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Indole Compounds Related to Auxins and Goitrogens of Woad (*Isatis tinctoria* L.)[1]

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ABSTRACT

Five conspicuous indole derivatives are present in leaves and other tissues of woad (*Isatis tinctoria* L.). They were identified as tryptophan, isatan B, glucobrassicin, neoglucobrassicin, and glucobrassicin-1-sulfonate. The latter three indole glucosinolates are present at levels of at least 260, 69, and 200 milligrams per kilogram fresh weight and were isolated as crystalline salts. Comparison of physical and chemical properties, particularly NMR spectral analysis, confirms that the 1-methoxyglucobrassicin structure suggested for neoglucobrassicin is correct, whereas further evidence for the even more unusual sulfonation of the ring nitrogen in glucobrassicin-1-sulfonate was obtained. Glucobrassicin-1-sulfonate has an enzymic degradation pattern identical to that of glucobrassicin. As it too releases thiocyanate, it must be added to the list of known plant goitrogens. These studies and the techniques described establish woad as exceptionally suitable higher plant material for metabolic studies of indoles related to goitrogens and auxins.

Following the demonstration by Linser (25) that *Brassica* plants were rich sources of indole compounds, a large number of simple indole derivatives were detected in extracts of *Brassica* and other Cruciferae (1, 5, 10, 17–19, 26–28, 31, 34, 35). In 1960 Gmelin *et al.* (12) reported the isolation of the crystalline TMA^+ salt[3] of an indole glucosinolate which was shown (14) to be 3-indolylmethylglucosinolate (glucobrassicin, Fig. 1a). They also demonstrated that the enzyme myrosinase, as well as exposure to acids or bases, caused degradation of glucobrassicin to yield a range of indolic products which included those described as natural endogenous indoles by the earlier workers. Virtanen (44) proposed that the presence of these compounds in the extracts was a consequence of the failure of the earlier workers to inactivate myrosinase before extraction, since it is present in special cells in the same tissues. Simple indole compounds could also have been produced as a consequence of the breakdown of glucobrassicin (or its enzymically produced degradation products) when extremely acidic or basic conditions were used during extraction or purification. Interest in these topics was heightened by the fact that one degradation product, indole-3-acetonitrile, has high auxin activity in some growth hormone assays (17), whereas other substances formed by glucobrassicin decomposition, notably thiocyanate, have high goiter-inducing activity when plants in the Cruciferae are included in animal diets and can even enter their milk (44).

At first, it was thought that glucobrassicin was the only natural indole occurring in large amounts in *Brassica* plants, but in 1962 Gmelin and Virtanen (15) isolated another indole glucosinolate, neoglucobrassicin, which was described as the 1-methoxy derivative of glucobrassicin (Fig. 1b). This structure has not been considered conclusive (9).

Previous studies in this laboratory (8, 40, 41) have established that the precursor of indigo in the woad plant is an unusual indoxyl derivative (isatan B), different from indoxyl-β-D-glucoside which is the precursor of indigo in *Indigofera* plants. The present paper identifies other natural indole compounds present in high concentration in the woad plant, establishes this plant as particularly suitable for studies of higher plant indole metabolism related to auxin and goitrogen formation, and describes techniques useful to these ends.

MATERIALS AND METHODS

Plant Material. Woad plants (*Isatis tinctoria* L.) were grown in Yale University's Marsh Botanic Garden from seed collected from plants of the previous year. The original sources of seed were the herb garden of Sissinghurst Castle, Kent, and Thompson and Morgan (Ipswich) Ltd. The plants were harvested and extracted when they were about 7 months old.

For sterile culture, seeds were sterilized by rinsing in 90% ethanol for 1 min and then soaking in 0.5% calcium hypochlorite solution for 4 hr. After washing with sterile water, the seeds were transferred to autoclaved 14-cm Petri dishes (about 130 seeds per dish) containing the inorganic solution used by Danckwardt-Lilliestrom (3) modified to include an Fe-EDTA preparation (43), 11 ml/liter, instead of ferric tartrate. The germinated seeds were allowed to grow in this solution in a constant temperature room at 25 C under 16-hr photoperiods of approximately 1000 ft-c of mixed incandescent and fluorescent light. After 7 days the seedlings were rinsed thoroughly with distilled water, then extracted with hot methanol as described below.

Extraction and Isolation. In order to check on the possibility of artifact formation during extraction, two different procedures were used for enzyme inactivation. In the first method of extraction (14, 23), the enzymes were inactivated by dropping the fresh plant material into boiling methanol (4 ml/g fresh weight) for 2 min and leaving it in the cooled methanol

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[3] Abbreviations: IAN: indole-3-acetonitrile; NMR: nuclear magnetic resonance; pDAC: *p*-dimethylaminocinnamaldehyde reagent; TLC: thin layer chromatography; TMA^+: tetramethylammonium.

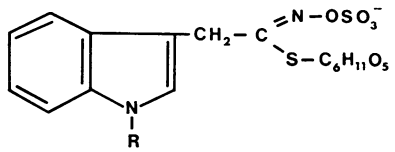

FIG. 1. Molecular structure of indole glucosinolates. a: R = H, glucobrassicin; b: R = OCH_3, neoglucobrassicin; c: R = SO^-_3, glucobrassicin-1-sulfonate.

overnight before filtration. The plant residue was ground and re-extracted twice with warm 80% methanol (3 ml/g original fresh weight). The filtrates were combined and concentrated under reduced pressure at 30 C and either subjected directly to paper chromatography or, for the isolation of crystalline specimens of the indole glucosinolates, the purification scheme shown in Figure 2 was used.

In view of the possibilites of esterification or transesterification occurring in boiling methanol (40, 41), we have compared the results obtained by this method with those obtained when an alternative extraction scheme was used (6, 42). Fresh plant material was lyophilized and subsequently macerated in cold (−15 C) 80% methanol (4 ml/g original fresh weight) and extracted for 18 hr before filtration. The plant residue was re-extracted twice with cold 80% methanol (3 ml/g original fresh weight) for 4 hr each time with frequent swirling. The filtrates were combined, concentrated under reduced pressure at 30 C, and subjected to paper chromatography.

Paper Chromatography. Separations were by descending development on Whatman No. 1 or 3 MM paper. Papers to be used for the isolation procedure were washed in 5% acetic acid followed by distilled water and dried at room temperature before use. The chromatographic solvents used were:

1. 1-Butanol-acetic acid-water (4:1:2, v/v).
2. 2-Propanol-10% ammonium hydroxide (8:2, v/v).
3. Pyridine-1-pentanol-water (7:7:6, v/v).
4. 1-Butanol-pyridine-water (1:1:1, v/v).
5. 20% Aqueous KCl (w/v).

Thin Layer Chromatography. Chromatography upon 300 μ thin layers of cellulose (binder free), using solvents 1 and 5 with a development distance of 15 cm, permitted very rapid separations of the indole glucosinolates or of their enzymic or chemical degradation products. In particular the use of solvent 1 gave excellent separation (glucobrassicin-1-sulfonate R_F 0.3; glucobrassicin R_F 0.6; neoglucobrassicin R_F 0.75) and was used to determine which column fractions contained indole glucosinolates.

For TLC on 300 μ Silica Gel G layers the following solvents were used:

6. Methyl acetate-2-propanol-25% ammonium hydroxide (45:35:20, v/v).
7. Chloroform (1% ethanol)-96% acetic acid (95:5, v/v).
8. Chloroform (1% ethanol)-methanol-96% acetic acid (75:20:5, v/v).
9. *n*-Hexane-2-butanone.

Paper Electrophoresis. Electrophoresis was carried out in a watercooled Warner-Chilcott electrophoresis apparatus using Whatman No. 3 MM paper and the following buffers:

1. 0.1 M Sodium phosphate buffer, pH 7.0 (4).
2. 0.1 M HCl-KCl buffer, pH 2.0.

A potential diference of 10.4 v/cm was applied for 3.5 hr.

Gas Chromatography. The gas chromatograph was a Pye series 104 Model 64 fitted with dual flame ionization detectors and a 2.1-m glass coil with a 4-mm bore packed with Chromosorb G.A.W.D.C.M.S. 85–100 mesh (Phase Separations, Rockferry, Cheshire, U. K.) coated with 5% Versamid 900. The column and injector head temperature was 240 C, and the detector oven temperature was 300 C. Nitrogen was used as carrier gas at a flow of 50 ml/min at 25 to 30 lb/in^2. Hydrogen and air flow rates were 50 and 500 ml/min, respectively. Samples were dissolved in acetonitrile.

Chromogenic Reagents. Compounds were routinely detected by means of *p*-dimethylaminocinnamaldehyde reagent, 10% solution (w/v) in a mixture of equal volumes of 37% HCl and ethanol. Other chromogenic tests involved the use of Ehrlich's reagent, after spraying the chromatogram was heated at 65 C for 3 min and examined under ultraviolet light as described by Poláček *et al.* (33), Procházka's reagent (16), ninhydrin reagent (39), ferric nitrate-nitric acid reagent (5% w/v solution of $Fe(NO_3)_3 \cdot 9H_2O$ in 1 N-HNO_3 [14]), 1% sodium bicarbonate (8), and aniline-phthalate reagent (4).

The release of SCN^- ions from indole glucosinolates was by the method of Gmelin and Virtanen (14), namely spraying with a 1-1 mixture of myrosinase solution and pH 7.0 buffer containing 0.002 M ascorbic acid, keeping the chromatogram between glass plates at room temperature for 2 hr, letting it dry, and spraying with ferric nitrate reagent.

Enzymatic Degradation of the Glucosinolates. Myrosinase was prepared from white mustard (*Sinapis alba,* L.) seeds according to the method of Neuberg and Wagner (32). Thiocyanate ions present in the enzyme preparation were removed by shaking for 30 min three times with Dowex 2-X4 in the chloride form (13).

The crystalline glucosinolate (between 30 and 50 mg) was dissolved in 1.5 ml of sodium phosphate buffer, pH 7.0, or citrate buffer, pH 4.0. One and one-half milliliters of myrosinase solution were added plus sufficient ascorbic acid to make the final solution 0.002 M. The mixtures were incubated at 37 C for 5 hr (pH 7.0) or 12 hr (pH 4.0). Appropriate controls lacking either the substrate or the enzyme were also included.

Estimation of Indole Glucosinolates. The glucosinolates were separated by paper chromatography in butanol-ethanol-water

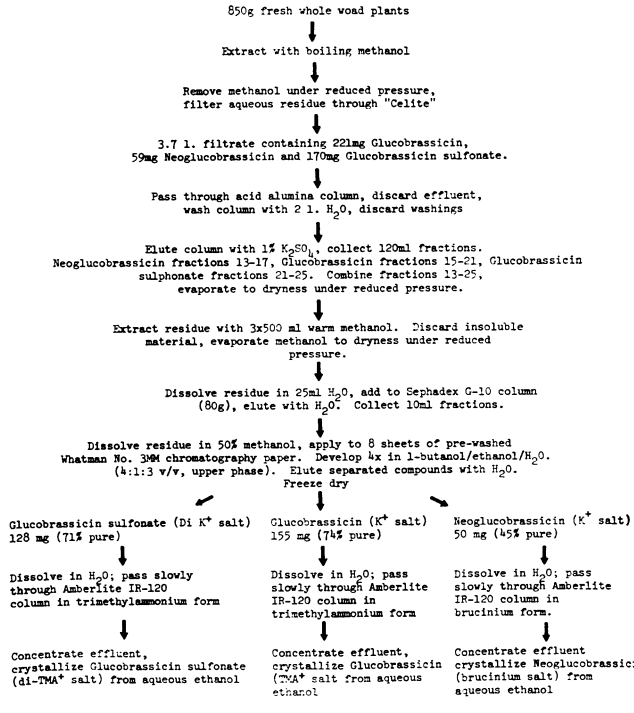

FIG. 2. Flow diagram of purification scheme used for isolation of the three indole glucosinolates of woad.

(4:1:3, upper phase), eluted from the paper by distilled water, and estimated by determining the quantity of thiocyanate ions released by degradation with myrosinase at pH 7.0 (29).

Ultraviolet Spectra. Absorbance values for the calculation of molecular extinction coefficients were determined on a Perkin-Elmer Model 350 spectrophotometer.

Infrared Spectra. Approximately 1 mg of crystalline glucosinolate was mixed with 300 mg of KBr and pressed into a disc which was used for determination of the spectra on a Perkin-Elmer Model 221 spectrophotometer.

Nuclear Magnetic Resonance Spectra. The material was dissolved in hexadeuterodimethylsulfoxide and the spectrum recorded on a Varian A60 spectrometer using tetramethylsilane as external standard.

RESULTS AND DISCUSSION

Paper chromatograms of the methanolic extract (at a loading equivalent of 80 mg fresh weight of plant material per cm) developed in solvent 1 revealed compounds reacting with pDAC at R_F 0.76, 0.63, 0.39, 0.34, and 0.18.

Compound 1. The compound at R_F 0.76 gave an instant blue color with pDAC, purple (rapidly fading) with Ehrlich's reagent, and blue with 1% sodium bicarbonate. The TLC behavior confirmed that the compound was isatan B which had previously been demonstrated in woad (8, 40, 41).

Compound 2. The compound at R_F 0.6 gave a rapidly developing blue-purple color with pDAC. The compound was eluted from the unsprayed part of the chromatogram, rechromatographed in solvent 2, and the eluate from this second chromatogram was examined further. The color reactions of the compound with pDAC, Ehrlich, Procházka, and ninhydrin reagents were identical with those of tryptophan. Solvents 1 to 9 (on paper or TLC as appropriate) failed to separate compound 2 from authentic tryptophan and finally the ultraviolet spectrum of compound 2 in distilled water (ϵ_{max} 273–274, 280, 288; ϵ_{min} 247, 286) was indistinguishable from that of tryptophan.

Compounds 3, 4, and 5. The compounds at R_F 0.39, 0.34, and 0.18 gave red-purple colors with pDAC reagent but the rate of development of the color at room temperature differed for each compound. Compound 3 gave a pink-purple color after 10 min, changing to red-purple after 25 min; compound 4 gave an instant pink-purple color, changing rapidly to red-purple, and compound 5 gave a pink-purple color after 12 min which became red-purple after 40 min.

The three compounds were eluted from the unsprayed portion of the chromatogram and their R_F values determined in solvents 1 to 5 (Table I).

The R_F values of compounds 3 and 4 suggested that they might be neoglucobrassicin and glucobrassicin (15) respectively, and their chromogenic reactions also supported these identifications (11, 33).

Compound 5, like compounds 3 and 4, gave a yellow-orange ultraviolet fluorescence after treatment with the Procházka reagent; this test is specific for compounds containing a skatyl residue (16). After heating the chromatogram sprayed with Ehrlich's reagent, the intense yellow-green ultraviolet fluorescence was found by Poláček *et al.* (33) to be characteristic of glucobrassicin and neoglucobrassicin among the indoles tested. Upon spraying with ferric nitrate reagent in test 4, the instant red-brown color indicates that thiocyanate ions have been released by the action of myrosinase upon the compounds. Although upon the basis of this chromatographic and chromogenic evidence, compounds 3 and 4 appeared to be neoglucobrassicin and glucobrassicin, respectively, whereas compound 5 might be a new indole glucosinolate of a more hydrophyllic character than glucobrassicin; conclusive identifications required that pure crystalline specimens of the indole glucosinolates be obtained. This was achieved by the scheme shown in Figure 2.

The melting points, ultraviolet spectra, and enzymic decomposition products of the isolated salts of compounds 3 and 4 confirmed them to be neoglucobrassicin and glucobrassicin. As Ettlinger and Kjaer (9) have observed, N-oxygenated compounds are unfamiliar to most chemists; however, they point out that two alkaloids from *Gelsemium* have been shown to be N-methoxyoxindoles (36, 45), and Morimoto and Oshio (30) have identified 1-methoxy-N,N-dimethyltryptamine (lespedamin) as a natural product from *Lespedeza bicolor* var. japonica. Ettlinger and Kjaer (9) concluded that the structure of neoglucobrassicin was probable but not proved. The isolation in this work of pure salts of glucobrassicin and neoglucobrassicin made possible a check on the position of the methoxy substituent in the neoglucobrassicin molecule by NMR spectroscopy. The complex brucinium salt of neoglucobrassicin was undesirable for NMR studies, so that cation was removed as described by Kutáček and Kefeli (20), by filtering a solution of the salt through an SE-Sephadex C-25 column in the K^+ form. The filtrate was freeze-dried and the neoglucobrassicin (K^+) powder used for NMR studies.

The low field portion of the NMR spectrum of glucobrassicin (TMA^+) dissolved in hexadeuterodimethylsulfoxide (Fig. 3a) contains a one-proton peak (presumed from comparison with spectra of other three-substituted indoles to be the indole N-H signal) at -0.9 τ (10.9 δ) and a five-proton group (remainder of the indole ring protons) between 2.2 and 3.0 τ

Table I. *Chromatographic Properties of Indole Glucosinolates in Woad*

Solvent	R_F Values of Compound		
	3	4	5
1-Butanol-acetic acid-H_2O	0.39	0.34	0.18
2-Propanol-10% NH_4OH	0.47	0.32	0.22
Pyridine-1-pentanol-H_2O	0.62	0.59	0.42
1-Butanol-pyridine-H_2O	0.68	0.64	0.50
20% KCl	0.76	0.76	0.81
Chromogenic Test	Color		
Procházka's (ultraviolet fluorescence)	Yellow-orange	Yellow-orange	Yellow-orange
Ehrlich's plus heat (ultraviolet fluorescence)	Yellow-green	Yellow-green	Yellow-green
$Fe(NO_3)_3$-HNO_3	Brownish	Blue-purple	Blue-purple
Myrosinase, pH 7.0 + ascorbic acid, then $Fe(NO_3)_3$-HNO_3	Red-brown	Red-brown	Red-brown

(7.0–7.8 δ). After addition of a drop of D_2O the signal at −0.9 τ disappeared, confirming that it represented the indole N-H, whereas the signals for the 2.2 to 3.0 τ group remained unchanged. The spectrum of neoglucobrassicin (K^+) in hexadeuterodimethsulfoxide (Fig. 3b) showed no peak in the region below 2.2 τ and therefore lacked the indole N-H proton, as is also true for glucobrassicin-1-sulfonate (Fig. 3c) (see "Discussion" in reference 7). Confirmation that the methoxy group was attached to the indole nitrogen was provided by the fact that the $-OCH_3$ signal of neoglucobrassicin (not shown in figure) occurs at 5.9 τ (4.1 δ) instead of at 6.2 τ (3.8 δ) which is characteristic of the more common 5- or 6-*O*-methyl indoles. The $-OCH_3$ signal of 1-methoxy-N,N-dimethyltryptamine dissolved in CCl_4 occurs at 6.0 τ (30). Thus figure 1b is correct for neoglucobrassicin, and Gmelin and Virtanen's (15) identification is confirmed.

The TMA^+ salt of compound 5, obtained crystalline as small white plates from aqueous ethanol, decomposed at temperatures above 156 C. The evidence demonstrating that it is unusual in being an N-sulfonated indole, namely glucobrassicin-

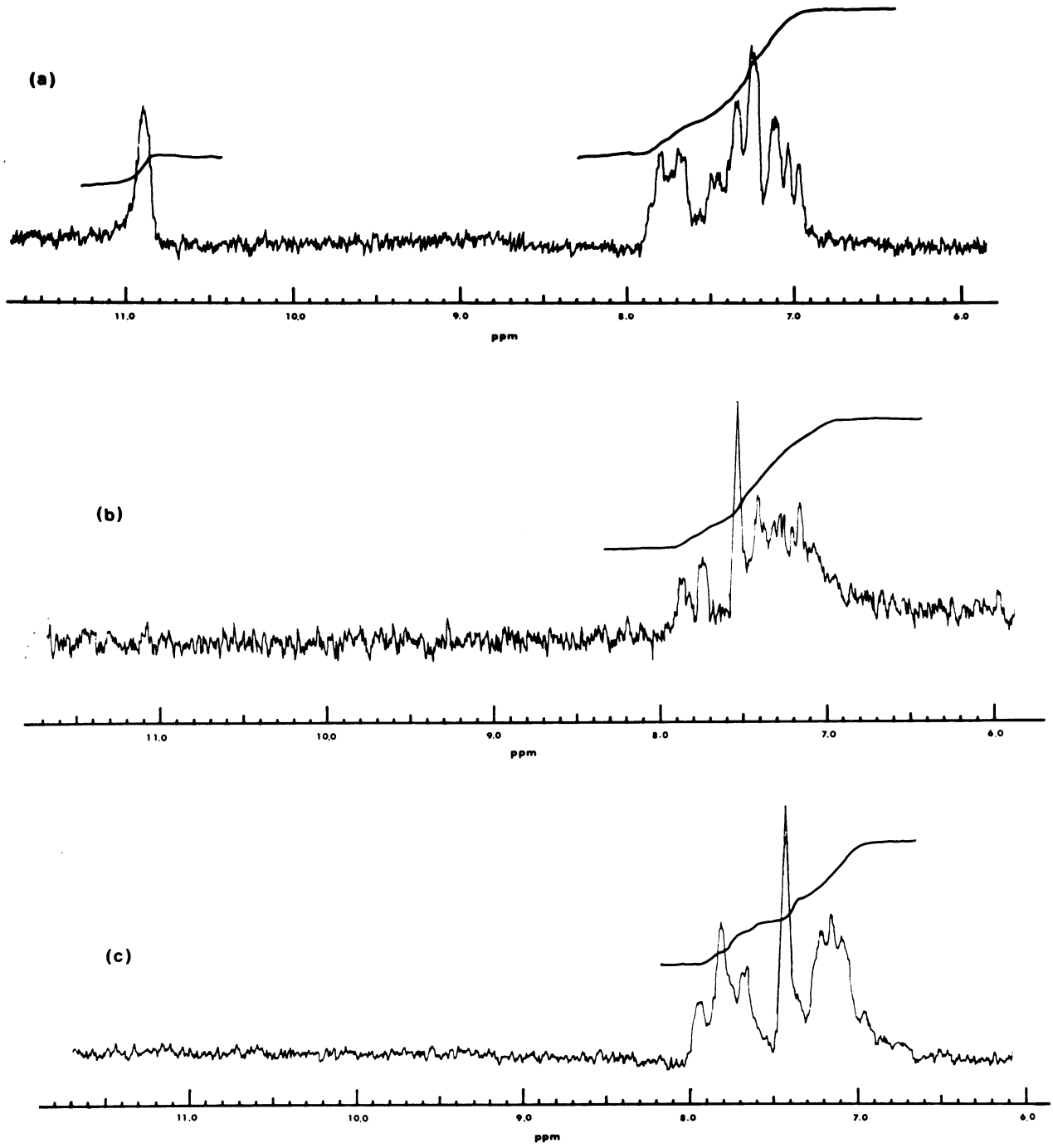

FIG. 3. Low field portions of nuclear magnetic resonance spectra in hexadeuterodimethyl sulfoxide of a: glucobrassicin (TMA^+); b: neoglucobrassicin (K^+); and c: glucobrassicin-1-sulfonate (di-TMA^+).

1-sulfonate (Fig. 1c), has been published elsewhere (7). Additional data supporting this structure assignment are given below.

Enzymic degradations of compound 5 with myrosinase were carried out, using 50-mg samples of the crystalline material, at pH 7.0 and pH 4.0. After incubation, 10-μl aliquots from each mixture were applied to Whatman No. 4 chromatography paper. Chromatograms were developed for 36 hr in each of solvents 1, 2, and 3. The products of the enzymic degradations at pH 7.0 and pH 4.0 each contained a single spot which reacted with aniline phthalate reagent. In each solvent the color and migration value were identical with that of the glucose marker.

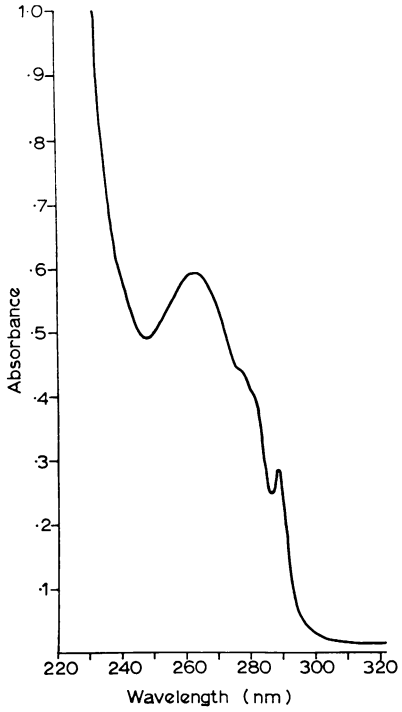

FIG. 4. Ultraviolet absorption spectrum of glucobrassicin-1-sulfonate (di-TMA$^+$) in water (9.75×10^{-5} M; 1-cm path length).

Table II. *Paper Electrophoresis of Indole Derivatives*

Buffer	Electrophoretic Migration[1]		
	Glucobrassicin	*Compound 5*	*IAA*
pH 7.0 Phosphate	7.5	14.5	11.5
pH 2.0 KCl/HCl	7.6	14.5	0.3

[1] Migration expressed in cm moved towards anode in 3½ hr at a potential gradient of 10.4 v/cm.

In order to demonstrate the release of SCN^- ions by the action of myrosinase on compound 5, 5-μl aliquots of the pH 7.0 incubation mixture were applied to cellulose thin-layer plates, and the chromatograms were developed in solvents 1, 2, and 3. In each case, upon spraying with ferric nitrate-nitric acid reagent, SCN^- was detected by an instant red-brown spot corresponding in color and R_F value with an SCN^- marker (applied as KSCN). The release of sulfate by the enzyme at pH 7.0 and pH 4.0 was demonstrated by formation of a white precipitate upon addition of $BaCl_2$ solution to a small sample of the incubation mixture after precipitation of the enzyme with ethanol.

The ultraviolet spectrum (Fig. 4) of glucobrassicin-l-sulfonate in water differs from those of the common three-substituted indoles (*e.g.*, glucobrassicin, tryptophan) in that it possesses an additional peak at 262 nm, and closely resembles spectra 3, 4 and 3, 7 disubstituted indoles. The infrared spectrum of compound 5 (Fig. 5) closely resembles that of glucobrassicin (14) but differs in the fingerprint region and in lacking the indole N-H stretching band at 3480 cm^{-1}.

Paper electrophoretic migration values for the compound (Table II) were in agreement with the concept that it differed from glucobrassicin by the possession of an additional strongly acidic group, since compound 5 migrated almost twice as fast towards the anode as did glucobrassicin at pH 7.0 and at pH 2.0. At the latter pH, ionization of weakly acidic groups such as the carboxyl group of IAA is almost completely suppressed.

Chromatograms of 20-μl aliquots of the myrosinase degradation products (pH 7.0 and pH 4.0) developed in solvent 1 and sprayed with pDAC reagent possessed spots at R_F 0.42, 0.64, and 0.75, and the pH 4.0 incubation mixture contained an additional and particularly strong spot at R_F 0.88. These degrada-

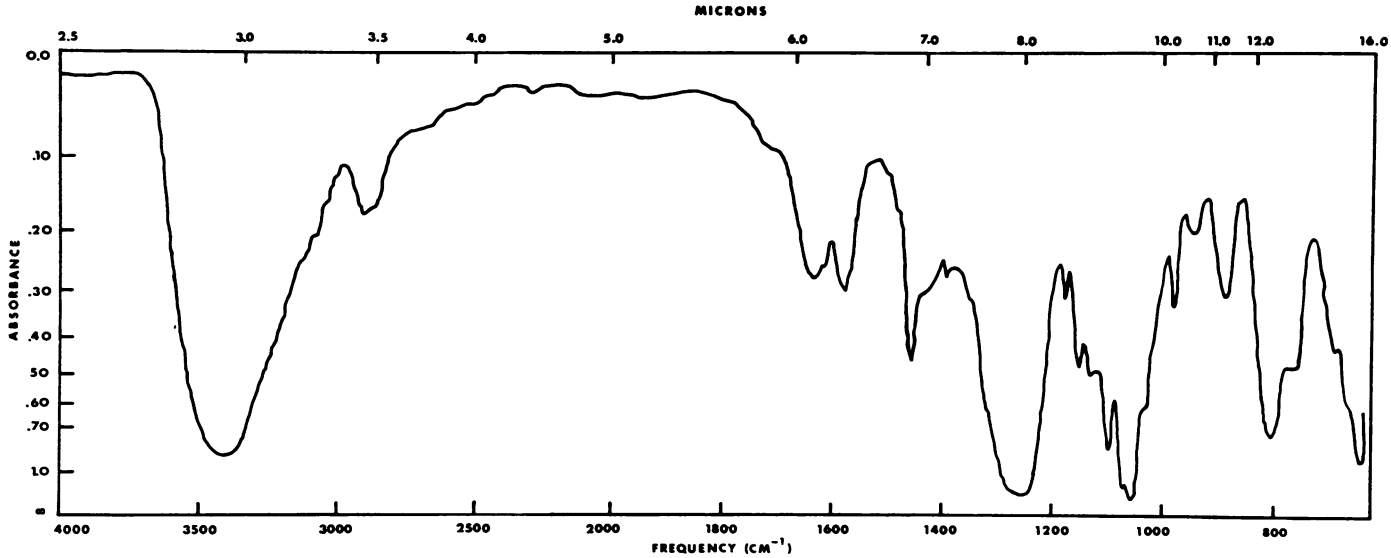

FIG. 5. Infrared absorption spectrum of glucobrassicin-1-sulfonate (di-TMA$^+$) in KBr.

tion products possessed ultraviolet spectra having the same shape as the spectrum of compound 5 itself. The compounds were clearly (on the basis of their R_F values) more hydrophyllic than the indolic products of glucobrassicin degradation by myrosinase and were shown by paper electrophoresis to bear a strongly acidic grouping, in contrast to the neutral degradation products of glucobrassicin (14).

Assuming that compound 5 was an indole ring-substituted glucobrassicin derivative, it was possible to calculate that the group constant, R_M (16), for the expected 1-sulfonic acid substituent in solvent 1 was +0.58. Using this value, and assuming that the indolic degradation products from compound 5 were analogous to those from glucobrassicin, the calculations indicated that the compounds were the 1-sulfono analogues of ascorbigen (R_F 0.42), of di-indolylmethane (R_F 0.64), of 3-hydroxymethylindole (R_F 0.75), and of IAN (R_F 0.88). The compounds were separated and purified by paper chromatography prior to further examination.

The putative IAN analogue was hydrolyzed in 0.1 N HCl at 100 C for 1 hr; at the end of this time the compound had been completely degraded to yield IAN (proved by identity of its ultraviolet spectrum, chromogenic reactions, R_F values in solvents 1 and 9, and gas chromatographic retention time with those of authentic IAN) plus free sulfate (identified by precipitation as barium sulfate and by its giving a positive rhodizonate reaction). The putative di-indolylmethane and 3-hydroxymethylindole analogues were found to yield di-indolylmethane and 3-hydroxymethylindole respectively (identified by TLC) on mild acid hydrolysis, and the putative ascorbigen analogue was found to yield ascorbic acid when acid hydrolyzed on the paper chromatogram (16). Thus, the degradation pattern of glucobrassicin-1-sulfonate appears to be completely analogous to that of glucobrassicin itself, and since this includes thiocyanate, glucobrassicin-1-sulfonate is a new goitrogen.

In view of recent demonstrations of the production of simple indole compounds by epiphytic bacteria (24), it should be stressed that the compounds described in this paper are readily detectable in extracts of light-grown sterile cultured woad seedlings. The concentrations of the glucosinolates vary considerably between the organs of the plant; neoglucobrassicin is high in the root and low in the shoot, as previously noted by Kutáček (20), whereas glucobrassicin-1-sulfonate is high in the shoot and absent from the root. These differences further support the independent natural occurrence of the three glucosinolates. Moreover, all three can be detected in boiling water extracts, hence under conditions where methanolysis or N-substitution is unlikely to occur. Observations of variations with age, time of year, and cultural conditions will be described elsewhere.

Our observations confirm the paper chromatographic evidence of Schraudolf (38), which appeared while this work was in progress, that glucobrassicin and neoglucobrassicin are present in *I. tinctoria*. It has now become clear that glucobrassicin and neoglucobrassicin are very widely distributed among the members of the *Cruciferae* (22) and, in fact, Schraudolf (38) found indoleglucosinolates in 49 out of the 50 species of crucifers examined. In addition, glucobrassicin and neoglucobrassicin have been detected in plants of the families *Capparidaceae, Tovariaceae,* and *Resedaceae* (37).

We are not yet able to say how widespread glucobrassicin-1-sulfonate is in higher plants. Schraudolf (38) in his study of the *Cruciferae* reported the widespread presence of low concentrations (below the limit of detection with pDAC reagent) of a compound which is labeled by feeding ^{14}C indole and ^{35}S sulfate and which has the same R_F as glucobrassicin-1-sulfonate in solvent 1. Further work may show that this compound is identical with glucobrassicin-1-sulfonate. It is noteworthy that Schraudolf failed to find his compound in *I. tinctoria*, but since he studied etiolated seedlings which we shall show elsewhere to synthesize very little glucobrassicin sulfonate as compared with light-grown tissues, this may explain the apparent absence of the compound. Woad itself, although it has been used in China as a food in time of scarcity (2), does not seem to have wide use as food or fodder at present. If, as Schraudolf's data implies, glucobrassicin-1-sulfonate is widely distributed among eminently edible plants, the goitrogenic properties of glucobrassicin-1-sulfonate may have nutritional significance.

The role of indole glucosinolates in plant metabolism remains uncertain. Kutáček and Kefeli (21) suggested that glucobrassicin may be able to act as a precursor of indole auxins at certain stages of the life cycle of the plant (*e.g.*, during very rapid growth such as "bolting" of the flower shoot). The studies reported here are preliminary to further consideration of this and other questions in a later publication.

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