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THERMAL DEGRADATION OF GLUCOSINOLATES

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Key Word Index—Cruciferae; glucosinolates; nitriles; isothiocyanates; thermal degradation.

Abstract—Three glucosinolates (allyl-, benzyl- and 2-phenethyl-) were shown to degrade thermally in a GC column to yield products identical with those obtained conventionally on enzymic decomposition, namely nitriles and isothiocyanates. Nitriles were formed more readily at 125° but the facility for isothiocyanate production varied slightly with the glucosinolate; 2-phenethylglucosinolate was the most labile of those studied yielding isothiocyanate at a column temperature of 150°. Temperature was confirmed as the cause of degradation by isolated heated-tube experiments. The results have significance both with regard to analytical methodology for glucosinolates and their products, and with regard to furthering understanding of the mechanisms of glucosinolate degradation.

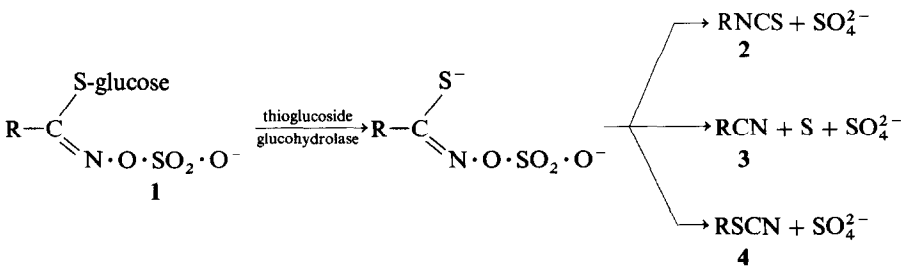
INTRODUCTION

Glucosinolates (**1**) are thioglucosides found mainly in the Cruciferae, which degrade enzymically (as shown in Scheme 1) to give three main products, isothiocyanates (**2**), nitriles (**3**) and thiocyanates (**4**). However, the elucidation of decomposition mechanisms and the possibility of secondary reactions are all complex problems which still require further work [1–4]. One aspect, relevant to this paper, is the now increasing evidence that nitriles can also be produced by non-enzymic degradation of glucosinolates [5–9], an origin which has not been reported for the other enzymic products.

Recently we synthesized 2-phenethylglucosinolate (**1**, R = $C_6H_5CH_2CH_2$) as a model compound [10] and observed that injection of an aqueous solution into a gas chromatograph provided one large peak, which was identified by GC/MS as 3-phenylpropionitrile (**3**, R = $C_6H_5CH_2CH_2$). This was not a contaminant from the synthesis and the most obvious explanation was that the glucosinolate was decomposing thermally in the GC column to give the nitrile. Clearly, if confirmed, this would have important ramifications not only with regard to analytical methodology for glucosinolates and their products, but also with regard to the causes and mechanisms of glucosinolate degradation in general. Consequently a survey was carried out to investigate the thermal decomposition of some pure glucosinolates.

RESULTS AND DISCUSSION

Table 1 gives details of the products and extent of degradation of three pure glucosinolates injected as aqueous solutions into a GC column at the temperatures given. Three different carrier-gas flow rates were employed but this variable did not significantly affect the amounts of products. Otherwise the gas chromatograph and operating conditions were the same throughout this series of experiments, and no injection-point heater was used (direct in-column injection was employed). Between six and nine injections were carried out for each



Scheme 1. Enzymic decomposition of glucosinolates.

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Table 1. Molar percentages of glucosinolate products formed on injecting aqueous solutions containing 0.2 μmoles of allyl-, benzyl-, or 2-phenethyl-glucosinolate into a gas chromatograph at different column temperatures

Column temp.	Allylglucosinolate		Benzylglucosinolate		2-Phenethylglucosinolate	
	Nitrile	Isothiocyanate	Nitrile	Isothiocyanate	Nitrile	Isothiocyanate
100°	—	—	—	—	—	—
125°	tr	—	tr	—	17 (±2)	tr
150°	14 (±1)	—	30 (±1)	—	22 (±1)	10 (±1)
175°	32 (±1)	17 (±1)	51 (±4)	—	31 (±4)	14 (±0.5)
200°	44 (±2)	32 (±2)	63 (±6)	13 (±4)	36 (±1)	18 (±2)

tr = trace.

glucosinolate solution at each column temperature, and the figures given in Table 1 are the averages of these measurements, rounded to whole numbers. The overall variation is also given within each set of data.

It can be seen that at a GC column temperature of 200° all the glucosinolates examined decomposed in the column to give relatively large quantities of both nitrile and isothiocyanate: no thiocyanate nor any other product was formed. In these conditions *ca* 76% of both allyl- and benzyl-glucosinolates was degraded in this manner and *ca* 54% of 2-phenethylglucosinolate. At lower column temperatures there was less decomposition and the amounts of degradation products obtained increased roughly linearly with temperature. Below 125° no decomposition occurred. For all three glucosinolates the nitrile was produced more readily and was detected at column temperature as low as 125°. The facility for thermal degradation to the isothiocyanate seemed to vary with the glucosinolate, the 2-phenethyl derivative being most labile.

It was thought that a finer assessment of the thermal degradation of glucosinolates by means of a GC technique could be obtained using an injection-point heater rather than relying on in-column decomposition. Therefore, adopting column temperatures at which it was known that no glucosinolate degradation could occur (i.e. < 125°), the data given in Table 2 were obtained. The results agree quite well with the previous series in that, for example, all three glucosinolates decomposed to give the same products as before, the amounts formed increased roughly linearly with temperature, and the nitrile was formed most readily. However, rather unexpectedly the effective temperatures varied between the two series of experiments. Overall, a temperature about 100° to 125° greater was necessary with the injection-point heater to show the same level of results obtained with the GC column. There was no obvious reason for this but it is almost certain that greater reliance can be placed on the temperature levels determined during in-column glucosinolate degradation.

It was possible that some factor other than temperature was responsible for glucosinolate degradation during GC, so experiments were carried out in which the solid glucosinolate was added into a small pre-heated glass tube. After 15 min the contents were dissolved in an aliquot of water and injected into the GC in conditions where glucosinolate degradation were known not to occur. Again all three glucosinolates gave similar results. At tube temperatures of 125° trace amounts of nitrile were

Table 2. Molar percentages of glucosinolate products formed on injecting aqueous solutions containing 0.2 μmoles of allyl-, benzyl-, or 2-phenethyl-glucosinolate into a gas chromatograph at different injection point heater temperatures

Injection-point heater temp.	Allylglucosinolate		Benzylglucosinolate		2-Phenethylglucosinolate	
	Nitrile	Isothiocyanate	Nitrile	Isothiocyanate	Nitrile	Isothiocyanate
200°	—	—	—	—	—	—
250°	23 (±2)	7 (±1)	29 (±1)	—	21 (±1)	tr
300°	39 (±2)	25 (±1)	56 (±3)	—	40 (±4)	19 (±3)
350°	49 (±3)	41 (±1)	77 (+2)	10 (±3)	52 (±2)	32 (±1)

tr = trace.

formed which increased with increasing temperature to about 33% at 175° and nearly 90% at 200°. Only traces of isothiocyanate were detected and then only at 200°. These results thus broadly confirm the behaviour and trends observed with the GC experiments. Finally, refluxing a glucosinolate in water for three hours gave about 30% nitrile and a trace of isothiocyanate but here, of course, the hydrolytic environment is a complication.

From these results and the data contained in Tables 1 and 2 it is clear that glucosinolates can degrade non-enzymically, purely thermally, to yield two of the normal products of enzymic degradation. Thus from an analytical point of view there is a distinct possibility of inaccurate results due to thermal decomposition, since neither the column temperatures nor the injection heater temperatures nor any other aspect of the GC analysis employed here were particularly extreme. Equally, there is similar risk of thermal degradation during extractions or work-up procedures prior to GC.

One of the most intriguing results is that the thermal degradation of glucosinolates yields products identical with those obtained on enzymic degradation. Clearly this need not have been the case as glucosinolates react chemically to yield a variety of other products [11, 12]. The non-enzymic degradation of glucosinolates to yield nitriles has been described previously [5–9, 13], but based largely on chemical reactions rather than thermal processes. However, MacLeod and MacLeod were probably the first to suggest the possibility of thermal degradation of glucosinolates to nitriles [7]. Previously isothiocyanates have only been obtained non-enzymically from glucosinolates by reactions involving silver nitrate. As long ago as 1899 Gadamer treated allylglucosinolate with silver nitrate and then with sodium thiosulphate, and isolated allyl isothiocyanate from the reaction mixture [14]. This finding was later confirmed by Schmid and Karrer with 4-methylsulphinylbut-3-enylglucosinolate [15]. This degradation is not based on thermal processes, the silver nitrate merely simulating the enzyme to give a product (a silver salt), which is effectively the aglucone of normal enzyme action (see Scheme 1), and which is thus obviously capable of isothiocyanate formation [16].

The results reported in this paper prove the thermal degradation of glucosinolates and could partly explain some previously observed variations in data regarding relative amounts of glucosinolate degradation products formed under different conditions of sample preparation. For example, whilst vacuum distillation of papaya volatiles gave benzyl cyanide and benzyl isothiocyanate in the ratio 1:30, steam distillation extraction reversed the ratio to 5:1 [17]. Thus, much more nitrile was formed during the procedure involving a higher temperature. Clearly, inaccurate data might have been obtained in the past, since steam distillation has been employed to collect glucosinolate products, and frequently only the isothiocyanate has been subsequently assessed. Low results would be expected for glucosinolate content based on such an assay. In conclusion, in contrast to earlier studies it has now been shown that both nitrile and isothiocyanate can be obtained non-enzymically from glucosinolates.

EXPERIMENTAL

Glucosinolates were synthesized as previously described [10]; 1-cyanoprop-2-ene, allyl isothiocyanate, phenylacetonitrile and benzyl isothiocyanate were purchased from commercial suppliers; 2-phenylpropionitrile and 2-phenethyl isothiocyanate were synthesized by conventional procedures. Standard solns of all compounds (glucosinolates, nitriles and isothiocyanates) were accurately prepared in distilled H_2O at the 0.2M level so that an injection vol. into the GC of 1 μl contained exactly 0.2 μmoles.

Gas chromatography. A heated FID was employed. A 0.3 m glass column packed with 10% Carbowax 20M coated on Diatomite C was used, but to confirm results with allylglucosinolate a similar 1.5 m column was also necessary due to the very short RR_t of allyl cyanide and of isothiocyanate through the shorter column. Carrier gas N_2, flow rate 30, 60 or 90 ml/min, and detector temp. 250°. Column temp. and injection-point heater temp. were varied as described in the text. Exactly 1 μl was injected of each glucosinolate soln and each product soln at every setting of column temp., injector temp. and flow rate. Replicate injections were made in all cases. As far as possible a particular series of expts was completed in one session and sensitivity settings were not varied. Peak areas were measured manually.

GC/MS. Products of glucosinolate degradation were identified by GC/MS using a Kratos MS 25 instrument equipped with a DS 50 data system. No significant differences were observed between conventional GC traces and those obtained using the TIC monitor on GC/MS, indicating that no further degradation or reaction occurred in the GC/MS interface or the MS itself. Significant operating parameters of the MS were: ionisation potential, 70 eV; ionisation current, 100 μA; source temp, 200°, accelerating voltage, 1.5 kV; resolution, 600; scan speed, 1 sec/decade (repetitive throughout runs). Spectra obtained from glucosinolate degradation agreed well with those of standard compds, also obtained via the GC inlet and the PEG 20M column.

Heated tube experiments. Small glass tubes were pre-heated in an oil bath to varying temps between 100° and 200°. Exactly 5 mg of the glucosinolate was added, the tube was gently stoppered and the temp. maintained for 15 min. After cooling, exactly 0.5 ml of water was added and the solid dissolved. Exactly 1 μl of the soln was injected into the GC at a column temp. of 100° and an injection-point temp. of 200°. The solns were not exactly 0.2 M with respect to glucosinolate or products so slight corrections to measurements were necessary.

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REFERENCES

1. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19,** 227.
2. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19,** 1369.
3. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19,** 1657.
4. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19,** 2547.
5. Austin, F. L. and Gent, C. A. (1967) *Chem. Commun.* 71.
6. Youngs, C. G. and Perlin, A. S. (1967) *Canad. J. Chem.* **45,** 1801.
7. MacLeod, A. J. and MacLeod, G. (1970) *J. Food Sci.* **35,** 739.
8. Kirk, L. D., Mustakas, G. C., Griffin, E. L. Jr. and Booth, A. N. (1971) *J. Amer. Oil Chem. Soc.* **48,** 845.
9. Gronowitz, S., Svensson, L. and Ohlson, R. (1978), *J. Agric. Food Chem.* **26,** 887.

10. Gil, V. and MacLeod, A. J. (1980) *Tetrahedron* **36,** 779.
11. Ettlinger, M. G. and Lundeen, A. J. (1956) *J. Amer. Chem. Soc.* **78,** 4172.
12. Friis, P., Larsen, P. O. and Olsen, C. E. (1977) *J. Chem. Soc. (Perkin I)* 661.
13. MacLeod. A. J. (1970) *Flavour Ind.* **1,** 665.
14. Gadamer, J. (1899) *Arch. Pharm.* **237,** 507.
15. Schmid, H. and Karrer, P. (1948) *Helv. Chim. Acta* **31,** 1017.
16. Miller, H. E. (1965) M.A. Thesis, Rice University, Houston, Texas, U.S.A.
17. Flath, R. A. and Forrey, R. R. (1977) *J. Agric. Food Chem.* **25,** 103.