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Note

Comparative study of the volatile aglucones of glucosinolates from *in vivo* and *in vitro* grown *Descurainia sophia* and *Alyssum minimum* using gas chromatography–mass spectrometry

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The occurrence of glucosinolates within particular plant families, notably the Cruciferae, is responsible for a pungent taste, as on crushing hydrolysis takes places. This autolysis brought about by contact of glucosinolates and the endogenous enzyme system, yields one or more of the following: isothiocyanate, nitrile, thiocyanate, cyanoepithiobutane, oxazolidinethione or thionocarbamate, along with glucose. Various workers have employed chromatographic separation techniques including paper[1], thin-layer[2] and gas chromatography (GC) for characterisation of the aglucone autolysis products. Owing to the volatility of most of these products and the resolution of GC, this latter method is frequently employed. Both packed[3] and capillary columns[4] have been used for separating the products and retention data and mass spectrometry used for identification. Previously used autolysis protocols involve treatment of large amounts of plant material (from 250 mg[5] to whole plants[3]) followed by defatting, then solvent extraction or distillation of the aglucones.

In order to determine the glucosinolates and their degradation products elaborated by plant cell cultures of the Cruciferae, we required a very sensitive method applicable to small sample sizes. Using material from *Descurainia sophia* and *Alyssum minimum* we decided to check a range of extraction procedures for qualitative and quantitative differences in the derived glucosinolate degradation products. Except one report of allyisothiocyanate in seeds of *Sisymbrium sophia*[6] (presently known as *Descurainia sophia*) there are no previous mentions of isothiocyanates in either of these two plants.

EXPERIMENTAL

Seeds of *D. sophia* and *A. minimum* were obtained from a commercial market in Isfahan, Iran, in 1984. They were grown, and the fully developed plants harvested, and identified at the Botany Department, University of Manchester Museum.

Seeds of *D. sophia* and *A. minimum* were surface sterilised in 15% (w/v) hydrogen peroxide containing Tween 80 for 2 min, then germinated on wet filter paper in Petri dishes in the dark at 25°C. Hypocotyls of 5-day-old seedlings were explanted onto Murashige and Skoog's revised Tobacco medium M & S[7] containing kinetin and 2,4-D. Callus was grown at 27°C in a 12-h dark/12-h light sequential regimen. Due to slow growth, callus was harvested at 20 weeks.

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Autolysis and collection of autolysis products

Method A. Crushed, defatted plant material (30 g) was mixed with distilled water (450 ml), covered by a layer of cyclohexane and left for autolysis at 25°C overnight (17 h). Autolysis products were collected by distillation. Plant material was defatted by shaking with four aliquots of ether.

Method B. Crushed, defatted plant material (1 g) was mixed with a 15 ml of phosphate buffer (pH = 7.4), covered by a layer of cyclohexane and left for autolysis at 25°C for 1 h. Then the mixture was shaken vigorously for 30 min. Autolysis products were collected by distillation.

Method C. The procedure was the same as for method B but with an overnight (17 h) autolysis.

Method D. Crushed, undefatted plant material (30 mg) was mixed with distilled water (7 ml) and left for autolysis overnight (17 h). Then dichloromethane was added and the mixture was shaken for 30 min. Centrifugation effected the separation of the organic solvent. The dichloromethane layer was concentrated and examined for the presence of autolysis products.

GC analysis

Samples were examined with a capillary GC system (Model HRGC 4130, Carlo Erba) equipped with a heated flame ionisation detector. The column used was a 25 m × 0.32 mm I.D. fused-silica bonded OV-1. The split ratio was 10:1 with a carrier gas (hydrogen) flow-rate of 2 ml/min. Temperature programme was 50°C initially followed by an increase at 5°C/min to 210°C. Injector and detector temperatures were 280°C and 290°C, respectively. Injection volume was 1–2 μl. Quantitation of chromatograms was via % peak area measurements obtained using a Hewlett-Packard 3392A integrator.

Identification of components by gas chromatography–mass spectrometry

A Kratos MS 25 instrument was used, equipped with a DS-55 computer data output. GC conditions were the same as used in the case of capillary GC, but with helium as carrier gas. The all-glass jet separator interface was operated at 250°C.

Mass spectrometer conditions were: ionisation potential, 70 eV; ionisation current, *ca.* 300 μA; source temperature, 250°C; resolution 600; scan speed, 1 s/decade.

RESULTS AND DISCUSSION

Seeds and dried callus cultures of both *D. sophia* and *A. minimum* were found to yield the same major isothiocyanates, namely 3-butenyl and allyl isothiocyanates. Table I lists the isothiocyanates, nitriles and epithiobutane derivative found in both seeds and callus cultures, and it is interesting to note the same major glucosinolate hydrolysis products in these plants from different genera; as in folkloric medicine they are both known to have the same effect when used as a febrifuge.

These results show the major glucosinolate degradation products released by seed when extracted by the four different methods used. As found previously[8] neutral pH and greater dilution with water favoured isothiocyanate production. Substitution of solvent extraction instead of distillation of autolysis products (especially after prior defatting), caused increased levels of isothiocyanate and markedly higher levels of epithiobutane derivative. Levels of isothiocyanate and epithiobutane derivatives ob-

TABLE I

LEVELS OF GLUCOSINOLATE AUTOLYSIS PRODUCTS IN *DESCURAINIA SOPHIA* AND *ALYSSUM MINIMUM* USING EXTRACTION METHODS A–D

Tr. = trace.

Compound	t_R *(min)*	*Concentration (μg/g)*				
		Seeds			*Callus cultures*	
		A	*B*	*C*	*D*	*D*
D. sophia						
Allyl isothiocyanate	2.2	1.82	3.54	3.61	0.39	0.43
3-Butenyl isothiocyanate	3.2	4.26	14.72	13.94	136.51	Tr.
1-Cyano-3,4-epithiobutane	5.4	0.34	1.86	1.69	991.36	0.08
5-Methylthiopentanitrile	8.3	0.81	–	–	0.20	Tr.
3-Phenylpropionitrile	8.9	0.24	–	–	16.82	0.03
4-Methylthiobutyl isothiocyanate	13.5	Tr.	–	–	–	–
2-Phenylethyl isothiocyanate	14.1	Tr.	Tr.	Tr.	–	–
A. minimum						
Allyl isothiocyanate	2.2	1.75	9.57	6.49	–	0.20
3-Butenyl isothiocyanate	3.2	1.75	4.22	16.35	70.71	Tr.
1-Cyano-3,4-epithiobutane	5.4	2.27	5.41	7.94	1139.22	Tr.
Phenylacetonitrile	5.8	0.21	–	–	–	–
6-Methylthiohexanitrile	10.5	Tr.	–	–	–	–
7-Methylthioheptanitrile	13.1	0.50	Tr.	Tr.	–	–
8-Methylthiooctanitrile	16.0	0.02	–	–	–	0.20

tained using method D (no prior defatting) appear substantially higher than any previously reported levels of glucosinolate aglucones released from seeds and whole plants of the same genera, *after* defatting[3,9]. This suggests either solvent inactivation of the myrosinase enzyme system or direct removal of aglucones. Under all conditions used, nitrile production in these plants was low, the epithiobutane levels being of much greater significance. Analysis of callus cultures revealed low levels of autolysis products, indicating either little accumulation or synthesis, or the ocurrence of autolysis during culture.

Allyl isothiocyanate is eluted from the column in 2.2 min at 61°C and breakdown is kept at a minimum 10.6–10.9% with six replicate injections. The standard deviation of six replicate estimations of a test extract was found to be 4.2%. GC has been used previously for similar analysis but never routinely on such small samples (*e.g.*, refs. 3 and 4) and never on plant cell cultures.

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