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ISOTHIOCYANATES, NITRILES AND THIOCYANATES AS PRODUCTS OF AUTOLYSIS OF GLUCOSINOLATES IN *CRUCIFERAE*

ROSEMARY A. COLE
National Vegetable Research Station, Wellesbourne, Warwick, England

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Key Word Index—*Cruciferae*, isothiocyanate; nitrile; thiocyanate; host-plant attractant; glucosinolates.

Abstract—Glucosinolates from seventy-nine 8-week-old plant species were hydrolysed and the volatile products identified by GC–MS and related to previous published findings. Known compounds, identified in new plant sources, were 4-methylthiobutyl thiocyanate in *Alyssum*, 4-methylthiobutyl isothiocyanate in *Diplotaxis* and *Eruca* and isopropyl isothiocyanate and 5-vinyl-2-oxazolidinethione in *Plantago*.

INTRODUCTION

Interest in the use of behaviour-inducing chemicals to assist in the control of insect pests has stimulated efforts to isolate feeding and oviposition attractants [1,2]. A close relationship often exists between attraction to the host plant and egg-laying behaviour of the insect. Females are attracted to suitable host plants which provide food for the larvae.

Glucosinolates are probably important in determining aspects of the behaviour of insects associated with cruciferous plants [1,3,4] and although not fully established it is most likely that the volatile hydrolysis products are involved in the attraction to the host plant. The type and proportions of these hydrolysis products from glucosinolates are known to be influenced by the conditions of hydrolysis, including the source of enzyme, pH, temperature [5–7]. For example, at intermediate pH and temperature, with the addition of thioglucoside glucohydrolase E.C.3.2.3.1 (myrosinase), the hydrolysis has given a high yield of isothiocyanates and a low yield of nitriles, whereas autolytic hydrolysis has given the reverse pattern. In addition, thiocyanates have also been observed as the end products of hydrolysis of glucosinolates and, under the mild conditions used, this rearrangement would appear to be enzymic as the isothiocyanate is the energetically favoured form [8].

The autolytic hydrolysis products from a range of wild and cultivated crucifers and related plant species were identified and quantified by GC-MS to characterize and relate these to assessments of their status as host plants of cabbage root fly and other pests of crucifers, as reported elsewhere [9]. The present paper lists the chemicals found and relates them to previous analyses of the chemical composition of plants of the Cruciferae.

RESULTS AND DISCUSSION

The aglucones—(isothiocyanates, nitriles and thiocyanates) identified in the hydrolysed plant extracts are shown in Table 1, and Table 2 shows the distribution and concentration (μg/g) of these compounds in the seventy-nine plant species analysed. The concentrations given are the means of the analyses from three separate plants of each species or variety, the range of concentrations being usually about $\pm$ 10% of each mean.

Only the alkyl isothiocyanates (**1,2**) were obtained from alkyl glucosinolates. There was, however, some evidence that methyl isothiocyanate was present in certain hydrolysed extracts, but, since this would represent the first occurrence of the parent methylglucosinolate in the Cruciferae, further work is being conducted to discover whether the methyl isothiocyanate detected originates as a decomposition product or whether it is evidence of the existence of the parent glucosinolate.

Alkenyl glucosinolates hydrolyse to give the alkenyl isothiocyanates (**3–5**) which can rearrange to give 1-cyanoepithioalkanes (**16–18**) by sulphur migration [10,11] Fig. 1.

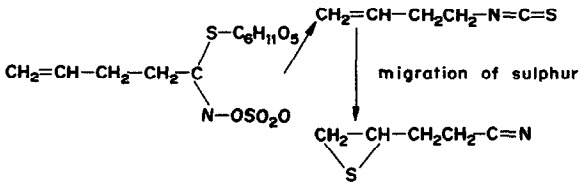

Fig. 1. Enzymic hydrolysis of an alkenyl glucosinolate.

In addition, some authors [12,13] have reported finding alkenyl nitriles in the volatiles released by the hydrolysis of glucosinolates. These could, however, be artifacts, induced by removing isothiocyanates from the hydrolysed extract when the medium has pH <5, and cleavage of the sulphur occurs giving rise to nitriles. In no case during the present analyses were alkenyl nitriles observed.

ω-Methylthioalkyl and ω-methylthioalkenyl isothiocyanates (**6–9**), particularly 3-methylthiopropyl isothiocyanate (**6**), were found after hydrolysis of many species. (**6**) is a lower homologue of the longer chain ω-methylthioalkyl isothiocyanates, known to be released from seeds of *Lunaria rediviva* [14] and *Erysimum* species [15,16] by hydrolysis. These compounds are formed by systematic biochemical chain extension by one carbon atom [17], oxidation of the sulphur then gives rise to the long chain ω-methylsulphinyl isothiocyanates pre-

Table 1. Aglucones from glucosinolates in crucifers

Alkyl isothiocyanates	
1. Isopropyl isothiocyanate	($rt^+.ce^+.ms^+$)
2. Secbutyl isothiocyanate	(rt.ce.ms)
Alkenyl isothiocyanates	
3. Allyl isothiocyanate	(rt.ce.ms)
4. 3-Butenyl isothiocyanate	(rt.ms)
5. 4-Pentenyl isothiocyanate	(rt.ms)
ω-methylthioalkyl isothiocyanates	
6. 3-methylthioalkyl isothiocyanates	(ms)
7. 4-Methylthiobutyl isothiocyanate	(ms)
8. 5-Methylthiopentyl isothiocyanate	(ms)
9. 4-Methylthiobut-3-enyl isothiocyanate	(ms)
Aromatic isothiocyanates	
10. Benzyl isothiocyanate	(rt.ce.ms)
11. 2-Phenylethyl isothiocyanate	(rt.ce.ms)
Aromatic nitriles	
12. Phenylacetonitrile	(rt.ms)
13. 2-Phenylpropionitrile	(rt.ms)
14. 2-Hydroxy-2-phenyl propionitrile	(ms)
15. *p*-Methoxyphenylacetonitrile	(ms)
1-Cyanoepithioalkanes	
16. 1-Cyano-2,3-epithiopropane	(ms)
17. 1-Cyano-3,4-epithiobutane	(ms)
18. 1-Cyano-4,5-epithiopentane	(ms)
Oxazolidinethiones	
19. 5-Vinyl-2-oxazolidinethione	(ms)
20. 4-Methyl-2-oxazolidinethione	(ms)
Thiocyanates	
21. 4-Methylthiobutyl thiocyanate	(ms)
22. Benzyl thiocyanate	(ms)

+ rt—retention time + ce—coelution + ms—mass spectra

viously recorded from species of *Arabis* [18,19]. The unsaturated 4-methylthio-but-3-enyl isothiocyanate (**9**) was found only in *Raphanus raphanistrum, R. sativus* and the related species *Rapistrum rugosum* [20]. Its presence was established by MS which indicated a molecular ion at *m/e* 159 and a strong peak at *m/e* 87 due to loss of Me–S–CH=CH. Although (**9**) was not detected in 8-week-old plants of the two *Mathiola* species analysed, it was found in 12-week-old plants [21]. 4-Methylthiobutyl glucosinolate also gave rise to a thiocyanate (**21**) which was observed only in the *Alyssum* species. This thiocyanate was first recognised in fresh parts of *Eruca sativa* and *Diplotaxis tenuifolia* [22], but no trace of it was found in the present analyses. Only the isothiocyanate was detected in these plants. The retention time of the thiocyanate (**21**) was slightly longer on Carbowax 20M than that of the corresponding isothiocyanate (**7**) but their mass spectra were similar, although the molecular ion was less pronounced in the thiocyanate than the isothiocyanate. A *m/e* 72 ion CH–N=C–S formed by the isothiocyanate was absent in the thiocyanate, but elimination of the *m/e* 27 H CN occurred with the thiocyanate.

Hydrolysis of aromatic glucosinolates yielded not only aromatic isothiocyanates but also aromatic nitriles due to loss of sulphur. 2-Phenylethyl isothiocyanate (**11**) and its decomposition product 2-phenylpropionitrile (**13**) had the widest distribution and were obtained particularly from the roots of *Brassica*. Benzyl isothiocyanate (**10**) and phenylacetonitrile (**12**) were found less frequently, occurring mainly in *Lepidium* species. In the same species, the thiocyanate (**22**), rather than (**10**), was the observed product from the enzymic hydrolysis as previously recorded by Gmelin and Virtanen [8,23] *p*-methoxyphenylacetonitrile (**15**) was observed after hydrolysis of *Aubrieta deltoides*, indicative of the loss of sulphur from *p*-methoxybenzyl isocyanate previously recorded [24].

β-hydroxy isothiocyanates are known to cyclize to oxazolidinethiones [25,26] and 4-methyl-2-oxazolidinethione (**20**) was found in the hydrolysed extract from the seven *Sisybrium* species tested. The seeds of *S. austriacum* are known to yield 4-ethyl-2-oxazolidinethione [26], but no traces were observed from plant tissue. 5-Vinyl-2-oxazolidinethione (**19**) occurred in most *Brassica* species and in *Cakile maritima*, although Rodman [27] makes no reference to finding (**19**) in an extensive study on volatile isothiocyanates in *Cakile* seeds. Small amounts of (**19**) with traces of (**1**) were also found after hydrolysis of *Plantago major* a member of the Plantaginaceae. The presence of glucosinolates in a family systematically remote from any previously known to contain glucosinolates was surprising, although plants of *P. major* are attractive to some insect pests of crucifers [9]. Previously the occurrence of the sulphoxide of (**9**) in *P. major* has been suggested [28], an observation subsequently questioned by Ettlinger and Kjaer [29]. Daxenbichler *et al.* [30–32] found that hydrolysing 2-hydroxy-3-butenyl-glucosinolate under selected conditions yielded 1-cyano-2-hydroxybutene and 1-cyano-2-hydroxy-3,4-epithiobutane, whereas under normal conditions the product was 5-vinyl-2-oxazolidinethione. With the conditions used in the present experiment, none of these nitriles was detected. 2-Hydroxy-2-phenylpropionitrile (**14**), was, however, observed as a product of hydrolysis, from the three species of *Barbarea* examined, without any evidence that the cyclized 5-phenyl-2-oxazolidinethione [33] was present. Neither compound was observed in the hydrolysis products from the two species of Resedaceae exam-

Table 2. Distribution and concentration (μg/g) of aglucones after autolysis of 79 crucifers and two related species

Plant species	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Alliaria petiolata (Bieb) Cavara & Grande			20							3												
Alyssum perenne L.	3			2													35				2	
A. saxatile L.	15			15	12	1		22									7				10	
Arabidopsis thaliana (L.) Heynh.	10		1													14						
Arabis alpina L.						2																
A. caucasica Willd						1																
A. hirsuta (L.) Scop.	4																					
A. turrita L.						1																
Aubrieta deltoides (L.) DC		4				1							1		15							
Barbarea intermedia Bor.	4		3			1					2			2								
B. stricta Andrz.	8		3								2			1								
B. vulgaris R.Br.			4			1					2			1								
Berteroa incana (L.) DC.					1			10										6				
Biscutella laevigata L.													1									
Brassica chinensis L	4		1			2					5		19				65	2	2			
B. juncea (L.) Czern & Cross	1		24	1		1					7					1						
B. napus L.	2			1	1						1		1					1	2			
B. nigra (L.) Koch	1		32								4					1						
B. oleracea L.	2		1								2		1			2			1			
B. rapa L.				1							3		1				4	1	1			
Bunias erucago L.	8																					
B. orientalis L.	8																					
Cakile maritima Scop.			1	1		2	1										8	23	3			
Camelina sativa (L.) Crantz.																						
Capsella bursa-pastoris (L.) Medic.			6																			
Cardamine flexuosa With		8																				
C. hirsuta L.		12																				
C. pratensis L.		95																15				
Cardaria draba (L.) Desv			2				2															
Cheiranthus cheiri L.							1															
Cochlearia anglica L.		6																				
C. danica L.		21																				
C. officinalis L.		9	3																			
Descurainia sophia (L.) Prantl		9																				
Diplotaxis erucoides (L.) DC.						10	94															
D. tenuifolia (L.) DC							48															
D. viminea (L.) DC.							18															
Draba aizoides L																						
Eruca sativa La Coste						2	24															
Erysimum aureum Timb-Lagr.		3				10																
E. cheranthoides L.		4				10																
E. hieraciifolium L.						100	80															
E. perofskianum Fisch & Mey						25																
E. repandum L.						6	24															
Hesperis matronalis L.							23															
Hirchfeldia incana (L.) Lagreze-Fossat			2	4		4					6		27				59	6				
Iberis amara L.						24																
Isatis aleppica Scop				12																		
I. tinctoria L.						2																
Lepidium graminifolium L.						3				1		8										
L. latifolium L						3																
L. ruderale L												103										51
L. sativum L.										1		14										
L. heterophyllum F. Schulz						2																
L. virginicum L.												52										10
Lobularia maritima (L.) Desv.													7				36					
Lunaria rediviva L.						1																
‡*Matthiola incana* (L.) R.Br.						1					2		3									
‡*M. sinuata* (L.) R.Br.												11	78									
Nasturtium microphyllum (Boenn) Rchb.											21											
***Plantago major* L.	1																		5			
Raphanus raphanistrum L.			5			2			1													
R. sativus L.			11			1			1		1											
Rapistrum rugosum (L.) All Coste						1			7													
†‡*Reseda lutea* L										2												
†‡*R. luteola* L.											34											
Rhynchosinapis monensis (L.) Dandy.										8												
Rorippa nasturtium-aquat. R.Br.											74											
Sinapis alba L		1			1	1	1			1	4											
Sinapis arvensis L.				5																		
Sisymbrium altissimum L						2					15							4		11		
‡*S. austriacum* Jacq						2														34		
S. irio L.	30	11																		33		
S. loesilii L			1																	24		
S. officinale (L.) Scop.			14																	28		
S. orientale L.						2					1	12	1							13		
S. strictissimum L.	47	15				2														45		
Tessdalia nudicaulis (L.) R.Br.											5	5										
Turritis glabra L.			4			4										4						

* Chemical numbers refer to compounds listed in Table 1; **Plantaginaceae; †Resedaceae; ‡analysed after 12 weeks also.

ined, although *Reseda luteola* has been reported [34] as a source of 5-phenyl-2-oxazolidinethione, particularly in the seed and inflorescence. This compound, or the corresponding nitrile, may not be present in significant amounts in less mature plants.

The concentrations of the hydrolysis products obtained from certain of the plant species, particularly *Brassica napus* and *B. rapa*, were rather less than expected possibly due to low enzyme activity during autolysis [7]. Generally the yield of hydrolytic products can be increased by the addition of ascorbic acid but, since the analyses were intended to reveal the proportions of volatile aglucones which may be released during natural cell breakdown, which must always occur during plant growth, ascorbic acid was not added during the present analyses.

Results in this experiment indicate that, generally, the presence of unsaturation in the aglucone facilitates both chemical rearrangement (c.f. alkenyl glucosinolates), and the loss of sulphur to form nitriles. The hydrolysis of aromatic glucosinolates to either isothiocyanates, nitriles or thiocyanates appears to occur in some species and not others [8]. Varietal differences may also occur as observed by Kirk and MacDonald [10] who found that two cultivars (Echo and Yellow Sarson) of turnip rape, *B. campestris* L., gave butenyl isothiocyanate and 1-cyano-3,4-epithiobutane respectively on hydrolysis. It may be that this rearrangement is under enzymic control as the formation of nitriles and thiocyanates is thought to be enzymic at pH 7–8 [5,6].

EXPERIMENTAL

Plant material. Plants of the species listed in Table 2 were grown throughout the year in 8 cm pots containing John Innes No. 1 Compost in a glasshouse. The glucosinolates from 8-week-old plants were extracted and hydrolysed and analyses were also made on some 12 week-old plants.

Isolation of autolytic hydrolysis products from glucosinolates in crucifers. Whole plants were macerated with 50 ml of water at pH 7–8 and allowed to autolyse by incubating for 1 hr at 40°. Fibrous material was removed by filtration and the filtrate extracted with 25 ml Et_2O. The emulsion was separated by centrifugation and the ether layer concentrated to 1 ml.

Gas chromatographic analysis. The GC procedure was modified from that of Daxenbichler *et al.* [35]. Analyses were carried out using 1.5 m × 4 mm i.d. glass columns packed with either 5% carbowax 20 M or 5% Apiezon L. on Gas Chrom Z. The N_2 carrier gas was maintained at 25 ml/min. Extracts were injected at 65° and the temperature was immediately programmed at 4°/min to 210°. Phenyl isothiocyanate replaced methyl palmitate as an internal standard. The peak areas were recorded with a Kent Chromolog 2 integrator.

Mass spectral analysis. Sample components were separated in a PYE 104 GC fitted with a 20 M column and their MS were measured at 70 eV on an AEI MS 902 mass spectrometer. Identifications deduced from the spectra obtained and were confirmed, where possible, by comparison with mass spectra of available reference samples or with published mass spectra [10,11,12,36,37].

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