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SYNTHESIS OF GLUCOSINOLATES

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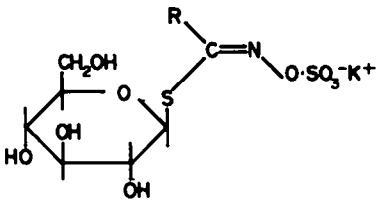
Abstract—A high yielding synthesis of 2-phenethylglucosinolate is described. The method should also be directly applicable to most (if not all) other glucosinolates.

Glucosinolates (1, R greater than 70 different possibilities) are important naturally-occurring thioglucosides mainly located in the botanical family Cruciferae. They decompose enzymically, and under some circumstances



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non-enzymically, to give a variety of products including isothiocyanates, thiocyanates, nitriles and epithiocyanoalkanes. It is important to elucidate in detail the mechanisms of glucosinolate degradations, but to date most work in this area has been carried out on natural systems. It is, however, clearly necessary to carry out in addition carefully controlled *in vitro* investigations on pure, synthesised glucosinolates. For this reason we synthesised recently for the first time large quantities (total 5 g) of 2-phenethylglucosinolate (1, R = $C_6H_5CH_2CH_2$) for such model system studies; this synthesis is reported here.

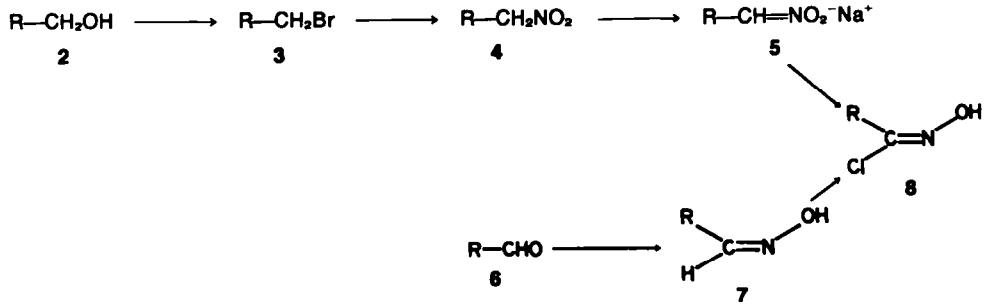
A number of glucosinolates have been synthesised previously but in milligram amounts purely for structural characterisation studies. Benzylglucosinolate (1, R = $C_6H_5CH_2$) was the first prepared by Ettlinger and Lundeen who reacted phenylacetothiohydroxamic acid with acetobromoglucose in the key stage of the synthesis.[1] Later Benn prepared the same compound but constructed the basic glucosinolate skeleton by the addition reaction of phenylacetonitrile oxide with acetothioglucose.[2] The nitrile oxide was generated from 1-chloro-2-phenylethanal oxime, and it is the preparation of this chloro-oxime, in this instance accomplished by direct chlorination of the oxime, which is the critical stage of the synthesis. Using this general procedure Benn has prepared a range of glucosinolates.[2-7] However, it is also possible to obtain the chloro-oxime from the appropriate nitro-compound using the method described by Kornblum and Brown,[8] and allylglucosinolate has been synthesised by Benn and Ettlinger employing this approach.[9]

The synthesis of 2-phenethylglucosinolate (1, R = $C_6H_5CH_2CH_2$) described here is based on these general methods employing the chloro-oxime as the key intermediate. Several significant modifications have been devised to render the synthesis more efficient and to improve some previously low-yielding stages, such that the method is now suitable for "large scale" preparations. A comparison has been made of the two broad methods previously adopted for the preparation of the chloro-oxime.

RESULTS AND DISCUSSION

Scheme 1 details the two general approaches to the synthesis of 1-chloro-3-phenylpropanal oxime (8). Scheme 2 gives the preparation of the necessary carbohydrate moiety and Scheme 3 shows the combination of the two components into the basic glucosinolate skeleton and the final simple transformations needed to obtain the required glucosinolate (1).

Preparation of 1-chloro-3-phenylpropanal oxime (8) (Scheme 1). The route to the sodium salt of the nitroparaffin (5) is conventional and was accomplished readily. However, conversion of 5 to the chloro-oxime (8) proved difficult. A number of previously reported methods for similar compounds were not very satis-



Scheme 1. Preparation of 1-chloro-3-phenylpropanol oxime (R = $C_6H_5CH_2CH_2$).

factory.[8-12] In all instances only low yields of product could be obtained. Usually a transient blue colour was observed as well as an unpleasant aroma, suggesting the temporary presence of a nitroso compound.[13] Sometimes the colour persisted and a blue oil was obtained, but which could not be made to crystallize. Eventually the chloro-oxime was prepared in reasonable yield using a modification of the method of Casnati and Rica.[14] A low temperature (−60°) would seem to be essential for crystallization of the product.

The alternative route to 8 by direct chlorination of the oxime (7) more readily gives much higher yields of a purer product. Several procedures have been reported in the literature,[7,15-17] but generally these gave only low yields in this instance. A modification of the Casnati and Rica method[14] was much more successful giving an excellent yield of a pure material with much higher m.p. than that reported in the literature. Again a low reaction temperature (−60°) was necessary and during treatment with chlorine a blue colour was observed. The identity of the prepared compound was confirmed beyond doubt from its mass spectrum and ^{1}H-NMR spectrum.

The IR spectrum of the synthesised 1-chloro-3-phenylpropanal oxime did not exhibit a band at about 3200 cm^{-1}, but an intense absorption was shown at 1195 cm^{-1}. The former indicates the lack of the oxime OH group, and hence the compound must be a dimer (17). Detailed studies have shown that most C-nitroso compounds do exist predominantly in the dimeric form,[18,19] and such dimers can be *cis* or *trans* because of the appreciable double bond character of the N=N bond.[20] *Trans*-dimers generally show high intensity bands in their IR spectra in the region 1190–1300 cm^{-1} while the *cis*-isomers do not, but have two bands at 1323–1344 and 1330–1420 cm^{-1}. Clearly therefore the compound synthesised in this work was the *trans*-dimer (17).

The solid dimer is colourless but when dissolved (e.g. in chloroform) it immediately gives a blue solution with strong absorbance at 320 nm (N=N, n→π*); the colour slowly fades in a few hours to a colourless solution of the monomer. This behaviour can be followed by UV and ^{1}H-NMR spectroscopy, and can be rationalised as shown in Scheme 4. The transient, blue nitroso compound 18 is presumably that observed during the preparation of the chloro-oxime.

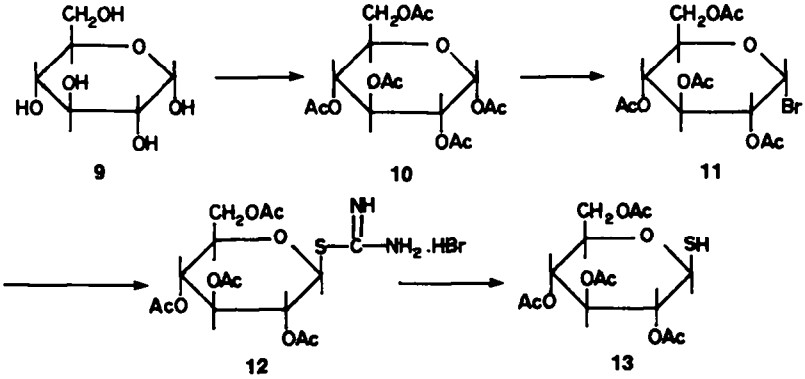
It may well be that this behaviour in solution is at least partly the reason for the preparative difficulties experienced with this compound. For the chloro-oxime to crystallize it is necessary for the monomers formed in solution during the reaction to dimerize, and it appears that this only occurs readily at low temperatures. However, in addition the compound is unstable at room temperature and has to be stored at −20°, so this thermal sensitivity may also partly explain why preparative methods not employing low temperatures were not very successful.

Preparation of 2,3,4,6 - *tetra* - O - *acetyl* - 1 - *mercapto* - β - *D* - *glucopyranose* (13) (Scheme 2). Tetraacetylbromoglucose (11) was prepared conventionally. Reaction with thiourea was easily achieved and is an elegant way of introducing a C–S bond into a sugar molecule.[21] Inversion of configuration occurs at C − 1 from α-D to β-D and the bromide ion is retained thus yielding a water-soluble thiouronium salt 12.[22] Generation of the thiol 13 from the salt was not as easy as might be expected and a number of recommended procedures gave poor yields of products of indifferent purity.[23-29] Ultimately a slight modification of previously reported methods was found to provide a reasonable yield of a product of high purity.

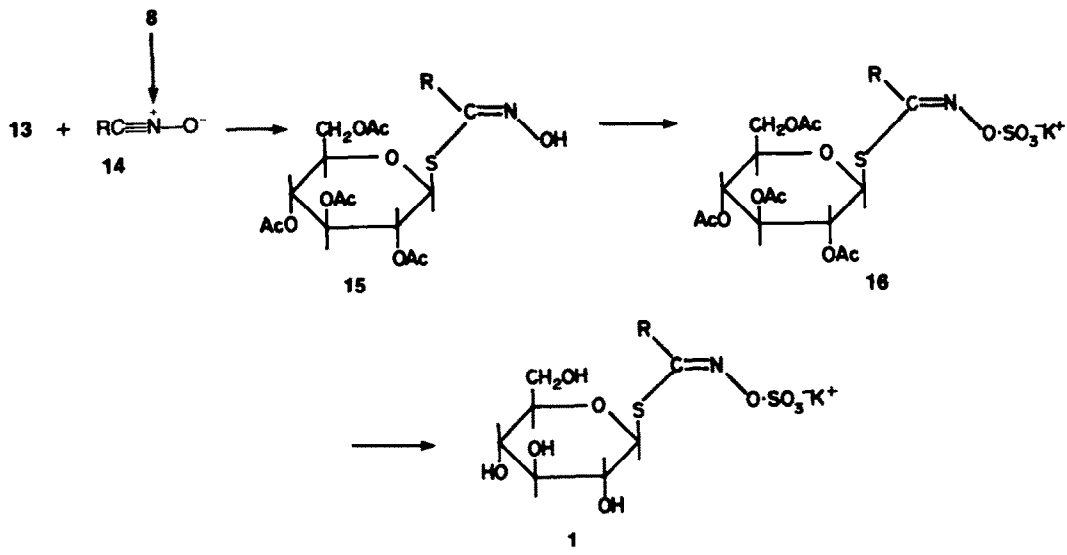
Final stages in the synthesis of 2-*phenethyl-glucosinolate* (1) (Scheme 3). The addition of thiols to nitrile oxides in the presence of base has been demonstrated to yield thiohydroximates.[30] In this synthesis tetraacetylthioglucose (13) was reacted with 3-phenylpropionitrile oxide (14) to yield the glucosinolate skeleton. The nitrile oxide was generated for reaction *in situ* by treating the chloro-oxime (8) with excess base (anhydrous trimethylamine). Overall the reaction is very high yielding and affords a product of good purity.

Preparation of tetraacetyl-2-phenethylglucosinolate (16) from the thiohydroximate (15) was accomplished by reaction with pyridine-sulphur trioxide reagent followed by displacement of the pyridine with a potassium salt. The commercial reagent was inadequate. The method of preparation adopted for the complex was based on that due to Sisler and Audrieth.[30] Under these conditions the product 16 was readily obtained in good yield and high purity.

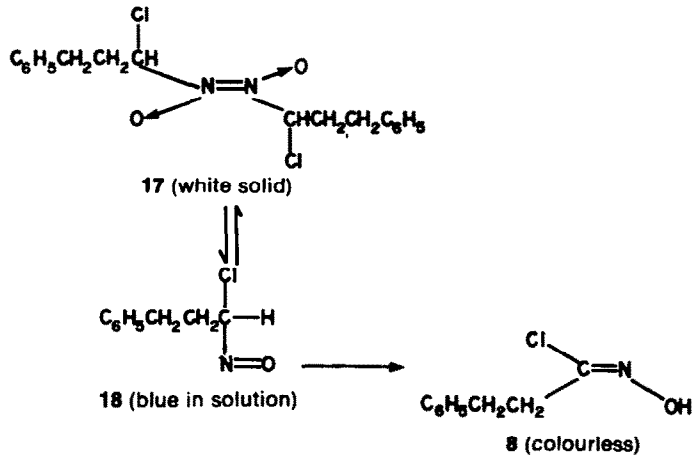
Removal of the protecting groups from the tetraacetyl compound (16) was accomplished without complications to yield 2-phenethylglucosinolate (1) in overall good yield. Confirmation of structure was provided by spectroscopic properties and by agreement of certain physical data with those previously reported for this compound.[7] In addition, its physical properties agree well with those reported for the natural product,[31] and fur-



Scheme 2. Preparation of tetraacetylthioglucose.



Scheme 3. Final stages in the synthesis of 2-phenethylglucosinolate (R = $C_6H_5CH_2CH_2$).



Scheme 4. Dissociation of 1-chloro-3-phenylpropanol oxime in chloroform solution.

thermore when treated with the appropriate crude thioglucoside glucohydrolase (E.C. 3.2.3.1) enzyme prepared from *Nasturtium officinale* it yielded expected products (2-phenethyl isothiocyanate and 3-phenylpropionitrile), identical with those obtained in natural systems from the natural material.

Since the properties of synthesised and natural 2-phenethylglucosinolate are the same, particularly with regard to their behaviour on enzymic decomposition, then it can be assumed that the stereochemistry of the synthesised compound is the same as that of the natural product. It has been shown by X-ray crystallography that natural allylglucosinolate (1, R = CH_2 = CH–CH_2) has the *anti*-configuration (with respect to the allyl substituent and the sulphate grouping about the C=N bond).[32] It is reasonable to assume that other glucosinolates have the same configuration, as shown in Scheme 3 for the 2-phenethyl-derivative (1). From that Scheme it can be seen that the configuration of the product 1 is determined by the configuration of the thiohydroximate (15) and hence by the stereochemistry of the addition reaction between nitrile oxide (14) and tetraacetylthioglucose (13). Presumably 15 must be *anti* also, although it would have been expected that the addition of a thiol to a nitrile oxide would produce, by *trans*-addition, a thiohydroximate with the *syn*-configuration. Since this is not obtained it must be deduced that the reaction does not proceed by this mechanism.

In conclusion, using the synthesis described here it is possible to obtain with little difficulty relatively large amounts of pure 2-phenethylglucosinolate, and it would be very surprising if the same procedure was not equally effective for most other glucosinolates as well.

EXPERIMENTAL

IR spectra were recorded on a Pye-Unicam SP 200 instrument; UV spectra on a Pye-Unicam SP800A; ^{1}H-NMR on a Perkin-Elmer R12B operating at 60 MHz; and mass spectra on an AEI

MS30 operating at 70 eV. A Pye-Unicam 104 with heated f.i.d. was used for gas chromatography. Microanalyses were performed by the unit at Queen Elizabeth College. All m.ps are uncorrected.

3-Phenyl-1-nitropropane **4**

Compound **3** (190 g, 0.95 mol), was poured into a stirred mixture of $NaNO_2$ (110 g, 1.6 mol), urea (54 g) and DMSO (1.5 l) immersed in a water-bath at room temp. Stirring was continued for 3 hr. The mixture was then poured into ice water (1.5 l) layered over with petroleum ether (b.p. 35–37°, 100 ml). The aqueous layer was extracted with 4 × 100 ml petroleum ether and then the combined extracts were washed with 4 × 100 ml water and dried over $MgSO_4$. Solvent was removed on the rotary evaporator, and then using a 50 cm Vigreux column under reduced pressure 25 g (16%) of nitrite ester distilled first, b.p. 80°/6 mm (lit.[33] b.p. 56°/1 mm). The required nitroparaffin (**4**) was then collected in 33% yield (52 g), b.p. 128–30°/4.5 mm (lit.[33] b.p. 123°/4 mm); IR (Nujol) 3600, 3005, 2900, 1600, 1550, 1490, 1460, 1440, 1380, 1185, 1015, 750, 700 cm^{-1}; ^{1}H-NMR δ 7.3 (s, 5 H), 4.2 (tr, 2 H), 2.5 (tr, 2 H), 2.2 (mult., 2 H); mass spectrum 165(1), 147(2), 135(3), 117(80), 108(25), 104(20), 91(100), 65(30).

Sodium salt of 3-phenyl-1-nitropropane **5**

Compound **4** (16.5 g, 0.1 mol), was treated with 1 M ethanolic NaOEt (100 ml, 0.1 mol) and then diluted to a volume of 500–600 ml with dry ether. The slurry thus formed was filtered and washed with dry ether. The white salt was pressed down in the filter funnel and partially dried by sucking N_2 through. Drying was completed *in vacuo* (12 hr) to yield 17.5 g (89%) of **5**; IR (Nujol) 3100, 1600, 1380, 1350, 1125, 1015, 950, 730, 650 cm^{-1}.

1-Chloro-3-phenylpropanal oxime **8**

Method A. Dry HCl gas was passed into a flask containing **5** (1 g, 0.005 mol) suspended in dry ether (25 ml) cooled to −60° in a solid CO_2/acetone bath. A ppt of NaCl formed which was quickly and immediately filtered. Passing HCl for a further few mins gave a blue soln which became deeper in colour and then a white ppt deposited. HCl treatment was continued for 20 min and then the solid was filtered at the pump. It was washed with cold dry ether and allowed to dry in the Buchner funnel to yield 0.6 g (64%) of of **8** as a white crystalline solid, m.p. 69–71° (lit.[7] 65–6°); IR (Nujol) 1595, 1580, 1500, 1495, 1195, 1000, 795, 710, 685, 650 cm^{-1}; UV λ_{max} 320 nm ($CHCl_3$); ^{1}H-NMR δ 8.6 (s, 1 H), 7.2 (s, 5 H), 2.8 (s, 4 H); mass spectrum 185(18), 183(59), 168(13), 166(37), 152(40), 147(12), 108(9), 104(5), 91(100), 65(12), 51(8).

Method B. The oxime **7** (20 g, 0.134 mol), was dissolved in dry ether (250 ml) and cooled to −60° in a solid CO_2/acetone bath. Dry Cl_2 was passed for 15 min during which the soln became blue and a solid was deposited. The mixture was allowed to warm to room temp. and quickly filtered. The product **8** was thus obtained as a white crystalline solid in 83% yield 20.5 g, m.p. 71–2° (lit.[7] 65–6°); IR (Nujol) 1595, 1580, 1500, 1490, 1195, 1000, 905, 795 cm^{-1}; IR (KBr disc) 790, 720, 695, 670, 470, 460, 430 cm^{-1}; ^{1}H-NMR and mass spectrum as above. (Found: C, 59.4, H, 5.6; N, 7.6. Calc. for $C_9H_{10}ClNO$: C, 58.9; H, 5.5; N, 7.6%).

2 - (2,3,4,6 - Tetra - O - acetyl - β - D - glucopyranosyl) - 2 - thiopseudourea hydrobromide **12**

A mixture of **11** (58 g, 0.14 mol) and thiourea (12 g, 0.16 mol) in dry acetone (60 ml) was refluxed for 15 min. Crystallization of product began during heating and was completed by cooling in ice. The crystals were filtered and washed with a little acetone to give **12** in 72% yield (49 g); m.p. 188° (lit.[34] 189°); IR(Nujol) 3600, 1760, 1660, 1350, 1060, 920, 815, 700 cm^{-1}.

2,3,4,6 - Tetra - O - acetyl - 1 - mercapto - β - D - glucopyranose **13**

$Na_2S_2O_5$ (15.2 g, 0.08 mol) in water (70 ml) was heated to 85° and **12** (39 g, 0.08 mol) was added. The mixture was kept at 85° for 5 min and then CCl_4 (60 ml) was added and the mixture refluxed for 10 min. After cooling the organic layer was removed and the aqueous layer extracted with 4×15 ml of CCl_4, filtered and kept overnight at −5 to −10°. Crystals were collected, dried and recrystallized from petroleum ether (b.p. 40–60)/isopropanol to give 12.1 g (42%) of **13**, m.p. 94–5° (lit. m.p. 75°,[26] 115°[28]); IR(Nujol) 3400, 2600, 1750, 1390, 1240, 1050, 900 cm^{-1}; $[\alpha]_D^{15}$ + 0.5° (c 0.2 in $CHCl_3$) (lit.[26] $[\alpha]_D^{15}$ + 0.5° IN $CHCl_3$). (Found: C, 46.3; H, 5.5. Calc. for $C_{14}H_{20}O_9S$: C, 46.1; H, 5.5%).

S - (2,3,4,6 - Tetra - O - acetyl - β - D - glucopyranosyl) - 3 - phenylpropanothiohydroximate **15**

Compound **13** (9 g, 0.026 mol), and **8** (4 g, 0.22 mol), were dissolved in dry ether (590 ml). Trimethylamine (4.8 g) in dry ether (100 ml) was added to the gently stirred blue soln. The colour was immediately discharged and a copious ppt of trimethylamine hydrochloride deposited, indicating the generation of **14**. After 30 min at room temp the mixture was shaken with ice cold *ca.* N H_2SO_4 (600 ml). About 400 ml of ether was decanted, replaced by EtOAc (800 ml) and the mixture shaken vigorously until all suspended solids dissolved. The soln was combined with the decanted ether extract and, after drying ($MgSO_4$), the solvents were removed under reduced pressure leaving a colourless crystalline residue. Recrystallization from EtOH gave **15** in 83% yield (9.2 g), m.p. 198° (lit.[7] m.p. 198°); IR(Nujol) 3300, 1740, 1710, 1600, 1260, 1040, 950 cm^{-1}; UV λ_{max} 238 nm ($CHCl_3$), 218 nm (EtOH); $[\alpha]_D^{20}$ −11.1° (c 0.2 in $CHCl_3$) (lit.[7] $[\alpha]_D^{24}$ −11° in aq. EtOH). (Found: C, 54.3; H, 5.9, N, 2.76. Calc. for $C_{23}H_{29}NO_{10}S$: C, 54.0; H, 5.7; N, 2.75%).

2,3,4,6 - Tetra - O - acetyl - 2 - phenethylglucosinolate **16**

Compound **15** (3.5 g, 0.00685 mol), was dissolved in dry pyridine (30 ml) and freshly prepared pyridine-sulphur trioxide complex (2.2 g, 0.014 mol) was added. The mixture was stirred for 24 hr at room temp and then poured into a soln of $KHCO_3$ (3 g in 100 ml water). The homogeneous soln was freeze-dried and the solid product extracted with 3×100 ml boiling 95% aqueous EtOH. The extracts were filtered hot, combined and stored at 0°. A solid deposited which was recrystallized from 95% EtOH to give **16** as fine white crystals in 70% (3.0 g) yield, m.p. 198–200° (lit.[7] m.p. 198–200°); IR(Nujol) 1740, 1580, 1240, 1050, 900, 790 cm^{-1}; UV λ_{max} 217 nm (EtOH); mass spectrum 169(12), 127(18), 109(14), 97(78), 91(100), 60(15), 45(27), 44(60), 43(58). (Found: C, 43.9; H, 4.4; N, 2.2. Calc. for $C_{23}H_{28}KNO_{13}S_2$: C, 43.9; H, 4.4; N, 2.2%).

2-Phenethylglucosinolate **1**

Compound **16** (4.5 g, 0.00715 mol), was dissolved in 150 ml anhyd MeOH previously saturated with ammonia. [Suitable anhyd MeOH was prepared by heating Analar MeOH with molecular sieve 4A for about 15 hr; decanting and standing over calcium hydride for about 24 hr; filtering and distilling]. The soln was maintained at room temp for about 20 hr and then evaporated under reduced pressure to a residual oil. The oil was dissolved in the minimum hot MeOH (in which **1** is soluble) and abs EtOH (in which **1** is insoluble) was added until the hot soln became turbid. The soln was then filtered and the filtrate diluted 5-fold with abs EtOH. On storage at 0° a solid deposited which was recrystallized from MeOH/abs EtOH as described above to give **1** as a slightly off-white crystalline solid in 67% (2.4 g) yield, m.p. 171° (lit.[7] m.p. 170–172°); IR(Nujol) 3400, 1660, 1610, 1505, 1440, 1270, 1080, 895, 800, 755, 700 cm^{-1}; UV λ_{max} 213 nm (228 nm–sh) (water); ^{1}H-NMR (D_2O) δ 7.3 (s, 5 H), 4.6 (s), 3.7 (s, 2 H), 3.4 (s, 3 H), 3.0 (s, 4 H); mass spectrum 333(3), 181(14), 165(11), 131(22), 105(39), 91(100), 77(14), 73(26), 65(11), 55(16), 44(32), 43(23); $[\alpha]_D^{20}$ −20.7° (*c* 1.0 in H_2O) (lit.[7] $[\alpha]_D^{24}$ −23° in H_2O). (Found: C, 36.1; H, 4.9; N, 2.7. Calc. for $C_{15}H_{20}KNO_9S_2$, $2H_2O$: C, 36.2; H, 4.8; N, 2.8%).

Authentication of synthesised 2-phenethylglucosinolate

An active extract of thioglucoside glucohydrolase (E.C. 3.2.3.1) was prepared from *Nasturtium officinale* seeds by a procedure based on that described by Schwimmer for mustard.[35] Seeds (100 g) were crushed to a fine powder in a coffee grinder. Subsequent preparation was carried out in a cold room (*ca.* 0°). The powder was defatted with cold acetone (10×300 ml) and then dried under vacuum. Cold water (800 ml) was added and the mixture stirred for 2 hr. After centrifugation for 30 min, 400 ml of

liquid were obtained and rejected. To the remaining slurry was added cold water (200 ml) and the mixture was stirred for a further 2 hr. Centrifugation as before provided 200 ml of extract. This was diluted with cold acetone (100 ml) and the resultant ppt collected by centrifugation and rejected. To the supernatant was added more acetone (400 ml) and centrifugation afforded a white starchy mass which was suspended in water (75 ml) and dialysed against distilled water for 40 hr. Lyophillization of the retentate gave a slightly off-white powder in 0.5 g yield which possessed strong enzyme activity.

A mixture of the synthesised 2-phenethylglucosinolate (0.1 ml of a soln in water containing 3.5 mg/ml) and 0.1 M L-ascorbic acid (0.25 ml) was treated with 0.1 ml of a suspension of the enzyme preparation in water (25 mg in 1.5 ml). After standing for 1 hr at room temp, 1 μl of the mixture was injected directly into the gas chromatograph. Two major peaks were obtained which by relative retention time measurements and by combined gas chromatography-mass spectrometry were shown conclusively to be 3-phenylpropionitrile and 2-phenethyl isothiocyanate. A blank without added enzyme gave no peaks on the gas chromatogram.

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