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1-CYANOEPITHIOALKANES: MAJOR PRODUCTS OF ALKENYLGLUCOSINOLATE HYDROLYSIS IN CERTAIN CRUCIFERAE

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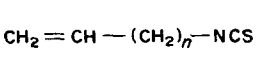
Key Word Index—Cruciferae; glucosinolate; 1-cyano-2,3-epithiopropane; allyl isothiocyanate; 1-cyano-3,4-epithiobutane; 3-butenyl isothiocyanate; 1-cyano-4,5-epithiopentane; 4-pentenyl isothiocyanate.

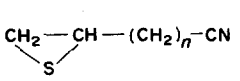
Abstract—Autolysis of glucosinolates in several crucifers produced 1-cyano-2,3-epithio-propane and 1-cyano-3,4-epithiopentane, in addition to the previously described 1-cyano-3,4-epithiobutane.

INTRODUCTION

The characteristic general reaction of glucosinolates is their enzymic hydrolysis to glucose, sulphate and isothiocyanates (e.g. **1–3**) with some additional nitriles and thiocyanates [1]. Previous results indicate that alkenyl glucosinolates produce alkenyl isothiocyanates on hydrolysis with only minor amounts of nitriles present. When 3-butenyl glucosinolate, in seeds of certain varieties of *Brassica campestris*, was hydrolysed under selected conditions, however, the main product was 1-cyano-3,4-epithiobutane (**5**) [2]. This paper reports the presence of other 1-cyanoepithioalkanes (**4**) (**6**) and their concentration in a range of crucifers which include cultivated vegetables and common weeds.



(**1**) $n = 1$
(**2**) $n = 2$
(**3**) $n = 3$



(**4**) $n = 1$
(**5**) $n = 2$
(**6**) $n = 3$

RESULTS

During a GLC survey of the autolysis products of glucosinolates in a range of crucifers, three unknown peaks, with much longer retention times than the alkenyl isothiocyanates, were observed. The MS data for the second unknown peak, found in the chromatogram of the hydrolysis products of e.g. *Alyssum perenne* was in good agreement with the published spectra for (**5**) [2]. It is postulated that the major fragments observed in the mass spectra are produced from 1-cyano epithioalkanes (**4**), (**6**); the relative intensities of the ten major peaks in the mass spectra of (**4**) and (**6**) are given in Tables 1 and 2 respectively. The MS data of the 1-cyanoepithioalkanes were distinct from that of the corresponding alkenyl isothiocyanates except for the molecular ion.

When the hydrolysed glucosinolates, extracted from crucifers, were chromatographed on Florisil, alkenyl isothiocyanates were eluted in the 5% Et_2O: hexane fraction while the corresponding 1-cyanoepithioalkanes emerged in elution with 50% Et_2O–hexane. This suggested that the rearrangement occurred during enzymic hydrolysis and not during GLC analysis.

Table 1. Mass spectrum of 1-cyano-2,3-epithiopropane (**4**)

m/e	Composition	Relative intensity
39	C_3H_3	100
99	C_4H_5NS	98·2
41	C_3H_5	97·2
72	C_4H_6S	31·9
67	C_4H_5N	15·1
40	C_2H_2N	11·8
59	C_2H_3S	10·9
38	C_3H_2	10·4
71	C_3H_3S	9·0
87	C_3H_2NS	9·0

Table 2. Mass spectrum of 1-cyano-4,5-epithiopentane (**6**)

m/e	Composition	Relative intensity
45	CHS	100
87	C_4H_7S	97·5
127	C_6H_9NS	42·9
53	C_4H_5	17·5
39	C_3H_3	14·3
59	C_2H_3S	12·6
73	C_3H_5S	12·4
71	C_3H_3S	11·7
47	MeS	11·5
41	C_3H_5	10·3

DISCUSSION

Because of the similarity to published spectral data on 1-cyano-3,4-epithiobutane it was considered that the two unknown compounds were 1-cyano-2,3-epithiopropane and 1-cyano-4,5-epithiopentane.

The factors which determine whether nitriles or isothiocyanates are the major end product of hydrolysis are still unknown. As the plants were grown and extracted under identical conditions, however, and still produced different end products (Table 3), it would appear that the differences in thioglucosidases are responsible for the different products.

EXPERIMENTAL

Plant material. Potted crucifer plants were grown in John Innes No. 1 Compost in a glasshouse. 8-week-old plants were used whole for the extraction and hydrolysis of glucosinolates.

Isolation of autolysis products of alkenyl glucosinolates. Plants were macerated with 50 ml of buffer (citric acid–phosphate pH 7·0) and incubated for 1 hr at 40°. No additional thioglucosidases, e.g. myrosinase, was added since recent work by Srivaslava and Hill [3] showed that different thioglucosidases produce different end products from the same substrate. Fibrous material was removed by filtration and the filtrate extracted with 25 ml Et_2O. The emulsion was separated by centrifugation and the Et_2O concentrated to 1 ml in a rotary evaporator.

Gas chromatographic analysis was carried out on a PYE 104 gas chromatograph. 1·5 m × 4 mm i.d. glass column were packed with either 5% carbowax 20M or 5% Apiezon L on 80-100, Gas Chrom Z. Carrier gas (N_2) flow was 25 ml/min.

Table 3. Occurrence of 1-cyanoepithioalkanes and alkenyl isothiocyanates in Cruciferae

Plant species	Isothiocyanates **1**	**2**	**3**	1-cyano-epithioalkanes **4**	**5**	**6**
Alliaria petiolata	1	3	—	4	—	—
Alyssum perenne	—	2	—	—	35	—
A. saxatile	—	16	12	—	7	—
Arabidopsis thaliana	1	—	—	14	—	—
Berteroa incana	—	—	1	—	—	6
Brassica chinensis	1	—	—	—	61	2
B. juncea	24	1	—	1	—	—
B. napus	—	1	1	—	—	1
B. nigra	32	—	—	1	—	—
B. oleracea	1	—	—	1	—	—
B. rapa	—	1	—	—	4	1
Cakile maritima	1	1	—	—	8	23
Cardamine pratensis	—	—	—	—	—	15
Hirchfeldia incana	2	3	—	—	59	6
Lobularia maritima	—	1	—	—	36	—
Sisymbrium altissimum	—	—	—	—	—	4
Turritis glabra	4	—	—	4	—	—

Quantities of chemicals, expressed in μg/g, are the means of three plants. Key for compounds, see formulae **1**–**6** in text.

Extracts injected at 65° and programmed from 65° to 210° at 4°/min with 1 μg phenyl isothiocyanate as an internal standard. Integration of peak areas was by means of a Kent Chromolog 2.

Mass spectral analysis was carried out on an AEI MS 902 mass spectrometer after being separated on line in a coupled PYE 104 GLC using a carbowax 20M column. Low resolution MS were obtained at 70 eV and a source temperature of 200°.

Column chromatography hydrolysis products of extracts in hexane were chromatographed on a 30 × 1 cm Florisil (60–100 U.S. mesh) column prepared with hexane and elution was with this solvent containing proportionally increased concentrations of di-ethyl ether.

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