Carbohydrate Research, 132 (1984) 323–329
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

N.m.r. spectra (^1H, ^{13}C) of glucosinolates

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(Received December 12th, 1983; accepted for publication, March 21st, 1984)

Glucosinolates are an important group of naturally occurring thioglycosides which are the flavour precursors of cruciferous vegetables (*e.g.*, cabbage, Brussels sprouts, and cauliflower), condiments (mustards), relishes (horse-radish), and salad vegetables (radish, cress)[1,2]. Almost one hundred glucosinolates have been described[1] and, with a single exception[3], they all possess the same general structure (Table I), comprising an amino acid-derived side-chain, a sulphonated oxime moiety, and a thioglucoside residue. Under the influence of myrosinase (EC 3.2.3.1), glucosinolates are hydrolysed to afford a range of organoleptically important products, notably volatile isothiocyanates and nitriles. Although myrosinase, apparently, is found in all glucosinolate-containing plants[4], enzyme and substrate are separated in the intact tissue and hydrolysis occurs following cellular disruption.

The disparate products of myrosinase-catalysed glucosinolate hydrolysis possess a wide range of sensory and physiological properties, both beneficial and deleterious. It is now evident that the chemical structure of the parent glucosinolate is an important factor in determining the nature of the hydrolysis products[5]. The analysis of the glucosinolate content in plant species, therefore, is mainly concerned with the identification and quantification of individual rather than total glucosinolates.

Recently, methods have been developed for the separation and purification of intact glucosinolates[6] and this, together with improvements in analytical methodology[7], will give added stimulus for the isolation and characterisation of glucosinolates.

The majority of glucosinolates have hitherto been characterised by consideration of their breakdown products[8], but such methods have limitations because of

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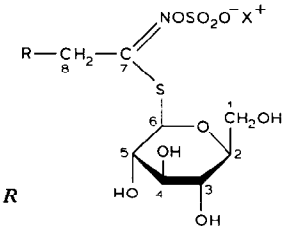
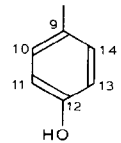
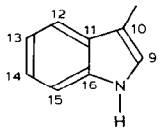
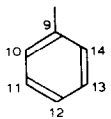
0008-6215/84/$ 03.00

TABLE I

STRUCTURE AND NOMENCLATURE OF GLUCOSINOLATES STUDIED



Glucosinolate	*R*	*Glucosinolate*	*R*
Prop-2-enylglucosinolate (**1**)	$CH_2=CH-$	*p*-Hydroxybenzylglucosinolate[a] (**8**)	
But-3-enylglucosinolate (**2**)	$CH_2=CH-CH_2-$	Indol-3-ylmethylglucosinolate (**9**)	
(2*S*)-2-Hydroxybut-3-enylglucosinolate (**3**)	$CH_2=CH-CH(OH)-$	1,3,4,5-Tetra-acetylbenzylglucosinolate (**10**)	
3-Methylsulphinylpropylglucosinolate (**4**)	$CH_3-SO-CH_2-CH_2-$	Desulpho-(2*S*)-2-hydroxybut-3-enylglucosinolate (**11**)	
4-Methylthiobutylglucosinolate (**5**)	$CH_3-S-CH_2-CH_2-CH_2-$		
3-Methylsulphonylpropylglucosinolate (**6**)	$CH_3-SO_2-CH_2-CH_2$		
Benzylglucosinolate (**7**)			

[a]Examined as tetramethylammonium, potassium, and sinapinium salts.

the small quantities in which certain glucosinolates may be isolated and the possible production of a range of hydrolysis products and their subsequent reaction. Pure glucosinolates are also important for analytical purposes and for physiological and toxicological studies; therefore, analytical techniques are required which can utilise the intact glucosinolate for structure elucidation. Some information can be obtained[9] by g.l.c.–m.s., but this is often insufficient for unique structural assignment and needs to be supplemented by other data. The results presented here suggest that the n.m.r. spectra of glucosinolates will be useful in this respect.

N.m.r. spectroscopy has been used only to a limited extent in the analysis of glucosinolates[3,10,11]. The ^{13}C-n.m.r. data for a selection of glucosinolates[12] have been reported, and the work presented here extends the survey and analysis. ^{1}H-N.m.r. spectra of glucosinolates are complex and difficult to resolve at low fields, and previous analysis has been very limited[3,10,11]. However, at higher fields, several characteristics are identifiable which provide a useful finger-print for the structural analysis of glucosinolates. MacLeod and Rossiter[13] reported the 200-MHz ^{1}H-n.m.r. spectrum of 2-hydroxybut-3-enylglucosinolate (**3**), but the complex spectrum was not analysed completely. Emphasis has been placed on the amino acid-derived moieties since, for the majority of glucosinolates, this is the location of structural differences.

The data for proton-noise-decoupled ^{13}C-spectra are summarised in Table II. The multiplicities observed in the single-frequency off-resonance-decoupled spectra helped to confirm the assignments based on known chemical shift rules

TABLE II

^{13}C CHEMICAL SHIFT VALUES (p.p.m. RELATIVE TO TETRAMETHYLSILANE) FOR GLUCOSINOLATES **1–11**

Carbon atom	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[a]	**8**[b]	**9**	**10**[c]	**11**[c]
1	61.6	61.7	61.4	61.6	61.8	61.5	61.3	61.2	61.4	61.4	62.9	61.7
2	80.9	81.2	80.8	81.0	81.3	80.8	80.8	80.7	80.9	81.0	76.3	81.1
3	70.1	70.2	69.9	70.1	70.3	70.0	69.8	69.7	69.8	69.8	70.0	70.1
4	78.1	78.3	77.9	78.1	78.4	78.0	78.0	77.9	78.1	78.1	75.0	78.1
5	73.0	73.2	72.8	72.9	73.2	72.8	72.9	72.9	73.0	73.0	71.0	73.2
6	82.4	82.8	82.8	82.6	82.9	82.5	82.2	82.2	82.8	82.3	80.5	82.6
7	163.2	163.4	161.2	162.7	163.8	162.5	162.4	163.3	162.8	162.0	158.7	152.9
8	37.1	32.4	40.1	31.7	32.7	31.4	39.0	38.3	38.1	30.3	40.4	40.1
9	133.0	31.8	70.5	20.6	26.9	20.2	136.0	127.6	127.7	124.8	136.4	71.2
10	119.2	137.6	139.3	52.5	28.5	53.4	130.0	130.4	130.3	109.2	130.0	139.5
11		116.6	117.0	37.5	33.7	40.7	129.0	116.7	116.6	127.3	129.1	117.0
12					15.1		128.4	155.7	155.9	119.4	128.5	
13							129.0	116.7	116.6	120.4	129.1	
14							130.0	130.4	130.3	123.0	130.0	
15										112.8		
16										137.2		

[a]Tetramethylammonium salt. [b]Sinapinium salt. [c]CD_3OD used as solvent because of insolubility in D_2O.

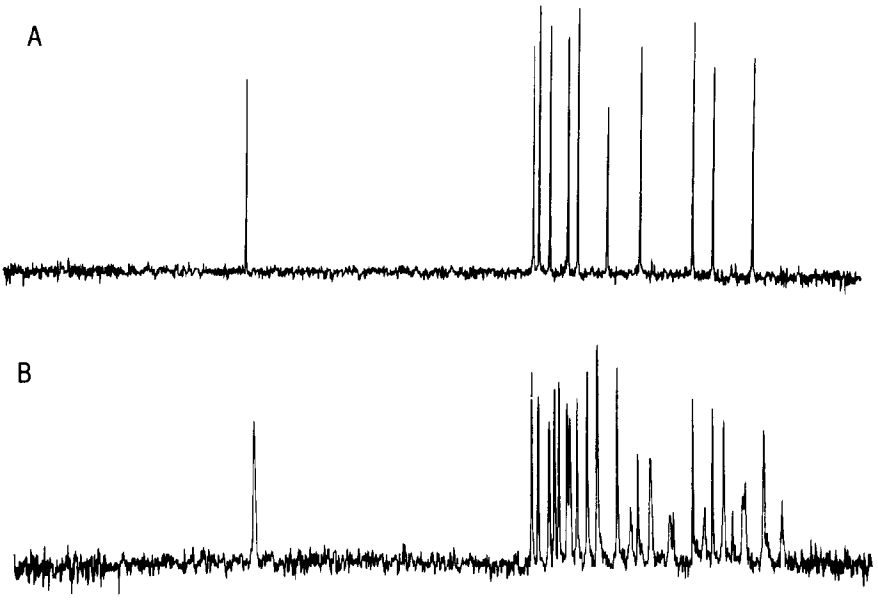


Fig. 1. ^{13}C-N.m.r. spectrum of 3-methylsulphonylpropylglucosinolate (**6**): A, fully decoupled; B, partially decoupled.

(Fig. 1). Where available, comparison with literature values confirmed the assignments[12].

The ^{13}C-n.m.r. spectra exhibit several characteristic features. The chemical shift values of the signals for C-7 (see Table II) and the D-glucose moiety are, within experimental error, independent of the nature of the side chain. In general, the positions of the peaks do not vary significantly when different counter-ions are present (*cf.* **8** and **9**) as noted previously[12,14]. Acetylation of the D-glucose moiety (a technique commonly used in the isolation of glucosinolates) reduces the chemical shift values of the signal for C-7 by ~4 p.p.m. and alters the values of the chemical shifts of the signals for the side-chain carbons by up to 4 p.p.m. (**7** and **10**). A comparison of the spectra produced by (2*S*)-2-hydroxybut-3-enylglucosinolate (**3**) and its desulpho derivative (**11**) shows that this particular modification, which is the first step in a number of h.p.l.c. techniques for glucosinolate isolation, principally affects the chemical shift value of the signal for C-7, reducing it by ~10 p.p.m.

The ^{1}H data are presented in Table III. Such data have not been assigned in detail hitherto because of the lack of resolution in the 100-MHz spectra[3,10,11]. At the higher fields employed here, the peaks are sufficiently resolved to permit individual identification of each of the protons in the side chain. These peaks have been characterised by the major splittings, although much fine structure is also apparent. The spectra typically have second-order characteristics, particularly in the glucose region. This area has not been fully assigned, but is a useful analytical fingerprint for glucosinolates since, with a single exception[3], all contain this grouping. The 500-MHz spectra of β-D-glucose has recently been fully assigned[15].

TABLE III

^{1}H-N.M R. DATA[a] FOR GLUCOSINOLATES **1–9** AND **11**

Atom	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[c]	**8**[c,d]	**8**[c,e]	**9**[c]	**11**[b]
H-6	5.00 (d, 9.3)	[f]	5.04 (d, 9.5)	5.07 (d, 9.3)	5.06 (d, 9.3)	5.06 (d, 9.3)	4.72 (m)	4.75 (m)	4.74 (m)	4.81[f] (m)	5.00 (d, 9.3)
H-8,8′	3.41[g] (m)	2.83 (t)	2.94 (m, (ABX))	3.01[h] (m)	2.75[i] (t)	2.95 (t)	4.16 (s)	4.07 (s)	4.06 (s)	4.28 (d, 16.3) 4.20 (d, 16.3)	2.82 (d, 6.7)
H-9	5.97 (ddt, 17.3, 10.3, 5.3)	2.43 (q)	4.65 (m)	2.20 (m)	1.76[h] (m)	2.26 (m)	—	—	—	7.36 (s)	4.57 (m)
H-10,10′	5.26 (dm, 17.3) 5.22 (dm, 10.3)	5.93 (ddt, 17.1, 10.3, 6.6)	5.97 (ddd, 17.3 10.5, 6.1	3.01[h] (m)	1.76[h] (m)	3.46[g] (m)	7.43[h] (m)	7.29 (d, 8.7)	7.28 (d, 8.8)	—	5.94 (ddd, 17.2, 10.5, 6.3)
H-11,11′		5.16 (dm 17.3) 5.04 (dm, 9.8)	5.35 (dt, 17.3) 5.25 (dt, 10.5)	2.73 (s)	2.60[i] (t)	3.12 (s)	7.43[h] (m)	6.93 (d, 8.7)	6.92 (d, 8.8)	— —	5.32 5.23 (dt, 17.2) 5.23 (dt, 10.5)
H-12					2.11 (s)		7.43[h] (m)	—	—	7.78[i] (dm, 7.4)	
H-13							7.43[h] (m)	6.93 (d, 8.7)	6.92 (d, 8.8)	7.25[h] (m)	
H-14							7.43[h] (m)	7.29 (d, 8.7)	7.28 (d, 8.8)	7.25[h] (m)	
H-15										7.55[i] (dm, 8.4)	

[a]Chemical shift, δ scale (J in Hz). [b]H-1–H-5: m, δ 3.26–4.06. [c]H-1–H-5: m, δ 2.94–3.79. [d]Tetramethylammonium salt. [e]Potassium salt. [f]Obscured by water peak. [g]Overlapping with signals for D-glucose moiety. [h]Peaks overlapping. [i]These assignments may be interchanged.

The ^{1}H-n.m.r. spectra are complicated by the presence of a chiral centre at C-6 which causes non-equivalence in adjacent protons. There is not sufficient resolution to detect second-order effects in all the compounds, and pseudo-first-order characteristics are sometimes observed. The protons on C-8 may therefore appear equivalent or non-equivalent, depending on the nature of the side chain. For example, (2*S*)-2-hydroxybut-3-enylglucosinolate (**3**) forms an ABX system in this region whereas, in the desulpho derivative (**11**) and benzyl glucosinolate (**7**), the protons on C-8 appear identical and, for **11**, a typical A_2X system is observed.

The glucose region has two distinct forms depending on the nature of the side chain. Those glucosinolates which contain an aliphatic side-chain (**1–6**) exhibit characteristics different from those having an aromatic side-chain (**7–10**). For the latter compounds, the fine structure is altered and the whole pattern is shifted to lower frequency possibly because of ring-current effects. Within each group, the pattern of glucose signals is similar and appears to be independent of the chemical structure of the aromatic or aliphatic side-chain.

The present study has, in addition to earlier work[11,12], demonstrated the utility of ^{13}C-n.m.r. spectroscopy for identifying glucosinolates and extended the high-resolution ^{1}H-n.m.r. data.

EXPERIMENTAL

The compounds were isolated by extraction from an appropriate plant source, and the crude plant extract was purified on a column of acidic alumina[16]. Further fractionation of glucosinolates, where required, was carried out by elution from a column of DEAE Sephadex A-25 with aqueous ammonium acetate[6]. The compounds were recrystallised as tetramethylammonium or potassium derivatives, and satisfactory chromatographic and spectroscopic data were obtained in all cases. In addition, the purity was assessed by enzymic release of D-glucose[17] and was >98% for all the compounds studied.

Spectra were obtained for 0.05M solutions in D_2O at room temperature. ^{13}C-N.m.r. spectra (25.05 MHz; internal CD_3OD, $\delta_{Me_4Si} = \delta_{CD_3OD} + 49.0$) were acquired, using both proton-noise decoupling and off-resonance decoupling, with a JEOL FX-100 spectrometer operating in the Fourier-transform mode. ^{1}H-N.m.r. spectra (internal 1,4-dioxane, δ for sodium 4,4-dimethyl-4-silapentane-1-sulphonate = δ for 1,4-dioxane + 3.76) were recorded with Bruker CXP 200 (200 MHz) and CXP 300 (300 MHz) spectrometers.

ACKNOWLEDGMENTS

We thank (the late) Professor R. Gmelin for supplying several of the samples used in this study, and Professor R. K. Harris for helpful discussions.

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