first to compare the uptake and translocation of root-exuded

BXs in several plant species. Several studies show the uptake root uptake of secondary metabolite and their transformation in roots (Buer et al., 2007, Selmar et al., 2019). In addition, recent studies have shown BXs taken up by root can be translocated to the shoots (Hazrati et al., 2020). However, no studies have intended to unravel and provide a complete picture of the uptake of exuded/decomposed organic compounds present in the rhizosphere using an untargeted metabolomics approach. Here we applied mass spectrometry-based metabolomics and observed that roots of rye plants growing with other plant species contain numerous compounds that most likely have been exuded by neighboring plant species.

Our results showed that neighboring plant species could absorb all 12 BXs. This is in accordance with a previous study by Hazrati et al. (2020), which reported root uptake of 12 BXs by hairy vetch plants growing together with one rye plant. The concentration and composition of absorbed BXs varied between neighboring plant species. For instance, Sinapis and subterranean clover roots absorbed the lowest concentrations of BXs while oat took up the highest amount of BXs. This could be due to differences in the architecture and volume of neighboring plant roots. Roots of Sinapis and subterranean clover were denser and occupied a smaller volume than the roots of the other neighboring plant species (based on visual observations at harvest). Less root volume may reduce the exposure of roots to BXs present in the rhizosphere. On the other hand, oat roots have a root architecture very similar to rye. Their roots grew alongside the roots of the rye plants, which was expected to increase the exposure of their roots to BXs exuded from rye roots.

Differences in the composition and concentration of BXs in the roots of neighboring plant species may be related to microbial degradation. Burns et al. (2015) suggested that plant species identity was the main determinator of microbial community composition in the rhizosphere. Moreover, plant-associated microorganisms have been shown to degrade organic compounds in the rhizosphere (McGuinness and Dowling, 2009). For instance, Friebe et al. (1996) found that root colonized bacteria converted BOA to 2-amino-H-phenoxazin-3-one and 2-acetylamino-H-phenoxazin-3-one in *Avena sativa* . In the present study, each of the neighboring plants may have shaped the microbial communities in the rhizosphere differently, resulting in different degradation patterns and, hence, different BX compositions and availability in the rhizosphere.

Simple diffusion across biomembranes has been suggested as a mechanism for the uptake of xenobiotics (Trapp and Legind, 2011). Similarly, it was shown that plant uptake of alkaloids is by simple diffusion through the plasmalemma of the root cells (Nowak et al., 2016, Yahyazadeh et al., 2017). Simple diffusion requires specific physicochemical properties of organic compounds, such as an appropriate Log Pvalue (Inoue et al., 1998). Previous studies demonstrated that compounds having a Log P value of -1 to 3 could be taken up by roots through diffusion (Limmer and Burken, 2014, Trapp, 2000). Our observations are in accordance with this conclusion. Log P values for the BXs taken up by neighboring plant roots and many flavonoids, which were the dominant class of secondary plant metabolite absorbed by rye roots, are within the range of -1.6 to 3 (https://pubchem.ncbi.nlm.nih.gov/). Log P values for the BXs studies in this experiment are shown in Table 2.

Only four BXs were translocated into the shoots of neighboring plants, and only one metabolite (wogonin) was translocated into the shoot of rye plants. This may be due to high limits of detection, soil degradation, and/or metabolization of metabolites in neighboring plants. Rapid metabolism in plants may decrease the concentration of BXs in the target plants. It is demonstrated that contaminants such as pesticides may be transformed inside the plant or on the leaf surface by the plants or microorganisms living on the plants (Trapp and Legind, 2011). Secondary metabolites in the plants may face a similar fate and, therefore, not reach the shoots. Briggs et al. (1982) showed that translocation of non-ionic pesticides from root to shoot is related to their polarity by a Gaussian curve distribution. They concluded that the translocation of pesticides to shoots is most efficient for pesticides with intermediate polarities (log P =1-3). In the present study, Log P for all the tested BXs was between -1.6 and 1, and we could not find a significant correlation between the polarity of BXs and their translocation to shoot. Lolium's shoot contained the highest amount of BXs, whereas shoots of Austrian pea, fodder radish, and Sinapis contained the lowest concentration of BXs. The aboveground biomass of Lolium was lower than of all the other neighboring plants (data not shown). Hence, it cannot be excluded that the lower biomass was partly the cause of the higher accumulation of

BXs in Lolium shoots. Wogonin and its glucuronide, wogonoside, have various biological effects such as anti-cancerogenic, anti-inflammatory, and anti-angiogenesis in humans (Wang et al., 2018, Kim et al., 2018, Lin et al., 2012). It is, therefore, very likely that they act as a defense compound against biotic and abiotic stresses in plants. In the rye root, wogonoside was found in a much higher concentration than wogonin. However, wogonin was detected in the rye shoot, and wogonoside was not detected. We assume that wogonin found in rye shoot originates from the wogonoside present in the root. Wogonoside likely undergoes deglycosylation through enzymatic reactions or microbiome metabolism and covert into wogonin in the rye shoot. It has been reported that deglycosylation of wogonoside enhances its bioactivity by inhibiting the growth of cancer cells (Wang et al., 2018, Yu et al., 2013). Conversion of wogonoside to wogonin in plant shoot may increase their growth inhibitory effects.

Conclusion

In the present study, extensive transfer of bioactive compounds between rye and several crops and weed species as neighboring plants has been revealed by applying mass spectrometry-based metabolomic. The results from targeted metabolomics demonstrated that all the neighboring plants in this experiment absorbed BXs by their root and translocated them to shoots. Meanwhile, we showed that the composition and concentration of absorbed BXs in tested plants were varied among different plant species. Furthermore, the results from untargeted metabolomics indicated that the rye plant roots took up compounds originating from neighboring plants. Wogonin, which is a phytotoxic flavonoid, was detected in both rye root and shoot. Elucidating the root absorption and root-to-shoot translocation mechanisms is essential for future utilization of the bioactive compounds as natural agrochemicals.

Funding

This study was funded by a project (28180) at the Graduate School of Science and Technology, Aarhus University (GSST, AU), Denmark.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that may influence the work reported in this paper.

Acknowledgments

We would also like to thank Kirsten Heinrichson and Bente Birgitte Laursen for their excellent laboratory technical assistance. The authors wish to thank Dr. Aaron John Christian Andersen, Mette Amfelt, and Xinhui Wang from the Department of Biotechnology and Biomedicine, Technical University of Denmark, for their valuable support.

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Table 1. Optimized Compound dependent MS parameters for BXs

Compound	**Q1 (m/z)**	**Q3 (m/z)**	**DP (V)**	**EP (V)**	**CEP (V)**	**CE (V)**	**CXP (V)**
BOA	134	42	-50	-9.5	-16	-40	0
HBOA	164	108	-30	-4.5	-12	-16	-2
HBOA-glc	326	164	-40	-10	-22.67	-20	-2
HBOA-glc-hex	488	164	-80	-10.5	-28.83	-36	-2
HMBOA	194	123	-35	-10.5	-16	-28	-2
HMBOA-glc	356	194	-45	-9.5	-23.81	-40	-2
DIBOA	180	134	-15	-8.5	-14	-8	-2
DIBOA-glc	342	134	-30	-11	-23.28	-22	-2
DIBOA-glc-hex	504	134	-45	-9.5	-29.43	-42	-0
MBOA	164	149	-30	-5	-14	-22	-2
DIMBOA	164	149	-12	-7	-16.52	-16	-1.2
DIMBOA-glc	372	164	-35	-10.5	-24.42	-20	-2

DP: declustering potential, EP: entrance potential, CEP: cell entrance potential, CE: collision energy, CXP: cell exit potential. The parameters for the second transition are not reported here because they were only used to confirm compound identification.

Table 2. Log P value of studies BXs from PubChem database

Compound	Log P	PubChem CID	Compound	Log p	PubChem CID
BOA	1.2	6043	DIBOA-glc	-1.6	58114415

Compound	Log P	PubChem CID	Compound	Log p	PubChem CID
DIBOA	0	28495	DIMBOA-glc	-1.6	4480305
DIMBOA	0	2358	HBOA-glc	-1.3	14605136
MBOA	1.1	10772	HMBOA-glc	-1.3	77195052
HBOA	0.3	322636	DIBOA-glc-hex	*	*
HMBOA	0.3	152213	HBOA-glc-hex	*	*

*Log P value is not reported in PubChem database. Due to the hexose presence in their structure, their Log P value should be smaller than their single glycoside form (DIBOA-glc and HBOA-glc).