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A NOVEL SULPHONATED NATURAL INDOLE

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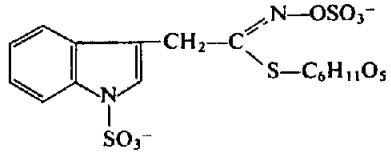
Abstract—A new indole glucosinolate isolated from *Isatis tinctoria* L., has been identified as 1-*sulpho*-3-*indolylmethylglucosinolate*.

INTRODUCTION

DURING studies of the indole metabolism of woad (*Isatis tinctoria* L., Cruciferae), crystalline specimens of 3-indolylmethylglucosinolate (glucobrassicin)[1] as the tetramethylammonium salt, 1 methoxy-3-indolylmethyl glucosinolate (neoglucobrassicin)[2] as the brucine salt and of a new indole glucosinate (as the ditetramethylammonium salt) were isolated. We present evidence here that the new substance has the novel structural feature of being an indole sulphonate and is 1-sulpho-3-indolylmethylglucosinolate (I).



1-sulpho-3-indolylmethylglucosinolate (I)

RESULTS AND DISCUSSION

The three indole glucosinolates were first detected on paper chromatograms of extracts of freeze-dried whole woad plants, prepared by immersing the plant material in cold (−15°) 80 per cent methanol for 18 hr. The filtered extracts were concentrated under reduced pressure and chromatographed by descent on paper (*n*-butanol/acetic acid/water, 4:1:2, v/v) without further purification. The chromatograms, sprayed with *p*-dimethylaminocinnamaldehyde reagent[3] (pDAC), revealed spots at R_f 0·34, 0·39 and 0·18, corresponding to glucobrassicin, neoglucobrassicin and the new compound (I). In subsequent studies, when the fresh plant material was extracted by boiling in absolute methanol for 2 min, an identical pattern of *p*DAC positive spots was obtained. These observations establish the presence of the three indole glucosinolates in the living plant and this was further substantiated when all three

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[2] R. GMELIN and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 1378 (1962).
[3] J. HARLEY-MASON and A. A. ARCHER, *Biochem. J.* **69**, 60P (1958).

compounds were found in sterile-cultured woad seedlings. Thus, they are natural constituents of the woad plant and are not produced by microbial contaminants.[4]

Glucobrassicin-sulphonate crystallized as its ditetramethylammonium (TMA^+) salt in white plates which decomposed at temperatures above 156°. The crystals were difficult to maintain in an analytically pure state because they rapidly darkened and became resinous in air. This behavior corresponds to that observed for the barium salt of indole-1-sulphonic acid synthesized by Terentiev and Zymbal.[5] After drying at 90° for 12 hr under high vacuum, the composition of the anhydrous substance was, according to elemental analysis: (Found: C, 42·1; H, 6·51; N, 7·78; S, 13·4. $C_{24}H_{42}N_4O_{12}S_3$ (674·81) required: C, 42·7; H, 6·27; N, 8·30; S, 14·2 per cent).

Its u.v. spectrum in water showed maxima at 288 ($\epsilon = 2850$), 277 ($\epsilon = 4440$), 262 ($\epsilon = 6100$) and 220 nm and minima at 286 ($\epsilon = 2330$) and 247 ($\epsilon = 5080$) nm and differed from that of glucobrassicin by the possession of the maximum at 262 nm. The spectrum did not change in acidic or basic solution.

The i.r. spectrum of I closely resembled that of glucobrassicin,[1] but lacked the indole N–H stretching band at 3480 cm^{-1} and showed minor differences in the finger-print region. Incubation of I with myrosinase at pH 7·0 resulted in the release of glucose, sulphate and thiocyanate ions, a clear indication of its glucosinolate nature.

All attempts to produce derivatives of I which would yield a parent peak in the mass spectrometer were unsuccessful. A comparison of the rates of elution of I and glucobrassicin through a Sephadex G-10 column indicated that I had a somewhat higher molecular weight. The paper chromatographic properties of I showed that it was considerably more hydrophilic than glucobrassicin and, upon paper electrophoresis at pH 7·0, the compound migrated about twice as fast as glucobrassicin toward the anode. The rates of migration were unchanged when the pH 7·0 buffer was replaced by a buffer of pH 2·0. These data suggest that the new compound bears a strongly acidic substituent.

The indolic products of degradation of I by myrosinase at pH 7·0 and pH 4·0 possessed u.v. spectra having the same general shape as that of I itself. They proved to be analogous to the indolic degradation products of glucobrassicin[1] but were more hydrophilic and were shown, by thin-layer electrophoresis, to bear a strongly acidic grouping (in contrast to the neutral degradation products of glucobrassicin).[1]

The congener of indoleacetonitrile (IAN) produced by incubation of I with myrosinase at pH 4·0, was separated from the mixture, purified and hydrolysed in 0·1 N HCl at 100°. After 1 hr the compound had been completely degraded to give IAN (plus a little indoleacetic acid) and sulphate (demonstrated by precipitation as barium sulphate).

The presence of a sulphur-containing moiety on the non-glucosinolate part of the molecule of I was confirmed by isolating I from plants which had been fed $^{35}SO_4^{2-}$. The action of myrosinase in pH 7·0 buffer in the presence of 0·002 M ascorbic acid on labelled I resulted in the release of $^{35}SO_4^{2-}$, $^{35}SCN^-$ and a ^{35}S-labelled analogue of ascorbigen.

The ease with which the SO_4^{2-}-producing moiety was completely removed from the IAN congener from I by acid hydrolysis precluded the possibility of the substituent being attached by covalent bonds to carbon of the benzene ring and suggested that the indole nitrogen was the site of attachment. This suggestion would explain the observation that, whereas glucobrassicin gives an instant pink-purple spot in the cold with *p*DAC reagent, I gives a colour only after standing for 15 min. Presumably the sulphate group prevents the migration

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[5] A. P. TERENTIEV and L. V. ZYMBAL, *Compt. Rendus Dokl. Acad. Sci. URSS* **55**, 833 (1947).

of the lone electron pair from the nitrogen atom which is required for the electrophilic attack of the *p*DAC on the 2 position of the indole ring.[6,7] The action of the concentrated acid in the reagent[3] will remove the substituent group slowly in the cold and permit the reaction to proceed.

Final confirmation of the position of substitution was provided by a comparison of the NMR spectrum of I with that of glucobrassicin. The spectrum of the tetramethylammonium salt of glucobrassicin dissolved in hexadeuterodimethyl sulphoxide revealed a one-proton peak (presumed from comparisons with spectra of other 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 (7·0–7·8 δ). After addition of D_2O the signal at –0·9 τ disappeared, confirming that this represented the indole N–H, whereas the signals for the 2·2–3·0 τ group remained unchanged. The spectrum of I in hexadeuterodimethyl sulphoxide showed no peak in the region below 2·2 τ and no change on addition of D_2O in the region below 4·5 τ, indicating the the indole N–H proton was absent. Furthermore, the peak at 2·74 τ in the glucobrassicin spectrum which is assigned to the 2-H of the indole ring (cf. Ref. 8) moved downfield to 2·62 τ in the spectrum of I, presumably due to the de-shielding effect of the sulphonic acid attached to the nitrogen atom.

This is the first identification of an indole-1-sulphonate from plants. Erspamer *et al.*,[9a,9b] in 1967, reported the isolation, from skin of *Bufo alvarius* Girard and other species, of two indole compounds from which sulphuric acid was released on mild acid hydrolysis; on this basis the compounds were tentatively identified as 5-*O*-methylbufotenine-1-sulphonic acid and bufoviridine (believed to be bufotenine-1-sulphonic acid).

The concentration of the new compound in the woad plant varies according to age, conditions of growth and the organs under examination (as we shall describe elsewhere). In the leaves of the plants from which the isolation was made, the concentration of glucobrassicin sulphonate was about 70 mg/100 g fresh weight.

In a publication which appeared during the progress of this work, Schraudolf[10] remarked on the widespread occurrence in the Cruciferae of a compound, R_f 0·20–0·22, in our solvent which incorporated ^{35}S from $^{35}SO_4^{2-}$ and ^{14}C from indole ^{14}C. This may indicate a broader distribution of I. However, he failed to detect his substance in *I. tinctoria*, possibly due to his use of etiolated tissue (this will be discussed elsewhere). The metabolic significance and widespread occurrence of glucosinolates in plants have been discussed in recent reviews.[11–13]

EXPERIMENTAL

Plant Material

Woad plants were grown in Yale University's Marsh Botanic Garden from seed obtained from the herb garden of Sissinghurst Castle, Kent, or from Thompson & Morgan (Ipswich) Ltd.

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[7] R. M. ACHESON, *An Introduction to the Chemistry of Heterocyclic Compounds*, p. 155, Interscience Publishers, New York (1967).

[8] Spectrum No. 582, in *High Resolution NMR Spectra Catalogue*, Vol. 2. Varian Associates, Palo Alto (1963).

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Isolation Procedure

Fresh plant material was immersed in boiling MeOH for 2 min and left in cooled MeOH overnight before filtration. The plant residue was ground and extracted with warm 80% MeOH (×2). MeOH was removed from the combined filtrates under reduced pressure and the solution passed through an acid alumina (Fisher Scientific, Inc.) column. The effluent was discarded and the indole glucosinolates eluted by 1% K_2SO_4. Fractions were examined by TLC and those containing the new compound (I) were evaporated to dryness under reduced pressure and the residue extracted with hot MeOH. After removal of the MeOH the residue was dissolved in H_2O applied to a Sephadex G-10 (Pharmacia, Inc.) column which was eluted with H_2O. The fractions containing I were freeze-dried and the solid purified by chromatography on Whatman 3MM paper with BuOH/EtOH/H_2O (4:1:3 v/v upper phase). The band of I was eluted by water and, after freeze-drying, gave a white powdery residue of the diK^+ salt of I. This material was dissolved in a small volume of water and the solution passed through an Amberlite IR-120 column in the TMA^+ form. The effluent contained the di-TMA^+ salt of I which crystallized from aqueous ethanol.

Enzymic Degradation

Myrosinase solution was prepared from mustard seeds[14] (*Sinapis alba*, L.) and incubations were carried out in buffer of pH 7·0 and pH 4·0 at 37°.

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