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^{1}H-N.M.R., ^{13}C-N.M.R., AND MASS SPECTRA OF GLUCOSINOLATES AND RELATED COMPOUNDS

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ABSTRACT

The mass spectra of per(trimethylsilyl) ethers of desulfated glucosinolates are dominated by ions derived from the glucose moiety. The ions at *m/e* 271 and 361 are much more abundant than in the spectra shown by the corresponding derivatives of hexoses and their simple glycosides. The individual glucosinolates are distinguished by a few ions originating from their respective aglycon groups. The ^{1}H- and ^{13}C-n.m.r. spectra of sinigrin and some closely related compounds have been analyzed.

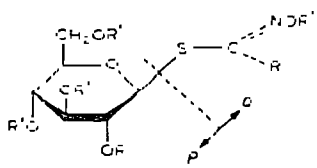
INTRODUCTION

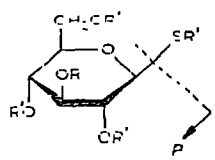
The general structure of glucosinolates (*e.g.*, **7**) was proposed in 1956[1], and verified a year later by synthesis[2]. Certain plants of the *Cruciferae* family are important in the food-oil industry, but their additional use as a protein source is hindered by their large content of glucosinolates, which may yield toxic products on enzymic hydrolysis[3]. Reliable methods are therefore needed for the detection and quantitative determination of glucosinolates, particularly when these have been partly removed by extraction or by plant breeding. We recently reported[4] a new method for determination of the total glucosinolate content and applied it to defatted seeds from some cultivars of rape or turnip rape. In addition, the individual compounds were determined[5] by g.l.c. as trimethylsilyl ethers (**1–5**), which are formed with concomitant desulfation[6]. Their identification was based only on the g.l.c. retention times, which matched those shown by authentic samples or found in the literature[5,6].

This comparison is now extended to the mass spectra. The ^{1}H- and ^{13}C-n.m.r. spectra of the simplest glucosinolate, sinigrin (**7**), and some related compounds are also analyzed. The spectra confirm the previous results[4], and such confirmation is imperative when analyzing complex samples or those having a low content of glucosinolate, such as protein concentrate[7] or isolate[8], which may yield numerous, large, but irrelevant, g.l.c. peaks. Knowledge of the general spectral features should also aid future identification of less-common glucosinolates for which g.l.c. data may not be available; some 50 representatives have already been found in *Cruciferae* plants[3].

RESULTS AND DISCUSSION

Trimethylsilylation[9] of pure glucosinolates, concentrated extracts of defatted seeds[4], and the sodium salt of 1-thio-β-D-glucopyranose yielded the respective derivatives **1**–**4**, **2**–**5**, and **6** (along with some α anomer), all of which were investigated by g.l.c.–mass spectrometry. Fig. 1 shows the g.l.c. analysis of a typical trimethylsilylated seed-extract, to which authentic sinigrin (**7**) had been added. The mass-spectral data for **1**–**6** are collected in Table I. The spectra of **2**–**4** were independent of their source, and those of **6** and its α anomer were identical. In Table I, abundance is given as a percentage of the base peak, disregarding the strong Me_3Si^+ peak at *m/e* 73. Ions are listed only if they are of diagnostic value, or if their relative abundance is ⩾ 20% in any spectrum and ⩾ 10% in the one considered. The larger, upper part of Table I contains all ions common to **1**–**5**. As most of these ions are also shown by **6**, they are largely derived from the glucose moiety (*P*), although trimethylsilylated fragments of the side-chain R in **4** and **5** may also contribute to the smaller ions. Most ions common to **1**–**6** are also shown by the trimethylsilyl ethers of glucose, other hexoses, and their simple glycosides, but the largest ions are much less prominent in the spectra of the latter compounds, or even absent[10]. The difference is most pronounced for the peak at *m/e* 361. This peak, and perhaps also that at *m/e* 271, may therefore be characteristic of 1-thiohexopyranosides or at any rate of 1-thioglucopyranosides. In any case, these peaks are very useful for distinguishing glucosinolates from sugars and ordinary glycosides often present in plants.





1 R = $CH_2CH{=}CH_2$
2 R = $CH_2CH_2CH{=}CH_2$
3 R = $CH_2CH_2CH_2CH{=}CH_2$
4 R = $CH_2CH(OR')CH{=}CH_2$
5 R = $CH_2CH(OR')CH_2CH{=}CH_2$

6

R' = $SiMe_3$

The smaller, lower part of Table I contains those ions which are unique for one particular compound. Apart from M and M − Me·, these ions are no doubt derived from the respective aglycon moiety (*Q*). Unlike *P* and its fragments, they generally appear at even *m/e* values, owing to the presence of one nitrogen atom in *Q*. The only exceptions are the odd-electron *P* fragment at *m/e* 204 and the *Q* fragment of **3** at *m/e* 67, which has probably lost the nitrogen and consists of $C_5H_7^+$. The *Q* fragments are therefore easy to recognize, and provide a simple means of identifying the individual glucosinolates. However, their small number and fairly low intensities may constitute a certain limitation, as indicated by the spectrum of **5**.

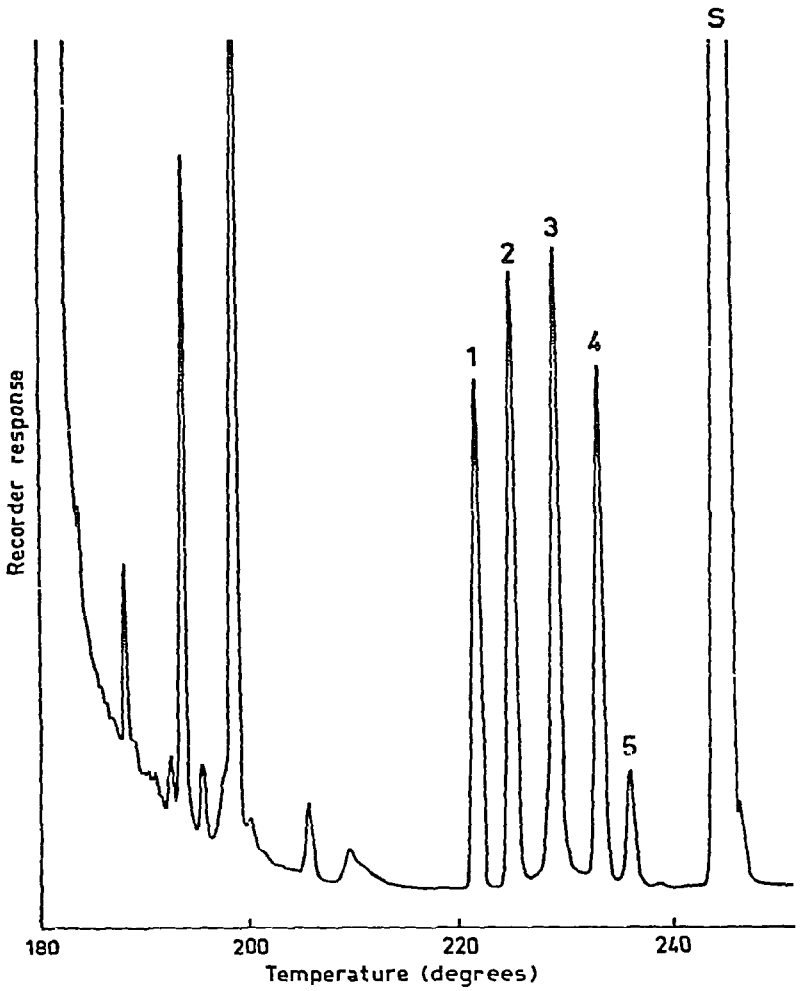


Fig. 1. G.l.c. analysis of a trimethylsilylated meal-extract, to which sinigrin (7) has been added, from the *Brassica campestris* cultivar "Bele" on 3% of OV-1 at 35 ml of N_2/min; S = octa-*O*-(trimethylsilyl)sucrose.

Table II shows the n.m.r. chemical shifts (δ) for desulfosinigrin (**8**), its per-(trimethylsilyl) and peracetyl derivatives (**1** and **9**, respectively), and sinigrin (**7**). Methyl β-D-glucopyranoside (**11**) and the peracetyl derivative (**10**) of 1-thio-β-D-glucopyranose are included for comparison. Even under anhydrous conditions, **1** decomposed fairly rapidly, as evidenced by a gradually increasing ^{1}H-n.m.r. singlet at δ 0.28, due to trimethylsilanol. Owing to the heat generated by the proton noise decoupler and the longer time needed, it was not possible to record a satisfactory ^{13}C-n.m.r. spectrum of **1**. The spectra of **1** and **7**–**9** each showed only one of the two geometrical isomers expected from the carbon–nitrogen double bond.

TABLE I

RELATIVE ABUNDANCE OF DIAGNOSTIC IONS IN THE MASS SPECTRA OF DESULFOGLUCOSINOLATE AND 1-THIO-β-D-GLUCOPYRANOSE PER(TRIMETHYLSILYL) ETHERS (1–6)

m/e	*Fragment*	1	2	3	4	5	6
59		39	55	33	18	26	24
73		[a]	[a]	[a]	[a]	[a]	[a]
75		75	90	81	68	76	70
89		25	20				
103		71	55	59	46	60	62
117		31	35	26	21	39	29
129		47	45	41	48	42	33
131		14	20	15	18	21	16
133		18	20	15	18	21	24
147		92	100	100	89	93	100
169		53	20	26	16	15	17
189		18	15	15	17	20	16
191		22	15	15	17	31	19
204	$[R'OCH{=}CHOR']^{+}$	14	20	15	19	26	67
205		18	10	15	18	25	31
217		71	45	59	56	75	89
243		25	20	15	16	20	
271	$P-2HOR'$	31	20	15	21	24	
361	$P-HOR'$	100	80	59	100	100	75
435		[b]	[b]	[b]	[b]		
451	P	[b]	[b]	[b]	[b]	[b]	4
	$Q-S-HOR'-HCN$			41			
	$Q-S-HOR'$		45	26			
	$Q-S$	31	15		14	10	
	Q		20		20		
	$M-Me^{\cdot}$	[b]	[b]	[b]	[b]		
	M	[b]	[b]	[b]	[b]		

[a] > 100. [b] Trace.

TABLE II

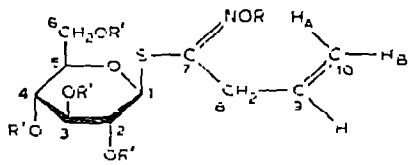
N.M.R. CHEMICAL SHIFTS (δ) FOR SINIGRIN AND RELATED COMPOUNDS IN CD_3OD (^1H) OR H_2O (^{13}C)

Nucleus	1[a]	7	8[b]	9[c,d]	10[c,e]	11[f]
H-1	4.70	4.92	4.88	5.49	5.38	4.18
H-2	~3.20	3.21	3.20	5.01	5.02	3.16
H-3	~3.3	~3.3	~3.3	5.38	5.37	~3.3
H-4	~3.3	~3.3	~3.3	5.06	5.05	~3.3
H-5	~3.3	~3.3	~3.3	~4.1	4.04	~3.3
H-6	~3.5	3.66	3.67	~4.2	4.07	3.68
H-6'	~3.8	3.89	3.90	~4.2	4.23	3.87
H-8,8'	3.66	3.54	3.40	3.57		
H-9	5.94	6.06	6.00	6.07		
H-A	5.20	5.32	5.24	5.31		
H-B	5.10	5.18	5.11	5.21		

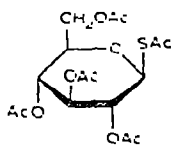
TABLE II (*continued*)

Nucleus	1[a]	7	8[b]	9[c,d]	10[c,e]	11[f]
CH_3	0.10			1.96	1.94	3.53
	0.15			2.00	1.96	
	0.16			2.00	1.99	
	0.18			2.02	1.99	
	0.20			2.12	2.38	
C-1		82.4	82.0	80.2	80.6	103.9
C-2		72.9	73.0	70.5	69.8	73.8
C-3		78.0	78.0	74.0	74.4	76.5
C-4		70.1	70.0	68.7	68.7	70.5
C-5		80.7	80.5	76.5	76.9	76.5
C-6		61.6	61.6	62.6	62.3	61.7
C-7		163.6	155.3	160.3		
C-8		36.9	36.5	37.3		
C-9		132.9	133.7	132.3		
C-10		119.1	118.5	118.9		

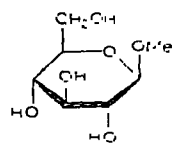
[a] In CCl_4. [b] NO*H*, 11.0 in Me_2SO. [c] In $(CD_3)_2CO$ (^1H) or $CDCl_3$ (^{13}C). [d] CH_3 19.1–20.6; C=O 169.0–169.4. [e] CH_3 20.4–20.7, 31.0; C=O 169.3, 169.5, 169.9, 170.3, 191.9. [f] CH_3 57.8.



1 R = R' = $SiMe_3$
7 R = $SO_3^-K^+$, R' = H
8 R = R' = H
9 R = R' = Ac



10



11

Assignment of all protons and determination of their mutual coupling constants (J) were possible only for **10**. This has been done previously with virtually the same results, acetone-d_6 being the most useful solvent[11]. Using the same solvent for **9** and successive spin-decoupling, first-order analysis of the whole spectrum was possible, apart from the strongly overlapping signals for H-5, H-6, and H-6'. The J values obtainable for the other compounds are not given, as they did not differ materially from the corresponding values for **9**. These are collected in the experimental section. The diastereotopic protons 8 and 8' in **1** and **7–9** appeared equivalent, or nearly so.

The ^{13}C-n.m.r. shifts for **11** accorded well with those reported by Breitmaier *et al.*[12], in view of their use of an external reference. For the other compounds, the assignment of carbons 6–10 followed immediately from their shifts and from the

multiplicities in the "off-resonance" spectra. In the latter spectra of **7** and **8**, the C-1 signal was clearly distinguished from those of C-2–C-5 by its smaller splitting. This observation was expected from the fact that the H-1 frequency was particularly close to that of the proton decoupler, which was centred at the proton resonance of external benzene. Using the same argument, the C-5 signal was identified by its large splitting in the off-resonance spectra of **9** and **10**. In chloroform-*d* solution, the $Pr(fod)_3$-induced ^1H- and ^{13}C-n.m.r. shifts[13] clearly showed preferential coordination of the shift reagent to the nitrogen atom in **9** and to the acetyl group at C-6 in **10**, but, in both compounds, the induced shifts for C-2–C-4 were too similar to permit their mutual assignment. The remaining carbons in **7**–**10** therefore had to be identified by shift comparison between the spectra, using that of **11** as a safe starting-point[12]. Such a procedure is not very satisfactory, but alternative assignments hardly seem reasonable in the present case.

As to the glucose signals, the only substantial shift differences between **7**–**8** and **11** concern H-1 and C-1, and this circumstance is not likely to change on simultaneous derivatization. Although the C-1 shift might therefore be used to distinguish glucosinolates from ordinary glycosides, the H-1 shift will not exclude α-glycosides, and other methods, *e.g.*, g.l.c.–mass spectrometry, will generally be more convenient. As indicated above, for identification of individual glucosinolates, the latter method may however be somewhat limited in scope. Confirmation by other methods is a minimal requirement when previously unknown glucosinolates are concerned. For that purpose, n.m.r. spectrometry should be an attractive alternative to degradation methods[1]. The aglycon signals are therefore of particular value, and their overlap by others should be minimized by choosing a suitable solvent and/or derivative. At least for sinigrin, such overlap is confined to the ^1H-n.m.r. spectra, where the strongly coupled, glucose protons obscure a wide, and important, frequency range. However, as seen from Table II, the signals for these protons are shifted downfield on acetylation in such a manner that no unaffected signal remains obscured. Thus, protons 8 and 8′ are partly obscured, but protons A and B are "free" in the spectra of **1**, **7**, and **8**, whereas the situation is reversed in that of **9**. Although a proton adjacent to a hydroxyl group may be hard to detect (*cf.* **4** and **5**), most, or all, aglycon signals should therefore also be revealed for more-complex glucosinolates if the ^1H-n.m.r. spectrum is recorded both before and after acetylation.

EXPERIMENTAL

General. — Melting points are corrected. Unless stated otherwise, evaporations were carried out to dryness *in vacuo* below 40°.

Chromatography. — P.c. was performed on Whatman No. 1 paper with ethyl acetate–acetic acid–water (3:1:1); detection was effected with silver nitrate–sodium hydroxide. T.l.c. was performed on silica gel (Merck, HF_{254}) with ethyl acetate–light petroleum (b.p. 40–60°) (1:5 for trimethylsilyl ethers and 7:3 for acetates); aqueous sodium dichromate–sulfuric acid was used for detection. G.l.c. was performed on a

Varian 2700 instrument equipped with dual flame-ionization detectors and glass columns (1.8 m × 2 mm i.d.). The nitrogen flow-rate was 35 ml/min. Trimethylsilyl ethers were analyzed on 3% OV-1/Varaport 30 (100–120 mesh) at 180–275°, programmed at 4°/min, and acetates on 3% OV-225/Gas-Chrom Q (100–120 mesh) at 200°.

G.l.c.–m.s. — The mass-spectral data (Table I) were obtained at 70 eV with a Varian MAT CH 7 mass spectrometer, equipped with the SpectroSystem 100 N 101/81 MS and a Varian 1740 gas chromatograph. This was fitted with the columns and operated in the manner indicated above for trimethylsilyl ethers, but with a helium flow-rate of 25 ml/min.

N.m.r. spectrometry. — ^{1}H-N.m.r. spectra were recorded at 100.0 MHz and ~30° on a Varian HA-100 D instrument, with ≤ 10% sample solutions in 5-mm o.d. tubes. ^{13}C-N.m.r. spectra were recorded at 25.14 MHz and ~50° on the same instrument (equipped with a Varian VFT-100 Fourier Transform System), with external benzene to provide the lock signal and ~15% sample solutions in 8-mm o.d. tubes. All n.m.r. shifts (Table II) were related to internal tetramethylsilane, apart from the ^{13}C-n.m.r. shifts for aqueous solutions. The latter shifts were related to internal sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate and converted into δ values by adding −1.75 p.p.m., which was the δ value for this compound in methanolic solution. Coupling constants for **9** are given below.

Starting materials. — Sinigrin (**7**, Koch-Light), methyl β-D-glucopyranoside (**11**, Sigma), and the sodium salt of 1-thio-β-D-glucopyranose (Sigma) were pure (p.c.) commercial samples. The glucosinolates gluconapin, glucobrassicanapin, and progoitrin, corresponding to **2**–**4**, respectively, and prepared from rapeseed meal[14], were kindly supplied by Dr. R. Björkman. Seed extracts were prepared and trimethylsilylated as described previously[4]. That used for Fig. 1 was obtained from seeds of the turnip rape (*Brassica campestris*) cultivar "Bele".

Penta-O-(*trimethylsilyl*)*desulfosinigrin* (**1**). — Sinigrin (**7**, 0.5 g) was trimethylsilylated[6], and the resulting mixture centrifuged. The clear supernatant was evaporated to dryness at 40°/760 torr in a stream of dry nitrogen. T.l.c. and g.l.c. of the residue (0.8 g) showed only **1** and trimethylsilanol (~5% by g.l.c.). The latter increased gradually in amount and was isolated by crystallization from chloroform; ^{1}H-n.m.r. data (CCl_4): δ 0.28 (s, 3 Me) and 4.47 (s, OH). Compound **1** had $[\alpha]_D^{20}$ −19.7° (*c* 2, chloroform).

1-Thio-tetra-O-(*trimethylsilyl*)-S-(*trimethylsilyl*)-β-D-*glucopyranose* (**6**). — The sodium salt (2 mg) of 1-thio-β-D-glucopyranose was trimethylsilylated[9]. G.l.c. of the product showed two peaks corresponding to **6** and its α anomer.

Desulfosinigrin (**8**). — A solution of **1** (0.8 g) in 60% methanol (20 ml) was boiled under reflux for 2 h and then evaporated[6]. The residue was treated with water (20 ml) for 10 min in an ultrasonic bath. The filtered solution was evaporated, yielding pure (p.c.) **8** (0.5 g) as a syrup, $[\alpha]_D^{20}$ −4.7° (*c* 1.5, water).

Penta-O-*acetyldesulfosinigrin* (**9**). — A solution of **8** (0.3 g) in dry pyridine (10 ml) and acetic anhydride (10 ml) was boiled under reflux for 15 min, and then

evaporated, first without, and then with, dry ethanol. Crystallization from methanol yielded pure (t.l.c.) **9** (0.3 g), m.p. 130–134°, $[\alpha]_D^{20}$ −0.1° (*c* 2, chloroform). ^{1}H-N.m.r. data (acetone-d_6):

x		1	2	3	4	8,8′	8,8′	9	9	A
y		2	3	4	5	9	A,B	A	B	B
$\lvert J_{x,y}\rvert$	(Hz)	10.0	9.0	9.0	9.1	6.0	1.6	17.5	10.1	1.7

Anal. Calc. for $C_{20}H_{27}NO_{11}S$: C, 49.1; H, 5.6; N, 2.9. Found: C, 48.7; H, 5.5; N, 2.6%.

*Tetra-O-acetyl-S-acetyl-1-thio-β-*D*-glucopyranose* (**10**). — The sodium salt (0.3 g) of 1-thio-β-D-glucopyranose was acetylated[15]. The product consisted of **10** and its α anomer (~10% by g.l.c.). Recrystallization from dry ethanol yielded pure (g.l.c.) **10** (0.35 g), m.p. 120–121°; lit.[15] m.p. 121°.

ACKNOWLEDGMENTS

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