Identification of Desulfoglucosinolates Using Positive-Ion Fast Atom Bombardment Mass Spectrometry

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A systematic study of 12 representative desulfoglucosinolates (RC(SG)NOH, SG = -S-β-D-glucose) using positive-ion fast atom bombardment (FAB) mass spectrometry has revealed a strong correlation between side chain (R) structure and fragmentation pattern. Molecular ions were obtained in all cases, and in addition the spectra contained fragment ions originating from the variable portion (R) of the molecule. Alkyl desulfoglucosinolates yielded a single major fragment ion, designated ion a, which corresponded to the aglycon moiety ($RC(S)NH_2OH$) and was the most abundant ion in the mass spectrum. In addition to ion a, the FAB mass spectra of aryl desulfoglucosinolates contained ions corresponding to (i) R and (ii) C_7H_7 (tropylium). The spectra of indolyl desulfoglucosinolates contained ion a with reduced abundance, an ion at m/z 130 corresponding to 3-methylindole, and fragment ions enabling a clear distinction between the isomers (1-methoxy-3-indolyl)methyl and (4-methoxy-3-indolyl)methyl desulfoglucosinolates. The mass spectrum of the single alkyl thioether desulfoglucosinolate studied contained ion a with additional fragment ions corresponding to a - H_2S and a - CH_3SH. Fragmentation schemes were deduced to facilitate structural analysis of new desulfoglucosinolates.

A total of 90 or more glucosinolates (Figure 1), differing only in the structure of the side chain, have been found in 11 families of the dicotyledonous angiosperms (Larsen, 1981). They are isolated from plant tissue as pyridinium (Olsen and Sorenson, 1980) or tetramethylammonium salts (Hanley et al., 1983) or more recently by enzymatic desulfation (Thies, 1979) on an ion-exchange resin followed by elution of the desulfoglucosinolates with H_2O. The quantitative determination of glucosinolate content of rape seed developed in this laboratory consists of HPLC analysis of these desulfoglucosinolate extracts (Minchinton et al., 1982). Mass spectra of desulfoglucosinolates were thus required for identification of compounds isolated and quantified in this manner.

Desulfoglucosinolates are hydrophylic and thermally labile so derivatization to increase volatility is essential prior to GC/MS analysis. However, derivatization with silylating reagents may result in multiple derivatives and degradation of some components (Heaney and Fenwick, 1982). Electron impact mass spectra of the TMS derivatives contain many abundant glucose-related ions, but molecular ions are absent and the single characteristic ion has low abundance (Olsson et al., 1977). In some cases it may not be possible to obtain molecular ions by chemical ionization (Eagles et al., 1981).

Abbreviated positive-ion fast atom bombardment (FAB) mass spectra for six desulfoglucosinolates have been previously reported (Fenwick et al., 1982). Abundant molecular ions are observed, and it was found that very little fragmentation occurs, with loss of oxygen being the major process. In contrast, we found that in addition to the $[M + H]^+$, the positive-ion FAB mass spectra of desulfoglucosinolates contained a variety of fragment ions with abundant structural information.

For this work 12 desulfoglucosinolates were isolated by enzymatic desulfation of the ion-exchange resin-bound glucosinolates and purified by high-pressure liquid chromatography (HPLC) (Minchinton et al., 1982). Positive-ion FAB mass spectra were obtained directly on these HPLC fractions without further purification. A wide range of side chain structures were examined including three previously analyzed by Fenwick et al. (1982) and the two new indolyl desulfoglucosinolates discovered in this laboratory (Truscott et al., 1982a,b). Since both $[M + H]^+$ and characteristic fragment ions were observed, the amount of structural information obtained was greater than that available from either the electron impact or chemical ionization spectra of the TMS derivatives.

EXPERIMENTAL SECTION

Allyl, 4-(methylthio)butyl, and benzyl glucosinolates were obtained from Roth Chemicals, Karlsruhe, West Germany, and were enzymatically desulfated (Thies, 1979). Phenylethyl desulfoglucosinolate was isolated from rape root and 3-butenyl-, (2-hydroxy-3-butenyl)-, (2-hydroxy-4-pentenyl)-, 4-pentenyl-, and (4-hydroxy-3-indolyl)methyl desulfoglucosinolates were isolated from rape seed. 3-Indolylmethyl-, (1-methoxy-3-indolyl)methyl-, and (4-methoxy-3-indolyl)methyl desulfoglucosinolates were isolated from cabbage leaf.

Desulfoglucosinolates were collected from a 100 × 8 mm Radial PAK C18 (10-μm particle size) cartridge fitted in a Z module, (Waters Associates Inc., Milford, MA), on a Spectra Physics SP8000 HPLC. The wavelength setting was 227.5 nm, and the solvent program was 100% H_2O for 10 min, 0–12% CH_3CN for 30 min, and then constant 12% CH_3CN. The previously identified desulfoglucosinolate fractions (Sang et al., 1984) were freeze-dried and resuspended in water to give an approximate concentration of 15 μg/μL based on calibrated peak area. A 2-μL solution (~30-μg sample) was then mixed with an equal volume of glycerol on the FAB probe tip.

FAB mass spectra were obtained with a Phrasor fast atom capillaritron source fitted to a Hewlett-Packard 5985A quadrupole mass spectrometer as described elsehere (Mahoney et al., 1983a,b; Faull, 1984). Xenon was used as the bombarding gas with a beam energy of 8000 V and current of 50 μA. The ion source temperature was 60 °C.

The FAB mass spectra of the desulfoglucosinolate/glycerol mixtures were computer-averaged for a selected part of the scan range. From these average spectra was subtracted the averaged spectrum of glycerol obtained the same way. Only those ions directly originating from glycerol were subtracted by this automated data processing; sodium adduct ions were not removed. However, for

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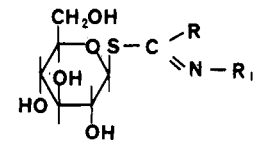


Figure 1. Structure of glucosinolates and desulfoglucosinolates. R = variable portion of glucosinolate that determines biological activity of the molecule. $R_1 = -OSO_3^-$ for glucosinolates as they occur in plants and –OH when the sulfate group is enzymatically removed during purification of plant extracts.

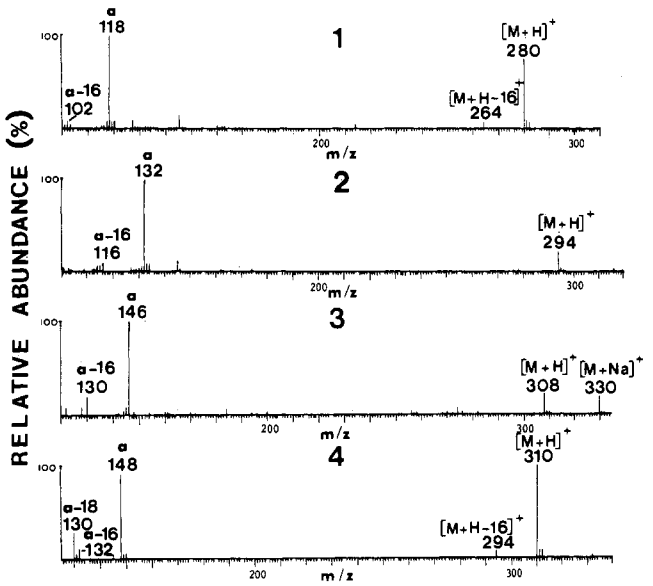


Figure 2. Positive-ion FAB mass spectra of allyl (1), 3-butenyl (2), 4-pentenyl (3), and 2-hydroxy-3-butenyl (4) desulfoglucosinolates. The presence of sodium in some desulfoglucosinolate preparations is indicated by $[M + Na]^+$.

clarity of presentation, glycerol–sodium adduct ions at m/z 115 and 207 were manually removed from spectra when these ions were due to sodium contamination as evidenced by the presence of $[M + Na]^+$.

RESULTS

A strong $[M + H]^+$ (where M = intact molecule) (10–100%) and a characteristic fragment ion corresponding to $[RCSNH_2OH]^+$ (50–100%) were observed in the FAB mass spectrum of each of the 12 desulfoglucosinolates studied (Figures 2–4). This aglycon fragment ion presumably arose by cleavage of the glucose–sulfur bond with hydrogen rearrangement and loss of the resultant dehydrogenated glucopyranose molecule ($C_6H_{10}O_5$), a process frequently observed in FAB mass spectra of glycosides and carbohydrates (Reinhold and Carr, 1983). This ion may also arise by thermal decomposition during desolvation from glycerol followed by ionization as these processes have also been observed in glycosides (Lin and Smith, 1979; Vine et al., 1979). Notwithstanding the uncertainty of its origin, the presence of the aglycon ion was diagnostic for the desulfoglucosinolates and confirmed the side chain formula weight. Further characteristic structural information was given by additional fragment ions in all spectra, with the greatest number of fragment ions observed for glucosinolates with cyclic side chains. There were no significant fragment ions originating from the glucose moiety.

In some spectra, glycerol–sodium adduct ions were observed but were removed for clarity of presentation. However, $[M + Na]^+$ ions were not removed since they confirmed the molecular weight of the desulfogucosinolate and revealed information concerning the purity of the

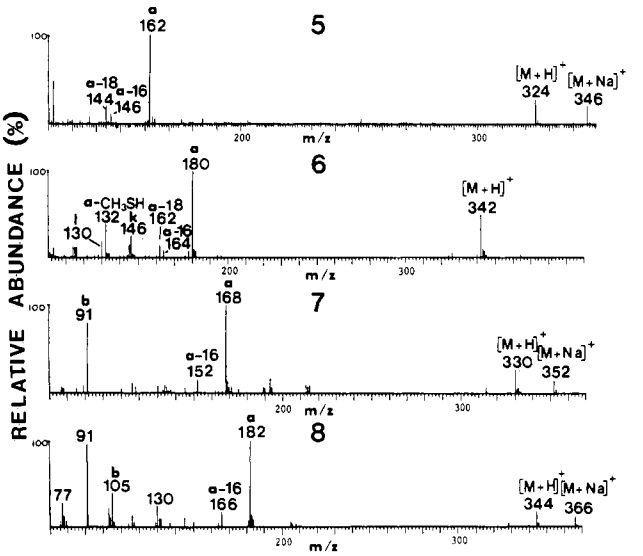


Figure 3. Positive-ion FAB mass spectra of 2-hydroxy-4-pentenyl (5), 4-(methylthio)butyl (6), benzyl (7), and phenylethyl (8) desulfoglucosinolates.

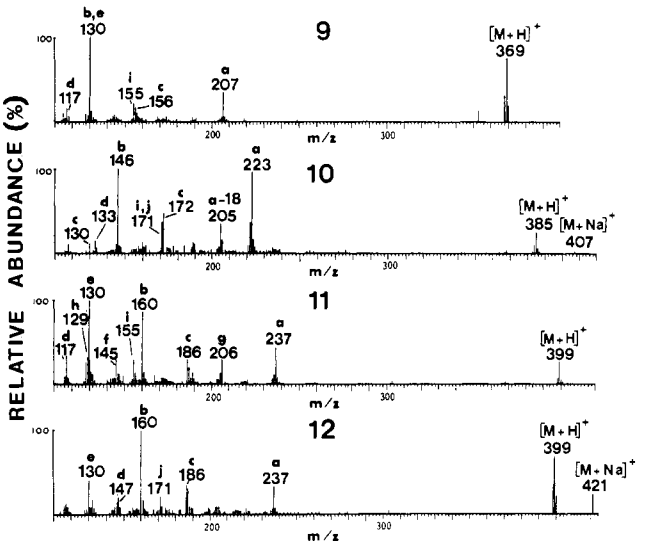
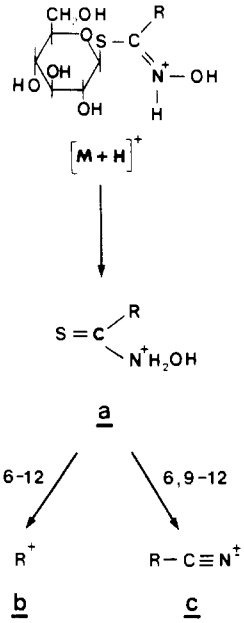


Figure 4. Positive-ion FAB mass spectra of 3-indolylmethyl (9), (4-hydroxy-3-indolyl)methyl (10), (1-methoxy-3-indolyl)methyl (11), and (4-methoxy-3-indolyl)methyl (12) desulfoglucosinolates. Note: Compound 9 was not contaminated with sodium (no $[M + Na]^+$ present) so m/z 207 originated from the analyte and not from $G_2 + Na]^+$; $[M + H]^+$ at m/z 369 was still present after glycerol ions were subtracted even though $[G_4 + H]^+$ occurred at this mass (G = glycerol).

compounds isolated by HPLC. A comparison of the spectra of 2 and 12 shows that the presence of sodium in the glycerol matrix did not alter the degree of fragmentation for these compounds relative to desulfoglucosinolates with side chains belonging to the same chemical group; thus, the spectrum of 2 contained only one major fragment ion even though no sodium was present (no $[M + Na]^+$), and the spectrum of 12 contained many fragment ions even though sodium was present.

Since the only structural differences between the compounds studied were in the side chain R group, it was possible to deduce the structures of fragment ions by spectral comparison and to propose common fragmentation pathways. This approach is analogous to the "shift technique" used by Biemann (1962) in the structural elucidation of indole alkaloids and was especially applicable to structural isomers such as (1-methoxy-3-indolyl)methyl and 4-methoxy-3-indolylmethyl desulfoglucosinolates. A

Scheme I

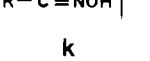


general fragmentation pathway for the desulfoglucosinolates is proposed in Scheme I.

Aliphatic Desulfoglucosinolates. Desulfoglucosinolates with aliphatic side chains had the simplest spectra; the only abundant ions were the $[M + H]^+$ and the aglycon ion a. The presence of a small a - 16 in these compounds (as with structures **5–8**) indicated that the specific oxygen lost in this process was the nitrogen-substituted atom, since that is the only oxygen present in ion a and the ion formed was thus $[RCSNH_3]^+$. The same fragmentation from the $[M + H]^+$ may be responsible for the small $[M + H - 16]^+$ observed in some spectra, and the corresponding ion structure would be $[RC(SG)=NH_2]^+$ where SG represents the thioglucose group. A small a - 18 was seen only for **4** and **5** both of which have a hydroxyl-substituted side chain, and was thus most likely due to loss of this substituent as H_2O.

Aryl Desulfoglucosinolates. The FAB mass spectra of the aryl desulfoglucosinolates **7** and **8** both contained the tropylium ion in similar abundance to a. This was ion b for **7**; **8** also contained ion b but at a lower abundance than the tropylium ion.

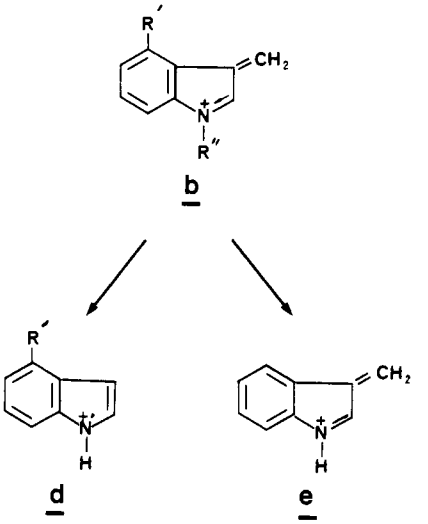
Thioether Desulfoglucosinolate. In addition to the three ions $[M + H]^+$, a, and b, the FAB spectrum of **6** contained two unusual ions at m/z 146 and 132. A possible structure for ion m/z 146 is shown as k, which could have been formed by elimination of H_2S from a at the hydroxamine carbon. Ion k was relatively small (~10%) in all other compounds studied and thus may be diagnostic for sulfur-containing side chains. The ion at m/z 132 was most likely derived by loss of CH_3SH from a and that at m/z 130 to loss of oxygen from k in a similar fragmentation to M + H - 16 and a - 16.



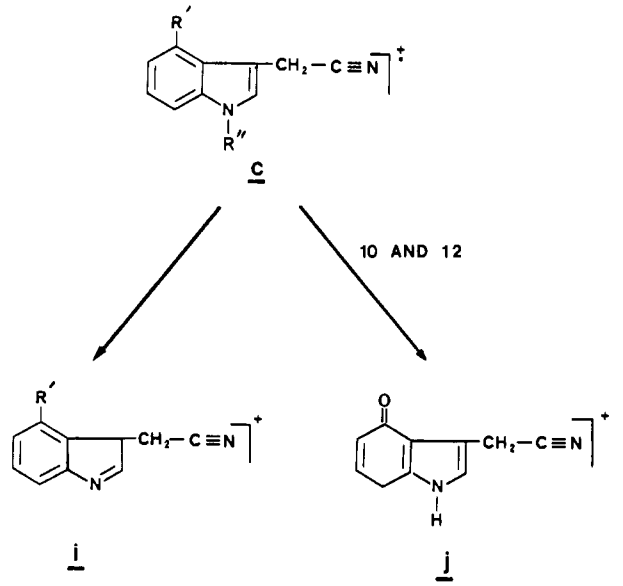
k

Indolyl Desulfoglucosinolates. The most striking feature in the spectra of indolyl desulfoglucosinolates **9–12** was the high abundance of ion b (R^+). Fragments of b, ions d and e, were observed for indolyl compounds as shown in Scheme II. An ion corresponding to b - 15 was observed solely in the mass spectrum of **11**, and the structure is proposed as ion f. Ions a - 31 and b - 31 were also observed only in **11**, reflecting the facile nature of the

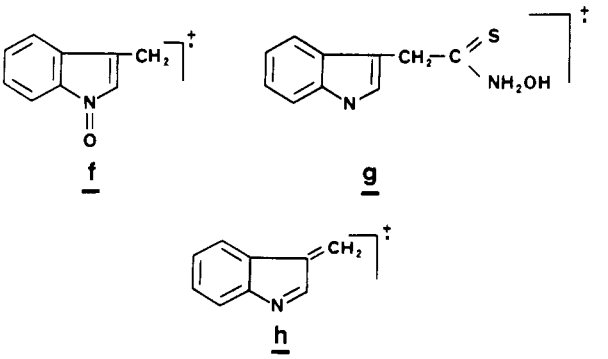
Scheme II



Scheme III



N···O bond, and their proposed structures are g and h, respectively. These fragment ions allowed ready differentiation between the 1-methoxy- and 4-methoxy-substituted indolyl desulfoglucosinolates **11** and **12**. Interestingly, an a - 18 ion was only observed in the single indolyl desulfoglucosinolate to contain hydroxyl substitution, paralleling the fragmentation of aliphatic side chains with this substituent and supporting the proposition that it was due to loss of H_2O.



Indolyl desulfoglucosinolates also formed the nitrile ion c in high abundance (18–46%), which was observed at the

center of a triplet of related ions in **9** (i.e. c − 1, c, c + 1) but as a doublet in the other three indolyl desulfoglucosinolates for the following reasons. Ion c − 1 in **11** corresponds to i (Scheme III) and was formed by loss of the indole nitrogen substituent from c. For **11** this substituent is OCH_3 not H as in **9**, **10**, and **12**, and thus the analogous fragment occurred at c − 31 (m/z 155) and not c − 1. For **12**, c − 1 is at m/z 185, the same mass value as $[G_2 + H]^+$ (where G_2 = glycerol dimer), and was subtracted from the spectrum with the rest of the glycerol ions. For **10**, c − 1 was observed at the same mass value as ion i. The ion c + 1 was observed in all spectra except for **10** where its mass value (173) coincided with a glycerol-derived ion and was substracted from the spectrum. Thus, ion c fragmented with loss of substituents from the indole nitrogen to form i and from the oxygen substituents on the indole nucleus of **10** and **12** to form j as shown in Scheme III.

DISCUSSION

Other than the alkyl desulfoglucosinolates, which were notable for the paucity of fragment ions, each type of side chain gave a readily identifiable fragment ion: tropylium for aryl, a − H_2S for alkyl thioether, and 3-methylindolyl for indolyl desulfoglucosinolates. These fragments reveal the type of side chain present and, combined with the formula weight derived from M + H and a, provide the basis for structural elucidation. Additional fragments may even be used to distinguish between structural isomers.

The variety of fragment ions observed in these FAB mass spectra contrasts with the results of Fenwick et al. (1982), who reported that the major process in FAB mass spectrometry of desulfoglucosinolates is loss of oxygen with occasional loss of $C_6H_{11}O_5$ and $C_6H_{11}O_5S$. In the work described above, the major process was formation of ion a by loss of $C_6H_{10}O_5$ with further fragmentation determined by side chain structure. Fenwick et al. used a sample concentration of 100 $\mu g/\mu L$ glycerol with a 3–7-kV argon beam whereas for the work detailed above only 15 $\mu g/\mu L$ was used with an 8-kV xenon beam. The greater amount of fragmentation observed in this work may be due to the use of a higher energy fast atom beam combined with a lower concentration of sample in the glycerol matrix, leading to an increased amount of energy imparted to sample molecules on desolvation from glycerol. The increased energy enabled the appearance of the aglycon ion with initiation of further fragmentation pathways. Other than this, an explanation of the differences is not readily apparent since it has been shown that interlaboratory reproducibility of FAB mass spectra is comparable to that of EI mass spectra (Clay et al., 1983).

Examples of the complete range of side chain classes with the exception of the oxidized sulfur side chains were examined. Since a molecular ion was obtained in each case in addition to abundant diagnostic fragment ions readily correlated with structure, this technique is a powerful method for determining glucosinolate structure and is ideal for structural determination of unknown glucosinolates. In addition to the wealth of structural information available with this technique, sample preparation was operationally economical since FAB mass spectra were obtained directly on HPLC fractions without further purification, thus eliminating the need for derivatization and gas chromatography and ensuring analytical relevance.

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Registry No. **1**, 5115-81-1; **2**, 31362-93-3; **3**, 109279-15-4; **4**, 80734-75-4; **5**, 109173-26-4; **6**, 77171-29-0; **7**, 5115-74-2; **8**, 77171-31-4; **9**, 43110-92-5; **10**, 109173-27-5; **11**, 85505-04-0; **12**, 85422-08-8.

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