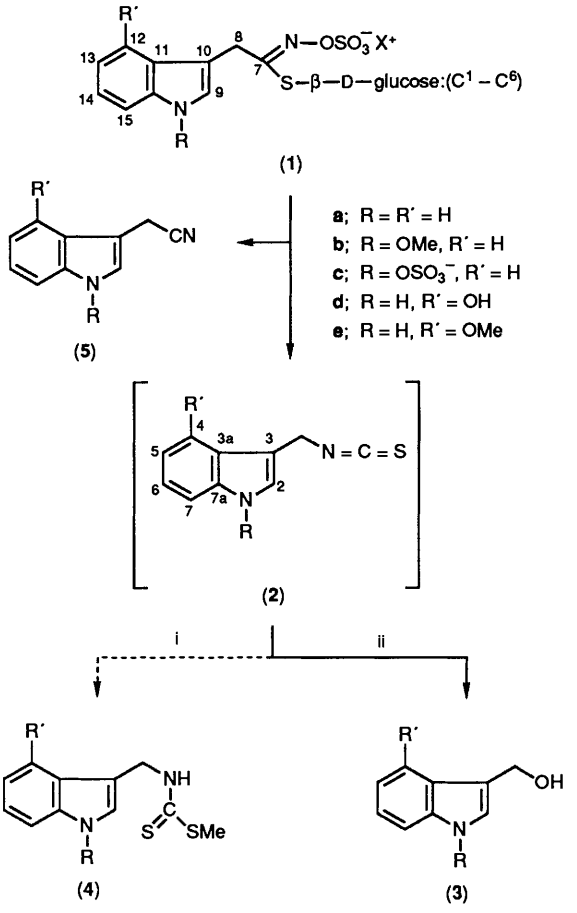
Chemistry of Indole Glucosinolates: Intermediacy of Indol-3-ylmethyl Isothiocyanates in the Enzymic Hydrolysis of Indole Glucosinolates

A. Bryan Hanley,*[a] Keith R. Parsley,[a] Jenny A. Lewis,[b] and G. Roger Fenwick[b]
Departments of Chemical Physics[a] and Molecular Sciences,[b] AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA

The enzymic hydrolysis of 1-methoxyindol-3-ylmethyl glucosinolate (**1b**) proceeds *via* the corresponding isothiocyanate (**2b**), thus providing evidence for a previously unsubstantiated breakdown pathway and establishing a link with 1-methoxycyclobrassinin (**4b**) and related indole phytoalexins.

Indole glucosinolates (**1a–e**) are members of a wider class of amino acid-derived secondary products which occur throughout the family Cruciferae.[1] Hydrolysis of the general class by a co-occurring thioglucosidase, myrosinase (thioglucoside glucohydrolase, E.C. 3.2.3.1), affords an unstable aglucone which spontaneously rearranges to either the corresponding nitrile or, following a Lossen-type rearrangement, the isothiocyanate.[2] These primary hydrolysis products can undergo a range of secondary reactions depending upon the hydrolysis conditions (*e.g.*, pH, presence of metal ions), or the nature of the side-chain.[1] In the case of the parent indole glucosinolate, glucobrassicin (**1a**), the postulated isothiocyanate (**2a**) has not been isolated; rather, spontaneous hydrolysis is considered to occur, furnishing indole-3-methanol (**3a**) (Scheme 1). While this pathway has been proposed for glucobrassicin (**1a**), evidence of a similar mechanism for the other indole glucosinolates is limited.[3,4] The intermediacy of indol-3-ylmethyl isothiocyanate (**2a**) itself is speculative and rests solely upon the isolation of indole-3-methanol (**3a**) as the first stable breakdown product.[5] It is alternatively possible that the alcohol (**3a**) could be formed from the aglucone without the formation of the isothiocyanate or, indeed, without the necessary preliminary of a Lossen rearrangement. A series of indole phytoalexins (**4a**), (**4b**), and (**4e**) has recently been isolated and characterised from members of the Cruciferae.[6,7] These compounds bear a pronounced structural similarity to indol-3-ylmethyl isothiocyanates and may formally result from the addition of methanethiol—a known breakdown product in Cruciferous plants[8]—to the isothiocyanate. Such a derivation implies that a Lossen-type rearrangement is a prerequisite for the formation of the indole phytoalexins (**4a**), (**4b**), and (**4e**) from indole glucosinolates (**1a–e**), although a stable isothiocyanate intermediate is not a necessity. We sought, therefore, to confirm the breakdown pathway of indole glucosinolates and to prove the intermediacy of the corresponding isothiocyanates, thereby providing evidence for a link between indole glucosinolates and phytoalexins.



Scheme 1. *Reagents:* i, MeSH; ii, water.

Results and Discussion

Given the susceptibility of the intermediate isothiocyanates to hydrolysis, the enzyme reactions were carried out in 'low water' systems comprising mainly hexane with the substrate and enzyme supported on Celite.[9] The presence of a 1-methoxy substituent is known to deactivate the 3-position in an indole nucleus[10] and this prompted us to examine the enzymic hydrolysis of both glucobrassicin (**1a**) and its 1-methoxy analogue, neoglucobrassicin (**1b**). Such comparison would also serve to confirm that the breakdown pathway of compound (**1b**) is identical with that of compound (**1a**).

Glucobrassicin and neoglucobrassicin were isolated from young leaves of Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*) and the root bark of swedes (*B. napus* L.), respectively, using modifications of methods described previously.[11,12] Glucobrassicin was recrystallised as its tetramethylammonium derivative while neoglucobrassicin was initially converted into the brucine salt for recrystallisation. Cation exchange subsequently yielded the potassium salt, which was used in subsequent reactions.

A number of 1-methoxyindole compounds were prepared both as standards for the breakdown studies and in an attempt

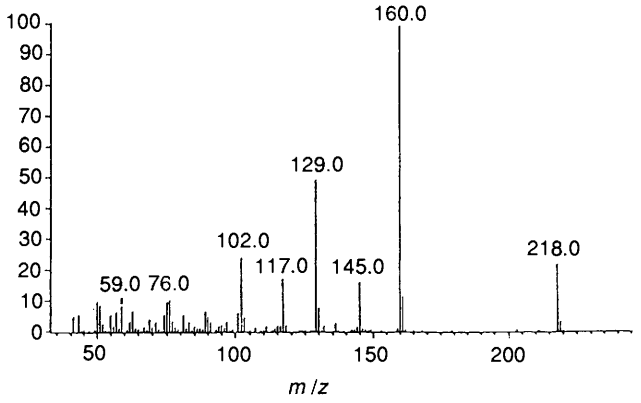
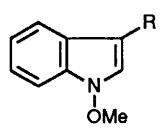


Figure. Mass spectrum (EIMS) of the 'low water' hydrolysis product of compound (**1b**) with myrosinase at pH 7.4.

Table. Mass spectral analysis of 1-methoxyindol-3-ylmethyl isothiocyanate (**2b**).

Measured mass	Formula	Fragment
218.0499	$C_{11}H_{10}N_2OS$	M^+
160.0741	$C_{10}H_{10}NO$	M − NCS
145.0546	C_9H_7NO	M − CH_3NCS
129.0577	C_9H_7N	M − (NCS + OMe)
117.0575	C_8H_7N	
102.0465	C_8H_6	
58.9788	CHNS	HNCS

to synthesize 1-methoxyindol-3-ylmethyl isothiocyanate (**2b**). 1-Methoxyindole (**6**) was prepared using established techniques[10,13] from 2-nitrotoluene *via* the enamine, and this indole was subsequently converted into 1-methoxyindole-3-carboxaldehyde (**7**) by reaction with phosphoryl trichloride and dimethylformamide (DMF). Reduction with sodium borohydride gave 1-methoxyindole-3-methanol (**3b**) as a yellow oil. Attempted tosylation of the alcohol (**3b**) gave either starting material or material tentatively identified as 1,1′-methoxy-3,3′-di-indolylmethane on the basis of mass spectrometry. The aldehyde (**7**) could also be converted into the oxime (**8**) but attempts to reduce this further to the amine under a range of conditions led only to loss of the 1-methoxy substituent. 3-(Dimethylaminomethyl)-1-methoxyindole was obtained from compound (**6**) by the method of Kuhn and Stein[14] and the corresponding methiodide was produced by reflux of this base with iodomethane in methanol. Attempted substitution to give the corresponding thiocyanate and isothiocyanate failed under a number of conditions with potassium thiocyanate.



(**6**) R = H
(**7**) R = CHO
(**8**) R = CH=NOH

Incubation of glucobrassicin (**1a**) with myrosinase in Tris buffer at pH 7.4 afforded a range of products including indole-3-methanol (**3a**), indole-3-acetonitrile (**5a**), and 3,3′-di-indolylmethane which were identified by comparison with authentic material. When the incubation was repeated in a Celite-supported, 'low water' system, however, only the nitrile (**5a**) was detected by TLC and MS.

Incubation of neoglucobrassicin (**1b**) with myrosinase in Tris buffer at pH 7.4 gave 1-methoxyindole-3-methanol (**3b**), 1-methoxyindole-3-acetonitrile (**5b**), and 1,1′-dimethoxy-3,3′-di-indolylmethane. The first two compounds were identified by comparison with authentic standards (TLC, MS) while the latter was compared on TLC with 3,3′-di-indolylmethane and its structure was tentatively confirmed by mass spectrometry. Incubation with myrosinase in a 'low water' system at pH 7.4 gave, principally, a single product possessing a different R_f-value from those of the products obtained under normal aqueous hydrolytic conditions. Analysis of this product by electron ionisation (EI) mass spectrometry gave a spectrum displaying an M^+ peak at 218 daltons and a base peak at 160 daltons (Figure). This is consistent with the expected fragmentation of 1-methoxyindol-3-ylmethyl isothiocyanate (**2b**). The ion at 160 daltons corresponds to a loss of NCS from the molecular ion, which is a characteristic fragment in the spectra of isothiocyantes produced by the hydrolysis of cruciferous glucosinolates.[15] The results of high-resolution analysis of the major ions in the spectrum (Table) confirm the formulae of the molecular and fragment ions expected from 1-methoxyindol-3-ylmethyl isothiocyanate (**2b**). The crude isolate exhibited a strong IR absorption at 2 200 cm^{-1}, characteristic of isothiocyanates. The isothiocyanate (**2b**) decomposed rapidly on silica to give polymeric material, but when stirred with water for 1 h, gave 1-methoxyindole-3-methanol (**3b**) (TLC), which was identified by comparison (TLC, MS) with an authentic sample.

Attempts to prepare stable derivatives of compound (**2b**) were unsuccessful. The crude isolate was treated with a solution of dry ammonia in ethanol in order to form the corresponding thiourea, but no reaction took place. While the link between indole glucosinolates (**1a–e**) and the indolic phytoalexins (**4a**), (**4b**), and (**4e**) *via* the corresponding isothiocyanates (**2a**), (**2b**), (**2e**) would appear likely, direct evidence is lacking. Incubation of compound (**2b**) with methanethiol in hexane gave a single product in low yield, with a mass spectral fragmentation pattern consistent with methyl 1-methoxyindol-3-ylmethyl sulphide (molecular ion *m/z* 207). This product may arise as a result of nucleophilic attack by the sulphur atom of methanethiol—analogous to the attack by water in the formation of the alcohol (**3b**)—on the α carbon at the 3-position in the indole ring.

While methanethiol appears not to add to isothiocyanate (**2b**) directly *in vitro*, the transfer of a thiomethyl group *in toto* has been postulated in the biosynthesis of other plant secondary products. Notably, an enzyme capable of catalysing the incorporation of the thiomethyl group as a unit has been detected in turnip leaves.[16] Granroth has observed[17] that the ^{14}C-methyl group and the ^{35}S atom of labelled methanethiol (or methionine) were incorporated into *S*-methylcysteine sulphoxide by *Allium* spp. at rates which suggested that the intact methylthio group was being transferred. A range of diacetylene compounds occurs in *Chrysanthemum* spp. together with the corresponding terminal methyl sulphides. The addition of methanethiol to the diacetylenes has been demonstrated *in vitro* and proposed *in vivo*.[8] Clearly, further studies are necessary to determine if the indole phytoalexins (**4a**), (**4b**), and (**4e**) are derived from indole glucosinolates (**1a**), (**1b**), and (**1e**) *via* the corresponding isothiocyanates (**2a**), (**2b**), and (**2e**) and if the formation involves intact incorporation of a methylthio moiety or if some other mechanism is operating.

Experimental

M.p.s. were obtained on a Gallenkamp m.p. apparatus and are uncorrected. IR spectra were run on a Perkin-Elmer 297

infrared spectraphotometer, and UV spectra were obtained on a Perkin-Elmer 550S UV–VIS spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker WH200 spectrometer operating at 200.13 MHz, and ^{13}C NMR spectra were obtained on the same instrument but at an operating frequency of 52.32 MHz. Mass spectra were obtained on a Kratos MS30 or MS60 mass spectrometer.

Isolation of Glucobrassicin (**1a**) *and Neoglucobrassicin* (**1b**).—*Source material.* Glucobrassicin was extracted from the apical leaves of Brussels sprouts (*Brassica oleacea* var. *gemmifera* cv Titurel) and neoglucobrassicin from swede rind (*B. napus* var. *napobrassica*).

Preliminary purification. The plant material (200 g) was stored at −40 °C, freeze dried, and ground, and portions (50 g) were extracted in boiling 70% methanol (1 l) for 15 min. The extract was filtered, the solids were re-extracted in fresh 70% methanol, and the extract was filtered; the filtrates were combined and evaporated under reduced pressure to *ca.* 200 ml. The solution was clarified by passage through Celite, the column being washed with water (100 ml). After further evaporation to *ca.* 150 ml, the extract was applied to a column of acidic alumina (45 × 7 cm, 900 g) and washed with water until the effluent was colourless. The glucosinolates were then eluted with 5% aq. potassium sulphate: the salt solution was collected until an orange band had almost reached the bottom of the column. The effluent was evaporated to dryness and desalted by dissolution in methanol and filtration to remove undissolved potassium sulphate (×3). The methanolic filtrate was evaporated to dryness and the residue was redissolved in a minimum volume of water (~10 ml).

Isolation of purified indole glucosinolates. The crude, partly desalted glucosinolate mixture was applied to a column (40 × 3 cm i.d.) of Sephadex G10 (preswollen in water) and eluted with water. Fractions (10^4 ml) were collected and monitored by TLC with butan-1-ol–acetic acid–water (4:1:2) as mobile phase and *p*-dimethylaminocinnamaldehyde[3,5] (10% w/v in equal volumes of 37% HCl and ethanol) as spray visualisation agent. Fractions containing indole glucosinolates were combined and freeze dried. In the case of neoglucobrassicin, the extract was reapplied to a second column of G10, as the source contains a wide variety of glucosinolates, to enable further separation.

Recrystallisation. Glucobrassicin was converted into the tetramethylammonium (TMA) salt by passage through an Amberlite IR-120 TMA column (15 × 2 cm i.d.), and freeze dried. Neoglucobrassicin was converted into the brucine salt by passage through an IR-120 (H^+) ion column, freeze drying, dilution with water, addition of brucine dihydrate (1 mol equiv.), stirring for 30 min, and freeze drying. Both indole glucosinolates were recrystallised from aq. ethanol.

Glucobrassicin (**1a**) (1.8 mg) had m.p. 145–147 °C (lit.,[5] 148–150 °C); δ_H(D_2O; external $SiMe_4$) 2.94–4.71 (m, 1–6-H), 4.28 (d, *J* 6.3 Hz, 8-H), 4.20 (d, *J* 6.3 Hz, 8-H), 7.36 s, 9-H), 7.78 (dm, *J* 7.4 Hz, 12-H), 7.25 m, 13- and 14-H), and 7.55 (dm, *J* 8.4 Hz, 15-H); δ_C(D_2O; external $SiMe_4$) 82.3–61.4 (C-1–C-6), 162.0 (C-7), 30.3 (C-8), 124.8 (C-9), 109.2 (C-10), 127.3 (C-11), 119.4 (C-12), 120.4 (C-13), 123.0 (C-14), 112.8 (C-15), and 137.2 (C-16); ν_{max}(KBr) 3 580, 2 930, 1 585, 1 485, 1 450m, 1 350m, 1 220m, 1 125m, 1 050m, 950s, and 780m cm^{-1}; *m/z* (FAB) 447 [M^-] (Found: C, 46.2; H, 6.3; N, 8.0. Calc. for $C_{20}H_{31}N_3O_9S_2$: C, 46.0; H, 6.0; N, 8.2%).

Neoglucobrassicin (**1b**) (520 mg) had m.p. 158–162 °C (decomp.); δ_H(D_2O; external $SiMe_4$) (selected resonances) 7.00 (s, brucine), 7.06 m, 13-H), 7.16 (m, 14-H), 7.32 (d, *J* 8.6 Hz, 12-H), 7.37 (s, 9-H), 7.56 (s, brucine), 7.63 (d, *J* 8.2 Hz, 15-H), and 3.95 (s, OMe); δ_C (D_2O; external $SiMe_4$) 82.5–61.2 (C-1–C-6), 66.9 (OMe), 163.5 (C-7), 30.0 (C-8), 123.7 (C-9), 99.7 (C-10), 124.1 (C-11), 121.4 (C-12), 123.7 (C-13), 119.9 (C-14), 106.4 (C-15), and 133.2 (C-16); ν_{max}(Nujol) 3 460w, 1 670, 1 285, 1 200, 1 220, 1 125m, and 1 050 cm^{-1}; *m/z* (FAB) 477 [M^-] (Found: M^+ − H, 477.0683. $C_{17}H_{21}N_2O_{10}S_2$ requires *m/z* 477.0638).

1-*Methoxyindole* (**6**).—2-Nitrotoluene was converted into (*E*)-*N*,*N*-dimethyl-2-(2-nitrophenyl)ethenamine by reaction with DMF dimethyl acetal in dry DMF by established procedures.[10] A solution of the enamine (10 g) in diethyl ether (300 ml) was treated with a solution of ammonium chloride (14 g) in water (50 ml) and finely divided zinc (50 g) and the suspension was stirred at room temperature for 4 h. The zinc was removed by filtration through Celite and the filtrate containing 1-hydroxyindole was treated with iodomethane (55 g), aq. sodium hydroxide (10%; 300 ml), and methyl trioctyl ammonium chloride (500 mg), then stored overnight at room temperature. The organic phase was separated, washed with brine (250 ml), dried (Na_2SO_4), and evaporated under reduced pressure to give crude compound (**6**). Flash column chromatography on silica gel with CH_2Cl_2–hexane as mobile phase gave essentially pure compound (**6**) (4.7 g), which had identical mass spectral, NMR, and IR data with those reported previously.[10]

1-*Methoxyindole*-3-*carbaldehyde* (**7**).—Fresh $POCl_3$ (20 ml) was added dropwise to stirred, ice-cold dry DMF (50 ml) and the mixture was treated with a solution of compound (**6**) (35 g) in dry DMF (20 ml) during 30 min. The mixture was stirred at room temperature for 2 h, then treated with ice–water (200 ml), washed with diethyl ether (2 × 200 ml), and basified with aq. sodium hydroxide (40 g in 200 ml). The reaction mixture was heated (100 °C; 1–2 min), cooled, and extracted (×3) with diethyl ether. The extracts were combined, dried (Na_2SO_4), and evaporated to afford crude aldehyde (**7**) as a brown oil (25 g), which was purified by flash chromatography on silica with EtOAc–hexane (2:3) as mobile phase to afford compound (**7**) as a yellow oil (18.4 g), δ_H($CDCl_3$; $SiMe_3$) 4.20 (OMe), 7.70 (2-H), 8.30 (m, 4-H), and 7.05–7.60 (m, 5-, 6-, and 7-H).

1-*Methoxyindole*-3-*carbaldehyde Oxime* (**8**).—A solution of aldehyde (**7**) (1.2 g) in ethanol (10 ml) was treated at 0 °C with a solution of hydroxylamine hydrochloride (2.5 g) in water (5 ml). A solution of sodium carbonate (1–5 g) in water (5 ml) and further ethanol (*ca.* 5 ml) were added. The reaction mixture was shaken for 5 min, then extracted with ethyl acetate (3 × 25 ml). The organic fractions were combined, dried (Na_2SO_4), and evaporated to dryness under reduced pressure to afford the crude product, which from TLC [EtOAc–hexane (2:3)] appeared to contain the oxime (**8**) and the corresponding demethoxy oxime. The crude product was purified by flash chromatography on silica with EtOAc–hexane (2:3) as mobile phase and the purified product was crystallised from EtOAc–hexane to afford oxime (**8**) (0.65 g), m.p. 94–96 °C; *m/z* (EI) 190 ($M^{+\cdot}$), 173 (M − OH)$^+$, and 160 (M − $OCH_2^{+\cdot}$); δ_H($CDCl_3$; internal $SiMe_4$) 4.06 (s, OMe), 7.20 (m, 6-H), 7.27 (m, 5-H), 7.40 (d, *J* 8.2 Hz, 4-H), 7.44 (s, 2-H), 7.75 (s, NOH), 8.00 (d, *J* 7.9 Hz, 7-H), and 8.26 (s, CH=N); δ_C($CDCl_3$; relative to $CDCl_3$, 77.000 ppm) 66.26 (OMe), 105.56 (C=NOH), 108.40 (C-7), 121.31 (C-3a), 121.61 (C-4), 122.10 (C-5), 123.71 (C-6), 124.81 (C-2), 132.64 (C-7a), and 145.35 (C-3).

1-*Methoxyindole*-3-*methanol* (**3b**).—A solution of aldehyde (**7**) (0.5 g) in methanol–ethanol (1:8; 5 ml) was treated with sodium borohydride (90.5 g) and the mixture was stirred at room temperature for 3 h. The solvent was then removed by evaporation under reduced pressure and the residue was treated with NaOH (0.1M; 5 ml), extracted with diethyl ether (3 × 15

ml), and the organic fractions were combined, dried (Na_2SO_4), then evaporated to dryness to afford crude alcohol (**3b**). Distillation (92–94 °C; 0.1 Torr) gave pure compound (**3b**) (0.23 g), $\delta_H(CDCl_3$; internal $SiMe_4$) 1.19 (t, *J* 7.0 Hz, OH), 4.03 (s, OMe), 4.80 (d, *J* 5.0 Hz, CH_2), 7.10 (m, 6-H, 7.23 (m + s, 5- and 2-H), 7.39 (d, *J* 8.2 Hz, 4-H), 7.65 (d, *J* 8.0 Hz, 7-H); $\delta_C(CDCl_3$; relative to $CDCl_3$, 77.000 ppm), 57.14 (CH_2OH), 65.86 (OMe), 108.41 (C-7), 119.29 (C-4), 119.69 (C-3a), 120.15 (C-5), 121.68 (C-3), 121.69 (C-6), 122.83 (C-2), and 133.40 (C-7a); *m/z* (EI) 177 ($M^{+\bullet}$), 160 ($M - OH)^+$, 145 ($M - CH_3OH)^{+\bullet}$, and 129 ($M - H_2O - CH_2O^{\bullet}$); ν_{max} 3 520w, 2 890, 2 150, 1 450, 1 220, and 1 100 cm^{-1} (Found: M^+, 177.0791. Calc. for $C_{10}H_{11}NO_2$: *M*, 177.0789).

Tosylation of 1-*Methoxyindole*-3-*methanol* (**3b**).—A sample of the alcohol (**3b**) (20 mg) in dry tetrahydrofuran (5 ml) was cooled in ice, then treated with triethylamine (0.1 ml) and toluene-4-sulphonyl chloride (20 mg). The mixture was stirred at room temperature overnight, then treated with ice–water and extracted into diethyl ether (3 × 50 ml). The organic fraction was dried (Na_2SO_4), and evaporated to dryness under reduced pressure at room temperature to afford an off-white solid. Examination of the crude material by TLC [EtOAc–hexane (2:3), R_f 0.9; *cf.* 3,3′-di-indolylmethane, R_f 0.88] and MS (EI) [306 ($M^{+\bullet}$), 276 ($M - OCH_3)^+$, and 245 ($M - CH_3OH - CH_2O$)] suggested that it was probably composed principally of 1,1′-dimethoxy-3,3′-di-indolylmethane.

Enzyme Incubations.—(a) *Aqueous.* Glucobrassicin (**1a**) or neoglucobrassicin (**1b**) (10 mg) was dissolved in aqueous buffer (Tris·HCl; pH 7.4; 100 mM; 5 ml) and incubated with myrosinase at 37 °C for 4 h. After this time, hexane (10 ml) was added, the mixture was shaken, and the organic layer was removed, dried (Na_2SO_4), and evaporated under reduced pressure at room temperature. The residue was taken up in EtOAc (100 μl) and examined by both TLC [EtOAc–hexane (2:3)] and MS. The alcohols (**3a**) and (**3b**) and acetonitriles (**5a**) and (**5b**) were identified by TLC comparison with authentic samples.

(b) *'Low water' system.* Compound (**1a**) (10 mg) or (**1b**) (10 mg) was dissolved in aqueous buffer (Tris·HCl; pH 7.4; 100 mM; 1 ml) and the solution was added to Celite (ca. 200 mg). Myrosinase in water (1 ml) was similarly adsorbed onto Celite and enzyme and substrate sample were then dried *in vacuo* at room temperature for 6 h. The resulting powders were then mixed and treated with hexane (5 ml) presaturated with water. An aliquot of water (10 μl) was added and the mixture was shaken for 30 min. The suspension was filtered through glass wool, dried (Na_2SO_4), and evaporated at reduced pressure at room temperature. The residue was taken up in EtOAc (100 μl) and examined by TLC and MS. Compound (**1a**) had produced principally the corresponding acetonitrile (**5a**) while 1-methoxyindol-3-ylmethyl isothiocyanate (**2b**) was detected by TLC [R_f 0.8; silica; mobile phase EtOAc–hexane (2:3)] and MS (EI) [218 (M^+), 160 ($M - NCS)^+$, and 59 (HNCS)] (Found: M^+, 218.0499. $C_{11}H_{10}N_2OS$ requires *M*, 218.0514) as the product from compound (**1b**).

Aqueous Hydrolysis of Compound (**2b**).—The crude isothiocyanate (**2b**) from hydrolysis of compound (**1b**) (200 mg) was taken up in ethyl acetate (2 ml) and the solution was stirred with water (5 ml) for 1 h; the organic phase was then separated and evaporated to dryness. TLC [silica plate; mobile phase EtOAc–hexane (2:3)] of the crude residue showed the presence of a spot corresponding to 1-methoxyindole-3-methanol (**3b**), which was purified by preparative TLC and identified by MS as being identical with authentic compound (**3b**).

Incubation of Compound (**2b**) *with Methanethiol.*—A sample of compound (**2b**) from hydrolysis of compound (**1b**) (200 mg) was taken up in hexane presaturated with methanethiol and the mixture was stirred gently for 1 h while methanethiol was bubbled through the mixture. The solvent was removed by bubbling N_2 through the reaction mixture and the crude material was then examined by MS. The principal ions present appeared to correspond to 1-methoxyindol-3-ylmethyl methyl sulphide—*m/z* (EI) 207 (M^+), 175 ($M - CH_3OH$), and 160 ($M - CH_3S$).

Acknowledgements

We are grateful to Dr. R. M. Acheson for 1-methoxyindole-3-acetonitrile. NMR spectra were run by Dr. I. J. Colquhoun of this Institute. This programme was supported by the Ministry of Agriculture, Fisheries and Food.

References

1 G. R. Fenwick, R. K. Heaney, and W. J. Mullin, *CRC Crit. Rev. Food Sci. Nutr.*, 1983, **18**, 123.
2 M. G. Ettlinger and A. J. Lundeen, *J. Am. Chem. Soc.*, 1956, **78**, 4172.
3 R. Gmelin and A. I. Virtanen, *Suom. Kemistil. B*, 1962, **35**, 34.
4 A. B. Hanley, P. S. Belton, G. R. Fenwick, and N. F. James, *Phytochemistry*, 1985, **24**, 598.
5 R. Gmelin and A. I. Virtanen, *Suom. Kemistil. B*, 1961, **34**, 15.
6 M. Takasugi, K. Moude, N. Katsui, and A. Shirata., *Chem. Lett.*, 1987, 1631.
7 M. Takasugi, N. Katsui, and A. Shirata, *J. Chem. Soc., Chem. Commun.*, 1986, 1077.
8 M. G. Ettlinger and A. Kjaer, 'Sulphur Compounds in Plants,' in 'Recent Advances in Phytochemistry,' eds. T. J. Mabry, R. E. Alston, and V. C. Puneckles, North Holland, Amsterdam, 1968, vol. 1.
9 A. B. Hanley and K. R. Parsley, *Phytochemistry*, 1990, **29**, 769.
10 R. M. Acheson, P. G. Hunt, D. M. Littlewood, B. A. Murrer, and H. E. Rosenberg, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1117.
11 A. B. Hanley, R. K. Heaney, and G. R. Fenwick, *J. Sci. Food Agric.*, 1983, **34**, 869.
12 A. B. Hanley, C. L. Curl, G. R. Fenwick, and R. K. Heaney, *Eucarpia*, 1984, **9**, 66.
13 M. Somei and T. Shoda, *Heterocycles*, 1981, **16**, 1523.
14 H. Kuhn and O. Stein, *Ber. Dtsch. Chem. Ges.*, 1937, **70**, 567.
15 G. F. Spenser and M. E. Daxenbichler, *J. Sci. Food Agric.*, 1980, **31**, 359.
16 J. F. Thompson and D. P. Moore, *Biochem. Biophys. Res. Commun.*, 1968, **31**, 281.
17 B. Granroth, *Ann. Acad. Sci. Fenn., Ser. AII, Chem.*, 1970, **154**, 1.

Paper 0/00631I
Received 12*th February* 1990
Accepted 13*th March* 1990