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NON-ENZYMIC DEGRADATION OF 2-HYDROXYBUT-3-ENYLGLUCOSINOLATE (PROGOITRIN)

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Abstract—The effect of a range of concentrations of Fe^{2+} on the non-enzymic degradation of pure, synthesized 2-hydroxybut-3-enylglucosinolate was assessed. For maximum activity, eight equivalents of Fe^{2+} were required, when 100% degradation was observed, yielding *ca* 51% of 3-hydroxypent-4-enethionamide and *ca* 47% of 1-cyano-2-hydroxybut-3-ene. Reduced amounts of products were obtained at lower pH, especially the nitrile. The effect of heat on 2-hydroxybut-3-enylglucosinolate (in aqueous solution) caused less degradation than previously reported, and only 35% decomposed in 5 hr at 100°. Allyl- and benzyl-glucosinolates were thermally more stable. Autoclaving the glucosinolates caused greater degradation. 2-Hydroxybut-3-enylglucosinolate was shown to decompose thermally during gas chromatography, but only to 1-cyano-2-hydroxybut-3-ene and no 5-vinyloxazolidine-2-thione was produced.

INTRODUCTION

One of the most important glucosinolates is 2-hydroxybut-3-enylglucosinolate, sometimes referred to by the trivial name progoitrin. Its importance resides in the fact that it is the major glucosinolate of rape and other economically important Cruciferae, and therein it is the natural precursor, on enzymic degradation, of goitrogenic 5-vinyloxazolidine-2-thione (goitrin) and other important products. As a result, a great deal of work has been carried out on the degradation of this particular glucosinolate in crucifers such as *Brassica napus* and *Crambe abyssinica*. Such work has provided valuable information regarding the natural formation of various glucosinolate products, and how these products vary, both quantitatively and qualitatively, depending upon conditions of autolysis, etc. within the plant. However, attempts to extrapolate the valid results of such studies to deductions regarding the chemistry of the mechanisms of glucosinolate degradation are liable to lead to inaccurate conclusions, in that they do not (cannot) take account of the numerous other constituents (mostly unknown) within the natural system which can affect the course of reaction. Some work has been performed on 2-hydroxybut-3-enylglucosinolate isolated from natural sources, but the product has invariably been impure and contaminated with co-extractives, so these studies also suffer the same limitations. Clearly, the only proper way to study the detailed chemistry of 2-hydroxybut-3-enylglucosinolate degradation is by using the pure compound, and recently we synthesized it [1], partly for this purpose.

In this paper we describe studies on some aspects of the non-enzymic degradation of synthesized 2-hydroxybut-3-enylglucosinolate, and in particular we selected parameters which have previously been shown to be important with regard to this specific glucosinolate's degradation in natural systems—namely the influence of ferrous ions and of heat.

The main product of enzymic degradation of a glucosinolate is usually the corresponding isothiocyanate (which, in the case of 2-hydroxybut-3-enylglucosinolate, spontaneously cyclizes to 5-vinyloxazolidine-2-thione), but the corresponding nitrile can also be formed, and especially at low pH. However, Youngs and Perlin found that refluxing allylglucosinolate for 15 min with equimolecular amounts of Fe^{2+} (i.e. non-enzymically) gave no isothiocyanate but a quantitative yield of allyl cyanide, together with *bis* (β-D-glucopyranosyl)disulphide [2]. On the other hand, under broadly similar circumstances, glucosinolates with a hydroxyl group in the 2-position of the side-chain were found to yield a thionamide as well as the nitrile [3–5]. Thus, 2-hydroxybut-3-enylglucosinolate gave 3-hydroxypent-4-enethionamide and 1-cyano-2-hydroxybut-3-ene, although an eight-fold excess of Fe^{2+} was necessary [3–5]. The formation of thionamide in this manner is interesting and unique in glucosinolate chemistry, but little further work has been carried out in this area, and little is known about the factors which control the reaction or about its mechanism. In view of this, some of the early work carried out [3–5] was re-examined, but using pure 2-hydroxybut-3-enylglucosinolate.

Relatively few studies have been carried out specifically on the effects of heat on glucosinolates, but behaviour would appear to vary to some extent depending on the glucosinolate side-chain and the nature of the heating. Gronowitz *et al.* studied the degradation of but-3-enyl- and 2-hydroxybut-3-enyl-glucosinolates, and found that after refluxing in water for 5 hr, only *ca* 36% of the original glucosinolate remained with *ca* 60% of the corresponding nitrile being produced [6]. Maheshwari *et al.* investigated the effects of conventional and microwave heating on the decomposition of allylglucosinolate [7].

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Unfortunately, only the loss of the glucosinolate was followed (by UV spectroscopy), and the products of degradation were not identified or considered. The thermal stability of some glucosinolates during GC has also been studied, and although nitriles were the major products of degradation in all cases, isothiocyanates were also produced but only at more extreme temperatures [8].

In the work reported here, a survey was carried out of the effects of heat on aqueous solutions of pure 2-hydroxybut-3-enylglucosinolate and comparison with some other pure glucosinolates. In addition, the thermal decomposition of 2-hydroxybut-3-enylglucosinolate during GC was also studied, and results compared with previous findings for other glucosinolates [8]. Finally, it will be appreciated that when cruciferous vegetables are cooked in a pressure cooker, the temperature will rise above that of normal cooking due to the increased pressure. Since the flavour of such vegetables will depend to a marked extent on the production of certain glucosinolate degradation products, a brief survey was therefore also carried out comparing the effects of autoclaving (equivalent to pressure cooking) on the degradation of glucosinolates, including 2-hydroxybut-3-enylglucosinolate.

RESULTS AND DISCUSSION

Considering first the effects of Fe^{2+} on the non-enzymic degradation of 2-hydroxybut-3-enylglucosinolate, Table 1 gives the results for a range of Fe^{2+} concentrations. Also given is the pH of the unbuffered medium at the end of the 18 hr period of the experiments; at the beginning the pH of the system was 6. The first interesting feature to note is that in agreement with previous work [3–5], eight equivalents of Fe^{2+} were necessary to provide maximum glucosinolate degradation. However, lesser extents of degradation were still observed at all other concentrations of Fe^{2+} assessed, so clearly, therefore, the degree of glucosinolate degradation under these circumstances is Fe^{2+} dependent. In the previous work [4, 5], only *ca* 55% degradation of 2-hydroxybut-3-enylglucosinolate was obtained with eight equivalents of Fe^{2+}, yielding *ca* 40% of 3-hydroxypent-4-enethionamide and *ca* 15% of 1-cyano-2-hydroxybut-3-ene. In the results presented here (Table 1), it can be seen that degradation was effectively quantitative (within experimental error), and whilst the level of thionamide produced was only a little higher (*ca* 51%), a much increased concentration of nitrile (*ca* 47%) was produced. It is particularly interesting that this ratio of thionamide to nitrile was reasonably constant at 1:1 throughout all concentrations of Fe^{2+} evaluated. This suggests a common intermediate in the formation of these two compounds from the glucosinolate precursor, but whose generation is, in turn, dependent on Fe^{2+} concentration. In summary, therefore, compared with previous work [4, 5] we obtained far greater degradation of 2-hydroxybut-3-enylglucosinolate with production of much higher concentrations of 1-cyano-2-hydroxybut-3-ene. Since experimental conditions were effectively the same, then these differences are most likely due to our use of a pure substrate in comparison with that used previously (only 90% pure) [4, 5].

As can be seen from Table 1, during the glucosinolate degradation the pH fell to a much lower level, and an explanation of this type of behaviour has been given previously [9]. However, the effects of Fe^{2+} on 2-hydroxybut-3-enylglucosinolate degradation at controlled pH gave slightly different results, and these are shown in Table 2. Thus, lower pH levels gave lesser

Table 1. Effect of various concentrations of Fe^{2+} on aqueous solutions of synthetic 2-hydroxybut-3-enylglucosinolate (60 μmol)*

Equivalents of Fe^{2+}	Yield of 1-cyano-2-hydroxybut-3-ene (μmol)	Yield of 3-hydroxy-pent-4-enethionamide (μmol)	Total yield (μmol)	Ratio of thionamide to nitrile	pH after reaction†
2	10.0	10.8	20.8	1.08	3.5
4	15.7	19.1	34.8	1.22	3.4
6	22.7	27.0	49.7	1.19	3.3
8	28.0	30.9	58.9	1.10	3.3
10	27.8	30.9	58.7	1.11	3.4

*Reaction time 18 hr at 25°.
†Initial pH 6.

Table 2. Effect of Fe^{2+} (10 equivalents) on aqueous solutions of synthetic 2-hydroxybut-3-enylglucosinolate (60 μmol) at various pH*

pH	Yield of 1-cyano-2-hydroxybut-3-ene (μmol)	Yield of 3-hydroxy-pent-4-enethionamide (μmol)	Total yield (μmol)	Ratio of thionamide to nitrile
3.8	14.9	14.9	29.8	1.00
4.6	21.6	16.7	38.3	0.77
5.2	23.3	13.5	36.8	0.58

*Reaction time 18 hr at 25°.

amounts of glucosinolate degradation products, with the same concentration of Fe^{2+}, but this was mainly due to decreased nitrile. As a result, the ratio of thionamide to nitrile increases with decreasing pH, and, in fact, a plot of the ratio against pH gives a straight line. Indeed, a point can also be incorporated, which reasonably fits the straight line, for reaction in unbuffered medium, averaged from the data in Table 1 for the final pH and the ratio of the two components. Overall, this implies that the relative formation of 3-hydroxypent-4-enethioamide and 1-cyano-2-hydroxybut-3-ene from the aforementioned common intermediate is pH-dependent. Again, the results obtained by Austin *et al.* do not agree with our results, and they obtained virtually identical amounts of the two products whether they carried out the reaction at pH 5.4 or 2.8 [4].

Regarding the mechanism of the Fe^{2+}-induced degradation of 2-hydroxybut-3-enylglucosinolate, it is possible that the common intermediate in thionamide and nitrile formation referred to above is a ferrous-glucosinolate complex of the type suggested by Youngs and Perlin [2], but also involving the hydroxyl group in the 2-position of the side-chain, since it is only glucosinolates which possess this feature which are capable of thionamide production. The existence of such a complex seems very reasonable. The complex, or a common product thereof, could then be the branch point for formation of nitrile or thionamide, dependent on pH. Benn has theorized that nitrile formation may proceed via a thiazirine and/or a thiocarbonyl nitrene [10]. Protonation of the latter could be envisaged as yielding a thionamide, which would fit in with the favoured formation of this product at lower pH (Table 2). However, since it is rather the concentration of nitrile which is suppressed at lower pH, then it may be more likely that nitrile is being lost by secondary reaction under these conditions, although no other product was detected in our assays.

Table 3 gives results for the degradation of 2-hydroxybut-3-enylglucosinolate (and allylglucosinolate) caused by heating the glucosinolate at 100° in aqueous solution for 5 hr. After this time, only *ca* 35% of 2-hydroxybut-3-enylglucosinolate had decomposed, whereas Gronowitz *et al.* recorded 64% degradation under the same conditions [6]. However, the isolated glucosinolate used by these workers was only 65–70% pure, and it is likely, therefore, that impurities accounted for the greater extent of decomposition. This shows again how erroneous results are obtained using impure glucosinolates. The product of this type of degradation was the corresponding nitrile, and again the pH dropped during the reaction as seen with Fe^{2+}-induced decomposition (Table 1), and presumably for the same reason [9]. At the conclusion of these experiments, it reached *ca* pH 5 from an initial level of 7.6.

As can be seen from Table 3, allylglucosinolate was more stable than 2-hydroxybut-3-enylglucosinolate to this type of heat treatment, but furthermore, it was found that benzylglucosinolate showed only 4.4% decomposition after 5 hr. These differences must reflect the differing stabilizing effect of the side-chain towards glucosinolate hydrolysis, and may well be related to the relative electron donating capabilities of the three groups.

Autoclaving (equivalent to pressure cooking) the glucosinolates at 119° increased the extent of decomposition as would be expected, but to a considerable extent (Table 4). For some unknown reason, the initially more stable benzylglucosinolate exhibited greatest degradation with time. Again the product of reaction was the corresponding nitrile, and clearly this non-enzymic degradation of glucosinolates could have consequences with regard to the flavour and, to a very minor extent, the toxicity of cruciferous vegetables prepared by pressure cooking.

Previous work has shown that pure glucosinolates injected into a GC degrade (non-enzymically) at column temperatures as low as 125° to yield traces of corresponding nitriles [8]. More extreme temperatures (200°) were necessary before isothiocyanate was also produced, but at this level more than 50% glucosinolate degradation was observed [8]. With 2-hydroxybut-3-enylglucosinolate, a column temperature of 150° was found to be necessary before any decomposition occurred, and this was very slight, but at 175° and 200°, *ca* 24% and *ca* 36%, respectively, of glucosinolate was degraded to nitrile. However, no 5-vinyloxazolidine-2-thione nor any other product was detected, even at 200°. Similarly, and again in broad agreement with previous findings [8], 2-hydroxybut-3-enylglucosinolate was also found to degrade in the injection port of the chromatograph, yielding *ca* 20% of nitrile at 200° increasing to *ca* 28% at 350°. However, unlike previous results [8], again 1-cyano-2-hydroxybut-3-ene was the only product and no oxazolidinethione (isothiocyanate) was produced.

In conclusion, it is worth emphasizing, therefore, that in all the work reported here, no 5-vinyloxazolidine-2-thione was ever produced non-enzymically from 2-hydroxybut-3-enylglucosinolate, either by the influence of various forms of heating or by using ferrous ions. In broad terms, this does not agree with some findings for other glucosinolates where isothiocyanates are similarly formed, and there may by something missing from the pure model system which is necessary specifically for oxazolidinethione formation and which does occur naturally.

Table 3. Effect of heat (100°) on aqueous solutions of allyl- and 2-hydroxybut-3-enyl-glucosinolates

Time (hr)	Glucosinolate remaining (%) allyl	2-hydroxybut-3-enyl
0	100	100
0.5	98.0	85.7
1.0	96.1	88.2
2.0	98.9	85.7
3.0	96.0	83.1
4.0	95.6	74.8
5.0	86.3	65.1

Table 4. Effect of autoclaving at 15 psi (119°) on aqueous solutions of allyl-, benzyl- and 2-hydroxybut-3-enyl-glucosinolates

Time (hr)	Glucosinolate remaining (%) allyl	benzyl	2-hydroxybut-3-enyl
0	100	100	100
0.5	81.4	92.9	77.4
1.0	77.2	57.3	75.8
2.0	71.0	37.3	47.6

EXPERIMENTAL

Action of Fe^{2+} *on 2-hydroxybut-3-enylglucosinolate. Synthesized* [1] 2-hydroxybut-3-enylglucosinolate (27 mg) was dissolved in deionized H_2O (3.75 ml), and aliquots containing 60 μmol of glucosinolate were pipetted into 50 ml screw-top bottles. Aq. solns of ferrous ammonium sulphate (2.5 ml, containing the appropriate relative amount of Fe^{2+}) were added. The mixtures were covered with N_2, stoppered, and kept at 25° for 18 hr. The solns were than satd with NaCl, extracted with $CHCl_3$ (5 × 15 ml), dried ($MgSO_4$) and the solvent removed in a rotary evaporator without the application of heat. The residues were then taken up in 1 ml portions of $CHCl_3$. Extraction efficiency was assessed by subjecting synthesized [11] 1-cyano-2-hydroxybut-3-ene (15.8 mg) to the whole procedure, giving a recovery of 74·5%. All assays were carried out in triplicate. Nitrile degradation product was estimated by FID GC using a 1.5 m × 4 mm i.d. glass column packed with 10% neopentyl glycol succinate (NPGS) coated on Celite. GC conditions were: column temp., 150°; inj. port temp., 150°; detector temp., 250°; flow rate, 30 ml N_2/min. Quantification was accomplished by injecting known amounts of pure, synthetic nitrile into the GC under the same conditions. 3-Hydroxypent-4-enethionamide was estimated by measuring the absorbance at 269 nm in $CHCl_3$ ($\varepsilon = 1.023 \times 10^4$).

Controlled pH expts were carried out in exactly the same manner as above, but in place of H_2O, NaOAc–HOAc buffers were used made by mixing 0.2 M solns to give the required pH.

Action of heat on glucosinolates. Portions of glucosinolate (10 mg) were dissolved in deionized H_2O (*ca* 3 ml) in stoppered test tubes and heated to 100°. At various time intervals, tubes were removed and the contents freeze-dried. Glucosinolate remaining was estimated as follows. The residue was heated at 110° for 40 min in a soln (1.5 ml) of hexamethyldisilazane, trimethylchlorosilane and pyridine (2:1:10). The TMSi-glucosinolate was then analysed by FID-GC using a 1.5 m × 4 mm i.d. glass column packed with 3% OV 17 coated on Chromosorb Q 80/100; temp. programme, 195–235° at 2°/min; injection port and detector temp., 250°; flow rate, 30 ml N_2/min. Quantification was by reference to the 100% standard at zero time. At the end of the 5 hr degradation period, samples were extracted with $CHCl_3$ (5 × 15 ml), dried ($MgSO_4$) and the solvent removed in a rotary evaporator without the application of heat. Residues were dissolved in $CHCl_3$ (1 ml), and degradation products of 2-hydroxybut-3-enyl- and benzyl-glucosinolates were analysed by FID-GC using a 1.5 m × 4 mm i.d. glass column packed with 5% Apiezon L coated on 80–100 BSS mesh acid-washed Diatomite C; temp. programme, 60–300° at 3°/min; injection port temp., 60°; detector temp., 250°; flow rate, 30 ml N_2/min. Allylglucosinolate degradation products were analysed by FID GC using a 1.5 m × 4 mm i.d. glass column packed with 10% PEG 20 M coated on Celite; temp. programme, 60–180° at 3°/min; injection port temp., 60°; detector temp., 250°; flow rate, 30 ml N_2/min. Products were identified by GC/MS and quantification was accomplished by injection of known amounts of appropriate synthetic standards into the GC under the same conditions. Assays were carried out in triplicate.

Identical expts were carried out as above but instead of conventional heating the glucosinolate solns were subjected to autoclaving at 15 psi (119°) for various periods of time.

Fresh, aq. solns of 2-hydroxybut-3-enylglucosinolate were also injected directly into the GC under the conditions noted above but maintaining the column temp. at 100°, 125°, 150°, 175° or 200°, or maintaining the injection port temp. at 200°, 250°, 300° or 350°. Again, any degradation products were identified by GC/MS and quantified by comparison with appropriate standards. Assays were performed in triplicate.

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