Synthesis of 2-Hydroxybut-3-enylglucosinolate (Progoitrin)

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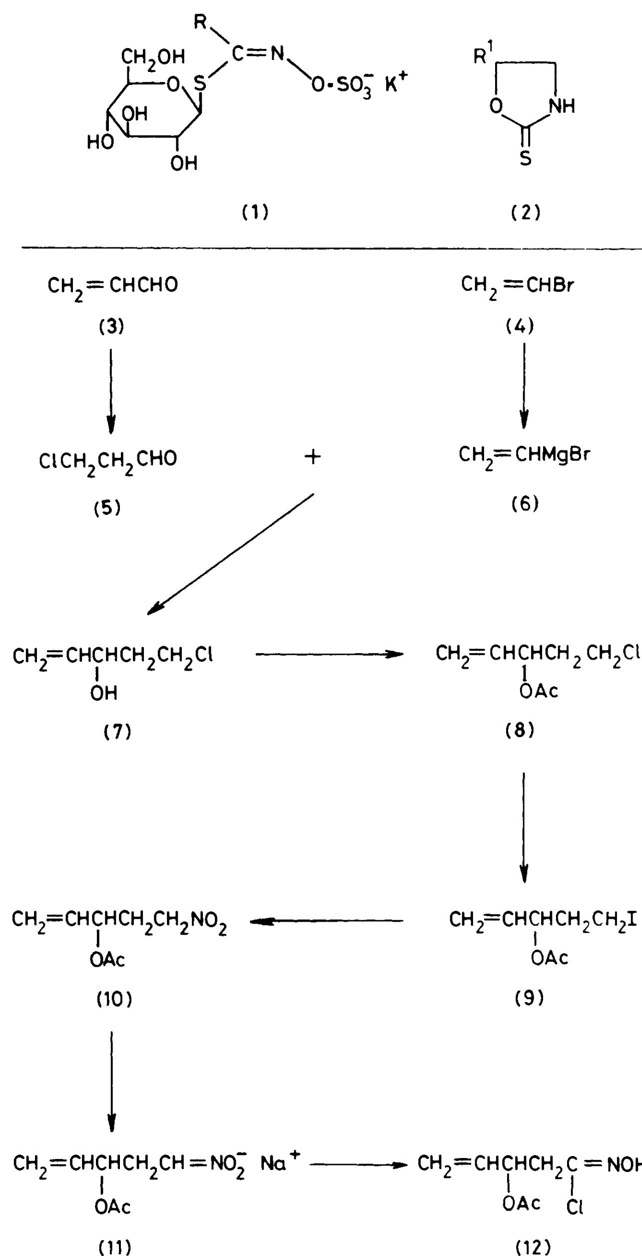
A synthesis of 2-hydroxybut-3-enylglucosinolate (progoitrin) is described. Its properties and authentication of its biological activity are discussed. This is the first time that this particular glucosinolate has been isolated in pure form by synthesis.

Glucosinolates (1) are naturally occurring thioglucosides mainly located in the botanical family Cruciferae. To date, over 80 glucosinolates have been identified, differing only in the nature of the side chain, R.

They degrade enzymically to give a number of important products including isothiocyanates, thiocyanates, nitriles, and epithiocyanoalkanes. Those with a hydroxy-group in the 2-position of the side chain yield isothiocyanates which are unstable, and which cyclise spontaneously to give toxic and goitrogenic oxazolidine-2-thiones (2). 2-Hydroxybut-3-enylglucosinolate [1; R = CH_2=CHCH(OH)CH_2], which has the trivial name progoitrin, is found in quite large amounts in rape (*Brassica napus*), and the consequent formation of the highly goitrogenic 5-vinyloxazolidine-2-thione (2; R′ = CH_2=CH), also known as goitrin, has constituted a serious problem in the economic utilization of this crop. The detailed mechanisms of progoitrin degradation are only partially understood, and to facilitate further study of these we have synthesized this glucosinolate. A few of the more common glucosinolates have previously been prepared,[1-9] mainly for structural studies, but the synthesis of 2-hydroxybut-3-enylglucosinolate is rendered more difficult by the extra reactivity of the functions in the side chain, R.

Glucosinolates are best synthesized *via* a reaction involving addition of tetra-acetylthioglucose to an appropriate nitrile oxide which is generated *in situ* from an α-chloro-oxime. The latter compound is the key intermediate, and generally it is obtained either by direct low temperature chlorination of the corresponding oxime or from a nitro-compound using the general procedure of Kornblum and Brown.[10]



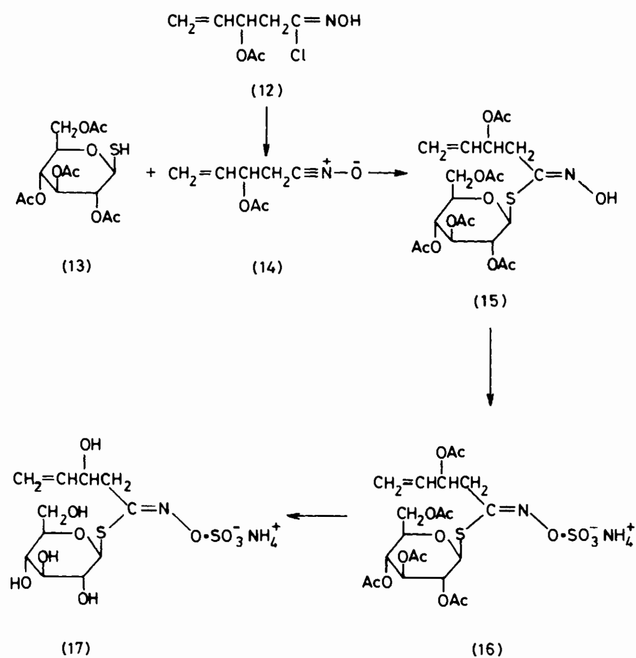
Scheme 1. Preparation of 3-acetoxy-1-chloropent-4-enal oxime

Results and Discussion

Scheme 1 details the synthesis of the intermediate α-chloro-oxime (12). Tetra-acetylthioglucose (13) was prepared as previously described,[1] and Scheme 2 shows the combination of the two components into the basic glucosinolate skeleton and the final simple transformations needed to obtain the required glucosinolate (17).

Using previously reported methods of preparation of (5),[9] a large proportion of cyclic trimer was also obtained, but by modification of this procedure it was possible to obtain (5) in good yield (*ca.* 65%), substantially free of trimer. Using equimolar amounts of (5) and (6), yields of *ca.* 31% have been obtained for (7),[9] but with a two-fold excess of (6), 65% has been claimed.[11] By means of the latter approach our yield was *ca.* 61%. Various possibilities were evaluated for the protection of the hydroxy-group of (7), but the most suitable function throughout subsequent stages was the acetate group.

Kornblum and co-workers have shown that a simple and effective method of preparing aliphatic nitro-compounds, such as (10), is by the reaction of an alkyl halide with sodium nitrite.[12-14] This procedure was adopted in this synthesis, but it was necessary to use the iodide (9), rather than the chloride (8), due to the low reactivity of chloro-compounds in this type of reaction.[13-15] A major by-product is the nitrite ester and this accounts for the generally low yields of nitro-com-



Scheme 2. Final stages in the synthesis of 2-hydroxybut-3-enylglucosinolate

pound by this method, but we found that improved yield could be obtained using high purity DMF as solvent rather than DMSO.

To prepare the sodium salt of the nitro-compound (11) it was necessary to employ sodium butan-2-olate rather than ethoxide [1] because of the susceptibility of the protecting acetate group to alcoholysis with methanol and ethanol. Owing to the sensitivity of (11) to air, it had to be used immediately in subsequent reaction and its physical properties were not characterized. The preparation and properties of α-chloro-oximes, such as (12), in the synthesis of glucosinolates have been previously described.[1] In this synthesis, (12) was best prepared from (11) using thionyl chloride in chloroform below −30 °C. It was not possible to isolate (12), and it was used directly in chloroform solution in the next step of the synthesis.

The addition of thiols to nitrile oxides in the presence of base has been demonstrated to yield thiohydroximates.[16] In this synthesis, tetra-acetylthioglucose (13), synthesised as previously described,[1] was treated with 3-acetoxypent-4-ene-nitrile oxide (14) to yield the glucosinolate skeleton. Treatment of (12) with excess of base generated (14) for reaction *in situ*. Preparation of tetra-acetyl-2-acetoxybut-3-enylglucosinolate (16) from the thiohydroximate (15) was accomplished by reaction with pyridine–sulphur trioxide reagent followed by displacement of the pyridine with an ammonium salt. As before,[1] it was found that the commercial reagent was unsatisfactory for this reaction, and it was necessary to use pure, freshly prepared complex.[17] A great improvement in the yield of this step [to 84% of (16)] was accomplished using DEAE Sephadex A-25 in the work-up procedure, rather than acidic alumina.

Removal of the protecting groups from the penta-acetyl compound (16) was readily accomplished to yield 2-hydroxybut-3-enylglucosinolate (17) in overall good yield as a white hygroscopic solid. This is the first time that this glucosinolate has been synthesised and isolated in pure form. The product was shown to be pure by gas chromatography (g.c.) of the trimethylsilylated (TMS) derivative and comparison with a quantitative standard (the TMS derivative of pure

Table. Summaries of the mass spectra of 2-hydroxybut-3-enylglucosinolate (17) and its TMS derivative

(17)				TMS-(17)			
m/z	% Rel. int.	m/z	% Rel. int.	m/z	% Rel. int.	m/z	% Rel. int.
41	30	80	8	59	18	189	5
42	25	97	4	73	(>100)	191	14
43	21	98	8	75	100	204	18
55	19	111	1	98	51	217	88
57	100	112	1.5	103	23	232	9
60	12	129	1	117	13	243	5
69	4	147	0.5	129	61	258	4
				131	9	271	6
				133	11	290	7
				147	77	361	28
				154	47	435	0.5
				169	8	450	5

allylglucosinolate). Confirmation of structure was provided by spectroscopic properties and by agreement of these properties and certain other physical data with those of a crude (*ca.* 70%) sample of the glucosinolate extracted from seeds of *Brassica napus* cv. Panter by Bjorkman's procedure.[18] Thus, for example, under identical g.c. conditions and using a 1.5 m column of 3% OV-17, the TMS derivatives of synthesised and extracted (17) both showed exactly the same retention behaviour (R_t, 17.95 min). Spectral data also agreed very well, although spectra of synthesised (17) were uncontaminated and therefore generally simpler.

The most useful diagnostic spectral data for synthesised (17) were provided by the electron impact mass spectra of both the glucosinolate itself and of its TMS derivative. Summaries of the significant fragment ion peaks in both spectra are given in the Table. For the glucosinolate itself, the base peak is due to $(CH_2{=}CHCHOH)^+$, but equally diagnostic peaks are seen at m/z 97 and 98 due to $[CH_2{=}CHCH(OH)CH_2CN]^+$ and its protonated species. Taken together these peaks confirm the nature of the glucosinolate side chain. Glucosinolates often show such indications of the elements of their normal (enzymic) degradation products in their mass spectra, and a small peak at m/z 129 is also seen in the spectrum of (17), due to 5-vinyloxazolidine-2-thione (2; R^1 = $CH_2{=}CH$). Typically the mass spectra of TMS derivatives show an intense base peak at m/z 73 due to Me_3Si^+. This is essentially meaningless, and in the Table peak intensities have been normalized to the second most abundant peak, which, in this instance, was the associated fragment at m/z 75. Similar diagnostic fragment ion peaks are seen in the spectrum of the TMS derivative as were observed in the spectrum of (17) itself. Thus the original base peak at m/z 57 is now seen at m/z 129 due to derivatization of the side chain OH group, and it is again of appreciable intensity. Similarly, the nitrile peak is shifted from mass 97 to mass 169, but, not surprisingly, the protonated analogue is not observed. However, a major peak is still given at m/z 98 and this may be due to the same protonated fragment as observed for (17), but after loss of the TMS grouping. Mass spectra of a few similarly derivatized, but desulphonated, glucosinolates have been reported previously,[19] although the glucosinolates were not synthesised and were extracted from natural sources.

In comparison with the mass spectral data, the ^1H n.m.r. spectrum of synthesised (17) was very complex, and it could not be fully interpreted. However, the resonances for all side-chain protons could be recognized in the spectrum, although the multiplicities were generally complex and could not be

entirely rationalised. Thus the vinyl protons were observed as complex multiplets at δ 5.05—5.40(CH_2) and at δ 5.85—6.05(CH). The 2-H signal was at δ 4.65, and the resonance had the appearance of a quartet. The multiplet due to the methylene protons was seen at δ 2.95, and it could be considered to represent two overlapping double doublets. The strongly coupled glucose protons comprised a complex series of multiplets between δ 3.40 and 4.00, but the anomeric proton was clearly distinguished as a doublet at δ 4.85.

There is a chiral carbon at position 2 in the side chain of (17) and both (*R*)- and (*S*)-forms are known in Nature. The synthetic compound (17) was a mixture of these forms but, nevertheless, it exhibited 100% biological activity, proving that the efficacy of the thioglucosidase is not affected, in this instance, by configuration within the side chain. In fact, (17) was a mixture of diastereoisomers, but from evidence previously described,[1] it would be expected that (17) would be exclusively in the β-D-glucopyranose form. The synthetic compound (17) thus exhibited optical activity, and its specific rotation was of the same order (*ca.* −22°) as that recorded for previously synthesised glucosinolates.[1-9] From arguments applied earlier,[1] it is reasonable to assume in addition that synthesised (17) had the (*Z*)-configuration, presumably as does the natural product.

The biological activity of synthetic (17) was assessed using the appropriate enzyme, thioglucoside glucohydrolase (E.C. 3.2.3.1), which was extracted in crude form from mustard using Schwimmer's method.[20] On mixing substrate and enzyme at pH 7 in a phosphate buffer, there was an immediate decrease in glucosinolate absorption at 228 nm in the u.v. region and a concomitant increase in absorption at 240 nm due to 5-vinyloxazolidine-2-thione (goitrin). The formation of the oxazolidinethione was quantified by gas chromatography using a standard synthesized by the general procedure of Ettlinger.[21] Complete hydrolysis of the glucosinolate occurred, and 100% of the theoretical yield of the oxazolidinethione was obtained (*i.e.* 7.63 μmol of glucosinolate gave 7.74 μmol of oxazolidinethione). No other volatile product was detected. The synthetic glucosinolate thus behaved in this respect in the same manner as the natural material.[22]

Experimental

I.r. spectra were recorded on a Pye-Unicam SP 200 instrument; u.v. spectra on a Pye-Unicam SP 800A; ^{1}H n.m.r. on a Perkin-Elmer R12B operating at 60 MHz and on a Nicolet NTC 200 operating at 200 MHz; and mass spectra on a Kratos MS25 operating at 70 eV and equipped with a Kratos DS50 data processing system. A Pye-Unicam 204 with heated f.i.d. was used for gas chromatography. Microanalyses were performed by the unit at Queen Elizabeth College. All m.p.s are uncorrected.

3-*Chloropropanal* (5).—Freshly distilled (3) (85 g, 1.52 mol) was added to dry ether (100 ml) in a Dreschel bottle equipped with a magnetic stirrer. The mixture was cooled to −42 °C in a solid CO_2-acetonitrile bath and dry HCl gas (bubbled through conc. H_2SO_4 and passed over $CaCl_2$) was passed through until 1.52 mol of HCl had been taken up. The reaction mixture was neutralized (anhyd. Na_2CO_3) and dried with powdered $CaCl_2$ (30 g). The ethereal solution of (5) was kept at < -40 °C until required. This procedure limits formation of cyclic trimer which can be detected by its ^{1}H n.m.r. spectrum: δ ($CDCl_3$) 5.2 (3 H, t, 3 × OCHO), 3.4 (6 H, t, 3 × CH_2Cl), and 2.1 (6 H, m, 3 × $CHCH_2CH_2$).

Vinylmagnesium Bromide (6).—Mg turnings (48 g, 2 mol), pre-washed with dry ether, were placed in a 2-l flat-flange flask equipped with stirrer, solid CO_2-acetone condenser, low-temperature dropping funnel, thermometer, and N_2 flow. Dry THF (600 ml, refluxed over calcium hydride for several hours, distilled and stored over calcium hydride) was added to the flask. Compound (4) (220 g, 2.06 mol) was diluted with an equal volume of dry THF and *ca.* 10 ml added to the stirred suspension of Mg. The mixture was warmed to *ca.* 60 °C and a few crystals of I_2 added. The solution of (4) was added slowly such that the temperature was maintained at 50—60 °C. After complete addition, the mixture was set aside for 30 min and then heated to 70 °C for 30 min. After the mixture had been cooled, dry ether (300 ml) was added, stirring being maintained.

5-*Chloropent*-1-*en*-3-*ol* (7).—Prepared (6) was cooled to −10 °C (ice-salt bath) and prepared (5) (at −42 °C) was added during 2 h, the temperature being kept < -5 °C. The reaction mixture was set aside overnight at room temperature, cooled to *ca.* 0 °C, and slowly added in portions to saturated aqueous NH_4Cl (2 l) at 0 °C. The mixture was filtered through glass wool and the organic layer separated. The aqueous layer was extracted with ether (2 × 500 ml) and the combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 × 100 ml) followed by water (2 × 100 ml) and then dried (Na_2SO_4). Distillation through a Vigreux column removed solvents and (7) was then distilled and obtained as a colourless liquid (74 g, 61%), b.p. 70—72 °C/10 mmHg (lit.,[11] 72—73 °C/11 mmHg); $\nu_{max.}$ (film) 3 400, 3 010, 2 853, 1 643, 1 416, 1 400, 1 050, 1 000, 930, and 720 cm^{-1}; δ ($CDCl_3$) 6.2—5.0 (3 H, m, $CHCH_2$), 4.3 (1 H, m, C*H*OH), 3.6 (2 H, t, CH_2Cl), 2.6 (1 H, s, OH), and 1.9 (2 H, q, CCH_2C); *m/z* 122 (7%), 120 (24; M^+), 103 (18), 105 (4), 71 (4), 43 (100), 67 (50), 41 (25), and 39 (21).

3-*Acetoxy*-5-*chloropent*-1-*ene* (8).—Acetic anhydride (84.7 g, 0.83 mol) was cooled to 0 °C in a three-necked 250-ml flask equipped with stirrer, thermometer, and dropping funnel. Catalyst (0.3 ml of 1 g 60% perchloric acid to 2.3 g acetic anhydride) was added and the mixture allowed to reach room temperature. Compound (7) (50 g, 0.415 mol) was added dropwise to the acetic anhydride with cooling so that the temperature did not rise above 30 °C. The reaction mixture was then poured onto ice-water (200 g : 200 g) and the organic layer was separated. The aqueous layer was extracted with ether (2 × 100 ml). Water (100 ml) was added to the combined organic extracts together with an excess of solid Na_2CO_3 and the mixture was stirred until evolution of CO_2 ceased. The organic layer was separated, washed with water (50 ml), and dried (Na_2SO_4). Distillation through a Vigreux column removed solvents and (8) was then distilled and obtained as a colourless liquid (57.4 g, 85%), b.p. 78—80 °C/14 mmHg (lit.,[9] 81—82 °C/12 mmHg); $\nu_{max.}$ (film) 3 010, 2 853, 1 740, 1 643, 1 416, 1 400, 1 030, 1 000, 930, and 720 cm^{-1}; δ ($CDCl_3$) 6.2—5.1 (4 H, m, $CHCH_2$ and C*H*OAc), 3.6 (2 H, t, CH_2Cl), 2.2 (2 H, m, CCH_2C), and 2.1 (3 H, s, CH_3COO); *m/z* 162 (1%; M^+), 122 (3), 120 (6), 67 (12), 53 (5), 43 (100), 41 (15), and 39 (10).

3-*Acetoxy*-5-*iodopent*-1-*ene* (9).—Sodium iodide (151.4 g, 0.99 mol), dried for 4 h at 100 °C in a vacuum oven, was added to a 1-l flask containing dry acetone (380 ml; held over anhydrous Na_2CO_3 for 24 h, distilled, and stored over 4A molecular sieve). Compound (8) (52.7 g, 0.324 mol) was added and the mixture refluxed overnight, with the exclusion of moisture. The bulk of the acetone was removed on the rotary evaporator and the residue was filtered and washed with a little water. The mixture was transferred to a separating funnel, shaken with water (100 ml) and the organic layer

separated. The aqueous layer was extracted with ether (2 × 100 ml) and the combined organic extracts were washed with 10% aqueous $Na_2S_2O_5$ (2 × 30 ml) followed by water (2 × 30 ml). The extract was dried ($MgSO_4$) and the solvents removed through a Vigreux column. Distillation of the residue yielded (9) as a slightly red liquid (64.6 g, 78%), b.p. 68—70 °C/1 mmHg (lit.,[9] 70—72 °C/1.2 mmHg); $\nu_{max.}$ (film) 3 010, 2 853, 1 740, 1 643, 1 416, 1 400 1 030, 1 000, 930, and 720 cm^{-1}; δ ($CDCl_3$) 6.2—5.1 (4 H, m, $CHCH_2$ and *C*HOAc), 3.1 (2 H, t, CH_2I), 2.2 (2 H, m, CCH_2C), and 2.0 (3 H, s, CH_3COO); *m/z* 254 (1%; M^+), 215 (4), 195 (12), 152 (3), 127 (32), 99 (7), 86 (6), 67 (95), 43 (100), 41 (27), and 39 (23).

3-*Acetoxy*-1-*nitropent*-4-*ene* (10).—Pure dry DMF (840 ml; distilled from CaO and redistilled) and dry $NaNO_2$ (49.4 g, 0.717 mol; dried *in vacuo* at 115 °C for 2 h) were placed in a 250-ml three-necked flask equipped with a stirrer, thermometer, and dropping funnel. The mixture was cooled to 0 °C and (9) (105 g, 0.409 mol) was added. The reaction was allowed to reach room temperature and then left for 2.5 h. The mixture was then poured into ice-cold water (1 l), layered with light petroleum (b.p. 40—60 °C)–$CHCl_3$ (3 : 2; 200 ml). After shaking, the organic layer was separated and the aqueous layer extracted further with solvent (4 × 200 ml). The combined extracts were washed with 10% aqueous $Na_2S_2O_5$ (2 × 100 ml) followed by water (2 × 100 ml). After drying ($MgSO_4$), solvents were removed on the rotary evaporator and the residue distilled through a Vigreux column. The first fraction (17.1 g, 24%), obtained as a light yellow liquid, b.p. 70 °C/0.5 mmHg, corresponded to the nitrite ester. The second fraction was the required nitro-compound (10) which was thus obtained as a light yellow liquid (27.6 g, 39%), b.p. 84—85 °C/0.5 mmHg (lit.,[9] 85—86 °C/0.6 mmHg) (Found: C, 48.6; H, 6.4; N, 7.8. Calc. for $C_7H_{11}NO_4$: C, 48.6; H, 6.4; N, 8.1%); $\nu_{max.}$ (film) 3 010, 2 853, 1 740, 1 643, 1 550, 1 416, 1 400, 1 375, 1 030, 1 000, 930, and 720 cm^{-1}; δ ($CDCl_3$) 6.2—5.2 (4 H, m, $CHCH_2$ and *C*HOAc), 4.5 (2 H, t, CH_2NO_2), 2.4 (2 H, m, CCH_2C), and 2.2 (3 H, s, CH_3COO); *m/z* 143 (5%), 128 (7), 127 (5), 126 (12), 114 (11), 99 (14), 83 (14), 67 (43), 57 (8), 55 (55), and 43 (100).

Sodium Salt of 3-*Acetoxy*-1-*nitropent*-4-*ene* (11).—Finely sliced sodium (0.33 g, 0.014 g-atom) was placed in a 500-ml three-necked flask equipped with a stirrer, dropping funnel and N_2 gas flow. Dry butan-2-ol (12 ml; distilled and stored over 4A molecular sieve) was added and the mixture gently warmed until the sodium had completely reacted. Compound (10) (2.5 g, 0.014 mol) in dry ether (25 ml) was added slowly to the butoxide. The sodium salt (11) was then precipitated by the addition of dry ether (250 ml). The salt was allowed to settle and the bulk of the supernatant liquid was decanted. The solid, in the remaining small volume of liquid, was quickly filtered through a sintered glass funnel under N_2. It was washed with dry ether (2 × 25 ml) and residual solvent was evaporated by N_2 gas flow for a short period. After drying over P_2O_5 for 1 h, (11) was thus obtained in 69% yield (1.95 g). It was used immediately in the next stage of the synthesis.

3-*Acetoxy*-1-*chloropent*-4-*enal Oxime* (12).—Thionyl chloride (4.8 ml, 0.066 mol) was added to pure, dry $CHCl_3$ (96 ml; extracted ×5 with equal volumes of water, pre-dried over $CaCl_2$, and distilled over P_2O_5) in a 250-ml three-necked flask equipped with stirrer, N_2 flow, and a modified drying tube for the purpose of adding the salt (11) in portions. The mixture was cooled to −60 °C in a solid CO_2-acetone bath and (11) (3.5 g, 0.018 mol) was added in portions such that the temperature did not rise above −30 °C. The mixture was then set aside for 5 min, filtered under N_2, and the filtrate poured into ice-cold water (160 ml). The organic layer was separated and dried ($CaCl_2$). It was not possible to isolate (12) as a stable product, so this solution was used directly in the next stage of the synthesis.

2,3,4,6-*Tetra*-O-*acetyl*-1-*mercapto*-β-D-*glucopyranose* (13).—This compound was prepared from α-D-glucose in four steps exactly as previously described.[1]

S-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*) 3-*Acetoxybut*-4-*enylthiohydroximate* (15).—Compound (13) (3.97 g, 0.011 mol) in pure, dry $CHCl_3$ (80 ml) was added to the prepared solution of (12) in $CHCl_3$ contained in a three-necked flask equipped with a stirrer, thermometer, and N_2 gas flow. Anhydrous triethylamine (9.6 ml) was then added slowly to the mixture at −10 °C. The reaction was left for 30 min at room temperature, and the solution was then washed with ice-cold *ca.* 0.5M-H_2SO_4 (2 × 400 ml), followed by water (2 × 400 ml). After drying ($MgSO_4$) the solvent was removed on the rotary evaporator at <40 °C to yield a syrupy residue (4.8 g). This was purified by passage through a silica gel column using benzene–ether (3 : 1) as eluant. Removal of solvent on the rotary evaporator at <40 °C gave (15) as a white amorphous solid (0.30 g, 20%), sintering at 75—90 °C (Found: C, 48.7; H, 5.5; N, 2.3. Calc. for $C_{21}H_{29}NO_{12}S$: C, 48.6; H, 5.6; N, 2.7%); $\nu_{max.}$ (Nujol) 3 350, 3 010, 2 853, 1 740, 1 720, 1 643, 1 600, 1 416, 1 400, 1 030, 1 000, 930, and 720 cm^{-1}; δ ($CDCl_3$) 8.9 (1 H, s, NOH), 6.0—5.0 (4 H, m, $CHCH_2$ and *C*HOAc), 2.9 (2 H, d, CCH_2C), and 2.1 (15 H, s, 5 × CH_3COO); *m/z* 169 (15%), 149 (100), 98 (6), 97 (9), 92 (60), 91 (90), 71 (35), 57 (73), and 43 (72).

2,3,4,6-*Tetra*-O-*acetyl*-2-*acetoxybut*-3-*enylglucosinolate* (16).—Compound (15) (0.85 g, 1.64 mmol) was added to dry pyridine (27 ml) containing freshly prepared [17] pyridine–SO_3 reagent (1.96 g, 12.3 mmol). The mixture was stirred overnight at room temperature, cooled to 0 °C in an ice-bath, and 10% aqueous $NaHCO_3$ was added, with stirring. The solution was extracted with ether (4 × 50 ml); the aqueous layer contained product (16) whilst the ether layer contained some unchanged (15). The ether phase was dried ($MgSO_4$), the solvent removed at <40 °C on the rotary evaporator, and the residue (0.8 g) resulphonated. Following concentration of the aqueous phase on a rotary evaporator at <40 °C, a syrupy residue was obtained which was combined with resulphonated material, taken up in hot absolute EtOH (2 × 40 ml), and filtered. EtOH was removed from the filtrate using a rotary evaporator at <40 °C to yield a brown residue (1.55 g) containing (16).

DEAE Sephadex A-25 (12 g) was swollen overnight in 0.2M-aqueous NH_4HCO_3 buffer (pH 8.0). It was then washed with water to remove excess of NH_4HCO_3, slurried with water, and packed into a Wright column (30 × 4.4 cm). The flow rate was adjusted to 100 ml h^{-1} with water. After equilibration of the column, an aqueous solution of the residue containing (16) was applied to the column, and it was rinsed with water for 4 h. Product (16) was eluted with 0.1M-aqueous NH_4HCO_3 solution, collecting 25-ml fractions and monitoring at 228 nm. Absorbing fractions were combined, concentrated to *ca.* 50 ml on the rotary evaporator at <40 °C, and freeze-dried to give (16) as a slightly yellow, amorphous solid (0.85 g, 84%), sintering at 160—170 °C; $\nu_{max.}$ (Nujol) 3400, 3 300, 3 010, 1 600, and 1 060 cm^{-1}. The i.r. spectrum inferred that slight partial deacetylation had probably occurred, so this compound was not further analysed.

2-*Hydroxybut*-3-*enylglucosinolate* (17).—Compound (16) (0.80 g, 1.32 mmol) was added at 0 °C to dry MeOH (50 ml)

saturated with NH_3, and the mixture left overnight at room temperature. The solvent was removed on a rotary evaporator at <40 °C to leave a gum-like residue. Dry EtOH (4 ml) was added to triturate (17) and a little dry ether was added to achieve complete precipitation. After centrifugation, the supernatant solvents were carefully decanted. This process was repeated and the small volume of solvent remaining after centrifugation was evaporated in a stream of dry N_2. Product (17) was thus obtained as a hard, white, hygroscopic solid (0.32 g, 59%), m.p. 136 °C [lit.,[18,23] for extracted (17) 137—139 °C, 135—140 °C]; $[\alpha]_D^{20}$ −22.2° (*c* 0.5, in H_2O) (Found: C, 28.2; H, 5.2; N, 6.1. Calc. for $C_{11}H_{22}N_2O_{10}S_2,3H_2O$: C, 28.7; H, 5.2; N, 6.1%); $\nu_{max.}$ (Nujol) 3 500—3 100 (br, OH, NH_4^+), 1 640 (C=C), 1 580 (C=N), 1 280 (C-O), 1 260 (C-O), and 1 068 cm^{-1} (OSO_3); $\lambda_{max.}$ (water) 228 nm; ^1H n.m.r. (DMSO) and mass spectrum—see text.

5-*Vinyloxazolidine*-2-*thione* (2; R′ = CH_2=CH).—This compound was prepared from 1,2-epoxybut-3-ene in three stages in overall *ca.* 16% yield by the method of Ettlinger.[21] It was obtained as slender white prisms, m.p. 64—65 °C (lit.,[21] 64—65 °C).

2-*Hydroxybut*-3-*enonitrile*.—This degradation product of (17) was synthesised by standard procedure in two steps from buta-1,3-diene and calcium hypochlorite, *via* 1-chloroprop-2-en-1-ol. It was obtained as a colourless liquid, b.p. 132 °C/30 mmHg.

Thioglucoside Glucohydrolase (*E.C.* 3.2.3.1).—A crude preparation of this enzyme was obtained from mustard as an off-white powder by the method of Schwimmer.[20] It possessed strong enzyme activity as assessed by its degradation of pure allylglucosinolate.

Extraction of 2-*Hydroxybut*-3-*enylglucosinolate* (17).—A crude (*ca.* 70%) extract of (17) was obtained from seeds of *Brassica napus* cv. Panter by the method of Bjorkman.[18]

Authentication of Synthesised (17).—A solution of synthesised (17) (3.1 mg, 7.63 μmol) in phosphate buffer (1 ml, pH 7.0) was treated with 1 mg of crude thioglucosidase, and u.v. spectra were recorded over the range 200—300 nm at 5 min intervals.

The same mixture was also assayed at *ca.* 15 min intervals by g.c. as follows. Aliquots (100 μl) were mixed with $CHCl_3$ (900 μl) and NaCl, and shaken for 0.5 min, after which 5 μl of the organic phase was analysed using each of two g.c. systems. For determination of 5-vinyloxazolidine-2-thione, an 0.3 m × 4 mm i.d. glass column packed with 5% Apiezon L coated on 80—100 BSS mesh acid-washed, DMCS-treated Chromosorb W was used, operated isothermally at 160 °C with carrier gas (N_2) flow of 30 ml min^{-1}. For determination of 2-hydroxybut-3-enenitrile, a 0.3 m × 4 mm i.d. glass column packed with 10% Carbowax 20M coated on 100—120 BSS mesh acid-washed Diatomite C was used, operated isothermally at 130 °C with carrier gas (N_2) flow of 30 ml min^{-1}. In each case, g.c. detector temperature was 250 °C and injector temperature was ambient. Extraction efficiencies and overall recoveries of the procedure were assessed using authentic, synthesised standards. G.c. peak areas were measured manually. Only one g.c. peak was obtained (with the Apiezon column; none with the Carbowax column), and this corresponded in R_t with 5-vinyloxazolidine-2-thione. This assignment was confirmed by combined g.c.–m.s. Quantification of this peak, in comparison with synthesised oxazolidinethione standard, showed that after 2 h 7.74 μmol (101.4%) had been produced by enzymic hydrolysis.

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