Phytochemistry, 1980, Vol. 19, pp. 1657–1660. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0801-1657 $02.00/0

DEGRADATION OF GLUCOSINOLATES OF *NASTURTIUM OFFICINALE* SEEDS

VICTOR GIL* and ALEXANDER J. MACLEOD

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

(*Revised received* 18 *December* 1979)

Key Word Index—*Nasturtium officinale*; watercress; *Lepidium sativum*; garden cress; *Barbarea praecox*; American cress; Cruciferae; glucosinolates.

Abstract—*Nasturtium officinale* contains four glucosinolates, the major representative being 2-phenethylglucosinolate. On autolysis of seeds or leaves, isothiocyanates were the main products of glucosinolate degradation but no thiocyanate was detected. The application of heat during extraction caused an increase in nitrile formation to dominance over isothiocyanates. A (benzyl) thiocyanate-forming extract of *Lepidium sativum* seeds did not provoke generation of any thiocyanate from glucosinolates of *N. officinale* (or *Barbarea praecox*), but it did impose accentuated nitrile-forming properties on the systems. The conclusion is reached that some glucosinolate-containing Cruciferae are predominantly nitrile-producing and some predominantly isothiocyanate-producing, all other factors being constant.

INTRODUCTION

Leaves of *Nasturtium officinale* (watercress) have previously been studied for their glucosinolate content, and the chief compound was 2-phenethylglucosinolate (*ca* 80% relative abundance) together with smaller amounts of 7-(methylthio)heptyl- (*ca* 12%) and 8-(methylthio)octyl- (*ca* 7%) glucosinolates [1]. Seeds invariably contain the same glucosinolates as plants therefrom, although sometimes in different relative proportions. However, with regard to the products of glucosinolate degradation, evidence has accumulated that benzylglucosinolate of *Lepidium sativum* (garden cress) decomposes to give benzyl thiocyanate in extracts of seeds but not of leaves, e.g. [2–8]. Therefore, although no thiocyanates were reported as glucosinolate products in extracts of *N. officinale* leaves [1] there remained a possibility of their production by seeds, which have not yet been studied in this context. Against this is the fact that to date only three of over 70 known glucosinolates have been proved to degrade to thiocyanates, namely allyl-, 4-(methylthio)butyl- and benzyl-glucosinolates, none of which was found in watercress leaves [1]. An objective of this project was thus to further test this peculiar limitation by a detailed examination of *N. officinale* seed extracts specifically for the presence of any thiocyanates.

At present there are two main theories to explain thiocyanate formation by glucosinolates; one, that it is caused by an enzyme or similar 'biological' factor or secondly that it is due to some specific chemical feature of a certain few glucosinolates (e.g. the ability to form particularly stable carbonium ions). To date all attempts to identify or isolate a thiocyanate-forming enzyme have failed, e.g. [2, 6] and efforts to substantiate the second suggestion have also been unsuccessful, e.g. [9, 10]. If no thiocyanates were detected in extracts of *N. officinale* seeds examined in this project, a second objective was to see whether an active (that is, benzyl thiocyanate-producing) extract of *L. sativum* seeds would cause any glucosinolate of *N. officinale* seeds to produce the corresponding thiocyanate. If this proved to be the case then this would support the contention that thiocyanate formation is enzymic (or 'biological') rather than dependent on some structural feature of the precursor. In support of this approach, Miller has studied mustard and *Thlaspi arvense* (both of which contain allylglucosinolate but only the latter forms allyl thiocyanate) using allylglucosinolate aglucone as substrate and has shown that an enzyme extract from *T. arvense* seeds is capable of producing thiocyanate whilst a similar one from mustard seeds is not [11].

RESULTS AND DISCUSSION

Table 1 lists the glucosinolate degradation products detected in various extracts of seeds and leaves of *N. officinale*. Products from a common precursor are grouped together and evidence has recently been presented that alcohols and aldehydes such as those listed here can also be of glucosinolate origin [12]. All identifications were made from GC–MS of extracts and in all instances spectra agree well with those previously published. In particular the spectra of the two long chain ω-(methylthio)alkyl nitriles have been reported before [1], as have those of the corresponding isothiocyanates [13, 14]. In all instances, except for these two isothiocyanates, identifications in Table 1 were further confirmed by comparison of GC RR_t data with those of authentic standards. Data from one type of sample were quantified by GC peak area measurements and comparison with reference standards, allowing for the recovery of the

* Present address: Chotiravi College, Nakorn Sawan, Thailand.

Table 1. Glucosinolate degradation products in various extracts of *Nasturtium officinale*

	Seeds			Leaves
	Autolysis extract		Likens and Nickerson extract	Autolysis extract
	(mg/g)	(%)	(%)	(%)
Phenylacetonitrile (benzyl cyanide)	tr	tr	tr	1
Benzyl isothiocyanate	—	—	—	tr
Benzyl alcohol			tr	—
Benzaldehyde	—		—	tr
3-Phenylpropionitrile (2-phenethyl cyanide)	0.18	9	36	1
2-Phenethyl isothiocyanate	1.79	90	44	91
2-Phenylethanol		—	tr	6
Phenylacetaldehyde		—	tr	
8-(Methylthio)octanonitrile		—	13	—
7-(Methylthio)heptyl isothiocyanate	0.02	1		tr
9-(Methylthio)nonanonitrile		—	6	—
8-(Methylthio)octyl isothiocyanate	0.01	0.5	—	tr

tr = trace.

extraction determined by the method of standard addition. Figures quoted refer to the defatted seed meal. However, for the purposes of this discussion the relative percentage figures are more appropriate.

Four glucosinolates were detected in *N. officinale* and all were found in both seeds and leaves (Table 1). 2-Phenethylglucosinolate is the major compound, and these results agree well with those from previous analyses of this plant [1] but with the additional detection here of trace amounts of benzylglucosinolate.

An important feature of these results is that no thiocyanates were detected in any samples although specific searches were made for all 4 possibilities, using the sensitive and specific technique of retrospective single ion monitoring using the GC–MS data system. If it had been formed, 2-phenethyl thiocyanate should certainly have been produced in detectable amounts bearing in mind the concentration of the glucosinolate precursor and the relative amounts of benzyl thiocyanate obtained in *L. sativum* seed extracts [12]. 2-Phenethyl thiocyanate was synthesised and its MS compared with that of the isothiocyanate. Summaries of the two spectra obtained are given below:

m/e	163 (M^+)	105	104	103	91	77	72
isothiocyanate, % rel. int.	37	11	8	7	100	15	8
thiocyanate, % rel. int.	20	21	28	13	100	20	1

As for the benzyl derivatives [15], noticeable differences between the MS of the two compounds are the slightly stronger M^+ and the peak at *m/e* 72 for isothiocyanate. Similar data for other thiocyanates and isothiocyanates are reported in the literature [16]. However, an additional more significant feature for this critical pair is the much more intense peak at *m/e* 104 for the thiocyanate. This is due presumably to the elimination of HSCN from the M^+. It was this highly characteristic ion which was used in single ion monitoring for the presence of 2-phenethyl thiocyanate in samples.

Since benzylglucosinolate is capable of forming thiocyanate, particular efforts were also made to locate this compound in extracts, but it is possible that in this case, if formed, it might have been below the detection limit of the analysis.

It is interesting to note from Table 1 that the relative amounts of glucosinolate products vary depending on the method of sample preparation. This type of behaviour has been observed before, e.g. [12, 17]. In this work, when genuine autolytic conditions were employed, the major glucosinolate products were isothiocyanates, both for seeds and leaves. Relatively small amounts were obtained of only two nitriles. Although not detailed, identical results were obtained whether the autolysis was carried out at 0° or at ambient temperature, and whether the reaction medium was distilled water or a citrate–phosphate buffer at pH 6.75. However, using the Likens and Nickerson extractor [18] as modified in this laboratory [19], nitriles became more important products and the only isothiocyanate detected was the 2-phenethyl compound. These results for the seeds agree well with those already reported for the leaves using this apparatus, when again only 2-phenethyl isothiocyanate was detected and the nitriles were dominant products [1]. The major difference between the two particular sample preparation methods is that heat is applied using the Likens and Nickerson apparatus. It must be concluded, therefore, that for *N. officinale* the effect of heat is somehow to promote nitrile formation. In our experience this has certainly not been the case with other plant systems studied, e.g. *Lepidium sativum* [12], *Farsetia* species [17].

Treating an *N. officinale* seed extract with a (benzyl) thiocyanate-producing seed extract from *L.sativum* failed to induce the formation of any thiocyanates from *N.*

officinale glucosinolates (although clearly conclusions cannot be drawn concerning benzylglucosinolate). Again, specific careful searches were carried out for these compounds. It can be deduced, therefore, that if there is a 'biological' factor such as an enzyme which does cause thiocyanate formation then it is highly specific either in respect of the particular plant system or in that it only functions on certain glucosinolates (the three previously mentioned). The latter is a more attractive suggestion since it combines the two present theories of thiocyanate formation, both of which have evidence in their favour.

During the course of this particular experiment some other interesting results were obtained and these are summarised in Table 2. It can be seen that the ratio of benzylglucosinolate products of *L. sativum* seeds did not vary appreciably in the presence of *N. officinale* seeds. On the other hand, the relative abundances of the 2-phenethylglucosinolate products from *N. officinale* seeds altered significantly in the mixed system, and in fact the ratio of isothiocyanate to nitrile was completely reversed. No heat was applied during the experiment (to explain the increased nitrile formation) and the results were confirmed by replicate analyses. Furthermore, exactly the same behaviour was observed with *N. officinale* leaves as with the seeds, and similarly with seed extracts of *Barbarea praecox* (American cress). On their own, seeds of *B. praecox* gave only the isothiocyanate from 2-phenethylglucosinolate, but in the presence of *L. sativum* seeds an almost equal amount of the nitrile was also obtained.

In our recent analyses of members of the Cruciferae we have observed that under standard, genuine conditions of autolysis some seeds or leaves give predominantly isothiocyanate products from their constituent glucosinolates (e.g. *Brassica oleracea* [14], *N. officinale* and *B. praecox* described here), whilst some give predominantly nitriles (e.g. *Lepidium sativum* [12, 15], some *Farsetia* species [17]). In some instances the same glucosinolate varies in its major product depending on the particular plant system (e.g. allylglucosinolate in *B. oleracea* [14] or *F. aegyptia* [17]), so it must be that some plants are mainly isothiocyanate-producing whilst others are mainly nitrile-producing. There is little evidence other than to speculate on the reason for this, but it is most likely that this variation in the major product of glucosinolate degradation is under enzymic control (in some support see reference [17]). It is highly unlikely that any difference in pH could alone be responsible for such a gross difference (see also [12]).

From this it follows that in a mixture of *L. sativum* seeds with *N. officinale* (or with *B. praecox* seeds) two mechanisms of glucosinolate degradation are in opposition. It would appear from the data in Table 2 that in this instance the nitrile-directing mechanism of *L. sativum* is the more powerful, imposing its influence over the normal degradation mode of 2-phenethylglucosinolate of *N. officinale*. The pH of the system is certainly not a factor here since in all instances it remained *ca* 5.7 during autolysis.

Thus, although the glucosinolates of *N. officinale* degrade under natural circumstances to give mainly isothiocyanates, this pathway appears to be particularly sensitive and can be readily subjugated in favour of nitrile formation either by simply applying heat to the system or by the incorporation of a member of the Cruciferae with natural nitrile-directing properties.

EXPERIMENTAL

Seeds (*Nasturtium officinale*, *Barbarea praecox*, *Lepidium sativum*) were obtained from Suttons Seeds Ltd., Reading, U.K., and where doubts existed were authenticated by microscopy. Watercress plants were purchased from a local retailer.

Sample preparation. (a) *Autolysis. N. officinale* seeds (50 g) were crushed in a coffee grinder and defatted with 5 × 75 ml dry hexane. After drying *in vacuo* 10 g of the seed meal were mixed with 100 ml of dist. H_2O (or citrate–Pi buffer, pH 6.75) and allowed to autolyse with shaking at room temp. (or 0°) for 2 hr. The mixture was then filtered, centrifuged and the supernatant extracted with 3 × 75 ml CH_2Cl_2. The extract was dried and carefully concd *in vacuo* to 1 ml. Exactly the same procedure was employed with seeds of *L. sativum* and *B. praecox*. With green parts of watercress, fresh leaves (80 g) were finely chopped in a Waring blender containing 100 ml of dist. H_2O (or buffer, pH 6.75). The mixture was then allowed to autolyse and the products extracted exactly as described for seed samples. In expts studying the influence of *L. sativum* seed extracts on other systems, 5 g of the defatted seed meal were mixed with 5 g defatted seed meal of *N. officinale* (or *B. praecox*) or 40 g macerated leaves of *N. officinale*, and the mixture allowed to autolyse in 100 ml of dist. H_2O or buffer exactly as above for single samples. (b) *Likens and Nickerson extraction.* Finely crushed, defatted and dried seeds of *N. officinale* (30 g) were placed in a round bottom flask containing 300 ml dist. H_2O (or citrate–Pi buffer, pH 6.75). The flask was fitted to the modified apparatus [19] and heated to boiling for 2 hr during which volatile components were steam distilled and extracted in the vapour phase into 2-methylbutane (40 ml). The extract was dried and concd carefully *in vacuo* to 1 ml.

Table 2. Approximate relative percentages of the main glucosinolate degradation products in some autolysed seed extracts

	L. sativum	*N. officinale*	*L. sativum* plus *N. officinale**
Phenylacetonitrile (benzyl cyanide)	58	tr	51
Benzyl isothiocyanate	7	–	5
Benzyl thiocyanate	35		43
3-Phenylpropionitrile(2-phenethyl cyanide)	tr	9	81
2-Phenethyl isothiocyanate	–	90	19
2-Phenethyl thiocyanate		—	–

* Figures refer to percentages for each individual glucosinolate.
tr = trace.

Sample analysis by GC. Extracts were analysed by conventional GC using a FID instrument. A 1.5 m × 4 mm id glass column was used packed with 10% Carbowax 20M coated on Diatomite C. With N_2 (30 ml/min) the temp. programme adopted was a 16°/min rise from an initial temp. of 60° for 5 min to a final level of 190° for the remainder of the run.

Sample analysis by GC–MS. Components in extracts were identified using a medium resolution instrument equipped with a data processing system. A silicone membrane separator was used with the same GC conditions as given above (but using He carrier gas). The operating 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 in particular the retrospective single ion monitoring facility of the data system were extensively employed.

Acknowledgements—We are grateful to Dr. J. G. Vaughan of the Botany Department, Queen Elizabeth College, for authenticating seed samples and to Mr. A. Cakebread for synthesis of 2-phenethyl thiocyanate. We thank also Mr. W. Gunn for carrying out the MS.

REFERENCES

1. MacLeod, A. J. and Islam, R. (1975) *J. Sci. Food Agric.* **26,** 1545.
2. Virtanen, A. I. (1965) *Phytochemistry* **4,** 207.
3. Saarivirta, M. and Virtanen, A. I. (1963) *Acta Chem. Scand.* **17,** (Suppl.) 74.
4. Gmelin, R. and Virtanen, A. I. (1959) *Acta Chem. Scand.* **13,** 1474.
5. Virtanen, A. I. (1962) *Arch. Biochem. Biophys.* **1,** (Suppl.), 200.
6. Saarivirta, M. (1973) *Planta Med.* **24,** 112.
7. Virtanen, A. I. and Saarivirta, M. (1962) *Suom. Kemistil. B.* **10,** 248.
8. MacLeod, A. J. and Islam, R. (1976) *J. Sci. Food Agric.* **27,** 909.
9. Benn, M. H. (1977) *Pure Appl. Chem.* **49,** 197.
10. Luthy, J. and Benn, M. H. (1977) *Can. J. Chem.* **55,** 1028.
11. Miller, H. E. (1965) M.A. Thesis, Rice University, Houston, Tx, U.S.A.
12. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **190,** 1369.
13. Kjaer, A. and Schuster, A. (1972) *Acta Chem. Scand.* **26,** 8.
14. Kaoulla, N., MacLeod, A. J. and Gil, V. (1980) *Phytochemistry* **19,** 1053.
15. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19,** 1365.
16. Jensen, K. A., Holn, A. and Wentrup, C. (1966) *Acta Chem. Scand.* **20,** 2117.
17. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19,** 227.
18. Likens, S. T. and Nickerson, G. B. (1964) *Proc. Am. Soc. Brew. Chem.* 5.
19. MacLeod, A. J. and Cave, S. J. (1975) *J. Sci. Food Agric.* **26,** 351.