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EVIDENCE FOR THE OCCURRENCE OF BUTYL- AND ISOBUTYLGLUCOSINOLATES IN SEEDS OF *BRASSICA OLERACEA*

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Abstract—Volatile glucosinolate products were collected from autolysed seeds of *Brassica oleracea* (cabbage), and identified by high sensitivity GC-MS. The detection in small amounts of 3-methylbutanonitrile and 2-methylpropyl isothiocyanate provides evidence for the occurrence of 2-methylpropylglucosinolate in cabbage. Similarly, the identification of pentanonitrile and butyl isothiocyanate provides evidence for the presence of butylglucosinolate. The former has not been previously reported in cabbage, and the latter has not been widely recognised as a cabbage component.

INTRODUCTION

In 1968 we reported for the first time the identification of butyl isothiocyanate as an aroma volatile, when we detected it in cooked cabbage [1]. Subsequently, we found it to be produced by cauliflower and Brussels sprouts as well [2]. Since all these vegetables are brassicas and since all *Brassica* species contain glucosinolates, it was most likely that the origin of the isothiocyanate was butylglucosinolate. In 1971, Kjaer and Schuster then detected butyl isothiocyanate in samples from the dried leaves of Jamaican *Capparis flexuosa* (caper), and they deduced a glucosinolate precursor [3]. Very much more recently in 1985, Hashimoto and Kameoka reported the identification of butyl isothiocyanate in plants of *Brassica campestris* (kabu), *Rorippa indica* (inugarashi) and *Armorasia rusticana* (wasabidaikon), all members of the Cruciferae [4].

However, since 1968 butyl isothiocyanate has not been confirmed as a cabbage volatile, especially in the detailed study by Buttery *et al.* [5], and doubt has been expressed concerning the validity of our earlier assignment [5, 6]. Therefore, we have re-examined some of the glucosinolate products of cabbage, in particular searching for evidence of butyl isothiocyanate. To maximise sensitivity, seeds were studied, which are far richer in glucosinolates than leaves, and high sensitivity GC-MS analysis was performed.

RESULTS AND DISCUSSION

Cabbage seeds were subjected to autolysis, and the autolysate then simply steam distilled to collect the volatile products of glucosinolate enzymic hydrolysis. It is well known that this approach does not detect all glucosinolate products (e.g. those from the indolylmethylglucosinolates), but it is certainly adequate, and indeed especially appropriate, for the glucosinolates yielding more volatile products, of which butylglucosinolate is an example. Distillates obtained in this manner were extracted with dichloromethane, and the extracts concentrated before analysis by GC-MS. Results are given in Table 1.

Evidence was obtained for the presence of seven glucosinolates in cabbage seeds (Table 1). The five major glucosinolates are the same as those that we have previously detected in cabbage seeds [7]. In total, *ca* 20 mg glucosinolates were obtained per gram of seeds, representing *ca* 45 μmol/g. Only one of the glucosinolates yielded a thiocyanate product, as well as the more normal isothiocyanate and nitrile. However, this is in agreement with previous work, and allylglucosinolate is one of only three of the *ca* 100 known glucosinolates which give a thiocyanate (not thiocyanate ion) [8].

Cyanoepithioalkanes are also well known products of degradation of glucosinolates (but only of those possessing terminal unsaturation in their side-chain), and such compounds have often previously been detected in cabbage samples [7, 9]. Although allyl and but-3-enyl products are listed here in Table 1, the expected corresponding cyanoepithioalkanes are not. However, when the samples were analysed following autolysis but without any steam distillation, then both 1-cyano-2,3-epithiopropane and 1-cyano-3,4-epithiobutane were readily identified, and in large amounts (*ca* 48 and 7% of the total detected glucosinolate products, respectively). Although it is well known that the epithiospecifier protein necessary for cyanoepithioalkane formation is thermally labile [10], these experiments suggest that the products themselves may also be susceptible to heat, in that they cannot withstand steam distillation. Either that, or they are not steam volatile, although taking into account their GC elution behaviour, this seems less likely.

With regard to the specific search for evidence of butylglucosinolate in cabbage, high sensitivity GC-MS of the autolysed samples did provide two mass spectra which matched literature spectra of butyl cyanide (pentanonitrile) and butyl isothiocyanate. However, examination of reference standards of these two compounds showed them to have the wrong GC R_ts under the same

Table 1. Volatile glucosinolate products from seeds of *Brassica oleracea*

*Compound	% of total volatile glucosinolate products	Amount (μg/g seeds)
But-3-enonitrile (allyl cyanide)	9.3	451
Allyl isothiocyanate	15.5	752
Allyl thiocyanate	2.4	116
But-3-enyl isothiocyanate	1.5	73
Pentanonitrile (butyl cyanide)	tr	0.07
Butyl isothiocyanate	tr	0.11
3-Methylbutanonitrile (isobutyl cyanide)	tr	3.58
2-Methylpropyl isothiocyanate (isobutyl isothiocyanate)	tr	3.01
4-(Methylthio)butanonitrile	39.2	1901
3-(Methylthio)propyl isothiocyanate	16.4	795
5-(Methylthio)pentanonitrile	1.4	68
4-(Methylthio)butyl isothiocyanate	2.1	102
3-Phenylpropanonitrile	8.9	432
2-Phenethyl isothiocyanate	3.2	155

*Compounds from a common glucosinolate are bracketed together.
tr = trace.

GC conditions as employed for GC-MS. Clearly the sample components were isomers of the butyl compounds, and further comparison with reference standards showed them to be isobutyl cyanide (3-methylbutanonitrile) and isobutyl isothiocyanate (2-methylpropyl isothiocyanate). R_ts and mass spectra of sample components agreed perfectly with those of the pure compounds. Sec. butyl isothiocyanate (1-methylpropyl isothiocyanate) has a different mass spectrum from that of butyl and isobutyl isothiocyanates, possessing a significant peak at *m/z* 86 due to β-fission (rather than at *m/z* 72).

Further, more detailed GC-MS of the cabbage samples, concentrating on the regions of known elution of butyl cyanide and butyl isothiocyanate, then disclosed another pair of mass spectra perfectly matching those of the reference butyl compounds. In this instance, the R_ts also matched perfectly. The butyl compounds were present at about 1/40th the level of the isobutyl isomers.

From these results, it can be deduced that both butyl- and isobutyl-glucosinolates are present in cabbage seeds. Some previous reports of butylglucosinolate products have already been outlined, and although isobutylglucosinolate (2-methylpropylglucosinolate) is also known, it is not very common, and it has not been reported previously in cabbage.

Quantification of the four butyl and isobutyl products in seed samples was performed (in triplicate) by direct comparison with the reference compounds and allowing for the recovery of the analytical procedure (assessed by standard addition) (Table 1). Assuming that the glucosinolates were quantitatively converted to their two products, then the amounts of butyl- and isobutyl-glucosinolates in cabbage seeds can be calculated at *ca* 0.74 μg/g (*ca* 1.8 nmol/g) and about 28.5 μg/g (*ca* 69 nmol/g), respectively. Although this assumption may not be entirely warranted, these data do, of course, give the minimum concentrations of the glucosinolates. The fact that they have not previously been detected in other analyses of cabbage would suggest that the methodology employed has not been sufficiently sensitive. Some workers, e.g. [11] denigrate GC-MS analysis of glucosinolate products, and claim that only analysis of intact glucosinolates is meaningful. However, until the techniques of analysis of intact glucosinolates approach the sensitivity routinely afforded by modern GC-MS of products, which is increasing all the time, then they merely provide an approximate and incomplete picture of the glucosinolate components of plants.

EXPERIMENTAL

Sample preparation. Cabbage seeds (*Brassica oleracea* cv. Golden Acre, from Suttons Seeds, Reading, U.K.) were ground to a powder in a coffee grinder. Distilled H_2O (*ca* 100 ml) was added to seed powder (*ca* 10 g, weighed accurately for quantitative work), and the mixture allowed to autolyse at room temp. for 1 hr with stirring. The autolysate was then submitted to conventional steam distillation and the distillate extd with 4 × 40 ml CH_2Cl_2. The combined extracts were dried (dry $MgSO_4$) and concd to < 1 ml using a rotary evaporator. Quantitative samples were accurately weighed just prior to analysis. Recovery of the procedure was determined (in duplicate) by adding a known weight of butyl isothiocyanate (e.g. 23.5 mg) at the beginning of autolysis.

GC. FID-GC: 25 m × 0.2 mm i.d. fused silica capillary column coated with BP1 bonded phase; hydrogen, 1.2 ml/min; temp. programme, 70° for 3 min then 6°/min to 170°; detector and injection point heaters, 250° and 200°, respectively; injection volume, typically 0.1 μl at 25:1 split.

GC-MS. A Kratos MS25 instrument was used, linked on-line to a Kratos DS55S data processing system. Capillary GC conditions as above were used, with He as carrier gas. The single-stage all-glass jet separator was at 250°. Significant operating parameters of the MS were: ionization voltage, 70 eV; ionization current, 100 μA; source temp., 225°; accelerating voltage, 1.33 kV; resolution, 1500; scan speed, 1 sec/decade (repetitive throughout run).

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