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MASS-SPECTROMETRIC CHARACTERISTICS OF SOME PERTRIMETHYL-SILYLATED DESULFOGLUCOSINOLATES

B. W. CHISTENSEN, A. KJÆR* and J. ØGAARD MADSEN
Department of Organic Chemistry, The Technical University of Denmark, 2800 Lyngby, Denmark

and

C. E. OLSEN, O. OLSEN and H. SØRENSEN*
Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, 1871 Copenhagen V, Denmark

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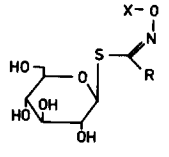
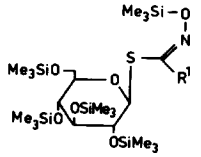
Abstract—The electron impact and chemical ionisation mass spectra of twelve pertrimethylsilyl derivatives of desulfoglucosinolates deriving from naturally occurring parent compounds are described and discussed. Series of characteristic ions, mostly comprising molecular ions, render mass spectrometry of such derivatives, in combination with gaschromatographic separation, a useful tool in the identification of known and novel glucosinolates. The scope of the method is wide though limitations exist. Thus, the extensive series of naturally occurring glucosinolates possessing methylsulphinyl-substituted side-chains undergo decomposition on attempted gas chromatography of their pertrimethylsilylated desulfoglucosinolate derivatives.

Glucosinolates (1) are anions with almost 80 structurally identified representatives discontinuously distributed within the Dicotyledoneae class of higher plants.[1a-c] Several of the glucosinolate containing taxa, notably of the family Brassicaceae, possess vast economic importance (e.g. rape, cabbage, radish); hence, much interest is associated with the analytical methodology for the separation, identification and quantitation of the individual glucosinolates in a given taxon. Today it is generally agreed that separation by chromatographic techniques of the genuine glucosinolates or their immediate derivatives, rather than their various degradation products, is the method of choice for this purpose. Among the various techniques, gas chromatography (GC) has attracted particular attention, due to ease and speed of performance, high sensitivity, and general availability of the equipment needed. Obviously, however, the anionic glucosinolates (1) must be non-destructively converted into volatile derivatives prior to GC separation and analysis.



1 X = SO_2O^-

2 X = H



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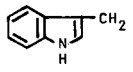
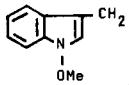
Ten years ago, Underhill and Kirkland,[2] described the successful separation of 11 glucosinolates by GC of the pertrimethylsilyl derivatives of the corresponding desulfoglucosinolates (2), produced by trimethylsilylation of the parent glucosinolates (1). Through subsequent modifications and refinement,[3a-d] this technique has become an efficient tool for the analysis of glucosinolate mixtures present in economically important food and fodder crops.[3d,4a-d] In most of the papers quoted, identification of the individual glucosinolates rests on retention times, matching those of authentic specimens. In one case, however, mass-spectrometric characteristics were suggested as an auxiliary diagnostic tool.[5] Its value was subsequently questioned, however, by British authors finding it 'impossible to obtain any structural information for the volatilised desulphoglucosinolate derivatives by mass spectrometry or combined gas chromatography-mass spectrometry'.[4c] The potential need for diagnostically useful mass-spectrometric data in connexion with gaschromatographic studies of naturally occurring, notably novel, glucosinolates remains, however, and prompted the present study of 12 individual glucosinolates. Spectra of the corresponding pertrimethyl-silylated desulfoglucosinolates (3) were recorded in both the chemical ionisation and the electron impact mode; the results are presented and discussed in the sequel.

RESULTS AND DISCUSSION

The individual glucosinolates (1), subjected to GC/MS(EI) analysis as their pertrimethylsilyl derivatives (3), are listed in Table 1. The various derivatives were produced from the potassium salts of the individual homogeneous glucosinolates (Table 1, Nos. 1, 2, 8–10) and/or by GC separation of the derivativatised mixtures of known glucosinolates present in rape seed (*Brassica napus* L. cv. Tower)[4b] (Table 1, Nos. 4–7, 9, and 10) or leaves of *Tovaria pendula* Ruiz and Pavon (Table 1, Nos. 3, 8, 11, and 12) (Experimental). Ionisation by electron impact of the individual GC fractions resulted in mass spectra containing a host of common ions, identical with those reported,[5] and arising from the derivatised glucose moiety; obviously, these ions are devoid of

Table 1. Glucosinolates (1), converted into pertrimethylsilylated desulfoglucosinolates (3), and subjected to GC/MS studies

No.	Glucosinolate, R in 1	No. of Me_3Si-groups in 3	MW of 3
1	CH_3	5	613
2	$CH_2{:}CHCH_2$	5	639
3	$(CH_3)_2CH$	5	641
4	$CH_2{:}CH(CH_2)_2$	5	653
5	$CH_2{:}CH(CH_2)_3$	5	667
6	$CH_2{:}CHCH(OH)CH_2$	6	741
7	$CH_2{:}CHCH_2CH(OH)CH_2$	6	755
8	$C_6H_5CH_2$	5	689
9	$C_6H_5(CH_2)_2$	5	703
10	(p)-$HOC_6H_4CH_2$	6	777
11		6	800
12		5	758

diagnostic interest in the present context. The various ions carrying structural information about the glucosinolate side-chains are presented in Table 2. Even when of low intensity the various peaks, taken together, constitute patterns uniquely characteristic for the individual, derivatised glucosinolates and hence deserve interest.

The same collection of derivatised glucosinolates (3) was subjected to GC, followed by chemical ionisation mass spectrometry [MS (CI)]; the characteristic ions observed in this mode are summarized in Table 3. It appears that MS(CI) offers certain advantages over the electron impact spectra. Generally, the M + 1-ions are of considerably higher intensity than the M-ions in the MS(EI), and the doubly protonated aglucone ions, as well as the protonated nitrile fragments, are consistently prominent, the only exception being the N–OMe compound (No. 12); this, however, affords the analogous fragment ions, with about the same intensities, lowered by 30 mass units, resulting from formal loss of formaldehyde from the N–OMe grouping.

The 12 glucosinolates discussed in the present paper represent only about one seventh of the total of such compounds known today as natural products. The generality of the method hence requires further study. Among the known side-chains, additional to the ones here studied, many are simple homologues or analogues.[1a–c] No complications are to be expected in the analysis of such glucosinolates. However, certain functionalities, as e.g. the sulfoxide grouping, would be suspected to cause complications due to the known propensity of sulfoxides to undergo thermally induced eliminations. This was indeed found to be the case. Hence, the remarkable group of naturally occurring glucosinolates (1) encompassing every member of the series with $R{=}MeSO[CH_2]_n$, $n = 3$–11, constitutes a serious limitation of the GC/MS technique in its present form. Other authors[3d,4c] similarly failed to gaschromatograph the widely distributed, derivatised 3-methylsulfinylpropylglucosinolate (1, $R{=}MeSO[CH_2]_3$, and two higher homologues,[4b] without extensive decomposition. Other limitations are unknown at present.

Mass spectra (EI) have recently been reported for pure glucosinolates (1) and desulfoglucosinolates (2), individually introduced through the solid inlet.[6] Apart from the $[R]^+$-ions (*cf* Table 2), the observed common ions have no counterpart in the characteristic ions listed in Tables 2 and 3. The fact that glucosinolates virtually always occur in mixtures, often of considerable complexity, makes their identification by GC/MS, with or without previous isolation and separation, a matter of continued interest.

EXPERIMENTAL

Initial GC/MS experiments were performed as previously reported;[4b] results described in the present paper were obtained as follows: GC was performed on a glass column (1,5 m × 2 mm i.d.), packed with 5% OV-101 on Chromosorb W, HP, 80–100 mesh. For efficient separation of the indoleglucosinolate volatiles, deriving from the parent compounds Nos. 11 and 12 (Table 1), the application of a more polar stationary phase, such as OV-17, proved mandatory. Again, preconditioning of the column by repeated injections of the silylating reagent, prior to GC analysis, was necessary to prevent desilylation during GC, notably of the N-silylated derivative arising from No. 11 (Table 1). He was used as carrier gas at a flow rate of 20 ml min^{-1}; the injection port was kept at 350°C and a linear gradient, 4° min^{-1}, was utilized within the range 230–280°.

Mass spectra were obtained on a VG 7070 instrument, attached, through an all glass jet separator interface system, to the gaschromatograph exit. For the electron impact spectra an IP of 70 eV and an ion source temp. of 220° were applied. Chemical ionization spectra were recorded with isobutane as the reactant gas.

Silylations were performed by treating the anhyd. glucosinolate samples, placed in pyridine soln in Teflon-capped vials, with a 10:11 mixture of BSTFA and TMCS, followed by heating to 120° for 15 min.

In order to obtain satisfactory mass spectra it proved essential to apply larger quantities of samples than usual; as much as about 1 μg of each component was needed in order to obtain acceptable spectra.

Glucosinolates of various provenance were employed in the present studies. Apart from pure glucosinolates (Table 1, Nos. 1–2, 8–10), and glucosinolates present in a purified rape seed fraction (cv. Tower) (Table 1, 4–7, 9, 10),[4b] a leaf extract of the monotypic, South American taxon *Tovaria pendula* Ruiz and

Table 2. Side-chain containing ions produced by electron-impact induced ionisation of pertrimethylsilyl derivatives of desulfoglucosinolates (3). *m/z* (% relative intensity); base peak: *m/z* 73

Compound No. (as in Table 1)	$M^{+\cdot}$	$(M-15)^+$	$(M-89)^+$	$(M-15-90)^+$	$(M-89-90)^+$	^+S—C(=NOSi(CH$_3$)$_3$)—R^1	$Me_3SiO\overset{+}{N}\equiv C-R^1$	R^{1+}	Other ions
1	613 (0)	598 (0.1)	524 (0.2)	X[a]	X	X	130 (13)		
2	639 (0.05)	624 (0.05)	550 (0.05)	534 (0.1)	X	X	156 (9)[b]	41 (3)	
3	641 (0)	626 (0.05)	552 (0.1)	536 (0.05)	462 (0.05)	X	158 (12)	X	
4	653 (0.1)	638 (0.1)	564 (0.1)	548 (0.2)	X	202 (10)[b,c]	170 (6)[b]	55 (3)	80 (5)[b,d]; 81 (3); 53 (3); 41 (7)
5	667 (0.05)	652 (0.1)	578 (0.05)	562 (0.1)	X	216 (0.5)	184 (0.5)	69 (2)	94 (4)[b,e]; 67 (4)[b,e]; 66 (4)[f]
6	741 (0.4)	726 (0.2)	652 (0.3)	636 (0.3)	562 (0.4)	290 (13)[b,c]	258 (7)[b]	X	
7[g]	755 (0.05)	740 (0.05)	666 (0.05)	650 (0.05)	576 (0.1)	X	X[b]	X	
8	689 (< 0.05)	674 (0.05)	600 (0.2)	584 (0.1)	X	X	206 (5)	91 (15)	
9	703 (0.05)	688 (0.1)	614 (0.4)	598 (0.2)	524 (0.2)	X	220 (1)	105 (6)	91 (11)
10	777 (0.5)	762 (< 0.05)	688 (0.3)	672 (0.05)	598 (0.1)	326 (0.5)	294 (1.7)	179 (33)	508 (1)
11	800 (2)	785 (0.1)	711 (0.3)	695 (0.05)	621 (0.2)	349 (2)	X	202 (29)	227 (16)[h]; 350 (1)
12	758 (0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	728 (3)[i]; 639 (0.7)[j]; 638 (0.3)[k]; 549 (0.3)[l], 157 (5); 156 (5)[m]; 155 (9); 130 (54)[n]

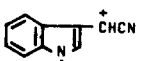
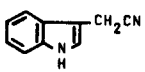
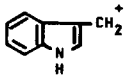
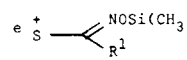
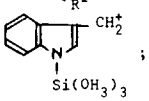
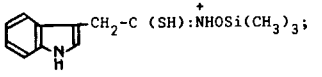
[a] X signifies an intensity too low to exclude its derivation from background signals; [b] previously recorded[5]; [c] probably stabilized as a quasi-cyclic six-membered sulfonium ion; [d] $[CH_2{:}CH(CH_2)_{2-3}CN-H]^+$; [e] $C_5H_7^+$; [f] $C_5H_6^{+\cdot}$; [g] very weak spectrum; [h]  ; [i] M-30 (CH_2O); [j] M-30-89; [k] M-30-90; [l] M-30-89-90; [m]  ; [n] 

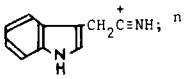
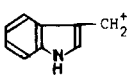
Table 3. Side-chain containing ions produced by chemical ionization, with isobutane as the reactant gas, of pertrimethylsilyl derivatives of desulfoglucosinolates (3). *m/z* (% relative intensity); base peaks: *m/z* 361, or *m/z* 91, as indicated

Compound No. (as in Table 1)	$(M+1)^+$	$(M-15)^+$	$(M-89)^+$	$(M-15-90)^+$	HS—C(=N$^+$HOSi(CH$_3$)$_3$)—R^1	$(CH_3)_3SiO\equiv C-R^1$	$HN\equiv C-R^1$	Other ions
1[a]	614 (1)	598 (0.1)	524 (0.1)	508 (0.1)	164 (15)	130 (8)	42[b]	
2[a]	640 (1)	624 (0.1)	550 (0.1)	534 (0.2)	190 (15)	156 (6)	68 (24)	
3[c]	642 (1)	X[d]	X	X	192 (24)	158 (2)	70 (7)	
4[a]	654 (1)	638 (0.1)	564 (0.1)	548 (0.2)	204 (4)	170 (3)	82 (4)	202 (3)[e]
5[c]	668 (1)	652 (0.2)	X	562 (0.5)	218 (7)	184 (< 0.5)	96 (19)	
6[a]	742 (13)	726 (1)	652 (1)	636 (1)	292 (9)	258 (5)	170 (5)	290 (6)[e]; 129 (10)[f]
7[c]	756 (3)	740 (0.3)	X	X	306 (5)	272 (3)	184 (13)	
8[c]	690 (3)	674 (0.1)	600 (0.1)	584 (0.2)	240 (11)	206 (4)	118 (54)	
9[c]	704 (2)	688 (0.2)	614 (0.2)	598 (0.3)	254 (6)	220 (2)	132 (28)	
10[a]	778 (7)	762 (0.2)	688 (0.3)	672 (0.1)	328 (6)	294 (2)	206 (12)	179 (30)[g]
11[c]	801 (17)	785 (0.6)	711 (0.5)	695 (0.3)	351 (10)	317 (1)	229 (11)	202 (95)[h]
12[a]	759 (0)	(0)	(0)	(0)	0	0	0	729 (20)[i]; 713 (0.3)[j]; 639 (1)[k]; 279 (13)[l]; 157 (29)[m]; 130 (100)[n]

[a] base peak: m/z 361; [b] m/z 42, hidden by reactant gas peak; [c] base peak: m/z 91 [$(CH_3)_3SiOH_2^+$]; [d] X signifies an intensity too low to exclude its derivation from background signals;

[e]  (cf. footnote c, Table 2); [f] $CH_2{:}CHCH{:}OSi(CH_3)_3$; [g] $(CH_3)_3SiO{\cdot}C_6H_4CH_2^+$;

[h]  ; [i] (M+1)-30 (CH_2O); [j] M-30-15; [k] M-30-89; [l]  ;

[m]  ; [n] 

Pavon (Tovariaceae) served as a glucosinolate source. Previous reports of the occurrence of the indoleglucosinolates (Nos. 11 and 12, Table 1)[7] and isopropylglucosinolate (No. 3, Table 1)[8] in this taxon were confirmed and supplemented with the finding of benzylglucosinolate (No. 8, Table 1). The ratio between the gluscosinolates Nos. 12, 11, 8, and 3 was estimated to be about 80:10:1:3.

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