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GLUCOSINOLATES AND AMINES IN *RESEDA MEDIA*

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Key Word Index—*Reseda media*: Resedaceae; glucosinolates; isothiocyanates; nitriles; phenols; amines; carboxylic acids; benzylglucosinolate; 2-phenethylglucosinolate; *m*-hydroxybenzylglucosinolate; (3-indoyl)-methylglucosinolate; benzylamine; hydroxybenzylamines; hydroxyphenylacetic acids; glucosinolate catabolism; amine biosynthesis.

Abstract—The content of glucosinolates and amines in green parts of *Reseda media* has been investigated. Benzylglucosinolate, 2-phenethylglucosinolate, and *m*-hydroxybenzylglucosinolate occur in appreciable amounts accompanied by minor amounts of other glucosinolates, benzylamine and *m*-hydroxybenzylamine. Isolation and identification of these compounds was made using ion-exchange chromatography, high voltage electrophoresis, GC, MS, and ^{13}C-NMR spectroscopy. The glucosinolates were transformed into corresponding nitriles and isothiocyanates by thioglucoside glucohydrolase-catalysed hydrolysis and to the corresponding carboxylic acids by acid-catalysed hydrolysis. The content of glucosinolates and amines in leaves and inflorescences of *R. media* has been determined by UV-spectroscopy and GC.

INTRODUCTION

In a previous communication, we described the isolation and identification of *o*-(α-L-rhamnopyranosyloxy)benzylglucosinolate (**1**) [1]. This glucosinolate is present in high concentration in the inflorescences of *Reseda odorata* together with the structurally related *o*-(α-L-rhamnopyranosyloxy)benzylamine (**1a'**) and *o*-hydroxybenzylamine (**1a**) [2]. *p*-Hydroxybenzylglucosinolate (**2**) has been isolated from rapeseed (*Brassica napus*) [3] and it is known that **2** and *p*-hydroxybenzylamine (**2a**) occur in large amounts in seeds of *Sinapis alba* [4]. The structural isomer of **2**, *m*-hydroxybenzylglucosinolate (**3**), has been detected in *Lepidium graminifolium* by identification of the hydrolysis products [5].

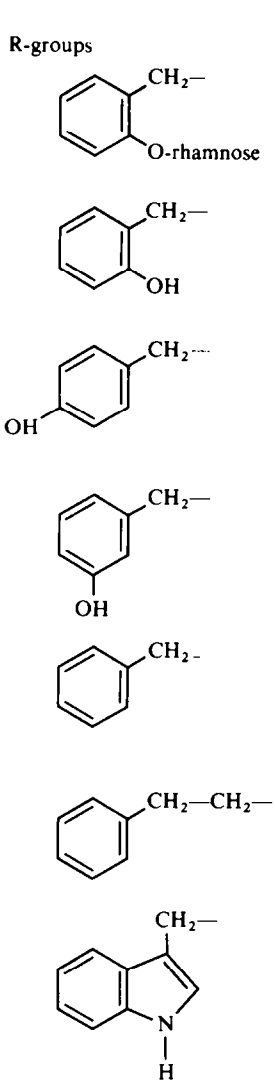
Reports on the co-occurrence of other glucosinolates and structurally related amines have appeared in the literature ([6] and refs. cited therein). These findings indicate a biogenetic relationship between glucosinolates and amines. Thus, some plants are able to produce amines by reactions different from the pyridoxal phosphate dependent decarboxylation of related amino acids or transamination of related aldehydes [7].

The present work is a continuation of our previous investigations of glucosinolates and amines and describes the isolation and identification of glucosinolates and amines in *R. media* including **3** and *m*-hydroxybenzylamine (**3a**). These two compounds are the dominant glucosinolate and amine in leaves and inflorescences of *R. media*. The glucosinolate **3** which is new to the Resedaceae is isolated and described for the first time as an intact glucosinolate. The amine **3a** is a new natural product. The *m*-hydroxy derivatives **3** and **3a** are structurally related to the *o*-hydroxy derivatives **1** and **1a** previously described as constituents of other Resedaceae [1]. However, *o*- and *m*-hydroxylations may be biosynthetically different and occurrence of these structurally related compounds may be of chemotaxonomic interest.

RESULTS

The total glucosinolates in leaves and inflorescences of *R. media* was isolated by the method described recently [1] with a slight modification. After ion-exchange chromatography on Ecteola-cellulose the glucosinolates were converted to their TMSi derivatives and investigated by GC. Furthermore, the glucosinolates were identified by NMR spectroscopy, PC, high voltage electrophoresis (HVE) and identification of the glucosinolate hydrolysis products (*vide infra*). The GC chromatograms revealed **3** as the major glucosinolate (R_t 19.4 min, 12.7 mg/g fr. wt) together with minor amounts of benzylglucosinolate (**4**) (R_t 14.4 min, 2.1 mg/g fr. wt), and 2-phenethylglucosinolate (**5**) (R_t 17.6 min, 0.2 mg/g fr. wt) and trace amounts of (3-indolyl)methylglucosinolate (**6**) (R_t 27.6 min, <0.01 mg/g fr. wt). Other unidentified glucosinolates were also present in trace amounts.

Thioglucoside glucohydrolase EC 3.2.3.1 (myrosinases) catalyzed hydrolysis of the naturally occurring mixture of glucosinolates gave *m*-hydroxybenzyl isothiocyanate (**3c**), benzyl isothiocyanate (**4c**), 2-phenethyl isothiocyanate (**5c**), *m*-hydroxyphenylacetonitrile (**3d**), phenylacetonitrile (**4d**), and 3-phenylpropionitrile (**5d**). The concentration ratio between (**3c** + **3d**): (**4c** + **4d**): (**5c** + **5d**) was as found for 3:4:5. The same ratio between isothiocyanate and nitrile existed in all three cases and no other hydrolysis products were detected. When high concentrations of ascorbic acid were used in the hydrolysis mixture (lower pH) the amount of nitriles relative to the amount of isothiocyanates were increased. The nitriles and isothiocyanates were identified by GC–MS [8] (see Experimental).



Hydrolysis in HCl of the glucosinolates purified by preparative HVE at pH 1.9 yielded *m*-hydroxyphenylacetic acid (**3b**), phenylacetic acid (**4b**) and 3-phenylpropionic acid (**5b**). Confirmation of the structure of these compounds was obtained by GC–MS of the TMSi derivatives. The results obtained for **3b** were compared with those for the corresponding structural isomers *o*-hydroxyphenylacetic acid (**1b**) and *p*-hydroxyphenylacetic acid (**2b**) [9]. GC R_t values, PC R_f values, and mobilities in HVE are presented in Table 1. The three hydroxyphenylacetic acids also produce different colours with Pauly's reagent [10].

Separation of **3**, **4** and **5** was performed by preparative PC and preparative HVE (Table 1). The glucosinolates were then investigated by NMR spectroscopy (Table 2). ^{13}C and ^{1}H chemical shift data for **1** [1], **2** [3] and **3** in neutral aqueous solutions are in complete agreement with the structures, exhibiting signals for all carbon—and hydrogen—atoms except those exchangeable with water. The ^{1}H-NMR spectrum of **3** showed an unsymmetrical pattern of signals in the aromatic region with 1 H at δ 7.4 – 7.1 ppm (*m*) and 3 H at δ 7.0 – 6.7 ppm (*m*) which was very different from the symmetrical pattern of the aromatic protons in **2** and the pattern obtained for the aromatic protons in **1**. The ^{13}C chemical shifts values obtained for the thioglucose part and C-1 (the glucosinolate carbon) in glucosinolates were similar for all of the investigated glucosinolates. However, substituents in the *R* groups bring about a characteristic influence on the chemical shift values for C-1 and C-2 in the glucosinolates [1]. The ^{13}C chemical shift values for the aromatic carbon atoms in **1**, **2**, and **3** confirmed the *o*-, *p*- and *m*-hydroxybenzyl structures. The UV spectra of **3** were similar to those previously described for **1a** [1] with λ_{max} nm: 274 in M HCl and 294 in M NaOH.

The amines **3a** and **4a** were isolated from *R. media* leaves and inflorescences by methods described in the Experimental including ion-exchange chromatography, preparative PC and extraction of **4a** with Et_2O from an aqueous alkaline solution. Both **3a** and **4a** have also been isolated under conditions where myrosinase activity was avoided and the glucosinolates were separated from other plant constituents by use of the Ecteola column as the first chromatographic purification step. This indicates that the compounds are not artefacts produced during isolation. If phenethylamine (**5a**) is produced from **5** in the same relative amount as **4a** from **4** and **3a** from **3** it is difficult to exclude **5a** as a constituent of *R. media* with the

Table 1. GC retention times, R_f values and ionic mobilities of glucosinolates, carboxylic acids and amines

		GC* (R_t) (min)	R_f values in solvent system† (1)	(2)	(3)	Distance in cm obtained by HVE in buffer system † (1) pH 1.9	(2) pH 3.6	(3) pH 6.5
Benzylglucosinolate	(**4**)	14.4	1.00‡		1.00‡	22.4	18.9	12.7
2-Phenethylglucosinolate	(**5**)	17.6	1.27‡		1.30‡	21.2	17.4	12.5
m-Hydroxybenzylglucosinolate	(**3**)	19.6	0.79‡		0.74‡	20.8	17.3	12.1
p-Hydroxybenzylglucosinolate	(**2**)	20.9	0.73‡		0.68‡	21.1	17.8	11.5
Phenylacetic acid	(**4b**)	9.2	0.96	0.63		−1	2.1	20.7
3-Phenylpropionic acid	(**5b**)	11.9	0.97	0.66		−1	2.0	20.3
o-Hydroxyphenylacetic acid	(**1b**)	15.8	0.94	0.65		−1	2.7	19.9
m-Hydroxyphenylacetic acid	(**3b**)	16.7	0.95	0.48		−0.5	3.5	18.4
p-Hydroxyphenylacetic acid	(**2b**)	17.2	0.94	0.46		−0.6	2.6	17.2
Benzylamine	(**4a**)		0.76	0.78			−17.5	−27.0
o-Hydroxybenzylamine	(**1a**)		0.69	0.68			−15.6	−23.9
m-Hydroxybenzylamine	(**3a**)		0.67	0.58			−15.5	−24.1
p-Hydroxybenzylamine	(**2a**)		0.56	0.56			−15.0	−22.9

* For GC conditions used for TMSi derivatives, see Experimental.
† For solvent and buffer system, see Experimental. The electrophoresis time for the amines was 40 min at pH 3.6 and 6.5.
‡ Mobilities relative to benzylglucosinolate.

Table 2. ^{13}C and ^{1}H chemical shifts (δ) for different atoms of glucosinolates and amines $\delta^{13}C/\delta^{1}H$

		Others*			Aromatic moiety						Glucose moiety					
Compound		1	2	3	1''	2''	3''	4''	5''	6''	1'	2'	3'	4'	5'	6'
Benzylglucosinolate	(**4**)	163.3	39.0 4.2		136.0	130.1 a†	128.9 a	128.4 a	128.9 a	130.1 a	82.2 4.9	72.7 c§	77.9 c	69.7 c	80.6 c	61.2 c
2-Phenethylglucosinolate	(**5**)	161.6	44.1 d‖	34.7 d	141.4	129.6 b‡	129.5 b	127.4 b	129.5 b	129.6 b	82.5 4.9	72.8 c	77.9 c	69.3 c	80.9 c	61.4 c
o-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate	(**1**)	163.7	34.7 4.19		120.8	154.2	115.2 7.25	131.2 7.37	123.5 7.13	130.0 7.37	82.4 4.9	72.7 c	77.8 c	69.5 c	80.6 c	61.1 c
m-Hydroxybenzylglucosinolate	(**3**)	163.4	38.9 4.1		137.6	115.3** 6.85	156.8	115.8** 6.85	131.4 7.25	121.1 6.85	82.2 4.9	72.8 c	77.3 c	69.5 c	80.6 c	61.2 c
p-Hydroxybenzylglucosinolate	(**2**)	163.7	38.4 4.1		127.6	130.4 7.4	116.8 7.0	155.7	116.8 7.0	130.4 7.4	82.3 4.9	72.8 c	77.9 c	70.1 c	81.1 c	61.8 c
o-Hydroxybenzylamine	(**1a**)		39.4 4.18		119.1	154.6	115.2 7.01	130.9 7.35	120.4 7.01	130.8 7.35						
m-Hydroxybenzylamine	(**3a**)		43.8 4.1		135.5	116.5** 6.85	157.3	117.0** 6.85	131.5 7.25	121.2 6.85						
p-Hydroxybenzylamine	(**2a**)		43.6 4.15		125.3	131.6 7.39	116.8 6.98	157.0	116.8 6.98	131.6 7.39						

* No. 1 corresponds to the glucosinolate carbon atom. No. 2 is the benzylic atom and No. 3 designates the other atoms in the aglucone part. For the rhamnose part in **1** and some of the other values in the table, see ref. [1].
† a = A peak with 4 protons at 7.6.
‡ b = A peak with 5 protons at 7.5.
§ c = A complex pattern at 3.2–3.9 characteristic of the glucose part.
‖ d = A multiplet with 4 protons at 2.9–3.3.
¶ Very weak signal.
** The $\delta^{13}C$ values assigned to the atoms 2'' and 4'' in **3** and **3a** are perhaps interchanged.

applied analytical technique, but we have not observed this amine in the extracts from the plant material. PC and HVE properties of the amines and some reference compounds are presented in Table 1. Both **1a**, **2a**, **3a**, and **4a** give a yellow colour with ninhydrin which changes to purple within different times. The colour change depends on concentration and temperature, but under identical conditions the time for colour change was *ca* 15 min for **4a**, 30 min for **3a**, 4 hr for **2a** and 20 hr for **1a**.

The ^{1}H- and ^{13}C-NMR chemical shifts for the amines **1a**, **2a**, **3a** and some reference compounds are presented in Table 2. As discussed above for the glucosinolates, these data for the amines are in complete agreement with the structures, exhibiting signals for all carbon—and hydrogen—atoms except those exchangeable with water. The structures of the amines have also been confirmed by MS, the *m/e* values obtained for **1a** [2], **2a** and **3a** were similar but the relative intensities of the peaks were

different. The UV spectra of **3a** deviated slightly from the corresponding spectra of **3** (see above) λ_{max}nm: 274 in M HCl and 292 in M NaOH. The amounts of **3a** and **4a** isolated from leaves and inflorescences of *R. media* were estimated from UV spectra and gave the following concentrations (in mg/g fr.wt): **3a** (0.3), **4a** (0.07).

DISCUSSION

The glucosinolate **3** is new to the Resedaceae, whereas **4**, **5**, **6** and some other glucosinolates have been reported previously as constituents of other Resedaceae species [1, 2, 11]. The amine **3a** is a new natural product and **4a** is new to the Resedaceae, but the co-occurrence of amines and structurally related glucosinolates have previously been reported ([1, 6] and refs. cited therein). In connection with the observation of **3** and **3a** as constituents of *R. media* it may be of interest to compare this finding with reports on structurally related phenolic glucosinolates and amines: **1** [1], **1a** [2], **2** and **2a** [4] as well as with reports on instability and problems with quantitative estimations of glucosinolates and isothiocyanates thereof ([3] and refs. cited therein).

The instability of isothiocyanates produced in the myrosinase-catalysed hydrolysis of e.g. **2** [12] and **6** [13] is one of the reasons for the difficulties encountered in quantitative estimations of these glucosinolates in different plants [3, 8]. It is generally agreed that this type of glucosinolate produces thiocyanate ions in quantitative amounts during myrosinase-catalysed degradation [14].

The nitrile and isothiocyanate proportions produced in the myrosinase-catalysed hydrolysis of isolated **3**, **4**, and **5** vary, depending upon the amount of ascorbic acid in the assay mixture (see Results). The reasons for this seemingly unique behaviour have previously been discussed in relation to a study of the production of volatile flavour compounds from different Cruciferae, e.g. **4c** and **4d** from **4** [15]. It was found that the ratio of isothiocyanate to nitrile varied according to both processing and upon the horticultural history of the plant, but no information of pH and ascorbic acid concentration in the mixtures producing nitriles and isothiocyanates was given [15]. The analytical methods of separation and quantitative determination of intact glucosinolates described in this paper improve the possibility for identification of both novel and well-known glucosinolates as **1**, **2**, **3** and **6**, which are difficult to detect by methods based on myrosinase-catalysed hydrolysis, especially if only relatively low concentrations are present.

The possibility of a carbonium ion-stabilized intermediate has been discussed in relation to the formation of **1a'**, **1a**, **2a** and **4a** from the structurally related glucosinolates [6]. However, this type of stabilization is less likely in the case of **3a**, and the results presented in this work as well as reports on the metabolic relation between glucosinolates and amines [2, 4, 6] show that some glucosinolate-containing plants produce the structurally related amines whereas other taxonomically related plants do not [1]. Therefore, these amines may be catabolic products of the related glucosinolates and not produced in the plants by the more well-known amine biosynthetic routes [7].

It may also be of interest to compare the biosynthetic sequences involved in the formation of **1**, **2** and **3**. The amino acid precursor involved in the biosynthesis of **2** may be tyrosine or phenylalanine [16]. The latter is certainly the precursor to **4**, **5**, and **1**, but the biosynthesis of **1** requires furthermore *o*-hydroxylation and formation of a rhamnoside. If phenylalanine is the precursor to **3**, an intermediate *m*-hydroxylation is required, but tyrosine and dopa may also be possible precursors to **3** if a reduction of the *p*-hydroxyphenyl group is involved in the biosynthetic sequence. No biosynthetic studies on the formation of **1** and **3** have been reported but it has been shown that *p*-coumaric acid and, especially, *p*-hydroxyphenylacetaldehyde oxime are efficient precursors in the biosynthesis of **2** [16].

EXPERIMENTAL

Plant material. R. media Lag. seeds, a gift from Institute Botanique de L'Université, Coimbra, Portugal, were sown in field plots at the Agricultural Experimental Station, Taastrup. Leaves and inflorescences were collected in September and used directly, or freeze-dried and stored at −20°, until extractions were carried out.

General methods and instrumentation. Methods and equipment used for GC, MS, ^{1}H-NMR, ^{13}C-NMR, prep. HVE and prep. PC have been described previously [1, 3]. PC was performed in solvents (1) *n*-BuOH–HOAc–H_2O (12:3:5), (2) *iso*-PrOH–H_2O–18M NH_4OH (8:1:1), (3) *n*-BuOH–EtOH–H_2O (4:1:4) (upper phase) by the descending technique on Whatman No. 1 paper or Schleicher and Schüll 2043b paper. HVE was carried out on Whatman 3 MM paper using a flat plate unit and the following systems: (1) buffer pH 1.9 (HOAc–HCO_2H–H_2O) (4:1:45), 2 hr at 3.2 kV and 90 mA; (2) buffer pH 3.6 (Py–HOAc–H_2O) (1:10:200), 2 hr at 3 kV and 90 mA; (3) buffer pH 6.5 (Py–HOAc–H_2O) (25:1:500), 50 min at 5 kV and 90 mA. Glucosinolates after HVE and PC were detected with $AgNO_3$ [17] and phenols with Pauly's reagent [10].

Isolation of glucosinolates and amines. Fresh plant material (750 g leaves and inflorescences) was freeze-dried and homogenized with an Ultra-Turrax homogenizer in 5 l. boiling MeOH–H_2O (7:3). The homogenate was boiled for an additional 5 min to ensure inactivation of myrosinase, cooled and filtered. The residue was washed twice with 100 ml MeOH–H_2O (7:3). The combined filtrates were evapd to dryness, redissolved in 200 ml H_2O, and extracted with 3 × 100 ml $CHCl_3$. The H_2O phase was concd to *ca* 50 ml and transferred to a column of Amberlite IR 120 (H^+, 2.5 × 90 cm) connected in series to a column of Ecteola-cellulose (AcO^-, 2.5 × 90 cm). The two columns were flushed with H_2O (1 l.) after which the glucosinolates were isolated from the Ecteola-cellulose column as previously described [1], whereas **3a** was isolated from the Amberlite IR 120 column as described below. The glucosinolate-containing fractions from the Ecteola column were pooled and taken to dryness, investigated by GC as previously described [3] and the glucosinolates were separated by prep. PC in solvent (3) and prep. HVE in (1) buffer pH 1.9 [1]. For PC and electrophoretic behaviour of **3**, **4** and **5**, see Table 1, UV spectra, see Results, ^{1}H-NMR and ^{13}C-NMR data, see Table 2 and Results.

Myrosinase-catalysed hydrolysis of glucosinolates and GC–MS analysis of products. After purification by prep.-HVE pH 1.9, 10 mg of the glucosinolates was dissolved in 3 ml 100 mM Pi buffer, pH 7 containing 25 mM ascorbic acid and 2 mg myrosinase [18] and left overnight at 25° in a sealed tube. Solvent–solvent extraction with 1 ml Et_2O was then carried out and the Et_2O extract analysed by GC–MS [8]. The following compounds were identified, R_t min [M^+/*e* (% of base peak), base peak]: phenylacetonitrile (**4d**), 12.1 [117 (100), 117]; benzyl isothiocyanate (**4c**), 15.3 [149 (13.2), 91]; 3-phenylpropionitrile (**5d**), 13.8 [131 (17.6), 91]; 2-phenethyl isothiocyanate (**5c**), 16.7 [163 (27.8), 91]; *m*-hydroxyphenylacetonitrile (**3d**), 17.3

[133 (100), 133]; *m*-hydroxybenzyl isothiocyanate (**3c**), 19.9 [165 (22.5), 107]. The results obtained for **4c**, **4d**, **5c** and **5d** were identical with those obtained for authentic material [8], and lit. values [19].

Hydrolysis of glucosinolates in 6 M HCl *and analysis of products as their TMSi derivatives by GC–MS.* The procedure was as previously described [3]. The following compounds were identified as TMSi derivatives: *m*-hydroxyphenylacetic acid (**3b**), R_t 16.7 min; phenylacetic acid (**4b**), R_t 9.4 min; 3-phenylpropionic acid (**5b**), R_t 12.3 min; and glucose, R_t 22.9, 23.5 and 25.5 min. R_t and MS data obtained for the compounds were identical with those obtained for authentic compounds and lit. values [9, 20]. For PC and electrophoretic behaviours of the carboxylic acids, see Table 1.

Isolation and semiquantitative determination of **3a**. **3a** was eluted from the Amberlite IR 120 column (see above) by use of M Py and 2 M NH_4OH and further purified by prep. PC in solvent 2 and prep. HVE as previously described [6]. For PC and electrophoretic behaviours, see Table 1. UV-spectra, see Results, ^{1}H-NMR and ^{13}C-NMR, see Table 2 and Results. MS of **3a** *m/e* (% of base peak): 124 (7), 123 (85), 122 (100), 107 (24), 106 (32), 95 (40), 93 (4), 79 (9), 78 (20), 77 (50), 67 (5), 66 (7), 65 (14), 64 (3), 63 (8), 53 (4), 52 (8), 51 (13). Corresponding data for **2a**: 124 (5), 123 (68), 122 (86), 107 (47), 106 (71), 95 (38), 93 (12), 79 (15), 78 (100), 77 (68), 67 (8), 66 (10), 65 (38), 64 (8), 63 (18), 53 (10), 52 (52), 51 (62), and for **1a**: 124 (8), 123 (28), 122 (21), 107 (6), 106 (10), 95 (10), 93 (10), 79 (22), 78 (74), 77 (100), 67 (8), 66 (20), 65 (10), 64 (7), 63 (16), 53 (13), 52 (48), 51 (50).

Isolation and semiquantitative determination of **3a** *and* **4a**. Freshly harvested inflorescences and leaves (5 g) were homogenized in boiling MeOH–H_2O as described above. After filtration and lyophilization, the amines and glucosinolates were separated on a Ecteola-cellulose column (AcO^-, 2.5 × 20 cm). The H_2O effluent (400 ml) was concd to 10 ml; 5 ml of this was diluted with M NaOH (2 ml) and extracted with Et_2O (3 × 10 ml) and re-extracted with 0.01 M HCl (2 × 5 ml), the other 5 ml was applied to a column of Amberlite IR 120 (H^+, 20 × 0.6 cm). The column was washed with 40 ml of H_2O, and eluted with 100 ml M Py followed by 200 ml 2 M NH_4OH. The NH_4OH eluate was concd to *ca* 1 ml and purified by prep. PC in solvent 1. The amount of **3a** was estimated by UV. The HCl extracts were concd to dryness, dissolved in H_2O and **4a** was isolated by prep. PC in solvent 1. The amount of **4a** was estimated by UV.

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REFERENCES

1. Olsen, O. and Sørensen, H. (1979) *Phytochemistry* **18**, 1547.
2. Sorensen, H. (1970) *Phytochemistry* **9**, 865.
3. Olsen, O. and Sørensen, H. (1980) *J. Agric. Food Chem.* **28**, 43.
4. Larsen, P. O. (1965) *Biochim. Biophys. Acta* **107**, 134.
5. Friis, P. and Kjær, A. (1963) *Acta Chem. Scand.* **17**, 1515.
6. Dalgaard, L., Nawaz, R. and Sørensen, H. (1977) *Phytochemistry* **16**, 931.
7. Smith, T. A. (1977) in *Progress in Phytochemistry* (Reinhold, L., Harborne, J. B. and Swain, T., eds.) Vol. 4, p. 27. Pergamon Press, Oxford.
8. Nielsen, J. K., Dalgaard, L., Larsen, L. M. and Sørensen, H. (1979) *Entomol. Exp. appl.* **25**, 227.
9. Mamer, O. A., Crawhall, J. C. and San Tjoa, S. (1971) *Clin. Chim. Acta* **32**, 171.
10. Jatzkewitz, H. (1953) *Hoppe-Seyler's Z. Physiol. Chem.* **292**, 99.
11. Cole, R. A. (1976) *Phytochemistry* **15**, 759.
12. Josefsson, E. (1968) *J. Sci. Food Agric.* **19**, 192.
13. Gmelin, R. and Virtanen, A. I. (1961) *Ann. Acad. Sci. Fenn. Ser. A* 2 **107**, 3.
14. McGregor, D. J. (1978) *Can. J. Plant. Sci.* **58**, 795.
15. Macleod, A. J. (1976) in *The Biology and Chemistry of the Cruciferae* (Vaughan, J. G., Macleod, A. J. and Jones, B. M. G., eds.) p. 307. Academic Press, London.
16. Kindl, H. Schiefer, S. (1969) *Monatsch. Chem.* **100**, 1773.
17. Von Schultz, O.-E. and Wagner, W. (1956) *Z. Naturforsch. Teil B* **11**, 73.
18. Appelqvist, L.-A. and Josefsson, E. (1967) *J. Sci. Food Agric.* **18**, 510.
19. Kjær, A., Ohashi, M., Wilson, J. M. and Djerassi, C. (1963) *Acta Chem. Scand.* **17**, 2143.
20. DeJongh, D. C., Radford, T., Hribar, J. D., Hanessian, S. (1969) *J. Am. Chem. Soc.* **91**, 1728.