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GLUCOSINOLATES IN *SESAMOIDES CANESCENS* AND *S. PYGMAEA*: IDENTIFICATION OF 2-(α-L-ARABINOPYRANOSYLOXY)-2-PHENYLETHYLGLUCOSINOLATE

OLE OLSEN, KIM WEDEL RASMUSSEN and HILMER SØRENSEN

Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Copenhagen V, Denmark

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Key Word Index—*Sesamoides canescens*; *S. pygmaea*; Resedaceae; 2-(α-L-arabinopyranosyloxy)-2-phenylethylglucosinolate; 2-hydroxy-2-phenylethylglucosinolate; 2-phenethylglucosinolate; *N*-(2-(α-L-arabinopyranosyloxy)-2-phenylethyl)thiourea; 3-(α-L-arabinopyranosyloxy)-3-phenylpropionitrile; 3-hydroxy-3-phenylpropionic acid; chemotaxonomy; metabolism.

Abstract—A new natural product, 2-(α-L-arabinopyranosyloxy)-2-phenylethylglucosinolate, has been isolated from *Sesamoides canescens*. This glucosinolate together with 2-hydroxy-2-phenylethylglucosinolate and 2-phenethylglucosinolate in a 1:1:1 ratio constitutes about 90% of the total glucosinolate pool in green parts of the plant. Phenethylglucosinolate constitutes about 70% of the total glucosinolate pool in green parts of *S. pygmaea* together with minor amounts of the two other glucosinolates. In addition, both plants contain at least seven other glucosinolates. The structure of the new natural product has been confirmed by transformations into D-glucose, L-arabinose, *N*-(2-(α-L-arabinopyranosyloxy)-2-phenylethyl)thiourea, 3-(α-L-arabinopyranosyloxy)-3-phenylpropionitrile and 3-hydroxy-3-phenylpropionic acid, respectively. The significance of this investigation is briefly discussed in relation to the methods used in glucosinolate analysis, chemotaxonomy and possible catabolic transformation of glucosinolates into amines.

INTRODUCTION

The Resedaceae consists of six genera among which are *Sesamoides* and *Reseda*. The nearest related families seem to be Capparidaceae and Cruciferae [1, 2]. In previous communications, *Reseda* species have been investigated for intact glucosinolates and amines, which are considered as catabolic products thereof [3–5]. Appreciable amounts of glucosinolates were found in all of the investigated *Reseda* species whereas appreciable amounts of amines, structurally related to the glucosinolates, were found in only a few of the plant species. The total pool of isolated glucosinolates was quantitatively dominated by one or a few compounds; some of them were new to the Resedaceae but others occurred in minor amounts in all of the investigated species. The quantitatively dominating glucosinolates identified in the genus *Reseda* are biosynthetically derived from aliphatic protein amino acids in some of the plants and aromatic protein amino acids in others, further alterations being side chain extensions, different types of hydroxylations and glycosylation of the side chains [4, 5]. Thus, the genus *Reseda* seems to be able to perform the biosynthesis of a range of glucosinolates which may be of chemotaxonomic interest.

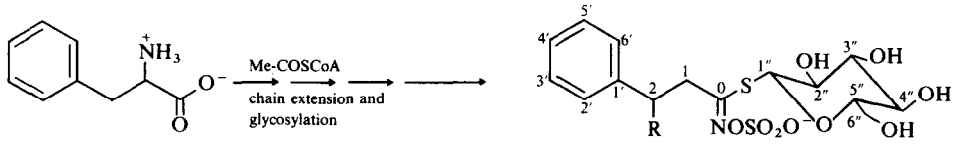
Most of the quantitatively dominating glucosinolates in *Reseda* species contain side chains which are β-hydroxylated, aromatic, and/or hydrophilic. Therefore, they are not readily detected by methods based on degradation products from myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) treatments, and the older literature concerning glucosinolates in this family is scattered and a little confusing [1, 6, 7]. Using the newly developed methods, it has been possible to isolate and identify nearly all of the glucosinolates previously reported as constituents of Resedaceae [6, 7] together with some new ones [4, 5]. The rather surprising reported occurrence of methylglucosinolate in *Reseda odorata* L. [8] has, however, not been confirmed [4].

The present work is a continuation of these studies. We now report on the intact glucosinolates in green parts of *Sesamoides canescens* (L.) O. Kuntze var. *canescens* and *S. pygmaea* (Scheele) O. Kuntze. The genus *Sesamoides* has not previously been examined for glucosinolates. It is shown that a new type of glucosinolate containing an arabinoside connected to the aliphatic part of the side chain is present in these plants.

RESULTS AND DISCUSSION

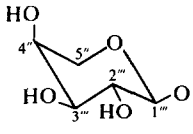
Isolation of the total pool of glucosinolates in green parts of *S. canescens* and *S. pygmaea* was performed by established methods including ion-exchange chromatography [4, 5]. Investigation of the isolated intact glucosinolates by high voltage electrophoresis (HVE) and PC, and of the TMSi derivatives of the desulphoglucosinolates by GC, revealed the presence of three major compounds in *S. canescens* which were identified as **1**, **2** and **3** by well established methods (Fig. 1). The GC traces revealed that these compounds, together with minor amounts of other glucosinolates, occur both in *S. canescens* and *S. pygmaea*. In the Experimental are shown the figures for the relative concentration ratios corresponding to the concentrations in mg/g fr. wt. The previously described methods for isolation and identification of amines structurally related to the dominating glucosinolates **1–3** were unable to reveal any in contrast to the results obtained in the investigations of other glucosinolate-containing plants [3–5].

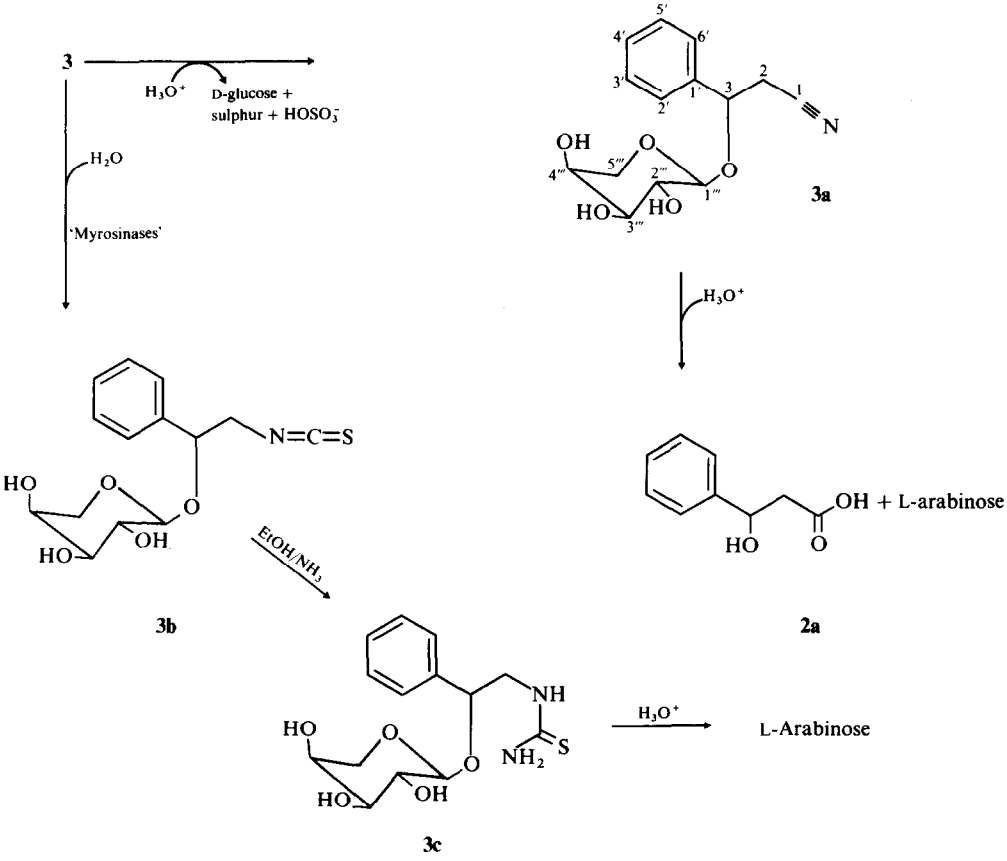
Fig. 1. Glucosinolates in *S. canescens* and *S. pygmaea* biosynthetically derived from phenylalanine; structures, names and degradation products.



Phenylalanine

Glucosinolates (**1**, **2**, and **3**)

No.	R-group in the side chain	Semisystematic glucosinolate name
1	H	Phenethylglycosinolate
2	HO	2-Hydroxy-2-phenylethylglucosinolate
3		2-(α-L-Arabinopyranosyloxy)-2-phenylethylglucosinolate



PC of the isolated glucosinolates revealed that **3** was much more hydrophilic than either **1** or **2**. HVE mobilities obtained at different pHs revealed, furthermore, a much higher MW (size) for **3** than for **1** and **2**, and by comparing the PC, HVE and GC results a diglycoside structure of **3** was indicated (Table 1).

Hydrolysis of **3** in acidic solution under mild conditions led to the release of D-glucose and 3-(α-L-arabinopyranosyloxy)-3-phenylpropionitrile (**3a**) in a 1:1 mixture together with intact **3** as shown by GC. Under more rigorous conditions (see Experimental), D-glucose and L-arabinose were released from **3** in a 1:1 mixture together with 3-hydroxy-3-phenylpropionic acid (**2a**), as shown by PC, TLC and GC (Table 1). Correspondingly, **3a** was transformed into **2a** and L-arabinose by hydrolysis in 6 M HCl. Quantitative determination of the carbohydrates by

use of D-glucose oxidase and D-galactose dehydrogenase after isolation by preparative HVE and preparative PC revealed the configuration of D-glucose and L-arabinose, respectively. The structure of **3a** has been revealed by GC, HVE (Table 1), and ^{13}C NMR (Table 2; see below). Treatment of **3** with myrosinase afforded the isothiocyanate **3b** (Fig. 1) which in ethanol–ammonia solution led to *N*-(2-(α-L-arabinopyranosyloxy)-2-phenylethyl)thiourea (**3c**). Purification of **3c** by preparative PC in solvent 1 followed by acid-catalysed hydrolysis resulted in arabinose, which was isolated by preparative PC and identified by co-chromatography with authentic arabinose in several systems and by GC (Table 1).

The ^{1}H NMR spectrum of **3** in D_2O solution closely resembled that of **2** and revealed an unsubstituted phenyl group with five protons at δ 7.6, as found for **2** [4], which was slightly different from that (δ 7.5) found for **1** [5]. The ^{1}H NMR spectrum of **3** also revealed the presence of additional protons other than those from the thioglucose part in the complex pattern at δ 3.2–3.9 characteristic for the glucose part [5].

The ^{13}C NMR spectra have been reported previously for **1** [5] and **2** [4]. The chemical shifts are included in Table 2 for comparison and show that **3** is a glucosinolate containing the same carbon skeleton as **2** and a pentose moiety with ^{13}C NMR chemical shifts in accordance with an α-L-arabinopyranosyloxy structure [9]. The shift value for C-1 in **3a** confirms the nitrile structure. The upfield shift of C-2 in **2** compared with the corresponding shifts in **3** and **3a** (C-2 and C-3, respectively, Fig. 1) revealed that the hydroxyl group at these carbon atoms is glycosylated in **3** and **3a** [10]. The configuration at this atom has not been established whereas the structures of L-arabinose and D-glucose have been determined by use of D-galactose dehydrogenase and D-glucose oxidase as mentioned above. L-Arabinose is also known as a constituent of other naturally occurring arabinosides ([9] and refs. therein).

The ^{13}C chemical shift values of the arabinose carbon atoms are nearly identical for corresponding atoms in **3** and **3a** (Table 2). These shift values indicate an α-L-arabinopyranoside structure and are quite different from those of both α-L-arabinofuranosides and β-L-arabinofuranosides [11]. Interpretation of the spectra of the pentoaldopyranosides is, however, complicated owing to the more variable conformational equilibria of these compounds compared to those of hexoaldopyranosides [12]. The ^{13}C chemical shift values reported for arabinose in different arabinosides are more or less variable [9, 12, 13], but the ^{13}C NMR spectra of **3** and **3a** support the α-L-arabinopyranoside structure (Table 2). Lacking the stabilization due to the bulky hydroxymethyl group, the pentopyranosides exist in conformations which are determined by the steric arrangement of the hydroxyl groups around the ring (Reeves effect) [13, 14]. The α-L-arabinopyranose is the mirror image to α-D-arabinopyranose which again is the mirror image to that of its homomorphous hexose, β-D-galactopyranose. Therefore, both L-arabinose and D-galactose are readily oxidized by D-galactose dehydrogenase, and it may be anticipated that α-L-arabinopyranosides occur in conformations as β-D-galactopyranosides. The resonances of carbon atoms 1, 2, 3 and 4 in these compounds would thus not be very different, as is found for **3**, **3a** and previously reported values for β-D-galactopyranosides [9, 15]. The chemical shifts of C-1‴ in **3** and **3a** indicate the presence of a bulky group at this atom as previously discussed for the β-D-galactopyranosides [15] (Table 2). Therefore, the ^{13}C NMR spectra of **3** and **3a** supports the proposed α-L-arabinopyranosyloxy structure although it is known that the variable conformational equilibria in some pentopyranosides give rise to large discrepancies in shift values [16].

The new compound (**3**) in *S. canescens* and *S. pygmaea* is the first example of a β-glycosylated glucosinolate. Arabinose has not previously been reported as part of a glucosinolate, but it is well known that some glucosinolates contain glycosylated side chains. An unknown glucosinolate in *S. canescens* with R_t 38.4 min

Table 1. R_f values from PC*, ionic mobilities from HVE*, retention times (R_t) from GC* of glucosinolates isolated from *Sesamoides* species and of hydrolysis products produced from the glucosinolates

	GC	PC					HVE		
		R_f values in solvent system					Distance in cm in buffer system		
Compound	R_t (min)	(1)	(2)	(3)	(4)	(5)	(1) pH 1.9	(2) pH 3.6	(3) pH 6.5
1	17.6	1.27†	—	—	0.98†	1.30†	21.2	18.5	12.5
2	18.3	1.02†	—	—	0.93†	0.91†	20.8	18.1	12.2
3	35.2	0.55†	—	—		0.45†	17.8	15.0	9.5
2a	16.4	0.95	0.76	0.47	—	—	−1	3.5	17.8
3a	41.9	—	—	—	—	—	−1	−1	−1
3c	—	0.50	—	—	—	—	—	—	—
Glucose‡	22,0, 23.5, 24.8, 25.5	0.20	0.41	0.24	—	—	−1	−1	−1
Arabinose‡	17.6, 18.4, 19.0, 19.6	0.23	0.56	0.34	—	—	−1	−1	−1

*For experimental conditions, solvent and buffer systems, see Experimental.

†R_B values (mobilities relative to benzylglucosinolate [4, 5]).

‡Identity of the isolated carbohydrates was confirmed by use of co-chromatography with authentic compounds and comparison with the reference compounds galactose, rhamnose, xylose and ribose in GC, PC and TLC on cellulose (Merck, DC-Alufolie Cellulose F-254, 20 × 20 cm) with mobile phase pyridine–EtOAc–HOAc–H_2O (9:9:2:5).

Table 2. ^{13}C chemical shifts (δ) for the different atoms in glucosinolates isolated from *Sesamoides* species and for some reference compounds

Atom No. (Fig. 1)		Glucosinolate 1	Glucosinolate 2	Glucosinolate 3	Nitrile 3a	Arabinopyranosides α-L*	Arabinopyranosides α-D†	Arabinopyranosides β-D†	B-D-galactopyranosides Me‡	B-D-galactopyranosides 2-Hy-*iso*-butyrat‡
	1″	82.5	82.6	82.8					103.7	98.8
	2″	72.8	72.7	72.8					70.8	71.7
Hexose	3″	77.9	77.9	77.5					72.9	73.7
moiety	4″	69.3	69.9	69.9					68.7	69.4
	5″	80.9	81.1	80.9					74.9	75.8
	6″	61.4	61.5	61.4					61.0	61.5
	1‴			101.1	100.6	101.8	107.0	102.7		
	2‴			71.5	71.5	71.7	73.8	71.7		
Arabinose	3‴			73.0	73.0	70.8	75.4	71.7		
moiety	4‴			68.9	68.9	65.9	71.2	71.0		
	5‴			66.9	66.6	64.1	68.9	65.4		
	1′	141.4	143.0	139.3	138.5					
	2′	129.6	129.8	129.8	129.6					
Aromatic	3′	129.5	127.0	127.9	128.5					
moiety	4′	127.4	129.2	130.0	129.8					
	5′	129.5	127.0	127.9	128.5					
	6′	129.6	129.8	129.8	129.7					
	0	161.6	161.0	161.1	—					
Aliphatic	1	34.7	42.1	40.9	119.9					
moiety	2	44.1	71.9	77.9	26.8					
	3				75.2					

*Quercetin-3-*O*-α-L-arabinopyranoside, from ref. [9].

†Methyl-α-D-arabinopyranoside and methyl-β-D-arabinopyranoside, respectively, from ref. [13].

‡Methyl-β-D-galactopyranoside and 2-(β-D-galactopyranosyloxy)*iso*-butyric acid, respectively, from ref. [15].

(see Experimental) most likely contains a carbohydrate side chain. *o*-(α-L-Rhamnopyranosyloxy)benzylglucosinolate (**4**) is the quantitatively dominating glucosinolate in *R. odorata* L. (Resedaceae) and in other *Reseda* species [4]. The isothiocyanates of both *p*-(α-L-rhamnosyloxy)benzylglucosinolate (**5**) and *p*-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzylglucosinolate (**6**) have been shown to occur in appreciable amounts in myrosinase-treated extracts of *Moringa oleiferae* Lam. and *M. peregrina* (Forssk.) Fiori (Moringaceae) [17, 18]. Glucosinolates with very hydrophilic side chains, such as **3**–**6** and/or other glucosinolates which lead to unstable isothiocyanates ([5, 19] and refs. cited therein), are very difficult or impossible to detect by traditional methods using myrosinase even with very sophisticated instrumentation [20]. The Resedaceae often contain these compounds as the quantitatively dominating glucosinolates [4, 5] which may as well be the case for other plants. They are more easily detected by isolation and examination of the intact glucosinolates [4, 5, 19, 21].

The two *Sesamoides* species considered here deviate drastically from each other in relative composition of the accumulated glucosinolates but obviously they produce the same types of phenylalanine-derived glucosinolates. Both plants contain the new natural product **3**. Compounds **1** and **2** occur in other genera of Resedaceae, **2** being the dominating compound in *R. luteola* L. whereas other *Reseda* species accumulate different phenylalanine-derived glucosinolates as the major compounds [5]. With respect to chemotaxonomy, it is well established that Resedaceae, like other families in the order Capparales, contain high concentrations of glucosinolates. Different structural types are found as the quantitatively dominating compounds in Resedaceae; both aliphatic and aromatic amino acids are found to be the biosynthetic precursors, and side chain extension as well as different types of hydroxylations and glycosylations occur within both the *Sesamoides* and *Reseda* genera [4, 5]. Some, but not all of the investigated *Reseda* species, produce the amines structurally related to the major glucosinolates [4, 5], whereas the investigated *Sesamoides* species do not contain amines structurally related to the glucosinolates identified. In chemotaxonomic studies of Resedaceae, reliable methods of glucosinolate analysis are required and the occurrence of other types of natural products, for example acidic amino acids [22, 23], must be taken into consideration in addition to glucosinolates and amines.

EXPERIMENTAL

Plant material. S. canescens var. *canescens* and *S. pygmaea* seeds, a gift from the Institute Botanique de L'Université, Coimbra, Portugal and Botanischer Garten der Landwirtschaftlichen Hochschule, Universität Hohenheim, Stuttgart–Hohenheim, Germany, respectively, were sown in field plots at the Agricultural Experimental Station, Taastrup, Denmark. Leaves and inflorescences were collected in August, freeze-dried and stored at −20°, until extractions were carried out.

General methods and instrumentation. Methods and equipment used for GC, ^{1}H NMR, ^{13}C NMR, HVE and PC have been described elsewhere [4, 19]. PC was performed in solvents (1) *n*-BuOH–HOAc–H_2O (12:3:5); (2) PhOH–H_2O–13 M NH_4OH (120:30:1) (w/v/v); (3) *iso*-PrOH–H_2O–13 M NH_4OH (8:1:1); (4) *n*-BuOH–pyridine–H_2O (6:4:3); (5) *n*-BuOH–EtOH–H_2O (4:1:4) (upper phase). HVE was performed in the buffer systems: (1) pH 1.9 (HOAc–HCO_2H–H_2O) (4:1:45); (2) pH 3.6 (pyridine–HOAc–H_2O) (1:10:200); (3) pH 6.5 (pyridine–HOAc–H_2O) (25:1:500).

Isolation and determination of glucosinolates. The previously described methods were used [5]. Leaves + inflorescences of *S. canescens* (150 g fr. wt; 50 g freeze-dried) resulted in glucosinolate fractions from the Ecteola ion-exchange column which were pooled and taken to dryness. A sample was trimethylsilylated and investigated by GC. The following results were obtained (R_t min, relative peak area): 7.0, 0.2; 7.5, 0.1; 9.6, 0.3; 10.9, 0.1; 14.1, 0.07; 16.3, 0.05; 17.6, 2.2; 18.3, 2.6; 27.2, 0.01; 30.0, 0.03; 35.2, 2.7; 38.4, 0.3. The corresponding results obtained for *S. pygmaea* (153 g fr. wt.; 50 g freeze-dried) were: 7.0, 0.1; 7.5, 0.05; 9.6, 1.1; 14.1, 0.1; 17.6, 5.6; 18.3, 0.7; 35.2, 0.4.

The glucosinolates were separated by prep. PC in solvent (4) and prep. HVE in (1) buffer pH 1.9 [4, 5, 19]. The glucosinolates **1** and **2** were identified by well established methods [4, 5], and the results from PC, HVE and ^{13}C NMR are included in Tables 1 and 2 for comparison with the results obtained for **3**.

Hydrolysis of **3** in 6 M HCl using the previously described procedure [19] resulted in an equimolar mixture of glucose and arabinose as shown by PC and GC of the TMSi derivatives [5] (Table 1). After isolation by prep. HVE at pH 6.5 and prep. PC in solvent (1), the glucose produced the quantitatively expected response in the D-glucose oxidase system and arabinose produced the quantitatively expected response in the D-galactose dehydrogenase system by using standards of D-glucose and L-arabinose as references, respectively. Hydrolysis of **3** in weakly acidic solution at room temp. for 2 weeks resulted in a nearly 1:1:1 mixture of **3**, **3a** and glucose as shown by ^{13}C NMR. Separation of **3** from **3a** and glucose was performed by prep. HVE at pH 1.9 (Table 1), after which **3a** was separated from glucose by prep. HVE at pH 6.5. Hydrolysis of **3a**, as described above for **3**, resulted in **2a** and L-arabinose. The ^{1}H NMR spectra of **3a** and **2a** dissolved in CD_3OD confirmed the structures for these compounds.

Hydrolysis of **3** with myrosinase catalysis and EtOH–NH_3 treatment was performed as previously described [4] and **3c** isolated by prep. PC in solvent (1). The isolated **3c** was investigated by chromatography as previously described for other thiourea derivatives [4]. Arabinose was released from **3c** by hydrolysis in 6 M HCl and identified by PC, TLC and GC (Table 1) after isolation as described above for the hydrolysis of **3**.

D-*Glucose oxidase* (*β*-D-glucose:oxygen oxidoreductase, EC 1.1.3.4) was used in conjunction with peroxidase for both qualitative and quantitative determination of D-glucose in an assay as described in the information sheet from Boehringer Mannheim GMBH.

D-*Galactose dehydrogenase* (*β*-D-galactose: NAD 1-oxidoreductase, EC 1.1.1.48) was used for qualitative and quantitative determination of L-arabinose in an assay as described in the information sheet from Boehringer Mannheim GMBH.

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