# Base-catalysed Neber-type Rearrangement of Glucosinolates [1-(β-D-Glucosylthio)-*N*-(sulphonato-oxy)alkylideneamines]

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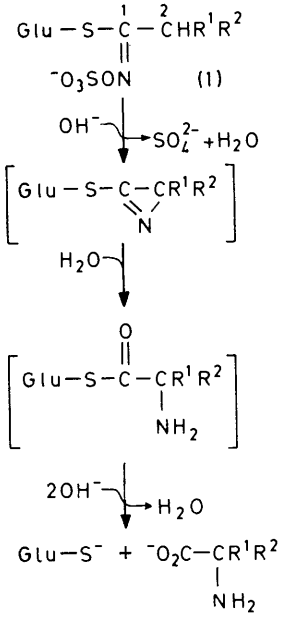
Allylglucosinolate in aqueous base produces vinylglycine (2-aminobut-3-enoic acid), thioglucose, and sulphate by a reaction of the first order with regard to both glucosinolate and hydroxide ion. No deuterium is introduced at C-2 of the amino-acid when the reaction is performed in $D_2O$. The reaction presumably takes place through a Neber-type rearrangement, starting with concerted loss of a hydrogen atom at C-2 in the aglucone, bond formation between C-2 and the nitrogen atom, and loss of sulphate, followed by fast addition of water to the resulting azirine, ring opening of the hydroxyaziridine produced, and finally hydrolysis of the thioester thus obtained. Benzylglucosinolate correspondingly yields phenylglycine, thioglucose, and sulphate in aqueous base but in this case other products, including benzaldehyde, benzyl cyanide, cyanide, and thiocyanate, are observed. Both vinylglycine and phenylglycine are produced in partly racemised forms but with an excess of the L-isomer. The reaction of the glucosinolates is facilitated by a high kinetic acidity of the hydrogens at C-2 in the aglucone, but glucosinolates other than the allyl and benzyl compounds can also give α-amino-acids with aqueous base, although only at elevated temperature.

GLUCOSINOLATE anions (1) with various side chains occur in many higher plants.[1-3] They are of general importance because of the flavouring and toxic properties of their enzymic degradation products and because of their role in plant–insect relationships. The enzymic degradation of glucosinolates to give isothiocyanates, sulphate, and glucose, or in special cases thiocyanates or cyanides and sulphur, has been studied in detail.[4-7] Also, the degradation of glucosinolates with strong acid to yield carboxylic acids and hydroxylamine is well established.[1] On the other hand, only a few studies have been made on base-catalysed degradations of glucosinolates (see later).

We now report the degradation with aqueous base of the allylglucosinolate (1a) (potassium allylglucosinolate has the trivial name sinigrin) and of the benzylglucosinolate (1b), with formation of the amino acids (2a and b) as the main products, by a Neber-type rearrangement.

The reaction of the allylglucosinolate was followed by $^{13}C$ n.m.r. spectroscopy. The Table shows the chemical shifts of the components of the reaction mixture produced from potassium allylglucosinolate (50 mg) in $D_2O$ (*ca.* 1 ml) 1 h after addition of 2N-sodium hydroxide (0.2 ml) (room temperature). Chemical shifts of potassium allylglucosinolate in $D_2O$, DL-vinylglycine in $D_2O$ and in $D_2O$–NaOH, and 1-thio-β-D-glucopyranose sodium salt in $D_2O$ are also given. The observation of only one set of signals for the anion of 1-thioglucose, both in the reaction mixture and in the spectrum of the pure compound, confirms that no equilibration between α- and β-anomers takes place in strong base.[8] No $^{13}C$ n.m.r. spectra of glucosinolates or of the anion of thioglucose have been reported previously. The assignment of signals to vinylglycine and to the aglucone of the glucosinolate can be made on the basis




of known chemical shifts for related compounds.[9] No attempts have been made to assign signals to individual carbon atoms in the glucose moiety, partly because of lack of suitable reference material. Some tentative

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[3] M. G. Ettlinger and A. Kjær, in 'Recent Advances in Phytochemistry,' eds. T. J. Mabry, R. F. Alston, and V. C. Runeckle, Appleton–Century–Crofts, New York, 1968, vol. 1, p. 58.
[4] M. G. Ettlinger and H. E. Miller in 'The Chemistry of Natural Products, Abstract Book,' International Union of Pure and Applied Chemistry, 4th International Symposium, Stockholm, Sweden, 1966, p. 154.
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[6] M. Schlüter and R. Gmelin, *Phytochemistry*, 1972, **11**, 3427.
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conclusions could, however, be made by comparison of values for the glucosinolate anion, the thioglucose anion, and *O*-glucosides.

Only signals from vinylglycine and thioglucose were found in the reaction mixture, indicating that the allylglucosinolate is transformed nearly quantitatively into these compounds. However, cyanide and thiocyanate (both less than 3%) were found by quantitative determinations, indicating that some side reactions were taking place. No further identification of the thioglucose was performed, whereas vinylglycine was isolated in high yield by adsorption to a strongly acidic ion-exchange resin ($H^+$ form), elution with aqueous pyridine, and evaporation. The crystalline residue was identical with synthetic vinylglycine [10] ($^1H$ n.m.r. spectrum and paper chromatography). The $^1H$ n.m.r spectrum showed. L-isomer. The $\Delta\varepsilon$ values were those expected from $[\alpha]_D$ and $\Delta\varepsilon$ values foi the D-isomer.[10]

The reaction of the allylglucosinolate was also followed by u.v. spectroscopy. The absorption maximum at 228 nm [11] disappeared, and a new maximum at 215 nm (1-thio-β-D-glucopyranose anion) arose. An isosbestic point was present at 250 nm. Kinetics were determined by measuring the absorbance at 228 nm. The reaction is of the first order with respect to both glucosinolate and hydroxide ion. In 0.091 9N-sodium hydroxide, adjusted to a total ion strength of 0.1 by addition of sodium chloride, the following bimolecular rate constants were obtained: 0.006 96 l $mol^{-1}$ $s^{-1}$ at 25.1 °C, 0.012 9 at 32.7 °C, and 0.023 6 at 40.1 °C. From these values the Arrhenius $E_a$ is calculated to be 63 kJ $mol^{-1}$, $\Delta S$ to be −92 J $mol^{-1}$ $K^{-1}$, and $t_{\frac{1}{2}}$ at 25.1 °C to be 18 min.

$^{13}C$ N.m.r. data for the reaction mixtures from potassium allylglucosinolate and sodium hydroxide, tetramethylammonium benzylglucosinolate and sodium hydroxide, and for various reference compounds

| | δ Values | | | |
|---|---|---|---|---|
| | C-1 * | C-2 * | Glucose carbon atoms | Others |
| 1-Thio-β-D-glucopyranose sodium salt in $D_2O$ † | | | 85.1, 80.6, 79.6, 77.9, 71.4, 62.3 | |
| DL-Vinylglycine in $D_2O$ | 173.6 | 58.5 | | C-3 131.2, C-4 122.2 |
| DL-Vinylglycine in $D_2O$–NaOH | 181.8 | 60.0 | | C-3 139.1, C-4 116.1 |
| Potassium allylglucosinolate in $D_2O$ | 163.8 | 37.1 | 82.5, 80.9, 78.1, 73.0, 70.1, 61.7 | C-3 in aglucone 133.0, C-4 in aglucone 119.3 |
| Mixture from potassium allylglucosinolate and NaOH in $D_2O$ after 1 h | ‡ | 60.0 | 85.2, 81.0, 79.7, 78.3, 71.8, 62.5 | 139.2, 116.1 |
| DL-Phenylglycine in $D_2O$–NaOH | 181.7 | 61.5 | | Aromatic 143.2, 129.7, 128.4, 127.7 |
| Tetramethylammonium benzylglucosinolate in $D_2O$ | 163.3 | 39.0 | 82.2, 80.6, 77.9, 72.7, 69.7, 61.2 | Aromatic 136.0, 130.1, 128.9, 128.4; $Me_4N^+$ 56.1 § |
| Mixture from tetramethylammonium benzylglucosinate and NaOH in $D_2O$ after 2 days | ‡ | 61.4 | 85.1, 80.7, 79.5, 77.9, 71.4, 62.2 | Aromatic 143.5, 129.7, 128.4, 127.7; $Me_4N^+$ 56.1 § |

Spectra recorded at 22.63 MHz with a Bruker HX 90E instrument (pulse technique with Fourier transformation). δ Values in p.p.m. downfield from $Me_4Si$. Dioxan used as internal standard [$\delta(Me_4Si) = \delta(\text{dioxan}) + 67.4$ p.p.m.].

* C-1 and C-2 in the aglucone parts of the glucosinolates, corresponding C atoms in reference compounds, and corresponding signals in the reaction mixtures. † Changes of not more than 0.5 p.p.m. were observed on adding NaOH to the solution. ‡ In the reaction mixtures signals of the carboxy groups in vinylglycine and phenylglycine were not observed, because of the large relaxation times of these C atoms and the short time lag between pulses. § Triplet due to $^{13}C$,$^{14}N$ coupling.

that no exchange of hydrogen in the α-position had taken place. Specific rotations ($[\alpha]_D$) of the products from three different experiments were +11, +38, and +38° (in water), to be compared with a value of at least −94° for the pure D-isomer.[10] Therefore the process involves a relatively high degree of optical induction. In both experiments paper chromatography showed that the reaction mixture contained only vinylglycine and traces of 2-aminobutyric acid (produced by chemical degradation of vinylglycine [10]) as ninhydrin-positive components. That the optical rotation was due to vinylglycine and not to impurities with high rotation was, in the latter case, also shown by measuring the c.d. curves for solutions both in water and in hydrochloric acid. Both $\lambda_{max.}$ and the shapes of the curves were identical with those of D-vinylglycine, whereas the sign of the Cotton effect was positive, as expected for the

No reaction intermediates were observed by u.v. spectroscopy. $^{13}C$ N.m.r. spectroscopy is not well suited for the identification of intermediates, because of its low sensitivity. It would be necessary to use a high initial concentration of glucosinolate and consequently of base (3 equiv. are consumed). However, this leads to a high reaction rate, which lowers the chance of observing any short-lived intermediates, since the spectrum is recorded over a long time (*ca.* 1.5 h). It was therefore attempted to follow the reaction by $^1H$ n.m.r. spectroscopy, which allowed the use of lower concentrations, and a much shorter recording time. However, because of overlapping of signals no useful results were obtained.

In the hope of stopping the reaction at the aziridine stage (see later) it was attempted to study the system under anhydrous conditions, by using sodium methoxide in methanol; the experiment was unsuccessful, however.

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[11] A. Kjær, R. Gmelin, and R. B. Jensen, *Acta Chem. Scand.*, 1956, **10**, 26.

$^{13}C$ N.m.r. spectroscopy was also used to study the reaction of the benzylglucosinolate (1b) in sodium hydroxide. The Table gives the chemical shifts obtained from the mixture produced from tetramethylammonium benzylglucosinolate (60 mg) in $D_2O$ (*ca.* 1 ml) 2 days after addition of 2N-sodium hydroxide (0.2 ml) (room temperature). The Table also gives chemical shifts for tetramethylammonium benzylglucosinolate in $D_2O$ and for DL-phenylglycine in $D_2O$–NaOH.

Signals from phenylglycine, thioglucose anion, and tetramethylammonium ion were identified. However, the aromatic signals occurred as a broad band, indicating that additional aromatic compounds were present in minor amounts. Phenylglycine was isolated in about 50% yield by adsorption to a strongly acidic ion-exchange resin, elution with aqueous pyridine, and evaporation. The $^1H$ n.m.r. spectrum of the residue again showed that no exchange of hydrogen in the α-position had taken place. The specific rotation of the sample was +42° in 5N-hydrochloric acid; *cf.* +168° for the pure L-isomer.[12]

The rate of the reaction of the benzylglucosinolate could not be determined by u.v. spectroscopy, since the changes in absorbance were too small. No isosbestic point was present. Small-scale preparative experiments indicate, however, that the rate is about 10 times smaller than that for the allylglucosinolate.

Because of the deficit in yield, attempts were made to identify other products. The reaction was performed with stirring with an equal volume of carbon tetrachloride. The $^1H$ n.m.r. spectrum of the organic phase after the reaction established the presence of benzaldehyde (*ca.* 22% yield) and benzyl cyanide *ca.* (8% yield), both when the reaction was performed without exclusion of oxygen and when performed in a nitrogen atmosphere. The $^1H$ n.m.r. spectrum of the aqueous phase showed a broad band centred at δ 7.5 in addition to the sharp singlet at δ 7.1 from phenylglycine. No benzoic acid or phenylacetic acid was present. G.l.c.–mass spectrometry of the organic phase gave additional proof for the presence of benzaldehyde and benzyl cyanide, and demonstrated the absence of benzyl alcohol.

When the reaction was conducted in a nitrogen atmosphere, cyanide and thiocyanate ions were formed in yields of 15 and 8%, respectively. Without exclusion of oxygen, or when the reaction mixture was subsequently exposed to air, the yield of thiocyanate increased at the expense of cyanide. This is presumably due to a reaction between the disulphide of thioglucose and cyanide with production of the thioglucose anion and β-D-glucopyranosyl thiocyanate, followed by hydrolysis of the latter to give glucose and thiocyanate anion (*cf.* ref. 13). In fact, when thioglucose sodium salt was oxidized with iodine and subsequently treated with cyanide, a *ca.* 60% yield of thiocyanate was obtained. However, atmospheric oxygen was not capable of oxidizing the thioglucose anion, not even in the presence of iron(II). The yield of $SCN^-$ was, in both cases, lower than 2% on treatment of thioglucose with cyanide in the presence of oxygen.

The reaction between benzylglucosinolate and cyanide was studied as a possible source of the originally formed (8%) thiocyanate, but the yield (5%) of $SCN^-$ was too low (theoretically 100% in this experiment) to account for the observed amount.

Finally, glucosinolate was found not to react with thioglucose anion to give benzyl cyanide and $SCN^-$ *via* the disulphide of thioglucose.

Both allyl- and benzyl-glucosinolate exhibit a high kinetic acidity of the first $CH_2$ group in the side chain. However, non-activated glucosinolates can also give α-amino-acids although under more severe conditions. Thus methyl- and 3-methylsulphinylpropyl-glucosinolate produce glycine and methionine *S*-oxide, respectively when kept in sealed tubes with 2N-sodium hydroxide for 16 h at 75 °C. The amino-acids in these cases were identified by paper chromatography; no information was obtained on yields. The two glucosinolates did not produce ninhydrin-positive components after prolonged periods in 2N-sodium hydroxide at room temperature.

The foregoing results indicate that glucosinolates are degraded in aqueous base through various competing reactions. One of these results in α-amino-acids. This reaction must take place through a number of steps; but no intermediates have been observed, and definite conclusions concerning the pathway have not been reached. Nevertheless it must be assumed that a Neber-type rearrangement is involved.

Neber rearrangements proper are transformations of arylsulphonyl esters of ketoximes into α-amino-ketones by the action of base.[14,15] A related reaction is the synthesis of α-amino-acids from nitriles *via* imino-esters and *N*-chloroimino-esters.[16-18]

Neber rearrangements most likely take place by loss of a proton from the carbon atom α to the C=N grouping, bond formation between the α-carbon atom and the nitrogen atom with loss of arene sulphonate ion to give an azirine, and addition of an alcohol molecule to give an alkoxyaziridine. The ring is then opened by addition of a second molecule of alcohol, and hydrolysis gives the α-amino-ketone.[14,15] In analogy, we propose that the rearrangement of the glucosinolates takes place by the mechanism outlined in the Scheme. The first step is presumed to involve concerted attack by hydroxide ion on C-2 in the aglucone to withdraw a proton, nucleophilic attack by this carbon atom on the nitrogen atom, and loss of sulphate ion. The concerted nature of this

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step is indicated by the absence of exchange of the C-2 hydrogen atoms. Addition of a molecule of water to the azirine will give a hydroxyaziridine, which may undergo fast ring opening. The resulting thiol ester is finally hydrolysed to give the α-amino-acid anion and thioglucose anion. Both the azirine and the aziridine must be unstable, and it is not surprising that they were not observed. The existence of the corresponding intermediate in the synthesis of α-amino-acids from nitriles [16] was similarly not demonstrated. Since thiol esters are hydrolysed much more rapidly than *O*-esters [19] it is not surprising that we did not observe this intermediate either, although the *O*-esters could be isolated in the synthesis of α-amino-acids from nitriles.[16]

The relatively high optical activity of the isolated amino-acids is surprising, since chiral centres are only present in the glucose moiety of the starting material. An analogy is, however, found in the synthesis of α-amino-acids from nitriles *via* imino-esters and *N*-chloro-imino-esters. When (−)-(1*R*,2*S*,5*R*)-menthol was used in the initial step, alcoholysis of the nitrile to give the imino-ester resulted in L-phenylglycine, L-alanine, L-phenylalanine, and L-leucine of optical purities 27, 33, 62, and 75%, respectively.[18]

The rearrangement reaction dominates with both allyl- and benzyl-glucosinolate, but in the latter case substantial amounts of benzaldehyde and cyanide are also produced. The pathway to these products has not been elucidated. One possibility is, however, that the azirine is attacked by hydroxide ion not at the unsaturated but at the saturated carbon atom, with simultaneous ring opening and elimination of the thioglucose anion. The resulting cyanohydrin would then easily liberate the aldehyde and cyanide.

The production of thiocyanate may be largely explained by the observed reaction between the disulphide of thioglucose and cyanide ion. No explanation is available, however, for the 8% of thiocyanate which is still formed when oxygen is excluded. The same applies to the production of benzyl cyanide.

Reports in the literature to a certain extent support our results. Thus it is known that treatment of allylglucosinolate with aqueous sodium hydroxide produces thioglucose [20] and with aqueous barium hydroxide produces barium sulphate.[21] In studies on the isolation of benzylglucosinolate by ion-exchange chromatography it was observed that α-phenylcinnamonitrile was formed from the glucosinolate in small amounts in basic fractions.[22] This is easily explained by the occurrence of benzaldehyde and benzyl cyanide in the reaction mixture from the benzylglucosinolate. The production of benzaldehyde from the tetra-*O*-acetyl derivative of benzylglucosinolate by treatment with sodium or barium hydroxide has also been reported, although with no information on yields.[23]

It has also been reported that allylglucosinolate in potassium methoxide is degraded to give thioglucose, isolated as a salt, together with merosinigrin in less than 11% yield.[20,24] According to the accepted formula (1) for glucosinolates merosinigrin must be formulated as a bicyclic compound formed by loss of sulphate and establishment of a bond between the nitrogen atom and O-2 in the glucose moiety.[2] This reaction has not been observed in our studies. Studies on the basic degradation of *p*-hydroxybenzylglucosinolate to give thiocyanate, of indol-3-ylmethylglucosinolate to give glucose, sulphate, hydrogen sulphide ion, thiocyanate, indol-3-ylacetic acid, indol-3-ylacetamide, indol-3-ylmethyl cyanide, 3-hydroxymethylindole, 3,3′-methylene-di-indole, indole-3-carbaldehyde, and indole, and of *N*-methoxyindol-3-ylmethylglucosinolate to give corresponding products have also been reported.[25-27]

## EXPERIMENTAL

Optical rotations were determined with a Perkin-Elmer 141 photoelectric polarimeter (1 dm tubes). C.d. curves were recorded with a Roussel-Jouan CD 185 Dichrographe (2 ml cells; *c* 0.5 mg ml$^{-1}$). $^1$H N.m.r. spectra were determined with a JEOL C-60 HL instrument. U.v. spectra were determined (1 cm cell) with a Zeiss DMR21 spectrophotometer. G.l.c.–mass spectrometry was performed with an A.E.I. MS3074 spectrometer. For $^{13}$C n.m.r. spectra see footnotes to Table. Potassium allylglucosinolate was obtained from Aldrich. Benzylglucosinolate was isolated from seeds of *Lepidium sativum* L. as the tetramethylammonium salt. The sodium salt of 1-thio-β-D-glucopyranose was obtained from Sigma.

*Degradation of Allylglucosinolate.*—Potassium glucosinolate (31 mg) in 2N-sodium hydroxide (1.2 ml) was left at room temperature for 2 h. The mixture was applied to a strongly acidic ion-exchange resin (Dowex 50W X 8; 200—400 mesh; 6 × 0.6 cm; $H^+$ form). After washing with water the column was eluted with aqueous pyridine (1M). The ninhydrin-positive eluate was evaporated to give crystalline DL- and L-vinylglycine (9.2 mg), identified by $^1$H n.m.r. spectroscopy and paper chromatography, and comparison with authentic material; [10] $[\alpha]_D$ +11° (*c* 0.9 in $H_2O$). In a similar experiment performed in $D_2O$ the $^{13}$C n.m.r. spectrum of the mixture was recorded (see Table). After purification a crystalline sample of vinylglycine showed $[\alpha]_D^{24}$ +38° (*c* 0.6 in $D_2O$); c.d. ($H_2O$) $\lambda_{max.}$ 207 nm ($\Delta\varepsilon$ + 3.2), (0.1N-HCl) 210 (+2.2); the $^1$H n.m.r. spectrum showed that no exchange of α-hydrogen had taken place. From a third run a sample with $[\alpha]_D$ +38° was obtained.

The reaction was followed by u.v. spectroscopy ([glucosinolate] *ca.* 0.1mM; [NaOH] 0.091 9M; total ion strength adjusted to 0.1 with NaCl) and the following rate constants were found: 0.006 96 l mol$^{-1}$ s$^{-1}$ (25.1 °C), 0.012 9 (32.7 °C),

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and 0.023 6 (40.1 °C). Boiled water was used for the preparation of all solutions.

*Degradation of Benzylglucosinolate.*—Tetramethylammonium glucosinolate (60 mg), $D_2O$ (1 ml) and 2N-sodium hydroxide (0.2 ml) were left at room temperature for 2 days. After recording the $^{13}C$ n.m.r. spectrum (see Table), the mixture was applied to a strongly acidic ion-exchange resin (Dowex 50W × 8; 200—400 mesh; 5.5 × 0.8 cm; $H^+$ form). After washing with water the column was eluted with aqueous pyridine (1M). The ninhydrin-positive eluate was evaporated to give crystalline DL- and L-phenylglycine (7.6 mg), identified by $^1H$ n.m.r. spectroscopy, paper chromatography, and comparison with authentic material; $[\alpha]_D$ +42° (*c* 0.4 in 5N-HCl).

In a second run the glucosinolate (30 mg) in water (0.5 ml), 2N-sodium hydroxide (0.1 ml), and carbon tetrachloride (0.5 ml) was stirred for 1 day at room temperature. The $^1H$ n.m.r. spectrum of the organic phase demonstrated the presence of benzaldehyde [δ 9.9 (s) and 7.3—8.0 (m)] in *ca.* 22% yield, and benzyl cyanide [δ 3.7 (s) and 7.4 (s)] in *ca.* 8% yield. The presence of these compounds was confirmed by g.l.c.–mass spectrometry. Only these two compounds were observed in the organic phase; no benzyl alcohol was present.

The same yields of benzaldehyde and benzyl cyanide were obtained when the reaction was performed in a nitrogen atmosphere.

*Quantitative Determinations of Cyanide and Thiocyanate.*—Cyanide was determined by the Aldridge method,[28] modified by use of the Conway distillation technique.[29] Thiocyanate was determined as the iron(III) complex.[30]

Tetramethylammonium benzylglucosinolate (15 mg) was dissolved in water (0.25 ml), and 2N-sodium hydroxide (50 μl) and carbon tetrachloride (0.25 ml) were added. The mixture was stirred in a nitrogen atmosphere for 20 h at room temperature; yields 16% $CN^-$, 8% $SCN^-$. Exposure of the mixture to the atmosphere for 5 days resulted in complete disappearance of $CN^-$ and in an increase in $SCN^-$ yield to *ca.* 21%.

Sinigrin (12 mg) was dissolved in water (0.25 ml), 2N-sodium hydroxide (50 μl) was added, and the mixture was set aside for 1.5 h at room temperature; yields $SCN^-$ 2.5%, $CN^-$ *ca.* 2%.

*Other Experiments.*—The sodium salt of thioglucose (2.14 mg) was dissolved in an aqueous solution (1.10 ml) containing potassium iodide (200 mg) and iodine (99 mg) in 100 ml. Potassium cyanide solution (0.707 g in 10 ml; 5 μl) was added, and the mixture was set aside for 18 h; yield of $SCN^-$ 60%.

A mixture of the sodium salt of thioglucose (1.14 mg), 2N-sodium hydroxide (5 μl), potassium cyanide solution (2 μl), and water (0.2 ml) was set aside for 24 h at room temperature; yield of $SCN^-$ 2%.

A mixture of the sodium salt of thioglucose (0.356 mg), potassium cyanide solution (5 μl) iron(II) sulphate solution (5 μl; 0.2 g in 100 ml), 2N-sodium hydroxide (20 μl), and water (0.2 ml) was set aside at room temperature for 45 h. Less than 1% of $SCN^-$ was formed.

Benzylglucosinolate (13.1 mg) was dissolved in pH 10.2 buffer (0.25 ml; 14.3 g $Na_2CO_3$, $10H_2O$ + 4.2 g $NaHCO_3$ in 100 ml; adjusted with 2N-NaOH). Potassium cyanide solution (50 μl) and carbon tetrachloride (0.5 ml) were added, and the mixture was stirred in a nitrogen atmosphere for 20 h; yield of $SCN^-$ 5%.

The sodium salt of thioglucose (7.1 mg) and benzylglucosinolate (15.5 mg) were dissolved in the carbonate buffer (0.25 ml). Carbon tetrachloride was added, and the mixture was stirred in a nitrogen atmosphere for 4 days. No benzyl cyanide was detected in the organic phase by $^1H$ n.m.r. spectroscopy. The glucosinolate remained unchanged in the aqueous phase.

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