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STUDIES ON GLUCOSINOLATE DEGRADATION IN *LEPIDIUM SATIVUM* SEED EXTRACTS

VICTOR GIL and ALEXANDER J. MACLEOD

Department of Chemistry, Queen Elizabeth College (London University), Campden Hill Road, London, W8 7AH, U.K.

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Key Word Index—*Lepidium sativum*; Cruciferae; glucosinolate degradation.

Abstract—Analysis of *Lepidium sativum* seeds showed the presence of allyl, 2-phenethyl and benzyl glucosinolates, the first two being reported for the first time from this source. The effects of temperature, pH of the extraction medium and the length of time allowed for autolysis were assessed on the benzyl glucosinolate degradation products in seed extracts. In particular benzyl thiocyanate was not produced at higher temperatures but at ambient and lower temperatures it exceeded isothiocyanate. Nitrile was always the major product under the conditions studied, ever at pH levels as high as 7.4. Five new possible benzyl glucosinolate degradation products were detected and evidence is presented that benzaldehyde and benzyl alcohol could be sceondary products formed thermally from isothocyanate and thiocyanate, respectively. Benzyl mercaptan and benzyl methyl sulphide also appear to be thermally produced.

INTRODUCTION

Glucosinolates (**1**) undergo enzymic decomposition, as shown in Scheme 1, to give two main types of products, isothiocyanates (**2**) and nitriles (**3**). In certain circumstances some glucosinolates are capable of forming thiocyanates (**4**) as well, but of more than 70 known glucosinolates only three appear to undergo this type of degradation. These are allyl, 4-(methylthio)butyl and benzyl glucosinolates. Many theories have been proposed to explain this odd limitation but none is entirely convincing. *Lepidium sativum* (garden cress) contains benzyl glucosinolate and many previous workers have identified benzyl thiocyanate in seed extracts [1–7]. Based on the ready availability of the seeds and the extent of previous work on *L. sativum*, this seemed an ideal system in which to study the mechanism of thiocyanate formation from glucosinolates. In addition, there is some slight disagreement in the literature with regard to nitrile formation by *L. sativum* seed extracts; whilst most workers report the production of benzyl cyanide, some do not [3, 4].

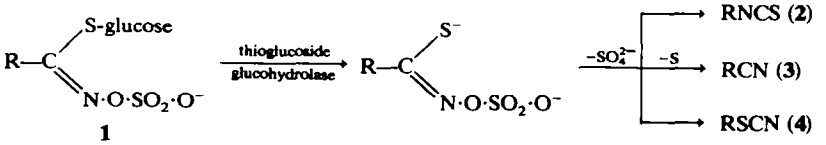
A detailed examination of the glucosinolate products in extracts of *L. sativum* seeds was thus undertaken and a survey was also carried out of the effects of certain variables (e.g. temperature, pH, time allowed for autolysis) on the nature and relative amounts of benzyl glucosinolate products.

RESULTS AND DISCUSSION

The compounds which were identified in extracts of *Lepidium sativum* seeds by GC–MS are listed in Table 1, together with their retention times and their relative abundances. All MS agreed well with those previously reported and retention times agreed exactly with those of reference standards where these were available (asterisks in the table). Compounds that were present in the extract at levels below 0.5% are indicated as trace (tr) components. A number of minor constituents remain unidentified but these comprise only *ca* 2% of the sample and those for which mass spectra were obtained were not glucosinolate products.

From Table 1 it can be seen that three glucosinolates were identified in *L. sativum* seeds, namely allyl, 2-phenethyl and benzyl glucosinolates. The first two are found only in trace amounts and have never been reported in *L. sativum* before, whilst the presence of benzyl glucosinolate is well documented [1–7]. Both benzyl isothiocyanate and thiocyanate were readily identified in most samples, as expected from previous investigations. However, the major product from benzyl glucosinolate was the nitrile, despite its omission from some previous analyses [3, 4].

In addition to the three main benzyl glucosinolate degradation products, five other benzyl derivatives were detected in the seed extracts, some or all of which could also be of glucosinolate origin. However,



Scheme 1. Enzymic decomposition of glucosinolates.

Table 1. Compounds identified by GC–MS in an extract of *Lepidium sativum* seeds

Compound	RR_t (min)	Approximate percentage
Acetone*	2.2	8
A xylene	7.8	tr
A xylene	8.3	tr
2-Methylbutan-1-ol	8.5	3
An ethyltoluene	9.5	tr
Pentan-1-ol	10.0	3
Styrene	10.2	tr
Isobutylbenzene	10.5	tr
An ethyltoluene	10.8	1
A C_4-substituted benzene	11.0	tr
2-Ethylhexanol	11.5	4
Allyl isothiocyanate*	11.9	3
Benzaldehyde*	12.5	7
Pentylbenzene	12.8	tr
Methyl benzoate*	13.5	tr
Benzyl methyl sulphide*	14.0	tr
Phenylmethanethiol (benzyl mercaptan)*	14.3	tr
Benzyl alcohol*	16.9	4
Phenylacetonitrile (benzyl cyanide)*	18.0	53
Benzyl isothiocyanate*	22.0	7
3-Phenylpropionitrile (2-phenethyl cyanide)*	22.5	tr
Benzyl thiocyanate*	24.0	ND

*RR_t agrees exactly with that of authentic compound.
tr = trace, ND = not detected.

none of these has been proved to be formed by glucosinolates but as a group they will be referred to here as the 'secondary products'. In previous work on glucosinolate degradations in the Cruciferae, we have found that benzaldehyde and benzyl alcohol often occur together with the main benzyl glucosinolate products [8]. However, the analysis of *L. sativum* seeds reported here is the only instance in which methyl benzoate, benzyl methyl sulphide and benzyl mercaptan have been identified as well. Forss [9] detected benzyl mercaptan in extracts of *Coronopus didymus* (Cruciferae), a plant in which benzyl glucosinolate occurs, but unfortunately he gave MS evidence which could not be interpreted since it derived from a mixture of components. However, important fragment peaks were recorded at *m/e* 77, 79, 91, 105, 106, 124, 138 [9]. In the light of our findings, the following possibilities can be suggested: (a) *m/e* 91 and 124(M^+) originate from benzyl mercaptan (100 and 25% relative abundance in our spectra); (b) *m/e* 77, 79, 105 and 106(M^+) from benzaldehyde (100, 15, 85, 83% in our spectra); (c) *m/e* 91 and 138(M^+) from benzyl methyl sulphide (100 and 35% in our spectra). Forss also identified benzyl cyanide and benzyl disulphide in *C. didymus* extracts [9] but we were unable to detect benzyl disulphide in our analyses of *L. sativum*. Similarly we could not locate benzylamine which has also been reported as a benzyl glucosinolate product [5]. To the best of our knowledge no one has suggested an ester as a glucosinolate product and this does seem the least likely genuine representative in the 'secondary products'.

Not all the compounds listed in Table 1 were found in all extracts of *Lepidium sativum* seeds nor in the same proportions. Table 2 summarises data showing

Table 2. The effects of some variables on the relative amounts of benzyl glucosinolate degradation products formed in extracts of *Lepidium sativum* seeds

Variable	Benzyl isothiocyanate (mg/g)	Benzyl thiocyanate (mg/g)	Benzaldehyde (mg/g)	Benzyl alcohol (mg/g)	Benzyl thiocyanate / Benzyl isothiocyanate
1. Temperature (2hr, distilled water)					
(a) Boiling	1.05	—	1.1	0.8	—
(b) Ambient	0.9	2.75	tr	tr	3
(c) 0°	0.95	3.65	tr	0.85	4
2. pH (2 hr, 0°)					
(a) 6.69	1.15	4.3	—	1.2	4
(b) 6.75	0.9	3.3	tr	0.65	4
(c) 7.42	1.25	4.7	—	0.7	4
3. Time of autolysis					
(i) Ambient, pH 6.75					
(a) 0.5 hr	0.2	2.0	—	—	10
(b) 1 hr	0.35	2.75	tr	tr	8
(c) 2 hr	1.0	3.9	tr	tr	4
(d) 4 hr	0.6	3.3	tr	tr	5
(ii) Ambient, distilled water					
(a) 1 hr	0.55	3.4	tr	tr	6
(b) 2 hr	0.9	2.75	tr	tr	3
(c) 4 hr	0.9	3.9	tr	tr	4
4. Heating					
(a) 3 hr reflux after 1 hr autolysis at pH 6.75 (cf. 3(i)d)	tr	—	0.9	tr	—
(b) 3 hr reflux after 1 hr autolysis in distilled water (cf. 3(ii)c)	tr	—	0.65	0.55	—

tr = trace. Benzyl cyanide is excluded (see text).

the effects of certain important variables on the relative amounts of the main benzyl glucosinolate degradation products (assuming that benzaldehyde and benzyl alcohol are of glucosinolate origin). Benzyl mercaptan, benzyl methyl sulphide and methyl benzoate are not included since they were detected only when one particular method was used to prepare the seed extracts, namely using a modified Likens and Nikerson apparatus [10, 11]. The results from this particularly sensitive analysis are given in Table 1. Benzyl cyanide is not included in Table 2, since in all instances it was by far the major glucosinolate product and quantitatively it did not appear to vary significantly whatever the conditions of analysis.

Effect of temperature

The amounts of benzyl cyanide and isothiocyanate that were produced in extracts were constant, within experimental error, whatever the temperature during autolysis. However, as can be seen from Table 2, aldehyde was produced in much greater amounts at 100° whilst thiocyanate is not obtained at all at such temperatures. It has been reported previously that benzyl thiocyanate formation is favoured at lower temperatures [1] and our results confirm this. However, large amounts are still readily obtained by room temperature autolysis, and it is not essential to employ temperatures near to 0° as has been assumed in the past [5]. Similar data to these shown in Table 2 were obtained in various buffer solutions.

Effect of pH

There was no great difference between the amounts of products obtained during autolysis over the narrow pH range 6.69 to 7.42 or between extracts prepared in distilled water or buffer (compare results under 1c) and it made no difference in these cases whether reaction was at 0° or at room temperature.

Effect of autolysis time

In buffer solution the ratio of thiocyanate to isothiocyanate produced by *L. sativum* seeds decreases regularly with the time allowed for autolysis. This is due to the fact that isothiocyanate production increases with time to a greater extent than thiocyanate, although the latter is always the major component of the pair. In other words more thiocyanate is formed and at a more rapid rate. The results obtained in distilled water are rather different and it is probable that the amounts produced are consistent whatever the period for autolysis.

A further slight difference between the behaviour in buffer and distilled water was observed if the samples, after 1 hr autolysis at room temperature, were then refluxed for 3 hr. Although in both cases less isothiocyanate was obtained and thiocyanate could not be detected at all, with distilled water alone an excess of benzyl alcohol was produced. Both systems produced extra benzaldehyde.

Benzyl cyanide formation

Under all conditions of extraction employed in this work the main benzyl glucosinolate degradation product was benzyl cyanide (phenylacetonitrile). This is still the case even at a pH as high as 7.4, at which level the main product expected from nearly all previous work on glucosinolate degradation is the isothiocyanate [12–20]. The generally accepted theory is that following enzymic fission of the sulphur–glucose bond by hydrolysis (see Scheme 1), the aglucone thus formed rearranges either to the isothiocyanate or the nitrile, depending on the pH of the medium. The nitrile is supposedly formed via a protonation mechanism and so is favoured at lower pH values. Between pH 3 and 5 a mixture is expected, but above pH 5 only the isothiocyanate should be obtained (by a Lossen rearrangement). The results described here for *L. sativum* seed extracts do not, therefore, conform, but we have recently suggested why the theory is not valid in this context [21], and indeed the data reported here further substantiate this suggestion. In addition, some other workers have also found nitrile to be a major product at these pH levels. Previous findings relevant to this study are that at pH 6.7 Saarivirta obtained more benzyl cyanide in *L. sativum* seed extracts than isothiocyanate and thiocyanate together [5], and in all *Lepidium* species examined by Cole at pH 7–8 the main product was the nitrile [12].

Nitrile formation can be promoted by some metal cations, ferrous being particularly effective [13]. This observation has been used to account for some of the high nitrile yields obtained under pH conditions expected to favour isothiocyanate formation [13]. In our extractions there was no possibility of any metallic contamination, but it is not known whether *L. sativum* seeds might not be exceptionally rich in appropriate metal ions, although this seems unlikely. However, a more detailed study of the mechanism of nitrile formation from glucosinolates is certainly warranted based on the results described here and other data [1, 2, 5, 7, 12, 21].

Thiocyanate and isothiocyanate formation

The mechanism of thiocyanate formation from glucosinolates is unknown, a problem rendered more intriguing by the fact that only three glucosinolates appear capable of this type of degradation. Gmelin and Virtanen originally suggested an enzyme-induced rearrangement of the glucosinolate itself [3]. Subsequently, Virtanen and Saarivirta proposed that an isomerase acted on the initially formed isothiocyanate [7, 22], but Saarivirta later abandoned this idea based on unsuccessful model system experiments [5]. To date all attempts to isolate such thiocyanate-forming enzymes have failed. An alternative is that thiocyanates are formed by a purely chemical mechanism and this could explain why only a few glucosinolates undergo this reaction, i.e. only those glucosinolates able to yield good, stable cations R^+ (see Scheme 1) can form thiocyanates via a type of ion-pair mechanism. This is an attractive theory considering the particular glucosinolates which do yield thiocyanates and the bidentate nature of the isothiocyanate–thiocyanate ion, but efforts to substantiate the suggestion have not been very successful [13, 23]. Also against any non-enzymic theory is the fact that sometimes benzyl thiocyanate is not obtained from benzyl glucosinolate although isothiocyanate and nitrile are formed. For example, this is true in *Tropaeolum majus* [4, 5, 7], and it might reasonably be expected that the same

compound would be capable of the same chemical degradation whatever the natural environment, other factors being constant.

In their original work on *L. sativum* seeds, Virtanen and Saarivirta found that the ratio of benzyl thiocyanate to isothiocyanate increased with time [1, 2, 5, 7, 22], and it was on these results that they proposed the isomerase theory. However, as can be seen from Table 2, our data show that this same ratio either decreases with time (in a pH-controlled medium) or seems to remain approximately constant (in distilled water). We would not necessarily suggest the reverse of the original Virtanen and Saarivirta proposal, namely that initially formed thiocyanate isomerizes to isothiocyanate with time, but it is a possibility. However, there is evidence from the data at pH 6.75 in Table 2 that both products reach a maximum of formation, and perhaps both subsequently react or decompose to secondary products. A major difference between the experiments performed here and those described by earlier workers [1, 2, 5, 7, 22] is that very short autolysis times (a few minutes) were used, whilst it is clear from the results in Table 2 that reaction can continue for very much longer. In any case, the present data do not support the isomerase theory with regard to thiocyanate formation. Independent routes to the two isomers seem much more likely.

Virtanen and Saarivirta also report that water is a better medium than citrate buffer for benzyl thiocyanate production by *L. sativum* seed extracts [1, 4, 5]. Indeed, they found that increasing ionic concentration in general favoured isothiocyanate production [4, 5]. Our data (Table 2) do not fully support this finding but a formalized survey was not carried out. Virtanen and Saarivirta also found that the pH of the reaction medium had no great effect on reaction products in the range 4.6 to 6.2 [4, 5] and here, using slightly higher pH levels, the data in Table 2 do agree.

Effect of heating on thiocyanate and isothiocyanate

It can be seen from Table 2 that if, after a 1 hr autolysis period, an *L. sativum* seed extract is subjected to heat treatment (reflux) for 3 hr there is a decrease in the amount of isothiocyanate detected, whether or not the reaction medium is a buffer solution or distilled water. In these circumstances no thiocyanate is detected, whilst if heat is not applied large amounts are produced. It has already been observed (Tables 1 and 2, 1a) that thiocyanate is not formed in extracts of *L. sativum* seeds obtained in boiling water and thus it can be deduced that either it is not formed at these temperatures or it reacts further thermally. From these other experiments (Table 2, 4a and 4b) it is obvious that the latter explanation is more likely, since the system was given 1 hr autolysis at ambient before heat treatment, ample time to produce reasonable amounts of thiocyanate [see Table 2, 3(i)b and 3(ii)a]. Similar reasoning also applies to the reduced amounts of isothiocyanate on such heat treatment.

It can be seen from Table 2 that during these experiments (4a and 4b) the concentrations of benzaldehyde and benzyl alcohol increase appreciably, so it is possible that they might be formed thermally from one or other (or both) of the sulphur-containing primary products. It was found that simply refluxing an aqueous solution of benzyl isothiocyanate does indeed yield some benzaldehyde, whilst thiocyanate conveniently gives some benzyl alcohol. However from Table 2 it can be seen that the amounts of products formed with and without heating do not correspond i.e. 3(i)d compared with 4a, or 3(ii)c with 4b, and if the sulphur compounds are reacting further to give other (secondary) products, compounds in addition to benzaldehyde and benzyl alcohol must be produced. In fact this is the case, and large amounts of two new unidentified components were obtained as a result of these experiments (4a and 4b). Their mass spectra are as follows:

Unidentified I *m/e*	180	165	137	77	91	181	39	122
% rel. int.	100	28	22	17	15	12	11	8
Unidentified II *m/e*	92	91	180	65	44	135	165	90
% rel. int.	100	99	52	31	22	20	15	12

There are some obvious similarities in the spectra and it could well be that both compounds have a MW of 180. One at least may be a benzyl compound but neither appears to contain sulphur. From their gas chromatographic behaviour neither is very volatile.

Comparison of the amount of isothiocyanate and thiocyanate 'lost' on heating with amounts of the two

Table 3. Benzaldehyde and benzyl alcohol formation in *Lepidium sativum* seeds at different temperatures and conditions of autolysis

Sample (distilled water medium)	Autolysis at 0° for 4 hr		Autolysis at ambient temp. for 4 hr		Autolysis for 1 hr at ambient temp. followed by 3 hr reflux	
	alcohol (mg/g)	aldehyde (mg/g)	alcohol (mg/g)	aldehyde (mg/)	alcohol (mg/g)	aldehyde (mg/g)
L. sativum seeds	0.9	tr	tr	tr	0.65	0.55
Seeds plus benzyl thiocyanate	0.65	tr	0.95	tr	1.40	tr
Seeds plus benzyl isothiocyanate	0.55	tr	tr	tr	tr	1.20
Benzyl thiocyanate					√	tr
Benzyl isothiocyanate						√

tr = trace.

unknowns, plus benzyl alcohol and benzaldehyde, that are formed on heating, gives a rough quantitative agreement (e.g. ~3.8 mg/g gives ~4.0 mg/g). It is therefore quite possible that benzyl alcohol, benzaldehyde and the two unknowns could well derive from the sulphur-containing primary products in heated *L. sativum* seed extracts. However, the two unidentified compounds were not obtained when the isothiocyanate or thiocyanate alone were refluxed.

Benzaldehyde and benzyl alcohol formation.

Table 3 summarizes the further data obtained after the experiments described above. For completeness some results already discussed are also included. Data are given only for experiments carried out in distilled water medium since those in buffer solution showed the same behaviour and did not differ over the pH range 6.69–7.42.

Considering first the formation of the aldehyde in extracts of *L. sativum* seeds, it can be seen that this is favoured by higher temperatures (i.e. refluxing in water). It must be produced thermally from one of the sulphur-containing primary products. Our results (Table 3) strongly suggest that the precursor is in fact benzyl isothiocyanate, since when this is added to seed extracts before heat treatment excess benzaldehyde is formed, whilst the same does not happen if benzyl thiocyanate is added. Furthermore, when an aqueous solution of isothiocyanate alone is refluxed for 3 hr a measurable amount of aldehyde is produced. Although when thiocyanate is refluxed under identical circumstances it gives a minute trace of benzaldehyde, it is most likely that benzyl isothiocyanate is the precursor for the thermal formation of the aldehyde in *L. sativum* seed extracts. Many extraction procedures do include the application of heat, although from Table 3 it will be noted that the aldehyde is still produced in its absence. The fact that benzyl isothiocyanate can react to give benzaldehyde could explain why Saarivirta and Virtanen found benzyl isothiocyanate decreased with time in their extractions of *L. sativum* seeds [2]. Large amounts of benzaldehyde are, in fact, produced by the base-catalysed hydrolysis of benzyl glucosinolate itself, but only at very high pHs (2 N NaOH) [24].

Turning now to benzyl alcohol production, there are many reports of glucosinolates forming alcohols, but this has always been associated with thiocyanate ion formation and is limited to the three indole glucosinolates [16, 25–32]. Thus the enzymically-formed isothiocyanates are unstable and spontaneously decompose to the corresponding alcohol and the thiocyanate ion. Benzyl alcohol has never been reported as a glucosinolate product. It can be seen from Table 3 that as with benzaldehyde, benzyl alcohol production is promoted at higher temperatures but the results are more variable and less clear. It seems most likely that the precursor for this thermal formation is the thiocyanate primary product, since addition of this to the seed extracts before heat treatment gives excess alcohol. In addition, heat treatment of the thiocyanate alone yields quantities of the alcohol. However, it would also appear that this reaction occurs at room temperature as well, since excess alcohol was produced by *L. sativum* seed extracts to which thiocyanate had been added, but which were not then submitted to any heat treatment.

The detection of excess benzyl alcohol in seed extracts prepared at 0°, a result confirmed by replicate analyses, is unexpected. Thus although benzyl alcohol can be produced thermally by hydrolysis of benzyl thiocyanate, the situation is more complex than for the aldehyde and other mechanisms probably pertain. Gmelin and Virtanen have suggested that the formation of *N*-methoxyindol-3-ylmethyl alcohol from the corresponding glucosinolate can occur either from the isothiocyanate or by hydrolytic splitting of the glucosinolate itself [16]. It may be that benzyl alcohol is also produced directly from the glucosinolate by a hydrolytic mechanism, not requiring any heat, and possibly even promoted at lower temperatures.

Formation of benzyl mercaptan and benzyl methyl sulphide

Trace amounts of these compounds were detected only in extracts of *L. sativum* seeds that had been heated, and they were particularly identified in extracts obtained using the sensitive Likens and Nickerson procedure [10, 11]. Thus it would appear that these may also be secondary products formed thermally from sulphur-containing primary products, but only in small amounts.

Forss identified the thiol in steam distillates of *Coronopus didymus*, which contains relatively large quantities of benzylglucosinolate, but he could not detect any benzyl thiocyanate [9]. The latter is not surprising considering the present results, which illustrate the thermal lability of the thiocyanate. It could be that the thiol determined by Forss originated from the thiocyanate, particularly since Park and Armitt did locate the thiocyanate in their extracts of *C. didymus* [6]. Park and Armitt also showed that the thiocyanate could be converted readily to thiol by cysteine, but they were of the opinion that when subjected to heat treatment isothiocyanate could also be the precursor of the thiol as well as of the thiocyanate [6]. Ohkawa and Casida have demonstrated that thiocyanates can be enzymically converted into thiols [33] and Tressl *et al.* found thiols as decomposition products of methylthioalkenyl ethers [34]. Kjaer has very reasonably concluded that thiols, as encountered in nature, are hardly uniform in their origin [35].

Park and Armitt also isolated benzyl methyl sulphide in butter fat from *Coronopus*-tainted milk as well as benzyl cyanide and isothiocyanate [6]. They thought that this could be a metabolite of benzyl thiocyanate, formed by methylation taking place inside the cow. The sulphide in *L. sativum* extracts could be a methylation product from the thiol, although Gmelin and Virtanen actually identified benzyl thiocyanate by its reductive conversion to the sulphide [3].

There is thus some circumstantial evidence that benzyl mercaptan and benzyl methyl sulphide are formed from benzyl thiocyanate or (less likely) from isothiocyanate. However, during the course of the experiments summarized in Table 3 no evidence was obtained to prove that either compound was derived from thiocyanate or isothiocyanate in *L. sativum* seed extracts.

EXPERIMENTAL

Lepidium sativum seeds were obtained from Suttons Seeds Ltd., Reading, U. K. ('curled cress', lot No. 6561) and were authenticated by basic seed microscopy studies.

Preparation of extracts. Seeds were ground to a powder in a coffee grinder. Dry hexane was added and the mixture shaken gently at room temp. for 15 min. After filtration the filtrate was rejected. More hexane was added to the seed powder and the procedure repeated ×5. The defatted seed meal was then dried *in vacuo*. 5 g of seed meal were added to 100 ml of dist. H_2O (or citrate–phosphate buffer at 1 of 3 pH values, 6.69, 6.75 or 7.42) and the mixture shaken at room temp. (or at 0° in a cold room), or refluxed with stirring, for 0.5 hr (or 1, 2, 3 or 4 hr). In some instances the mixture was shaken at room temp. for a specified length of time (e.g. 1 hr, 2 hr) and then refluxed with stirring for another period (e.g. 3 hr, 2 hr). In some expts an aliquot of either benzyl isothiocyanate or benzyl thiocyanate was added to the seed meal mixture at the begining (i.e. before autolysis). At the conclusion of the autolysis period the aq. layer was separated from the starchy mass by centrifugation. *Ca* 20 ml H_2O (or appropriate buffer) were added to the solid residue and the mixture shaken for a few min before centrifugation. The supernatant was separated, added to the 1st aq. fraction and extracted with 3×75 ml CH_2Cl_2 (centrifugation aided separation of any emulsion). The organic layer was collected, dried and concd to 1 ml. The most conc extract was obtained using a Likens and Nickerson apparatus [10] which had been suitably modified [11]. Finely crushed, hexane-defatted seeds (170 g) were mixed with H_2O (1700 ml) in a round-bottomed flask attached to the Likens and Nickerson extractor [11]. The mixture was heated and the volatile components continuously extracted for 4 hr with isopentane (35 ml). The extract was concd to 1 ml by low temperature–high vacuum distillation as previously described [11].

Analysis by gas chromatography. Extracts were examined first by routine GC with a heated FID and a 1.5 m×4 mm i.d. glass column packed with 10% Carbowax 20M coated on 100–120 BSS mesh acid-washed Diatomite C. N_2 was employed (30 ml/min), and the temp. programme was a 16°/min rise from an initial setting of 60° for 5 min to a final temp. of 190°. Typically 2 μl of the extract were injected at an attenuation setting of 1000 (i.e. 1×10^{-9} A f.s.d.). RR_ts were measured from the onset of the solvent peak and internal standards employed were allyl isothiocyanate and 3-phenylpropionitrile. Peak area measurements were accomplished manually. Absolute amounts produced were determined by standard injections of pure references (indicated by asterisks in Table 1) and corrected to allow for the recovery of the particular extrn used. This was assessed by the method of standard addition.

Identification of components by GC–MS. Components in the conc extracts were identified by GC–MS using the same GC conditions as above but with He as carrier gas. MS conditions were: ionization potential, 70 eV; ionization current, 300 μA; source temp. 230°; resolution, 1500; scan speed, 3 sec/decade (repetitive throughout run). The background subtraction facility and the retrospective single ion monitoring facility of the GC–MS data system were extensively employed in evaluating the MS results.

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