Experiment, Georgia. Included were one accession of *Sorghum aethiopicum*, three of *almum*, three of *arundinaceum*, three of *bicolor*, four of *caudatum*, one of *controversum*, one of *halepense*, two of *hewisonii*, one of *japonicum*, one of *miliaceum*, three of *nigricans*, one of *niloticum*, three of *notabile*, one of *plumosa*, one of *propinquum*, one of *pugionifolium*, four of *saccharatum*, two of *subglabrescens*, seven of *sudanense*, one of *versicolor*, three of *verticilliflorum*, two of *virgatum* and four accessions designated only as *Sorghum* sp. In addition, cv. 65D (*S. bicolor*) was obtained from L. M. Mazhani, Department of Agricultural Research, Gaborone, Republic of Botswana. In total, 50 entries representing 22 species plus four entries without species designation were included in the study. Seedlings were grown as previously described [2]. Samples usually consisted of a bulk of five shoots from 1-week-old seedlings.

Sample treatment and spectral scanning. Samples were weighed, dried at 75° for 2.5 hr, pulverized and extracted with 20 ml H_2O at room temp. for 2 hr. The tissue residue was removed by filtration and filtrates were diluted 10-fold with 0.1 M NaOH for spectral scanning.

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RING OXYGENATED INDOLE GLUCOSINOLATES OF *BRASSICA* SPECIES

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Key Word Index—*Brassica napus* subsp. *napobrassica*; *Brassica napus* subsp. *oleifera*; Cruciferae; swede; rape; structural confirmation; indole glucosinolates.

Abstract—Detailed chemical, degradative and spectroscopic analysis of two ring oxygenated indole glucosinolates isolated from *Brassica* species has confirmed these to be substituted in the 4- rather than the 5-position, although the latter had been suggested on biosynthetic grounds.

INTRODUCTION

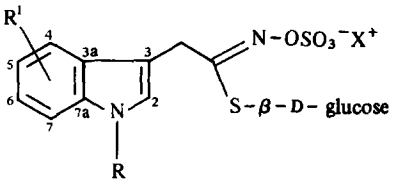
Indole glucosinolates, derived from tryptophan, are commonly found in the genus *Brassica* and have recently attracted attention as the precursors of anticarcinogenic factors [1, 2]. Five such compounds (**1a**–**1e**) are now known including two (**1d** and **1e**) only recently reported by Truscott *et al.* [3, 4]. On the basis of limited ^{1}H NMR analysis of their desulpho derivatives the new glucosinolates were both claimed to be substituted in the 4-position. Subsequently other workers [5] have suggested that 5-substitution is more likely.

In this paper we present unambiguous evidence from both enzymic degradation and physical techniques that the two novel compounds are 4-substituted.

RESULTS AND DISCUSSION

Compound **1d** was isolated from the root bark of swede (*B. napus* subsp. *napobrassica* cv. Purple Top). CC on acid-washed alumina followed by passage through Sephadex G-10 or DEAE-A25 Sephadex afforded chromatographically pure material. The compound, when desulphated, had identical TLC characteristics to earlier reports [3, 5] and the ^{1}H NMR spectrum, with shifts possibly due to the use of different solvents and standards for the sulpho and desulpho derivatives had a similar appearance to that reported earlier [6].

Detailed examination of the ^{1}H NMR spectrum reveals characteristics not considered previously [3] supporting an aromatic system containing three adjacent protons rather than the interrupted pattern required by 5-substitution. The upfield double doublet (6.85 ppm) shows *ortho* and *meta* coupling (8 Hz and 2 Hz, respectively) and irradiation of this proton eliminates these couplings from the multiplet at 7.46 ppm. The remaining almost first order structure corresponds to an *ortho* coupling between two protons with similar chemical shifts. The ^{13}C NMR spectrum of **1d** and of authentic 4- and 5-oxygenated



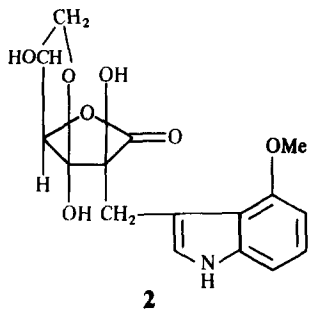
1a R=H, R¹=H

1b R=OMe, R¹=H

1c R=OSO_3^-, R¹=H

1d R=H, R¹=OMe

1e R=H, R¹=OH



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indole derivatives provided further support for the 4-methoxy structure (Table 1). Unambiguous assignments of all the aromatic carbons is not yet possible so further studies are continuing. However the pattern of shifts is strikingly different in the 4- and 5-substituted compounds and **1d** clearly resembles the former.

Final confirmation was obtained by controlled hydrolysis of the glucosinolate with myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) in the presence of ascorbic acid. The major product was chromatographically and spectroscopically (^{1}H NMR, UV and IR) identical to synthetic 4-methoxyindole-3-acetonitrile and the minor to 4-methoxyascorbigen (**2**).

Compound **1e**, isolated by the same method from rapeseed (*B. napus* subsp. *oleifera* cv. Midas), also exhibited a complex ^{1}H NMR spectrum and showed generally similar shifts. Comparison of this and of the ^{13}C NMR spectrum with those of authentic 4- and 5-hydroxy indole compounds strongly suggested a 4-hydroxy derivative.

Confirmation of structure again came from controlled hydrolysis experiments. Since the initial hydrolysis products were too unstable for direct analysis, the mixture was methylated with iodomethane and potassium carbonate. Two of the resultant products were shown to be identical with authentic 4-methoxyindole-3-acetonitrile and 4-methoxyascorbigen (**2**).

EXPERIMENTAL

TLC was carried out on silica gel plates using three solvent systems: 1, BuOH–EtOH–H_2O (4:2:3); 2, EtOAc–60/80° petrol (2:3); 3, toluene–EtOH (9:1). *p*-Dimethylaminocinnamaldehyde spray reagent was used to visualise the indole compounds. NMR spectra were determined at 300 MHz (^{1}H), 75.46 MHz (^{13}C) and 25 MHz (^{13}C). Spectra were recorded in CD_3OD or D_2O–CD_3OD using CHDOD (^{1}H) or CD_3OD (^{13}C) as int. standard. Chemical shift values are quoted relative to TMS for ^{1}H($\delta_{TMS} = \delta_{CHDOD} + 3.49$) and ^{13}C($\delta_{TMS} = \delta_{CD_3OD} + 49.0$) spectra. IR spectra were recorded in KBr and UV spectra in MeOH. MS were recorded at 200° for EI (70 eV) and at ambient for FAB (6 kV).

Methoxyindolyl-3-methylglucosinolate **1d**. The compound was extracted from *B. napus* subsp. *napobrassica* cv. Purple Top using techniques described previously [7]. The chromatographically pure product was isolated by freeze drying to afford a gummy white solid. $R_f = 0.77$ (system 1), UV λ_{max} nm: 267, 282, 292. IR ν_{max} cm^{-1}: 3450, 2940, 2825. ^{1}H NMR (D_2O) (aromatic region): δ7.49 (1H, *s*, H-2), 6.85 (1H, *dd*, J = 2 Hz, 8 Hz, H-5), 7.46 (2H, *m*, H-6, H-7). Irradiation at 6.85 ppm collapsed the multiplet at 7.46 ppm to an AB quartet. MS *m/z*: 477 $[M]^+$

Hydroxyindolyl-3-methylglucosinolate **1(e)**. The product was isolated similarly from *B. napus* subsp. *oleifera* cv. Midas as described earlier [7]. The purified product readily oxidised in the presence of air and light and so was kept below 0° under N_2 in the dark. $R_f = 0.77$ (system 1); UV λ_{max} nm: 267, 284, 293. IR ν_{max} cm^{-1}: 3650, 2940, 2825. ^{1}H NMR (D_2O) (aromatic region): δ7.45 (1H, *s*, H-2), 6.84 (1H, *m*, H-5), 7.64 (2H, *d*, H-6, H-7) MS *m/z*: 463 $[M]^+$.

Synthetic 4-methoxyindole-3-acetonitrile. Following synthesis from 3-methyl-2-nitrophenol [8], the compound was purified by prep. TLC (system 2) and recrystallised from EtOAc–hexane to afford colourless crystals. Mp 139–141° (lit. [7] 140–142° UV λ_{max} nm: 220, 265, 279, 290. IR ν_{max} cm^{-1}. 3480, 2240. ^{1}H NMR (CD_3OD) (aromatic region): δ7.25 (1H, *s*, H-2), 6.65 (1H, *dd*, J = 2 Hz, 8 Hz, H-5), 7 20 (1H, *dd*, J = 8 Hz, 8 Hz, H-6), 7.12 (1H, *dd*, J = 2 Hz, 8 Hz, H-7). MS *m/z* (rel. intensity): 186 ($[M]^+$, 74), 171 (100), 143 (20), 116 (18), 89 (23).

Synthetic 4-methoxyascorbigen (**2**). The product from reaction of 4-methoxyindole-3-carbinol with ascorbic acid at pH 4 [9] was purified by prep. TLC (system 3) and isolated as a yellow

Table 1. ^{13}C NMR spectra of the aromatic region of certain 4- and 5-substituted indole compounds

	2	3	4	5	6	7	3a	7a
1e	123.1	104.6*	150.3	104.0*	123.1	109.7	115.6	138.5
4-Hydroxytryptamine hydrochloride	123.9	104.8	153.1	104.3*	123.9	109.6	117.4	140.8
5-Hydroxytryptamine [11]	123.6	108.4	102.0	150.3	111.4	111.7	127.4	130 7
1d	123.1	105.6	153.9	100.2	123.1	108.5	115.9	137.9
4-Methoxyindole-3-acetonitrile	123.0*	105.4	154.2	100.5	124.1*	108.9	120.7	139.5
5-Methoxyindole-3-carbinol	125.4	112.9*	101.7	155.2	112.9*	116.1*	133.5	128.5

*These assignments may be interchanged.

hygroscopic solid. $R_f = 0.30$ (system 3); UV λ_{max} nm: 282, 291. IR ν_{max} cm^{-1}: 3350, 2830, 2950. ^1H NMR (CD_3OD) (aromatic region): 7.25 (1H, *s*, H-2) 7.20 (1H, *dd*, $J = 8$ Hz, 8 Hz, H-6) 7.10, (1H, *dd*, $J = 8$ Hz, 2 Hz, H-7), 6.55 (1H, *dd*, $J = 8$ Hz, 2 Hz, H-5) MS *m/z*, 336 ($[M+1]^+$), 205, 160.

Enzymic breakdown of methoxyindole-3-methylglucosinolate. A soln of the natural methoxyindole-3-methylglucosinolate (10 mg) in buffer (pH 4) was incubated with myrosinase (2 mg) and ascorbic acid (2 mg) at 30° for 2 hr, centrifuged to remove proteinaceous material and the supernatant extracted (× 3) with EtOAc. The organic fractions were combined, dried (Na_2SO_4) and the solvent removed by evapn under red. pres. The residue was purified by prep. TLC (system 3) to afford a chromatographically pure sample of 4-methoxyascorbigen with identical MS, UV and ^1H NMR characteristics to the synthetic sample. Repetition of the above procedure followed by prep. TLC (system 2) gave a small yield of material with identical chromatographic, UV and MS characteristics to synthetic 4-methoxyindole-3-acetonitrile.

Enzymic breakdown of hydroxyindole-3-methylglucosinolate. A sample of the natural hydroxyindole-3-methylglucosinolate treated under similar conditions gave an unstable product with the ^1H NMR and MS characteristics of a hydroxyindole-3-acetonitrile. Immediate methylation by reaction with iodomethane and K_2CO_3 in Me_2CO [10] gave a product with identical chromatographic and spectral characteristics to authentic 4-methoxyindole-3-acetonitrile.

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IDENTIFICATION OF ALLIIN, A CONSTITUENT OF *ALLIUM CEPA* WITH AN INHIBITORY EFFECT ON PLATELET AGGREGATION

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Key Word Index—*Allium cepa*; Alliaceae; onion; platelet aggregation; alliin; *S*-allyl-L-cysteine sulfoxide.

Abstract—A component of *Allium cepa* which inhibits platelet aggregation *in vitro* was isolated. The active compound was identified as alliin, (+)-*S*-allyl-L-cysteine sulfoxide. Alliin was synthesized and found to exert the same activity on platelet aggregation as the natural compound.

INTRODUCTION

Baghurst *et al.* [1] first reported the effect of onions on platelet aggregation in people who had consumed a high fat diet. This observation was confirmed by others showing that chloroform extracts of the essential oils of *Allium cepa* and *A. sativum* inhibit platelet aggregation *in vitro*, induced either by ADP or by arachidonic acid [2, 3]. Philips and Poyser [4] have found that ethanol extracts of *Allium cepa* cause the same effect, while Ariga *et al.* [5] have isolated from garlic the methyl allyl trisulphide with inhibitory activity on platelet aggregation.

In this paper we describe the isolation and elucidation of the structure of the constituent of *Allium cepa* with inhibitory activity on platelet aggregation. The active