Collision-Induced Dissociation of the A + 2 Isotope Ion Facilitates Glucosinolates Structure Elucidation by Electrospray Ionization-Tandem Mass Spectrometry with a Linear Quadrupole Ion Trap

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An approach is presented that can be of general applicability for structural elucidation of naturally occurring glucosinolates (GLSs) in crude plant extracts based on the fragmentation of isotopic A and A + 2 peaks. The most important fragmentation pathways were studied by tandem mass spectrometry (MS^n, n = 2, 3) using a linear quadrupole ion trap (LTQ) upon GLSs separation by optimized reversed-phase liquid chromatography (RPLC) and electrospray ionization (ESI) in negative ion mode. As the LTQ MS analyzer ensures high sensitivity and linearity, the fragmentation behavior under collision induced dissociation (CID) of the isotopic peaks A and A + 2 as precursor ions was carefully examined. All GLSs (R−C$_7$H$_{11}$O$_9$NS$_2^-$) share a common structure with at least two sulfur atoms and significant isotopic abundance of ^{34}S. Thus, dissociation of the +2 Da isotopomeric ions results in several fragment ion doublets containing a combination of ^{32}S and ^{34}S. Accordingly, their relative abundances allow one to speed up the structural recognition of GLSs with great confidence, as it produces more structurally informative ions than conventional tandem MS performed on A ions. This approach has been validated on known GLSs bearing two, three, four, and six sulfur atoms by comparing expected and measured isotopic peak abundance ratios (I_A/I_{A+2}). Both group- and compound-specific fragments were observed; the predominant pathway of fragmentation of GLSs gives rise to species having the following m/z values, [M − SO$_3$ − H]$^-$, [M − 196 − H]$^-$, [M − 178 − H]$^-$, and [M − 162 − H]$^-$ after H rearrangement from the R− side chain. The present strategy was successfully applied to extracts of rocket salad leaves (*Eruca sativa* L.), which was sufficient for the chemical identification of a not already known 6-methylsulfonyl-3-oxohexyl-GLS, a long-chain-length aliphatic glucosinolate, which contains three sulfurs and exhibits a deprotonated molecular ion at m/z 494.1.

Glucosinolates (GLSs) are sulfur-rich secondary metabolites found in certain dietary vegetables and condiments.[1] They exhibit a common chemical structure consisting of a β-D-1-thioglucopyranose moiety bearing on the anomeric site an O-sulfated thiohydroximate function; different glucosinolates have different side groups (−R), and the variation in the side group is responsible for the variability of their biological activities.[1−4] GLSs are classified as aliphatic, aromatic, and indole glucosinolates, depending on whether they are derived from methionine, phenylalanine or tyrosine, or tryptophan, respectively. More than 120 different glucosinolates are known to occur naturally in plants that belong mainly in the order Capparales, principally in the families Brassicaceae, Resedaceae, and Capparidaceae, although their presence in other families has also been reported.[1,5,6]

As already mentioned, several natural GLSs occur in plants, and their analysis requires chromatographic separation. Generally, liquid chromatography (LC) is preferable and is more accurate for determining glucosinolate content. However, some compounds have very similar chemical behavior and may coelute. The introduction of mass spectrometry (MS) as a detecting means has enabled the acquisition of mass spectra, which are of great help to identify peaks and characterize unresolved compounds by specific mass spectral features. The mass spectrum has also served as a "confirmatory test" for resolved chromatographic peaks since two methods of identification, that are fundamentally different, may be generated, i.e., chromatographic retention time and mass spectrum. With the addition of a new and properly tailored dimension in tandem MS, the LC-MS/MS offers a very powerful and important tool in examination of GLSs that will go beyond most common sulfur containing plant metabolic compounds. The facile ionization of these secondary metabolites

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obviously renders them amenable to negative ion mass spectrometry. To study the mass spectrometric behavior of GLSs, a range of ionization methods has been explored, including fast atom bombardment (FAB),[7−10] thermospray,[11] atmospheric pressure chemical ionization,[12] matrix-assisted laser desorption ionization,[13] and electrospray ionization (ESI).[14−19] However, the characterization of intact GLSs was insufficient, as no extensive fragmentation was carried out. Recently, a fully validated LC-ESI-MS method was developed for the identification and quantification of glucosinolates naturally occurring in the plant of *Eruca sativa*, which enables a complete structure elucidation of such phytochemicals by direct LC-MS^n (n = 3 to 4).[20] Furthermore, Fabre et al.[21] have extended the analysis of GLSs using both negative and positive ion ESI-tandem mass spectrometry, which resulted suitably not only for the characterization of a single compound but also for resolving complex mixtures. Despite improvements made by these experimental approaches, structural interpretation of the data is still a major bottleneck in the glucosinolate identification process because it is very time-consuming.

All glucosinolates share a common structure and possess at least two sulfur atoms.[1] Therefore, a prominent isotopic A + 2 peak (i.e., ∼2 mass heavier than monoisotopic ion A) can be observed in most MS spectra of these compounds which represents a sum of contributions from isotope combinations differing by a few mDa (e.g., one ^{34}S, one ^{18}O, two ^{13}C, two ^{15}N, one ^{13}C and one ^{33}S, etc.) and mainly attributable to ^{34}S, as the natural occurrence of this isotope is relatively high.[22] Of all the LC-MS approaches, the possibility to extract further information from tandem MS applied both on the isotopic A and A + 2 peaks has not been most utilized within compounds containing one or more sulfurs in the chemical formula. Indeed, the possibility of the use of tandem MS on individual isotopic peaks (e.g., A + 1, A + 2, etc.) has not widely been addressed in the literature, and yet, it is of great significance as regards achieving potentially useful information for mass spectra interpretation, leading to chemical composition and structure elucidation. The first report was that of Singleton et al.[23] in which chlorinated and brominated organic compounds in simple and complex mixtures were investigated by tandem MS of their A + 2 isotope ions upon electron impact ionization. In 1995, Lehmann and co-workers reported the identification of cholesterol−sulfate by tandem MS of its A + 2 molecular anions by direct infusion and ESI/MS,[24] and later, Lehmann demonstrated the brutto formula determination of fragment ions based on tandem MS of A + 1 and A + 2 ions, along with a comparison between calculated and observed fragment ion isotopic patterns in a phospholipid species.[25] Most recently, the innovative and interesting works of Rockwood et al.[26] and Ramaley and Cubero Herrera[27] added something new in the panorama of tandem MS, resulting from the dissociation of single isotopic peaks. In particular, Rockwood et al.[26] reported a general theory for predicting the isotopic distributions providing several examples for comparisons between experimental and calculated patterns. Ramaley and Cubero Herrera[27] described the use of a polynomial expansion algorithm to calculate the isotope patterns for a precursor ion, neutral loss, and MS^n product ion tandem mass spectra, and experimental examples of the software application were presented. All these contributions are fine examples of what it is possible to glean from a molecular ion cluster and, specifically, from precursor ion spectra of nonmonoisotopic A + 1, A + 2, etc. isotopomers whereby decomposition pathways are sampled to produce structurally more useful information and a comparison between expected and experimental abundance ratios among isotopic peaks.

Here, a comprehensive fragmentation pattern of GLSs is illustrated which accounts for several of the diagnostic ions formed upon collision induced dissociation (CID) in a linear quadrupole ion trap. Such an added selectivity is extremely important when performing identification of GLSs by MS, as it can make the difference between obtaining an interpretable spectrum or not. Experimental data discussing isotopic peak abundance ratios for several GLSs having two, three, four, and six sulfur atoms are presented. The structural information obtained by the MS/MS experiments are used in combination with retention time to characterize unknown components in crude plant extracts. We wish to highlight that tandem MS experiments on isotopic A + 2 ions can be set up directly on the instrument without any further manipulation. Indeed, this approach is very helpful and works efficiently in a mixture analysis with all investigated known and unknown GLSs. Examples are given that illustrate the capability of the method for the characterization of representative vegetative extracts of rocket salad leaves (*Eruca sativa* L.).

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MATERIALS AND METHODS

Chemicals. Sinigrin monohydrate from horseradish (99%) was obtained from Sigma-Aldrich (Steinheim, Germany). Progoitrin/epiprogoitrin, glucoraphanin, glucoerucin, gluconapin, and glucoiberin were purchased from C2 Bioengineering Aps (Karlslunden, Denmark). Methanol and acetonitrile (ACN), both LC-MS grade, and formic acid (99%) were from Carlo Erba (Milan, Italy). Ultrapure water was produced using a Milli-Q RG system from Millipore (Bedford, MA).

Instrumentation and Separation Conditions. All experiments were performed using a LC system coupled to a linear quadrupole ion trap (LTQ) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). LC separation was performed at ambient temperature on a Discovery C18 column, 250×4.6 mm i.d., 5 μm particle size (pore size, 180 Å), equipped with a Discovery C18 (20×4 mm i.d.) security guard cartridge (Supelco Inc., Bellefonte, PA). Standard solutions and plant extracts were injected into the column via a 20 μL sample loop. The mobile phase was a gradient prepared from the proportional mixing of two solvents: 0.1% HCOOH water solution (component A) and ACN (component B). The gradient program was as follows: 90%:10% (A/B, v/v) linear gradient to 76%:24% (A/B, v/v) in the first 10 min; increased to 40%:60% in the next 2 min; and changed to 90%:10% (A/B, v/v) in the subsequent 3 min; finally, the column was reconditioned for 5 min to the start conditions. Cycle time was 20 min, and flow rate was 1.0 mLmin^{-1}. A flow split of 1:3 was allowed to enter to the ESI source.

The negative ion mode for ESI-MS and tandem MS analyses was selected, working under the following optimized experimental conditions by direct infusion of standard solutions into the ionization source: ESI needle voltage of -4.60 KV, capillary voltage of -22 V, temperature of the heated capillary of 350 °C, and the sheat gas (N$_2$) flow rate of 80 (arbitrary units). Systematic MS/MS experiments were performed by changing the relative collision energy and monitoring the intensities of the fragment ions. All analyses were achieved under automatic gain control (AGC) conditions using helium as the damping as well as the collision gas for MS/MS experiments. The collision energy was varied according to the ion of interest, typically 20−35% (100% corresponding to a 5 V excitation voltage), and was adjusted to get a precursor ion peak of 20% relative intensity or less (when possible). Isolation width was set at 1.5 Da. Normally, an activation $q = 0.25$ (to allow detection of sulfate ion at m/z 97), and activation time of 30 ms was applied for MS^2 and MS^3 acquisitions. Data acquisition and analysis were accomplished using Xcalibur software package (version 2.0 SR1 Thermo Scientific). The chromatographic raw data were imported, elaborated, and plotted by SigmaPlot 9.0 (Systat Software, Inc., London, UK). ChemDraw Pro 8.0.3 was employed to draw chemical structures (CambridgeSoft Corporation, Cambridge, MA).

Plant Material and Sample Preparation. Samples of rocket salad leaves (Naturfresco, COF Srl, Porto Salvo, Italy) purchased from a local market were frozen at -20 °C to inactivate myrosinase and then lyophilized and ground in a laboratory mill to a fine powder. The extraction procedure was based on a previous report from our group.[20] In brief, 500 mg of ground sample was extracted with 8 mL of 70% v/v aqueous methanol solution at 70−80 °C for about 10 min and sonicated at 80 °C for 10 min. Then, the extract was centrifuged at 5000 rpm (3100 g) at 4 °C for 10 min, and the supernatant was removed using a syringe and filtered through a 0.22 μm nylon filter (Whatman, Maidstone, UK). The extraction procedure was repeated again with 5 mL of solvent; the supernatants were combined and concentrated by evaporation to dryness at 40 °C on a rotary evaporator (Laborota 400-efficient, Heidolph Instruments) and subsequently redissolved in 3 mL of 70% v/v aqueous methanol. When necessary, the extracts were diluted and injected into the LC/MS system without further pretreatment.

Isotopic Ratio Calculation between A and A + 2 Peaks. Isotopic peak abundance ratios between peaks A and A + 2 (I_A/I_{A+2}) of the most characteristic fragment ions of GLSs were calculated by considering all possible combinations of two atoms of ^{13}C, one atom of ^{18}O, and one atom of ^{34}S that can arise in the intact molecule and in the fragment ions. The mathematical aspects of such an algorithm are outlined elsewhere.[27] The free-of-charge Windows-based IsoPatrn (Version 2.00) program[28] was used which does not require large memory or disk space. Such a simple software is able to calculate the isotope cluster pattern of an ion in simple or tandem mass spectra (MS^n for n up to 4).

RESULTS AND DISCUSSION

LC/ESI/LTQ MS analysis of GLSs. Recently, the reversed-phase liquid chromatography mass spectrometry (LC-MS) with electrospray ionization (ESI) of glucosinolates from leave extracts of rocket salad (*Eruca sativa*) was investigated after the appropriate selection of mobile-phase composition and nature and concentration of the acidic modifier.[20] ESI in the negative ion mode was chosen for a detailed analysis of the fragmentation pattern of naturally occurring GLSs. In such a study, a conventional 3-D ion trap was used as a mass analyzer whereby multistage fragmentation processes were performed. Considering the relevant increase in sensitivity offered by the LTQ linear ion trap and the possibility to perform multistage mass spectrometry (MS^n),[29−32] a confirmatory procedure was developed in order to investigate the occurrence of major and minor and known and unknown GLSs in plant extracts (see below). To begin, a standard solution of GLSs (5 mg/L each) was analyzed by LC-ESI-LTQ mass spectrometry. The extracted ion chromatograms (XICs) of glucoiberin, glucoraphanin, progoitrin/epiprogoitrin, sinigrin, gluconapin, and glucoerucin at m/z 422.0, 436.0, 388.0, 358.0, 372.0, and 420.1, respectively, are shown in Figure 1. All GLSs exhibit [M − H]$^-$ as the predominant ion, which corresponds to the deprotonation of the sulfate group.

As the number of GLSs identified is continuously increasing and it is not uncommon to find compounds with the same molecular weight, which possess different chemical formulas or different chemical structures, there is the need for an additional confirmation of GLSs. Moreover, dissociation of [M − H]$^-$ yields

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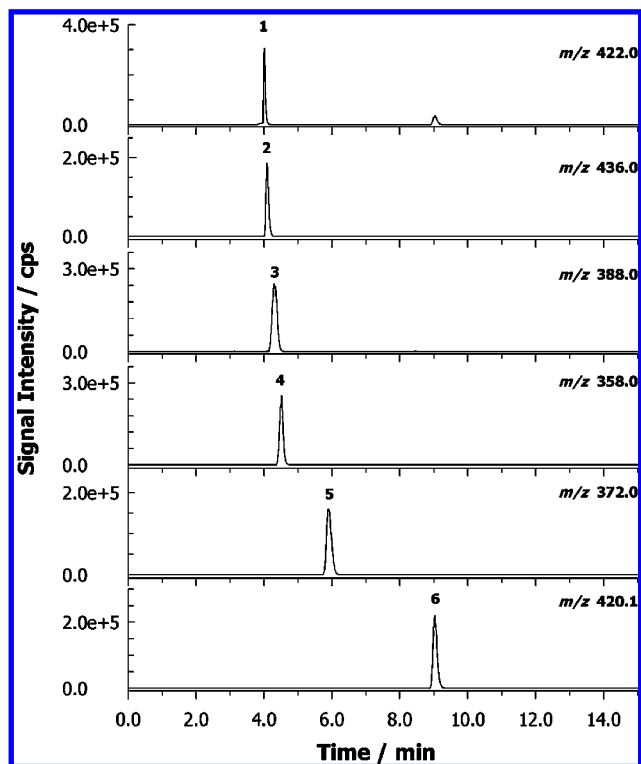(32) Schwartz, J. C.; Senko, M. W.; Syka, J. E. P. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 659–669.

Figure 1. Extracted ion chromatograms (XICs) by LC/ESI-LTQMS acquired in negative ion mode ([M − H]⁻) of a 5 ppm standard solution of intact GLSs. Peak identity: (1) glucoiberin at m/z 422.0; (2) glucoraphanin at m/z 436.0; (3) progoitrin/epiprogoitrin at m/z 388.0; (4) sinigrin at m/z 358.0; (5) gluconapin at m/z 372.0; and (6) glucoerucin at m/z 420.1. The chromatographic and mass spectrometric conditions are reported in the Materials and Methods.

abundant product ions and side chain structurally informative fragments, which would be of great value for a correct assignment of known and unknown GLSs.

Hidden Gold of A + 2 Isotopic Peaks. The groundwork for intact GSL fragmentation studies was laid by Bojesen and Larsen,[7] who analyzed a series of compounds by FAB. Most of these fragments were also observed under electrospray ionization in negative ion mode (ESI−).[20,21,33−35] Proposed structures of some of the fragments detected in the LTQ analyzer are given in Figure 2; the majority of these fragments were of elemental importance for a correct interpretation of fragmentation patterns and molecular identification. The above proposal for the formation of common fragments is not new but, to our knowledge, it has not been systematically explored. The full scan mass spectra of all GLSs show a signal at m/z 259, indicating a common fragment ion resulting from CID within the linear ion trap mass spectrometer. This fragment is well-known as a glucosinolate specific fragment; it corresponds to the neutral loss of R−N=C=S from the deprotonated molecule [M − H]⁻. Other significant fragments, usually present in most MS spectra of GLSs, were found at the following m/z values, [M − 196 − H]⁻, [M − 178 − H]⁻, and [M − 162 − H]⁻ after H rearrangement from the R

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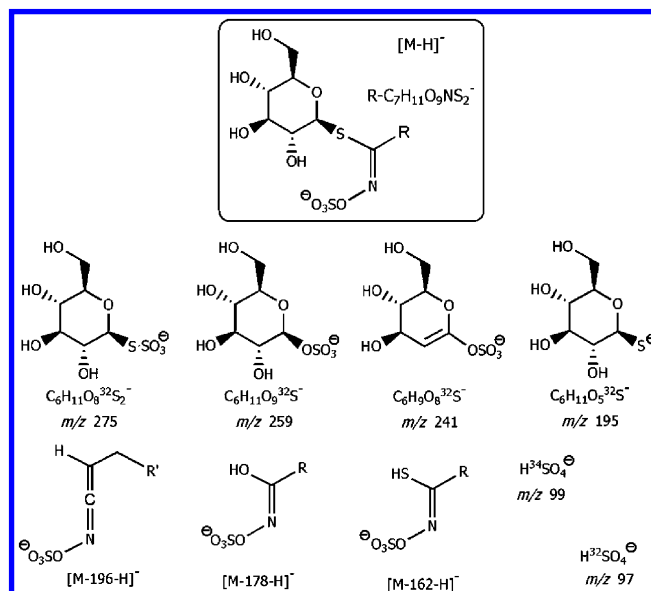
Figure 2. Molecular structure of GLSs (*cis-N*-hydroximinosulfate esters) in the anionic form and suggested structures of the most common fragment ions obtained upon CID of deprotonated glucosinolates, [M − H]⁻, in the LTQ linear ion trap. These fragments were observed for most of the GLSs investigated.

group by cleavage of the bond on either side of the sulfur and with charge retention on the sugar aglucone.[8,9] The suggested structures of these fragments are depicted in Figure 2, being very informative for the side chain of the glucosinolate. Moreover, it was possible to ascertain the presence of the peak at m/z 195, corresponding to the fragment ion of the D-thioglucose group $C_6H_{11}O_5S^-$. The peak at m/z 275, which is very interesting from a diagnostic point of view of targeted and untargeted GLSs, corresponds to the neutral loss of R−N=C=O from the [M − H]⁻ ion. Note that all these ions at m/z 275, 259, and 241 are most likely formed through intramolecular rearrangements in which the sulfate group is transferred to the thioglucose moiety. Frequently, the MS/MS spectrum of GLSs contained also peaks corresponding to the loss of SO_3 (80 Da) from the [M − H]⁻ ion.[21,35] Finally, the peak at m/z 97 represents the fragment ion HSO_4^- from the glucosinolates.

All GLSs contain at least two sulfur atoms, and the peak of each deprotonated molecule is accompanied by an isotopic peak at (A + 2). Its relative intensity is about 8.4% (i.e., 2 × 4.2%) if the contribution of other element isotopes is neglected. Figure 3 shows the benefit of acquiring the spectra of the ions derived from fragmentation of the deprotonated gluconapin at m/z 372 (plot A) and its isotopic ion at m/z 374 (plot B). This last spectrum was acquired using the second isotopic peak (A + 2) as precursor ion. In simple terms, the (A + 2) ion contains one ³⁴S and one ³²S. If both sulfur atoms are kept in a fragment, then this fragment also contains one ³⁴S and one ³²S. Such a result can be seen with fragments at m/z 275 and 277 which exhibit an intensity ratio I_A/I_{A+2} ca. 0 S:1 S (zero intensity means only noise signal). If the fragment contains one of the two sulfur atoms, the probability that the atom being retained is ³⁴S is the same as the probability that the atom is ³²S; the signal intensity ratio between the light and heavy fragments I_A/I_{A+2} should be roughly 1 S:1 S. This is the case of peaks at m/z 292 and 294, peaks at m/z 259 and 261, and more interestingly
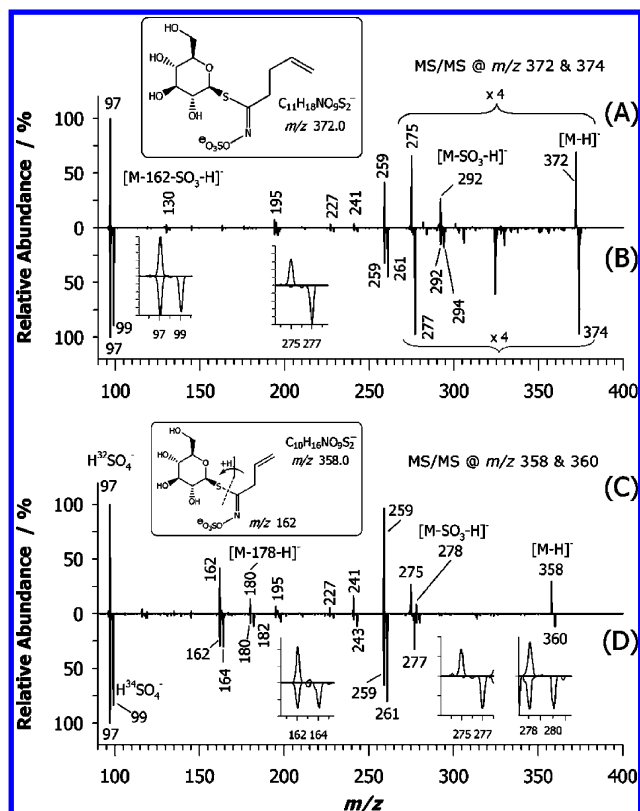
Figure 3. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of (A and B) gluconapin and (C and D) sinigrin. The precursor ion base peaks of gluconapin and sinigrin at m/z 372 (A) and m/z 358 (C), respectively, and the corresponding A + 2 isotope peaks at m/z 374 (B) and m/z 360 (D) were fragmented at 20% collision energy. The expanded views in plots (C) and (D) show, as profile spectra, some examples of isotopic peak doublets. See data in Table 1.

for peaks at m/z 97 and 99 as well. We can assign this last couple of peaks to the sulfate group $H^{32}SO_4^-/H^{34}SO_4^-$, respectively (Figure 3B inset), i.e., a single sulfur in the fragment. Therefore, the remainder of the molecule also contained a single sulfur, two in total. As reported by Fabre et al.,[21] all deprotonated molecules fragment with a characteristic loss of SO_3 (80 Da); with regard to gluconapin, this fragment is at m/z 292. Note that, if the fragment does not contain sulfur atoms, it shows up at the same mass-to-charge ratio in both spectra. This is not the case of mass spectra displayed in Figure 3A,B.

An advantage of performing CID experiments on (A + 2) isotopomeric ions is that they provide more information for structural elucidation compared to tandem MS on A type ions (see below). An additional example of isotopic peak fragmentation is illustrated for sinigrin in Figure 3C,D. Precursor ions of sinigrin at m/z 358 produced some well-defined product ions under a relatively low collision energy, 20%. At least four product ions of m/z 275, 259, 162, and 97 occur in the spectra (see plot C). Fragmentation of the satellite ion (A + 2) at m/z 360 offers the signature for a compound containing two sulfur atoms into the molecular formula. Indeed, looking at the intensity ratios of light and heavy fragments, 259:261, 162:164, and 97:99 with measured ratios 0.74 ± 0.05, 1.04 ± 0.03, and 1.18 ± 0.05, respectively, it is suggested that each fragment contained nearly half the total sulfur. The occurrence of peak at m/z 277 and the absence of peak at

m/z 275 (intensity ratio 0.03 ± 0.02) is again in good agreement with a compound belonging to the glucosinolate family.

Our general strategy was based on the isotopic patterns within each cluster upon MS/MS fragmentation of the A + 2 ion of deprotonated molecules. The key points of the pattern were the relative heights of each isotope cluster. By the postulated elemental compositions, we compared the expected and observed height ratios of each isotope (A and A + 2 ions) within a cluster as a means of finding informative features. Measured ratios represent the average of five replications (±standard deviation), which accounts for scan-to-scan variability of ion intensities. While an accurate evaluation is made by comparing measured and expected ratios, columns fifth and third, respectively (see Table 1), a first approximate comparison can be made between measured and expected ratios in which the simple contribution of ^{34}S was taken into account, columns fifth and fourth, respectively. Interestingly, a good agreement was found with all isotopic clusters; as an example, the doublet at m/z 162/164 exhibits a measured abundance ratio (I_A/I_{A+2}) of 1.04 ± 0.03 (ca. 2% as RSD) which is very close to the calculated value of 1.06.

It is interesting to note that the collision energy does not strongly affect the fragmentation behavior of compounds changing the relative intensity of product ions. In Figure 4A,B are illustrated the product ion spectra of progoitrin/epiprogoitrin at m/z 388 along with the corresponding spectra of the isotopic peak (A + 2) at m/z 390 using a relatively low collision energy, namely, 20%. Although the spectrum in Figure 4A yields seven to eight ions useful for structural characterization, the precursor ion is not fully fragmented. Whereas the couple of MS peaks 332:334 is due to the neutral loss of acrylic aldehyde (CH_2CHCHO, 56 Da) from the side chain, being in accordance with an intensity ratio 0 S:1 S (see spectrum B), the peak at m/z 136 corresponded to the loss of both neutral D-thioglucose ($C_6H_{11}O_5SH$, 196 Da) and acrylic aldehyde from the [M − H]$^-$ ion. Two fragment peaks, at m/z 210 and 212, rationalized as [M − 178 − H]$^-$, exhibit roughly equal intensity and account for the side chain of the GLS. As the collision cell potential energy was increased up to 30%, no significant changes in the already spectral-rich product ion spectrum were observed (Figure 4C,D). Most importantly, the signal intensity ratio between the lighter and heavier fragments is approximately respected within almost all the examined couples: 332:334 (0 S:1 S), 259:261 (1 S:1 S), 210:212 (1 S:1 S), 136:138 (1 S:1 S), and 97:99 (1 S:1 S). In both MS/MS spectra, the peak at m/z 97 represents the fragment ions HSO_4^- from the glucosinolate. Further examples of GLSs (i.e., indole glucosinolates) investigated using the present procedure are given in supplemental figures (Figures S1 and S2, see Supporting Information).

When More Sulfurs Make a Difference. As this investigation was undertaken to verify the capability of tandem MS performed on the A + 2 isotopic peaks, more data were acquired upon an LC-MS running a crude extract of rocket salad (*E. sativa*) leaves, which notoriously contains at least two major GLSs, glucoerucin and glucoraphanin.[36,37] Glucoraphanin is an aliphatic glucosinolate which is the precursor of sulforaphane, a potent anticancer

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(37) Barillari, J.; Canistro, D.; Paolini, M.; Ferroni, F.; Pedulli, G. F.; Iori, R.; Valgimigli, L. *J. Agric. Food Chem.* **2005**, *53*, 2475–2482.

Table 1. Experimental and Calculated Ratios (I_A/I_{A+2}) for the Isotope Abundances of the Fragment Ions As Observed by MS/MS of the +2 Da Isotopomer of Deprotonated Sinigrin [M − H]⁻ at m/z 360 ($C_{10}H_{16}NO_9S_2^-$ + 2)[a]

product ions (m/z)	molecular formula	expected ratio	expected ratio counting only the contribution of ^{34}S	measured ratio ($n = 5$)
97−99	HSO_4^-	1.17	1 S:1 S	1.18 ± 0.03
162−164	$C_4H_4NO_4S^-$	1.06	1 S:1 S	1.04 ± 0.03
259−261	$C_6H_{11}O_9S^-$	0.70	1 S:1 S	0.74 ± 0.05
275−277	$C_6H_{11}O_8S_2^-$	0.03	0 S:1 S	0.03 ± 0.02
278−280	$C_{10}H_{16}NO_6S^-$	0.79	1 S:1 S	0.80 ± 0.04

[a] Expected data were calculated using the software named IsoPatrn Version 2.00 offered by Ramaley.[28] Values represent the average of five replications ± one standard deviation. The relative error between expected (theoretical) and measured ratios for all ion clusters was below 10%.
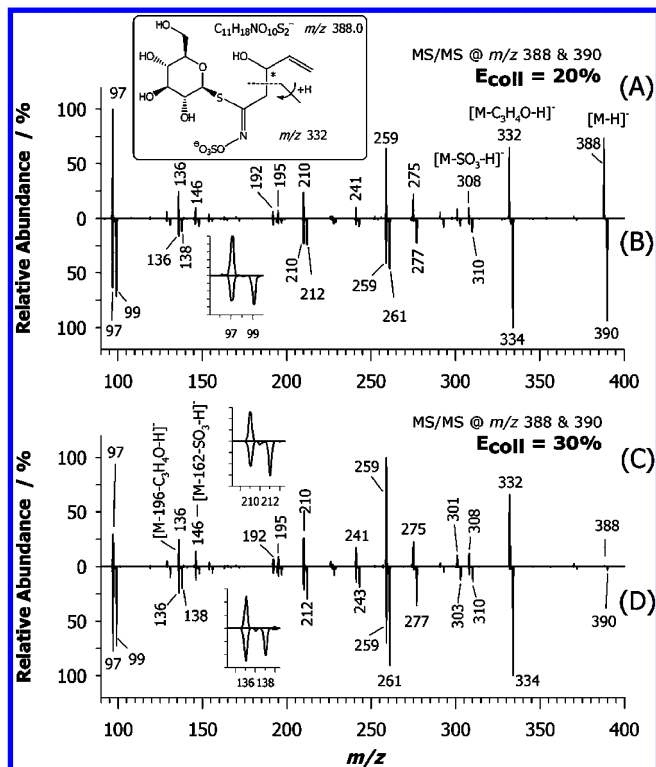

Figure 4. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of deprotonated progoitrin/epiprogoitrin molecules, [M − H]⁻, fragmented at two collision energies: (A and B) at 20% and (C and D) at 25%. At both collision energies, the signal intensities of isotope pairs are in good agreement with expected values, namely, approximately 1:1 ratios of isotope pairs 97:99, 136:138, 210:212, and 259:261 and 0:1 ratios of isotope pairs 275:277 and 332:334 in the A + 2 spectra, thus confirming that the original molecule contained two sulfur atoms.

isothiocyanate. For the deprotonated glucoraphanin (Figure 5A), the relatively low activation barrier for cleavage of the side chain C(4)−S bond makes it susceptible to fragmentation and leads to the loss of methanesulfenic acid (CH₃SOH, 64 Da) from the precursor ion at m/z 436 and generation of a single product ion at m/z 372 (see inset in Figure 5). Indeed, the spectrum is nearly bare of product ions, presenting the need for more stages of MS to confirm the molecular structure (see below). Figure 5B shows the corresponding MS² spectrum of the +2 parent at m/z 438. CID of the [M − H + 2]⁻ satellite ion of glucoraphanin results in a splitting of the [M − CH₃SOH − H]⁻ fragment ion signal into a doublet separated by 2 mass units. The generation of an ion doublet at m/z 372 and 374 consisting of two peaks exhibiting an intensity ratio equal to 0.39 ± 0.02 (mean ±
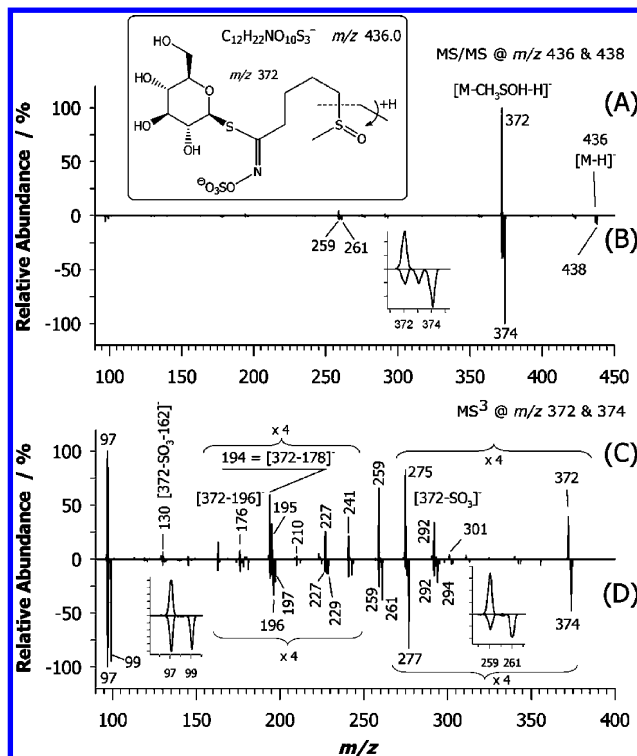
Figure 5. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of glucoraphanin. (A) Precursor ion of glucoraphanin [M − H]⁻ at m/z 436 was fragmented at 20% collision energy. The main product ion is due to the characteristic loss of methanesulfenic acid (CH₃SOH, 64 Da) from the side chain of glucoraphanin. (B) The A + 2 isotopic peak at m/z 438 was fragmented at the same collision energy. The near 1S:2S ratio of isotope pairs 372:374 shows that the original molecule contained three sulfur atoms. Ions at m/z 372 and m/z 374 were selected as precursor ions for a further stage of fragmentation (MS³) at the same collision energy, and the resulting mass spectra are illustrated in plots (C) and (D), respectively. See data in Table 2.

standard deviation, $n = 5$) is due to the fact that the ion at m/z 372 contains about equal amounts of ^{32}S and ^{34}S along with the additional isotopic contributions of ^{13}C, ^{18}O, and ^{15}N. The estimation of the isotopic composition of the ion at m/z 374 of glucoraphanin and of the corresponding fragment ions is given in Table 2.

An exact calculation gives a ratio of 0.40 of the fragment at m/z 372 relative to one at m/z 374, which is in very good agreement with the experimental data. As this ratio may not be sufficient for a conclusive structure elucidation, an additional fragmentation stage was performed on both ions at m/z 372 (A)

Table 2. Experimental and Calculated Ratios (I_A/I_{A+2}) for the Isotope Abundances of the Fragment Ions As Observed by MS/MS of the +2 Da Isotopomer of Deprotonated Glucoraphanin [M − H]⁻ at m/z 438 ($C_{12}H_{22}NO_{10}S_3^-$ +2) and Subsequent Fragmentation (MS/MS/MS) of the Prominent A + 2 Ion at m/z 374

product ions (m/z)	molecular formula (A)	expected ratio	expected ratio counting only the contribution of ³⁴S	measured ratio ($n = 5$)
372−374	$C_{11}H_{18}NO_9S_2^-$	0.40	1 S:2 S	0.39 ± 0.02
	MS³ at m/z 374 ($C_{11}H_{18}NO_9S_2^-$ + 2)[a]			
97−99	HSO_4^-	1.19	1 S:1 S	1.14 ± 0.04
227−229	$C_6H_{11}O_7S^-$	0.82	1 S:1 S	0.88 ± 0.06
259−261	$C_6H_{11}O_9S^-$	0.71	1 S:1 S	0.68 ± 0.05
275−277	$C_6H_{11}O_8S_2^-$	0.03	0 S:1 S	0.05 ± 0.02
292−294	$C_{11}H_{18}NO_6S^-$	0.78	1 S:1 S	0.75 ± 0.06

[a] Note that the expected ratios counting the contribution of ³⁴S look like that of a GLS exhibiting two sulfur atoms in the molecular structure.

and 374 (A + 2) (see Figure 5C,D). The observed fragment ion at m/z 292 resulted from the loss of SO_3 from the [M − CH₃SOH − H]⁻ ion, analogous to all glucosinolates. The full-scan MS/MS product ion spectrum is rich with product ions and the most abundant one is at m/z 97. Conceivably, the same fragmentation behavior of GLSs exhibiting two sulfur atoms was observed. Indeed, the occurrence of a peak at m/z 277 and the low intensity of the peak at m/z 275 (Figure 5D) with an intensity ratio I_A/I_{A+2} equal to 0.05 ± 0.02 is in good agreement with the expected ratio (i.e., 0.03 see Table 2). Most of the recognized fragment ions are also present, including those at m/z 259, 227, 195, and 97. Note that the original fragment at m/z 259 is now accompanied by nearly equal amounts of peaks at m/z 261. Even without knowing the identity of the fragment, it turns out that the fragment included at least one sulfur, from a parent ion at m/z 372 that contained at least two S. Thus, the isotope-selective tandem MS is a useful tool for determining the elemental composition of fragment ions originated from precursor ions of known elemental composition (see Tables 1 and 2). There are some small discrepancies in our results; for example, the expected ratio of doublet ions at m/z 227−229 ($C_6H_{11}O_7S^-$) should be 0.82 while the measured value is slightly higher (0.88 ± 0.06) but still reasonably in good agreement. The same applies to doublet ions at m/z 97−99. A plausible explanation is the occurrence of isobaric interferences, which increase the abundance of one fragment ion, A or A + 2. Apparently, when the absolute intensity was relatively high (e.g., >10−15%) a good agreement was found between the expected and experimental ratios. Further examples of GLSs containing three sulfur atoms (i.e., 4-mercaptobutyl-GLS, glucoerucin, and glucoiberin) are given in the Supporting Information (Figures S3, S4, and S5).

The strategies detailed in this report are not meant to exhaustively identify every GLS present within plant extracts but rather to rapidly and accurately identify the occurrence of biologically active phytochemicals. Therefore, not only major metabolites were identified. Figure 6 shows the ion trap MS/MS spectrum of a GLS containing four sulfur atoms. Kim et al.[38] have demonstrated the occurrence of this secondary metabolite in plant extracts of *E. sativa* after liquid chromatographic separation. The precursor ion of 4-(β-D-glucopyranosyl-disulfanyl)butyl-GLS at m/z 600 fragments in the linear ion trap to three abundant ions at

(38) Kim, S.-J.; Jin, S.; Ishii, G. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2444–2450.
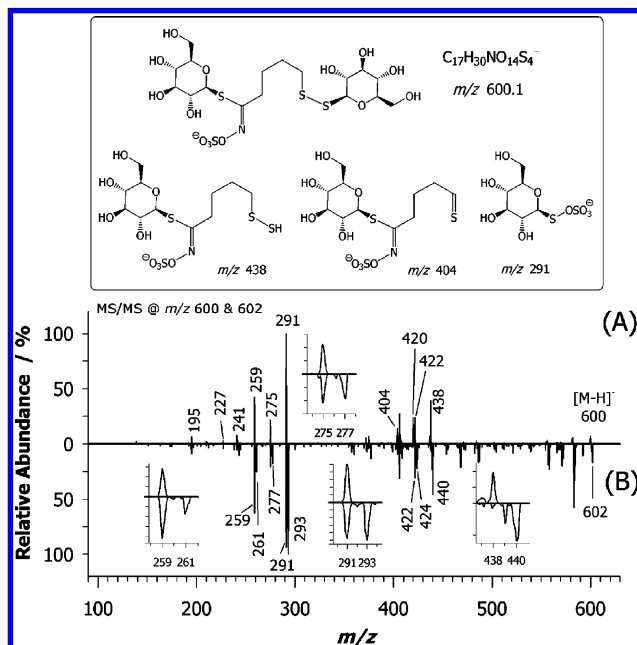
Figure 6. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of 4-(β-D-glucopyranosyl-disulfanyl)butyl-GLS. The precursor ions [M − H]⁻ at m/z 600 (A) and the A + 2 isotope peak at m/z 602 (B) were fragmented at 25% collision energy. The deprotonated GLS and specific fragments that explain the occurrence of some intense tandem MS peaks are shown in the top panel of the figure. See data in Table 3.

nominal masses of m/z 438, 291, and 259. Less abundant ions are also observed at m/z 422, 420, 404, and 275. Although the activation q was fixed at the lowest possible value of 0.19, tandem MS spectra do not show the occurrence of a peak at m/z 97. The presence in this GLS of a second thioglucose moiety confers two similar sites of bound cleavage. Yet, this compound is preferentially cleaved at the C−S bond of the β-glucopyranose residue linked to the N-hydroxyamine sulfate ester. Although the signal intensity of dimeric GLS is far lower than that of other glucosinolates in extracts of *E. sativa*, the mass spectra were clean enough (i.e., low baseline noise) with well recognizable peak signals.

To count the number of sulfur atoms, we need to consider the fragmentation of the precursor ion at m/z 602 which gives a well-defined doublet at m/z 291 and 293 with an intensity ratio I_A/I_{A+2} very close to 1:1 and the well recognized doublet 259:261 which shows an intensity ratio roughly equal to 2.41 ± 0.06

Table 3. Experimental and Calculated Ratios (I_A/I_{A+2}) for the Isotope Abundances of the Fragment Ions As Observed by MS/MS of the +2 Da Isotopomer of Deprotonated 4-(β-D-Glucopyranosyl-disulfanyl)butyl-GLS [M − H]⁻ at m/z 602 ($C_{17}H_{30}NO_{14}S_4^-$ + 2)

product ions (m/z)	molecular formula	expected ratio	expected ratio counting only the contribution of ³⁴S	measured ratio (n = 5)
259−261	$C_6H_{11}O_9S^-$	2.35	3 S:1 S	2.41 ± 0.06
275−277	$C_6H_{11}O_8S_2^-$	1.02	1 S:1 S	1.12 ± 0.08
291−293	$C_6H_{11}O_9S_2^-$	0.98	1 S:1 S	0.95 ± 0.05
404−406	$C_{11}H_{18}NO_9S_3^-$	0.35	1 S:3 S	0.31 ± 0.06
438−440	$C_{11}H_{20}NO_9S_4^-$	0.06	0 S:1 S	0.05 ± 0.02

(Figure 6B and Table 3). Further evidence of its chemical identity was found by looking at the m/z 438 and 440 doublet with an experimental ratio of 0.05 ± 0.04, which is consistent with both precursor and product ion containing the same number of sulfur atoms. Note that the ion at m/z 291 contains two sulfur atoms, as depicted in the top panel of Figure 6. As a whole, the signal intensity of isotope peaks (i.e., 259:261, 275:277, 291:293, and 438:440) confirms that this dimeric GLS contained four sulfur atoms. In the Supporting Information (Figure S6), the additional fragmentation of the cluster at m/z 438 and 440 is reported. Of course, it is not possible to exploit the fragmentations of peaks (A) at m/z 420 or 422 as the fragmentation of the corresponding ions A + 2 at m/z 422 or 424 would be affected by isobaric interferences.

Figure 7A is the full-scan mass spectrum of an other dimeric GLS, 4-mercaptobutyl-GLS (DMBG), a compound recently dis-

Figure 7. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of dimeric 4-mercaptobutyl-GLS at m/z 811. (B) The precursor doubly charged ion [M − 2H]²⁻ (base peak) at m/z 405 was fragmented with the following parameters: activation q of 0.190; isolation width of 0.7 Da; the percentage relative collision energy was 20%. (C) The A + 2 isotope peak at m/z 406 was fragmented at the same collisional energy. Product ion spectra were extracted from the apex of the chromatographic peak. See data in Table 4.

covered as being present in methanol extracts of *E. sativa*.[20,38,39] This is a case demonstrating a doubly charged precursor ion; Figure 7B shows the MS/MS of DMBG at a fixed relative collision energy of 20%. MS parameters optimized for this analysis included an activation time of 30 ms and a relatively low activation q of 0.19 which results in an increase in the coverage of lower m/z values. The predominant species in spectrum A of Figure 7 is the 405.1 m/z ion [M − 2H]²⁻/2 with a small [M − H]⁻ peak at m/z 811.1. These findings and the occurrence of fragment ion signals at m/z 731 and 569, which correspond to [M − SO₃ − H]⁻ and [M − SO₃ − 162 − H]⁻, respectively, are in agreement with the presence of several sulfurs in the precursor ion. In regard to tandem MS spectra of the doubly charged precursor ion at m/z 405 (spectrum B), it gives major fragment ions at m/z 519 and 97. A proposed fragmentation pathway for this dimeric GLS is shown in the top panel of Figure 7. Fragmentation of the precursor m/z 406 ion, which was isolated with no detectable contamination (Δm = 0.7) by m/z 405, gave two well-defined doublets at m/z 519 and 521 and m/z 97 and 99 with intensity ratios very close to 1 S:2 S and 5 S:1 S (experimental I_A/I_{A+2} values are 0.50 ± 0.02 and 5.10 ± 0.12), respectively (insets of Figure 7B,C). Accordingly, these intensity ratios are in good agreement with a precursor ion which contains six sulfur atoms because only in such a situation the probability of one ³⁴S belonging to m/z 99 of HSO₄⁻ ion is one-fifth of that of ³²S of its isotopomer at m/z 97 (see Table 4). Further evidence was found by looking at the following doublets m/z 259−261, m/z 275−277, and m/z 535−537 with signal ratios 3.98 ± 0.07, 1.92 ± 0.05, and 0.48 ± 0.05, respectively, which are in good agreement with 3.94, 1.98, and 0.50, respectively. Again, as the peak at m/z 259 contains one sulfur atom, an intensity ratio 5 S:1 S is appropriately fulfilled with a total number of S equal to six. It is evident that the abundances of the heavy ions increase in proportion to the number of sulfurs. We believe this is the first systematic report combining comprehensive fragmentation analysis of deprotonated molecules (A) and their isotopomeric ion (A + 2) for counting the number of S in GLSs. Together with the formation of specific ions during tandem MS, all these benefits are important in obtaining dependable results regardless of whether easy samples or the most challenging matrixes are being analyzed.

Identification of an Unknown GLS. This study included an evaluation of any potential gain in the ability to establish a correct identification in vegetable extracts of unknown GLSs by screening out a chromatographic file acquired by LC-ESI-MS in conjunction
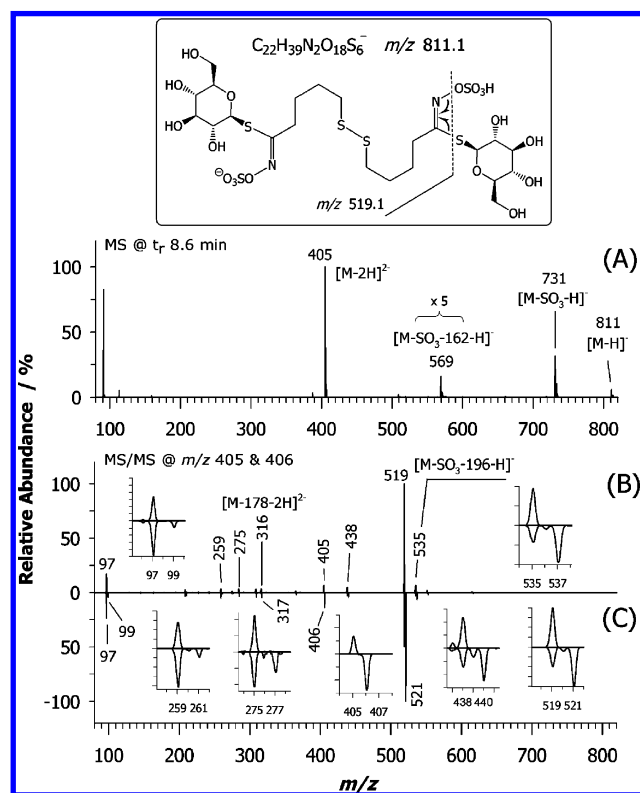
(39) Kim, S.-J.; Kawaharada, C.; Jin, S.; Hashimoto, M.; Ishii, G.; Yamauchi, H. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 114−121.

Table 4. Experimental and Calculated Ratios (I_A/I_{A+2}) for the Isotope Abundances of the Fragment Ions As Observed by MS/MS of the +2 Da Isotopomer of Deprotonated Dimeric 4-Mercaptobutyl-GLS, Which Is a Doubly Charged Ion $[M - 2H + 2]^{-2}$ at m/z 406[a]

product ions (m/z)	molecular formula	expected ratio	expected ratio counting only the contribution of ^{34}S	measured ratio ($n = 5$)
97−99	HSO_4^-	4.76	5 S:1 S	5.10 ± 0.12
259−261	$C_6H_{11}O_9S^-$	3.94	5 S:1 S	3.98 ± 0.07
275−277	$C_6H_{11}O_8S_2^-$	1.98	2 S:1 S	1.92 ± 0.05
519−521	$C_{16}H_{27}N_2O_9S_4^-$	0.52	1 S:2 S	0.50 ± 0.02
535−537	$C_{16}H_{27}N_2O_{10}S_4^-$	0.50	1 S:2 S	0.48 ± 0.05

[a] The doubly charged ion $[M - 2H]^{-2}$ at m/z 405 is the most prominent peak in the mass spectrum of the precursor ion at m/z 811 ($C_{22}H_{39}N_2O_{18}S_6^-$).
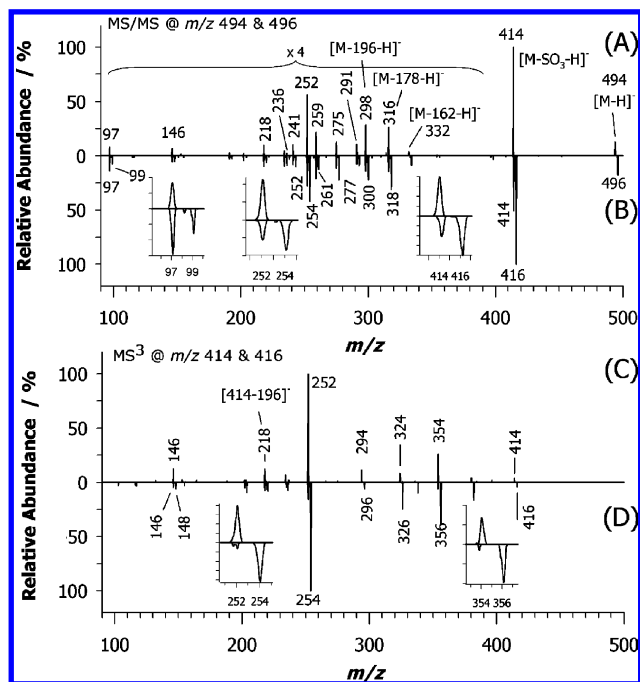

Figure 8. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of an unknown GLS exhibiting an $[M - H]^-$ ion at m/z 494.1 with a potential identity as 6-methylsulfonyl-3-oxohexyl-GLS. The precursor ion at m/z 494 and the A + 2 isotope peak at m/z 496 were fragmented at 25% collision energy. Fragment ions (25% collision energy) at m/z 414 and m/z 416 were selected as precursor ions for a further stage of fragmentation (MS^3), and the resulting mass spectra are illustrated in plots (C) and (D), respectively. See data in Table 5.

with tandem MS. The above presented strategy was successfully applied to recognize an unknown GLS which was found in extracts of *E. sativa* and exhibits a retention time of 4.77 min under the same experimental conditions described in Figure 1. The identification was obtained through the following data: (i) a deprotonated molecule $[M - H]^-$ with a base peak at m/z 494.1, (ii) isotope peak at m/z 496.1 with ca. 13% of relative intensity, and (iii) the observed CID spectra of isotopic A and A + 2 ions. On the basis of the fragmentation behavior of the precursor ion $[M - H]^-$, a pattern resembling that of a GLS was observed (Figure 8A). We wish to highlight that neither standard is commercially available for a GLS having the same molecular weight, nor is the structural indication present in the literature.[1,18] Fragmentation of the precursor m/z 496.1 ion (A + 2) gave a well-defined doublet at m/z 414 and 416 with an intensity ratio equal

to 0.43 ± 0.03 (inset of Figure 8B). If the fragment ion at m/z 414 results from the loss of SO_3 from the $[M - H]^-$ ion, it turns out that the precursor ion must contain three sulfur atoms in the chemical formula. Accordingly, this allows for relationships between product and precursor ions to be established. Thus, this compound belongs to a group of long-chain-length aliphatic GLSs because the side chain contains an additional sulfur.[40] The estimation of the isotopic composition of the ion at m/z 414 of this unknown GLS and the corresponding fragment ions is given in Table 5. Further evidence was found by looking at the m/z 97−99 and m/z 275−277 doublets with signal ratios of 2.09 ± 0.12 and 0.52 ± 0.05, respectively, which confirms an odd number of three sulfur atoms in the precursor ion at m/z 494. Moreover, the calculated ratio between the light and heavy fragments at m/z 252 and 254 is 0.62, a result which agrees well with the measured value (0.61 ± 0.05, $n = 5$). For the same reason as described for the MS/MS spectra of glucoraphanin, the doublet ions at m/z 275−277 contain two sulfur atoms. Other significant ions in the MS/MS spectrum of Figure 8A at m/z 316 and 298 can be explained by two neutral losses, i.e., −178 and 196 Da (D-thioglucose), from the precursor ion at m/z 494, respectively. These data are all consistent with a minor glucosinolate containing three sulfur atoms.

In Figure 9A, a suggested structure is proposed along with the major and minor product ions observed and their respective assignments. The deprotonated molecule corresponds to an aliphatic GLS never described before: 6-methylsulfonyl-3-oxohexyl-GLS. Upon consecutive CID, the resultant product ion spectra (MS^3) of ions m/z 414 and 416 exhibit a complete fragmentation of precursor ions and the generation of two major ion doublets at m/z 354−356 and at m/z 252−254 (see Figure 8D and Table 5). The peak at m/z 252 (254) corresponded to the molecular ion with the loss of $C_6H_{10}O_5$ (162 Da) from the precursor ion $[M - SO_3 - H]^-$ at m/z 414. Because such a fragment contains both sulfur atoms, the intensity ratio I_A/I_{A+2} between peaks at m/z 252−254 is close to zero, which is a markedly different ratio compared to that observed in the spectrum of Figure 8B (i.e., 0.61 ± 0.05). As expected, no presence of HSO_4^- ions was observed because the precursor ions at m/z 414 and 416 had already lost the SO_3 moiety. Both doublets at m/z 324−326 and 354−356 (spectrum 8D) exhibit an intensity ratio I_A/I_{A+2} equal to 0.04 ± 0.03, which is in good agreement with the expected ratios, thus suggesting that the heavy fragments retained both sulfur atoms. Low energy CID spectra

(40) Clarke, D. B. *Anal. Methods* **2010**, *2*, 310−325.

Table 5. Experimental and Calculated Ratios (I_A/I_{A+2}) for the Isotope Abundances of the Fragment Ions As Observed by MS/MS of the +2 Da Isotopomer of an Unknown Glucosinolate at m/z 496 Identified as 6-Methylsulfonyl-3-oxohexyl-GLS ($C_{14}H_{24}NO_{12}S_3^-$ +2) and Subsequent Fragmentation (MS/MS/MS) of the Prominent A + 2 ion at m/z 416

product ions (m/z)	molecular formula	expected ratio	expected ratio counting only the contribution of ^{34}S	measured ratio ($n = 5$)
97−99	HSO_4^-	2.28	2 S:1 S	2.09 ± 0.12
146−148	$C_5H_8NO_2S^-$	2.29[a]	2 S:1 S	1.06 ± 0.05
218−220	$C_8H_{12}NO_4S^-$	1.90	2 S:1 S	1.92 ± 0.05
252−254	$C_8H_{14}NO_4S_2^-$	0.62	1 S:2 S	0.61 ± 0.05
259−261	$C_6H_{11}O_9S^-$	1.53	2 S:1 S	1.59 ± 0.06
275−277	$C_6H_{11}O_8S_2^-$	0.53	1 S:2 S	0.52 ± 0.05
291−293	$C_6H_{11}O_9S_2^-$	0.50	1 S:2 S	0.62 ± 0.05
298−300	$C_8H_{12}NO_7S_2^-$	0.53	1 S:2 S	0.59 ± 0.05
316−318	$C_8H_{14}NO_8S_2^-$	0.50	1 S:2 S	0.47 ± 0.05
414−416	$C_{14}H_{24}NO_9S_2^-$	0.42	1 S:2 S	0.43 ± 0.03
MS³ at m/z 416 ($C_{14}H_{24}NO_9S_2^-$ + 2)				
146−148	$C_5H_8NO_2S^-$	1.27[b]	1 S:1 S	0.81 ± 0.05
218−220	$C_8H_{12}NO_4S^-$	1.00	1 S:1 S	0.99 ± 0.03
252−254	$C_8H_{14}NO_4S_2^-$	0.12	0 S:1 S	0.10 ± 0.04
294−296	$C_{10}H_{16}NO_5S_2^-$	0.08	0 S:1 S	0.06 ± 0.04
324−326	$C_{11}H_{18}NO_6S_2^-$	0.06	0 S:1 S	0.04 ± 0.03
354−356	$C_{12}H_{20}NO_7S_2^-$	0.04	0 S:1 S	0.04 ± 0.03

[a] Value which is much greater than that measured for the isotopic ion pair at m/z 146 and 148; a more reasonable agreement can be obtained if such a pair is considered as a result of incomplete fragmentation of the ion at m/z 252 or at least a combination of both ions as precursor ions: m/z 252 and m/z 416. [b] The same rationale can be applied here where the effective precursor ion may be considered at m/z 252. In both cases with a hypothetical precursor ion at m/z 252, the expected ratio of ion intensity is equal to 0.98.
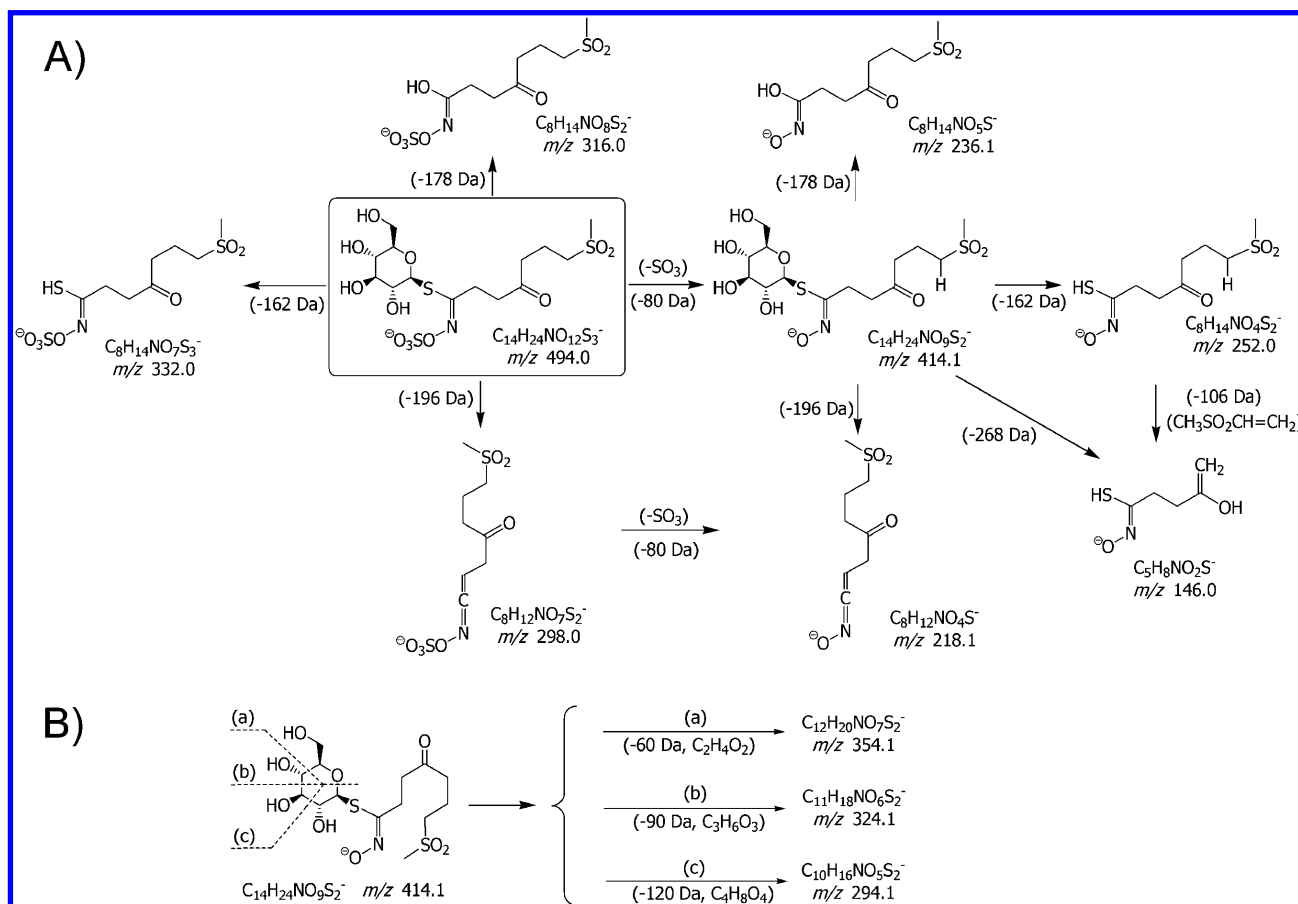

Figure 9. (A) Proposed fragmentation scheme of the long-chain-length aliphatic GLS exhibiting an [M − H]⁻ ion at m/z 494.1 based on information from Figure 8 data. (B) Proposed fragmentation of thioglucose moiety from the precursor ion at m/z 414.1.

of this ion were almost identical and were dominated by consecutive losses from the sugar moiety. In particular, the ions at m/z 354 and 324 are probably due to the fragmentation of thioglucose from the precursor ion at m/z 414, with

the neutral loss of glycolaldehyde (−60 Da) and 1,3-dihydroxyacetone (−90 Da), [414 − $C_2H_4O_2$]⁻ and [414 − $C_3H_6O_3$]⁻, respectively, so leaving the side chain unmodified (see panel B of Figure 9).

Even more interesting and informative is a compound-specific fragment, that is ion doublet at 146−148 which seems to be related to the side chain of this minor GSL. Indeed, the product ion at m/z 146 (148) is due to the neutral loss of $CH_3SO_2CH=CH_2$ (106 Da) through a McLafferty rearrangement from the most intense ion at m/z 252, which exhibits a suitably acidic proton in the γ position.[41] Most likely, the first step of this process is the abstraction of a proton by the proximate keto-group which undergoes β-cleavage of the $C(4)−C(5)$ bond. However, evidence of possible discrepancies can be seen when the measured and expected intensity ratios between peaks at m/z 146 and 148 are compared. (See data in Table 5.) Although for some low-intensity ions, the standard deviation could be considered the main reason of this disagreement; we speculate that the reason lies on the effective ion that gives rise to the fragment at m/z 146. Looking at the suggested fragmentation scheme of Figure 9A, the main route of 146 formation should be from the ion at m/z 252 and not directly from the precursor ion m/z 494. It is noticeable that with a hypothetical precursor ion at m/z 252 the expected isotope ratio of ion intensity I_{146}/I_{148} is equal to 0.98, which is in reasonable agreement with measured ratios in both MS/MS and MS/MS/MS experiments, 1.06 ± 0.05 and 0.81 ± 0.05, respectively (Table 5). To test this hypothesis, there is the need of an additional fragmentation of ion at m/z 254 which is unfortunately of very low intensity. Any further confirmation of the ion structure at m/z 146 would be left to NMR and LC/NMR.

CONCLUSIONS

Isotope-selective tandem MS of GLSs in a linear ion trap exhibiting exemplary fragmentation patterns is discussed. The presence of certain residues and/or chemical moieties tends to induce selective cleavage and prevent the formation of a homologous ion series via CID. Because in these compounds a significant isotopic contribution exists, the fragmentation of precursor ions generates rich spectra that are useful to help determine the chemical formula of common and uncommon fragments along with the number of sulfur atoms. As the method is very informative, it is suitable for large-scale investigations in vegetative extracts, and because of its ease of implementation in tandem MS experiments, the method is very attractive for detailed structural characterization and confirmation of glucosinolates. The new direction for glucosinolate identification is the integration of automated data interpretation with LC/MS/MS in combination with the protocols described in this report. This approach will significantly accelerate GLS identification.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

(41) Grossert, J. S.; Cook, M. C.; White, R. L. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1511–1516.