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TWO NATURAL INDOLE GLUCOSINOLATES FROM BRASSICACEAE

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Key Word Index—Cruciferae; Brassicaceae; callus culture; indole glucosinolates; HPLC; mass spectrometry.

Abstract—Four indole glucosinolates were isolated from different cruciferous tissues and tissue cultures. After separation in the form of their desulfo derivatives, they were separated by HPLC and analysed by mass spectrometry. Besides 3-indolylmethylglucosinolate and 1-methoxy-3-indolylmethylglucosinolate, two new compounds were identified as 5-hydroxy-3-indolylmethylglucosinolate and 5-methoxy-3-indolylmethylglucosinolate, respectively. Feeding experiments revealed that [ring-2-^{14}C]tryptophan was a precursor of all of these glucosinolates and that [Me-^{14}C]methionine serves as the methyl donor of the methoxylated derivatives.

INTRODUCTION

Indole glucosinolates (Fig. 1) have been found in most seedlings of Brassicaceae (Cruciferae), Capparidaceae, Tovariaceae and Resedaceae [1], whereas other glucosinolate-containing taxonomic groups (e.g. Moringaceae [2], Tropaeolaceae, Caricaceae [3]) are free of hetero aromatic mustard oil glucosides. The distribution of these secondary plant products has provided useful information for chemotaxonomic considerations. It has been postulated, however, that the content as well as the distribution patterns of these compounds may depend on the plant organs analysed [4] and the developmental state of the investigated plant [5].

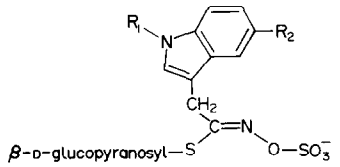

Fig. 1. Structures of natural indole glucosinolates.

Selection for low contents of glucosinolates in seeds and vegetative tissues is of high priority in breeding programmes since these compounds may possess undesirable physiological properties [6–8]. The determination of individual and total glucosinolate contents in plant materials is thus of high importance and a variety of different analytical methods have been proposed (see reviews refs. [9, 10]).

Three indole glucosinolates have been identified to date. Besides glucobrassicin [11] and its *N*-methoxy derivative, neoglucobrassicin [12], Elliott and Stowe [13] have isolated sulfoglucobrassicin from woad tissues. We here report on the isolation and identification of two other indole glucosinolates present in different species of the Brassicaceae.

RESULTS AND DISCUSSION

In order to find suitable material for a reinvestigation of indole glucosinolate biosynthesis, different tissues of brassicaceous plants have been analysed for their ability to synthesize indole glucosinolates or their presumed precursors. By analogy with previous observations, TLC analyses of methanolic extracts from various Brassicaceae were found to contain L-tryptophan, glucobrassicin (3-indolylmethylglucosinolate = GL) and neoglucobrassicin (1-methoxy-3-indolymethylglucosinolate = NGL). Additionally, the indole derivatives sulfoglucobrassicin (1-sulfo-3-indolylmethylglucosinolate = SGL) and isatan B were detected in woad leaves. Under the experimental conditions (amount of extracted tissues, specific activity of precursors) no other indole derivatives reported to occur in Brassicaceae could be detected.

Generally, TLC analysis of crude extracts is insufficient because of the high proportions of other compounds present. We, therefore, pretreated the extracts by a combination of ion exchange chromatography and sulfatase-treatment as described by Thies [14] and Heaney and Fenwick [15]. By this method, pure preparations of the corresponding desulfoglucosinolates were obtained, with the exception of SGL which remained bound to the DEAE-Sephadex due to its negative charge. The pre-purified material could be further analysed by TLC and HPLC (Table 1). Following this method, TLC separation of extracts from broccoli and cauliflower callus cultures, as well as from kohlrabi seedlings, revealed the existence of an unknown compound with an R_f value of 0.50 in the CEL 400 system.

Table 1. Chromatographic properties of desulfo indole glucosinolates

	TLC (R_f values)		HPLC (R_t min)
	Si gel	CEL 400	
DGL	0.50	0.65	8.0
DNGL	0.55	0.80	16.5
DSGL	0.30	—	—
DHGL	0.40	0.50	4.0
DMOGL	0.50	0.65	12.0

For details of chromatographic solvents see Experimental.

Table 2. Partial data of the mass spectra (EI, 70 eV) of desulfo indole glucosinolates

	M^+	a	b	c
DGL	368(2)	130(93)	156(70) 155(91)	172(18)
DNGL	398(4)	160(64)	186(80)	202(64) 171(31) 145(33) 155(93) 204(9)
DHGL	—	146(14) 145(47)	172(40) 171(45)	188(32)
DMOGL	—	160(34)	186(91)	204(7) 171(100) 145(15) 202(5)

The intensity relative to the major ion is in parentheses. **a** = R^+; **b** = R–C ≡ N^+; **c** = R–CH = N–OH^+; R = indolylmethyl.

For the preparative isolation and identification of the desulfo indole glucosinolates a HPLC system was developed. Using reversed phase chromatography, with a water–methanol system as described in the Experimental, an excellent separation of desulfo indole glucosinolates was possible. Under these conditions, four peaks were detected and two of these were identified as the desulfo derivatives of GL and NGL, respectively, by comparison with authentic compounds.

All four substances were isolated and subjected to mass spectrometry. Since thermal instability prevented the formation of M^+ EI-mass spectrometry, fragment ions have to be used for interpretation [16, 17]. Mass spectra and intensities of the isolated desulfo glucosinolates are summarized in Table 2. For DGL, they correspond with those reported for glucobrassicin [16]. NGL is characterized by the existence of a methoxy group (+30 a.m.u.), methyl fragmentations (*m/z* 145 $[\mathbf{a}-15]^+$ and *m/z* 171 $[\mathbf{b}-15]^+$, respectively), and a formaldehyde fragmentation (*m/z* 155 $[\mathbf{b}-31]^+$). This formyl fragmentation does not occur with DMOGL which otherwise corresponds to DNGL. Thus, it is highly probable, that a methoxylation take place on a heterocyclic carbon. Since the *m/z* of DHGL differs from that of DGL by 16 a.m.u., the existence of a heterocyclic hydroxyl group corresponding to the methoxyl group of DMOGL is reasonable. We, therefore, postulate the existence of *C*-hydroxyglucobrassicin (HGL) in Brassicales. The existence of the predominating MOGL (Table 1) was obscured by the fact that its R_f corresponds to that of GL in most chromatographic systems; the very low amount of HGL in most investigated tissues (Table 3) prevented its identification by the analytical procedures applied in indole glucosinolate analysis until now.

Table 3. Content of indole glucosinolates in cruciferous tissues (nmol/g fr. wt)

	DGL	DNGL	DHGL	DMOGL
Broccoli				
roots	120	140	10	150
callus	130	80	40	250
Cauliflower				
callus	220	10	20	110
Kohlrabi				
seedlings	110	320	100	50
callus	180	70	6	370
Woad				
callus	70	60	—	10

D, L-[ring-2-^{14}C]tryptophan was incorporated into all indole glucosinolates found in Brassicaceae, whereas L-[Me-^{14}C]methionine labeled exclusively NGL and MOGL. Since we have good indications that the indole glucosinolate-synthesizing enzyme system has only low specificity for variously substituted precursors (e.g. an *in vivo* synthesis of 7-bromoglucobrassicin after application of DL-7-bromotryptophan is possible in *Sinapis* seedlings), the biogenetic pathway tryptophan → hydroxytryptophan → HGL → MOGL seems reasonable. Since to date the hydroxylation in position 5 of the indole ring is proven for many tryptophan derivatives [18–20], and since even the existence of 5-methoxy derivatives has been shown in plant tissues (e.g. 1,5-dimethoxygramin [21]), it is very probable that the new compound described here is also hydroxylated at position 5 of the indole ring.

EXPERIMENTAL

Plant material. Seeds on kohlrabi (*Brassica oleracea* L. cv *acephala* var. *gongylodes* L.) were supplied by Ruggaber, broccoli (*Brassica oleracea* L. cv *botrytis* var. *italica* Plenk) and cauliflower (*Brassica oleracea* L. cv *botrytis* var. *botrytis* L.) by Maier's Samen. Seeds of woad (*Isatis tinctoria*) were a gift of the Botanical Garden, Giessen.

Tissue cultures were isolated by standard procedures from seedlings, leaves and tubers, respectively, and cultivated on an agar medium according to ref. [22] as modified in ref. [23]. Root cultures were isolated from root regenerates of the respective tissue cultures and kept in a liquid medium without auxins.

Extraction. Tissues (1–10 g fr. wt) were extracted in boiling MeOH [4]. After addition of 9 ml H_2O to 1 ml MeOH extract, the glucosinolates were adsorbed on a 10 × 5 mm column of DEAE-Sephadex A-25 [14, 15]. After hydrolysis with sulfatase (*Helix pomatia*, Sigma Chemical Co.) the resulting desulfo glucosinolates were eluted with H_2O and evaporated under red. pres. The desulfuration product of sulfoglucobrassicin could be eluted with 3% K_2SO_4.

TLC. Plates of MN-CEL 400 (solvent: *n*-BuOH–HOAc–H_2O, 4:1:2) and MN Sil G [solvent: *iso*-PrOH–MeOAc–NH_4OH (27%), 7:9:4] were used. All indoles were detected by means of *p*-dimethylaminocinnamaldehyde reagent (1%) in a 1:1 mixture of conc. HCl and EtOH.

HPLC. The column (300 × 3.9 mm) was packed with μBondapack C_{18} (Waters Associates). Separations were accomplished by gradient elution: 15 min linear from 20 to 40% MeOH in H_2O with a flow rate of 2 ml/min. Indole compounds were

detected by UV (280 nm) and radioactive compounds with a heterogenous monitor system using a 50 μl glass scintillator cell.

Mass spectra were EI and recorded at 70 eV.

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NOTE ADDED IN PROOF

After submission of the manuscript, Truscott *et al.* [Truscott, R. J. W., Burke, D. G. and Minchinton, I. R. (1982) *Biochem. Biophys. Res. Commun.* **107**, 1258 and 1368) reported the isolation of two novel indole glucosinolates (4-hydroxy-3-indolylmethyl- and 4-methoxy-3-indolylmethylglucosinolate) from cabbage and rape seed, respectively, which probably are identical with the compounds described here.