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ISOLATION OF GLUCOSINOLATES AND THE IDENTIFICATION OF *o*-(α-L-RHAMNOPYRANOSYLOXY)BENZYLGLUCOSINOLATE FROM *RESEDA ODORATA*

OLE OLSEN and HILMER SØRENSEN

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

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Key Word Index—*Reseda alba*; *R. luteola*; *R. odorata*; Resedaceae; glucosinolates; *o*-(α-L-rhamnopyranosyloxy)-benzylglucosinolate; 2-hydroxy-2-phenylethylglucosinolate; 2-hydroxy-2-methylpropylglucosinolate; *N*-(*o*-(α-L-rhamnopyranosyloxy)benzyl)thiourea; ion-exchange chromatography; electrophoresis; glucosinolate catabolism.

Abstract—A method has been developed for the quantitative isolation of glucosinolates by ion-exchange chromatography and high voltage electrophoresis avoiding strongly alkaline and acidic conditions. The compounds were identified by ^{1}H and ^{13}C NMR spectroscopy and through the products arising from enzymatic, acid and alkaline hydrolysis. The method is well suited for the isolation and identification of glucosinolates containing aglucone parts which produce non-volatile compounds on enzymatic hydrolysis. The method has been used in the isolation and identification of 2-hydroxy-2-methylpropylglucosinolate from *Reseda alba*, 2-hydroxy-2-phenylethylglucosinolate from *R. luteola* and a new glucosinolate, *o*-(α-L-rhamnopyranosyloxy)benzylglucosinolate, occurring in *R. odorata*. The glucosinolate content in different parts of this plant has been determined and the metabolism of glucosinolates is briefly discussed.

INTRODUCTION

The glucosinolates constitute a well-known group of natural products comprising more than 80 different compounds [1–3]. They are biosynthetically derived from the related amino acids [4] and are found in appreciable amounts only in a few plant families [5]. Plants containing glucosinolates also contain β-thioglucosidases EC 3.2.3.1 (myrosinases) [5, 6]. On autolysis or damage of the plant cells, these lead to the formation of isothiocyanates or other degradation products such as thiocyanates, nitriles, oxazolidinethiones and cyano-epithioalkanes [7]. Some of these enzymatic degradation products often possess strong physiological effects, but it is not believed that they occur in undamaged cells [3, 5]. The presence of these compounds in plant extracts subjected to autolysis is often used as evidence for the presence of the corresponding glucosinolates in the intact plant [8, 9]. However, isothiocyanates, and products thereof, have been identified in organisms not known to contain glucosinolates [3, 10]. Furthermore, recent investigations on amines as catabolic products of structurally related glucosinolates [11, 12], as well as studies on beetles feeding on glucosinolate-containing plants [13], have revealed the necessity of a simple method for isolation of the total amount of glucosinolates including compounds with aglucone parts containing hydrophilic groups.

This paper is a continuation of previous work on the study of a possible biogenetic relationship between amines and structurally related glucosinolates [11, 12], and the concurrent investigation on biosynthesis of glucosinolates and amines in *Reseda odorata* (Resedaceae). The isolation of a new glucosinolate, *o*-(α-L-rhamnopyranosyloxy)benzylglucosinolate, from *R. odorata* has been performed at mild conditions by ion-exchange chromatography, high voltage electrophoresis (HVE) and PC, avoiding destruction caused by acid-catalysed hydrolysis [14] or base-catalysed Neber-type rearrangement [15]. Glucosinolates of this type are not detectable by traditional methods [9, 16].

RESULTS

Investigation of a crude MeOH–H_2O extract from the inflorescences of *R. odorata* by PC and HVE revealed the presence of an unknown glucosinolate in appreciable amounts. Several attempts to identify this glucosinolate by use of the traditional methods, including myrosinase-catalysed hydrolysis followed by extraction of the aglucone part to organic solvents and subsequent GC and MS [13, 16], were unsuccessful. It became clear that the aglucone part of the glucosinolate in question is very hydrophilic, as the isothiocyanate produced with myrosinase remained in the water phase.

The plant material was homogenized in boiling aqueous methanol to prevent the compound from myrosinase-catalysed degradation [13]. The concentrated extract was applied to a strongly acidic cation-exchange resin resulting in a water effluent containing the glucosinolate, which after neutralization was concentrated *in vacuo* and transferred to a weakly basic anion-exchange column in the acetate form (Ecteola-Cellulose). The functional groups of this material have a pK_a of *ca* 7.5. The glucosinolate was quantitatively retained by the Ecteola column, and after thoroughly washing the column with water, it was released by elution with M pyridine. The weakly basic pyridine solution removed the charges on the ion-exchange resin, leaving the glucosinolate in a few fractions. On evaporation a nearly pure pyridinium salt of the glucosinolate was obtained. Further purification was accomplished by preparative PC and preparative

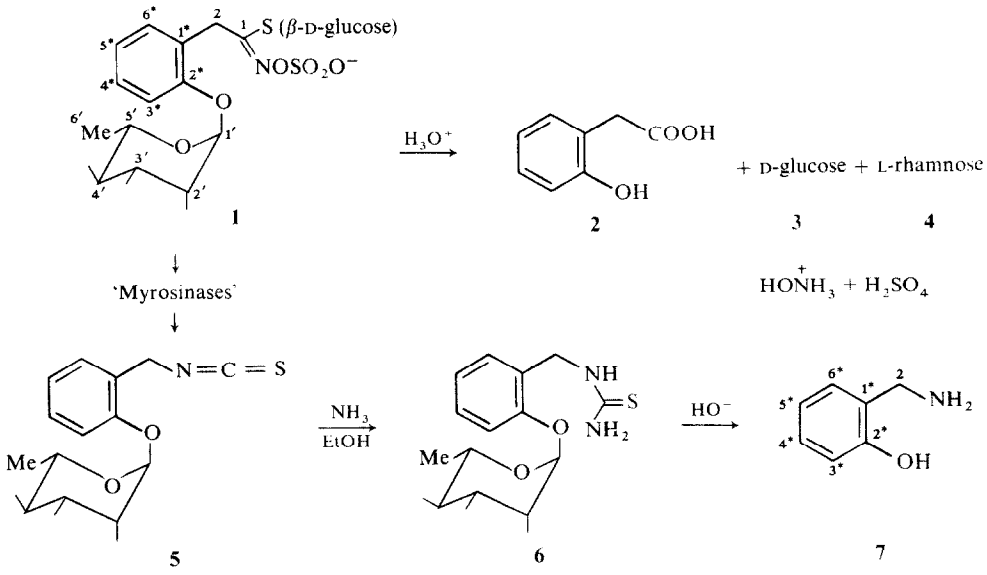

Fig. 1.

HVE. The hitherto unknown glucosinolate could then be identified as *o*-(α-L-rhamnopyranosyloxy)benzylglucosinolate (**1**). **1** was transformed into different known compounds as shown in Fig. 1.

Acid hydrolysis of **1** resulted in *o*-hydroxyphenylacetic acid (**2**), D-glucose (**3**) and L-rhamnose (**4**). The compounds **2**, **3** and **4** were present in the hydrolysis mixture in equal amounts as shown by GC and PC. After isolation, **2**, **3** and **4** were identified by co-chromatography with authentic compounds using PC and solvents 1, 2 and 3 (see Experimental) and by HVE for **2** at pH 3.6 and 6.5. The GC R_ts of the TMSi derivatives of **2**, **3** [17] and **4** were identical with those obtained for the corresponding reference compounds. Also MS of the compounds corresponding to these peaks were identical with those reported in the literature [17–19].

Myrosinase-catalysed hydrolysis of **1** gave *o*-(α-L-rhamnopyranosyloxy)benzylisothiocyanate (**5**) and **3**. The isothiocyanate **5** was transferred into the thiourea derivative (**6**) on treatment with ammonia in ethanol, and furthermore, **6** was converted to *o*-hydroxybenzylamine (**7**). R_f values from PC and mobilities in HVE of the isolated glucosinolates and some reference compounds are presented in Table 1, and R_f values of some thiourea derivatives obtained by TLC are shown in Table 2. The mobilities of the glucosinolates in HVE in 3 different buffer systems are in accordance with their net charge (low pK_a for the sulphate group) and molecular size, i.e. nearly linear relationship between log MW and log mobilities. However, careful analysis of this relationship requires an internal standard of nearly the same molecular size. Preparative HVE at pH 1.9 is efficient in separation of glucosinolates from most other plant constituents since few of the latter have a negative net charge at this pH. Immediately after the electrophoresis at pH 1.9, the papers must be dried in an atmosphere of NH_3 to prevent acid-catalysed hydrolysis of the glucosinolates. The R_f values obtained for **1**, **2**, **6** and **7** are in agreement with the proposed structures, and as expected [11], R_f values of the *p*-derivatives are smaller than those of the corresponding *o*-isomers.

^{13}C and ^1H chemical shift data for some of the compounds in neutral aqueous solutions are given in Table 3. The ^{13}C chemical shift for the carbon atoms in the glucose part of **1**, 2-hydroxy-2-methylpropylglucosinolate (**8**) isolated from *R. alba* and 2-hydroxy-2-phenylethylglucosinolate (**9**) isolated from *R. luteola* is in agreement with the shift obtained for the corresponding atoms in sinigrin and glucotropaeolin [15, 20]. These shift values together with the values for C-1 (Table 3) establish the glucosinolate and benzyl structure of **1**. The ^{13}C shift values obtained for the rhamnose part of **1** compared with literature values [21, 22] indicate the L-rhamnose structure. The α-L-rhamnopyranoside structure is confirmed

Table 1. R_f values and ionic mobilities of glucosinolates

Glucosinolates	R_f values [34] in solvent system* (1)	(a)	(b)	Distance in cm obtained by HVE in buffer system* (1) pH 1.9	(2) pH 3.6	(3) pH 6.5
(Sinigrin) Allylglucosinolate	65	62	50	24.2	21.3	13.1
(Glucoiberin) 3-Methylsulphinylpropylglucosinolate	35	22	31	21.6	19.3	12.5
2-Hydroxy-2-methylpropylglucosinolate (**8**)	59	65	49	22.8	21.2	14.4
(Glucotropaeolin)Benzylglucosinolate	100	100	100	22.4	18.9	12.7
2-Hydroxy-2-phenylethylglucosinolate (**9**)	102	93	91	23.3	20.0	12.5
(Sinalbin) *p*-Hydroxybenzylglucosinolate	73	81	68	21.1	17.8	11.5
o-(α-L-Rhamnopyranosyloxy)benzylglucosinolate (**1**)	69	60	67	18.8	15.2	10.8

* For solvent and buffer systems, see Experimental.

Table 2. R_f values on Si gel plates of thiourea derivatives

Thiourea derivatives	Solvent system* (1)	(c)
N-Allylthiourea	0.86	0.60
N-Isopropylthiourea	0.86	0.46
N-*sec*-Butylthiourea	0.86	0.51
N-Phenylthiourea	0.90	0.67
N-Benzylthiourea	0.94	0.52
N-(*o*-(α-L-Rhamnopyranosyloxy)benzyl)-thiourea (**6**)	0.77	0.03

* For solvent systems, see Experimental.

by the values obtained for authentic *o*-(α-L-rhamnopyranosyloxy)benzylamine (**10**) [11]. The ^{13}C chemical shift values for the aromatic and benzylic atoms of the compounds shown in Table 3 confirm the *o*-(α-L-rhamnopyranosyloxy)benzylglucosinolate structure of **1**.

The ^1H NMR chemical shifts presented in Table 3 substantiate the structure of the glucosinolates **1**, **8** and **9** isolated from *R. odorata*, *R. alba* and *R. luteola*, respectively. In the 60 MHz spectra of **1**, **7** and **10**, the signals from the aromatic protons show a nearly symmetrical pattern as previously described [11], whereas the 270 MHz spectra of these compounds reveal the unsymmetrical pattern and coupling constants corresponding to *o*-substituted benzyl derivatives.

The UV data (see Experimental) for **1**, **6**, **7** and **10** also confirm the proposed structures and these data are used in the semi-quantitative estimation of **1** in different parts of *R. odorata*. The following results are obtained for **1** (mg/g fr. wt): roots (1.2), stems and leaves (1.4), inflorescence (4.7). **1** is the major glucosinolate in green parts of *R. odorata* but chromatographic evidence indicates that another glucosinolate is present in low concentration.

DISCUSSION

Glucosinolates have previously been found in some species of the family Resedaceae [5, 23] as well as in the allied Capparidaceae, Crucifereae and Moringaceae; thus *p*-(α-L-rhamnopyranosyloxy)benzylglucosinolate occurs in *Moringa oleifera* (see [11] and refs cited therein). Phenylethylglucosinolate and **9** have previously been identified in extracts from *R. luteola* [24–26], and **8** has previously been found in seeds of *R. alba* [27]. Therefore, the isolation of **8** and **9** from green parts of these plants is not surprising. *R. odorata* and other Resedaceae species have been reported to contain 3-indolymethylglucosinolate (glucobrassicin) [23, 28]. We have found that different parts of *R. odorata* contain appreciable amounts of the hitherto unknown glucosinolate **1** and small amounts of an unknown glucosinolate, which does not seem to be the indole derivative but rather *o*-hydroxybenzylglucosinolate.

The presence of the new glucosinolate **1** together with the amines **7** and **10** in *R. odorata* is in agreement with our previous reports on amines as catabolic products of the corresponding glucosinolates [12]. We have observed (unpublished results) the occurrence of **7** and **10** in other Resedaceae species, for which reason **1** should also be expected in these plants. However, the amines corresponding to the glucosinolates **8** and **9** could not be

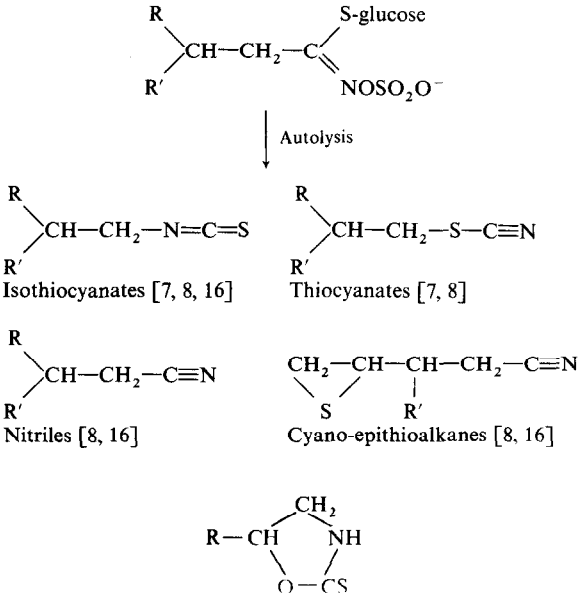

Fig. 2.

detected in extracts from *R. alba* and *R. luteola*, respectively. The occurrence of different glucosinolate autolysis products [29, 30] shown in Fig. 2 depends, among other things, on the structure of the glucosinolate [31] and the presence of other plant constituents than β-thioglucosidase [13, 32, 33]. Therefore, the production of amines from glucosinolates [12] can also be subject to variation.

EXPERIMENTAL

Plant material. Reseda odorata L. "grandiflora" seeds were purchased from J. E. Ohlsens Enke, Copenhagen and sown in field plots at the Agricultural Experimental Station, Taastrup. *R. alba* L. and *R. luteola* L. were collected in the Botanical Garden of the University of Copenhagen. The plants were collected in September, freeze-dried and stored at −20° until extractions were carried out.

General methods and instrumentation. The ^1H NMR spectra were determined in D_2O soln at 60 and 270 MHz. The chemical shifts are in ppm downfield from Na 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate used as internal standard; coupling constants are in Hz. The ^{13}C NMR spectra were recorded at 67.889 MHz using the pulse technique with Fourier transformation. The chemical shifts are in ppm downfield from TMS; dioxane was used as internal standard [δ(TMS) = δ(dioxane) + 67.4 ppm]. GC was carried out using a FID detector maintained at 280°. The carrier gas was N_2. GC–MS were obtained at 70 eV ionizing energy. The carrier gas was He. GC and GC–MS conditions were: glass column, 1.5 m × 4 mm i.d. packed with 3% OV1 on Chromosorb W-HP, programmed from 70° for 1 min followed by an increase of 5°/min to the final temp. 280°. The injection port was 300° and the carrier gas flow was 40 ml/min. Chromatography was performed in solvents (1) *n*-BuOH–HOAc–H_2O (12:3:5), (2) PhOH–H_2O–conc NH_3 (120:30:1) (w/v/v), (3) *iso*-PrOH–H_2O–conc NH_3 (8:1:1), (a) BuOH–Py–H_2O (6:4:3), (b) BuOH–EtOH–H_2O (4:1:4) and (c) $CHCl_3$–EtOH (9:1). PC was performed by the descending

Table 3. ^{13}C and ^{1}H chemical shifts (δ) for the different atoms of glucosinolates and related compounds $\frac{\delta^{13}C}{\delta^{1}H}$

		Compound											
			8		**9**	**1**		**4**	**7**		**10**		**6**
Atom No.†		Sinigrin‡	2-Hydroxy-2-methylpropyl-glucosinolate	Glucotropaeolin§	2-Hydroxy-2-phenylethylgluco-sinolate	*o*-(2-L-Rhamnopyranosyloxy)-benzylglucosinolate	1-Thio-β-D-glucopyranose, Na-salt‖	α-L-Rhamnose‖	*o*-Hydroxybenzylamine, HCl	*p*-Hydroxybenzylamine, HCl	*o*-(2-L-Rhamnopyranosyloxy)-benzylamine	*p*-(2-L-Rhamnopyranosyloxy)-benzylamine	*N*-(2-L-Rhamnopyranosyloxy)-benzylthiourea
Glucose moiety	1′	82.4 4.88	83.0 4.9	82.2 4.9	82.6 4.9	82.4 4.92	85.1						
	2′	72.9 3.20	72.9 ¶	72.7 ¶	72.7 ¶	72.7 ¶	77.9						
	3′	78.0 3.3	77.9 ¶	77.9 ¶	77.9 ¶	77.8 ¶	79.6						
	4′	70.1 3.3	70.1 ¶	69.7 ¶	69.9 ¶	69.5 ¶	71.4						
	5′	80.7 3.3	81.0 ¶	80.6 ¶	81.1 ¶	80.6 ¶	80.6						
	6′	61.6 3.39	61.5 ¶	61.2 ¶	61.5 ¶	61.1 ¶	62.3						
Rhamnose moiety	1′					98.4 5.62		95.1			98.6 5.61	5.61	5.61
	2′					71.0 **		71.9			71.5 **	**	**
	3′					70.4 **		71.1			71.1 **	**	**
	4′					72.8 **		73.3			73.1 **	**	**
	5′					70.4 **		69.4			70.7 **	**	**
	6′					17.7 1.28		17.9			17.9 1.24	1.24	1.24
Aromatic moiety	1*			136.0	143.0	120.8			119.1	125.3	118.3		
	2*			130.1 ††	127.0 ††	154.2			154.6	131.6 7.39	154.5	7.46	
	3*			128.9 ††	127.0 ††	115.2 7.25			115.2 7.01	116.8 6.98	115.4 ‡‡	7.21	‡‡
	4*			128.4 ††	129.2 ††	131.2 7.37			130.9 7.35	157.0	131.2 ‡‡		‡‡
	5*			128.9 ††	127.0 ††	123.5 7.13			120.4 7.01	116.8 6.98	123.8 ‡‡	7.21	‡‡
	6*			130.1 ††	129.8 ††	130.0 7.37			130.8 7.35	131.6 7.39	131.2 ‡‡	7.46	‡‡
Others	1	163.6	161.4	163.3	161.0	163.7							
	2	36.9 3.4	44.8 2.95	39.0 4.2	42.1 3.26	34.7 4.19			39.4 4.18	43.6 4.15	40.2 4.21	4.17	4.2
	3	132.9 6.0	71.7		71.9 5.36								
	4	119.1 5.24–5.11	29.5 and 28.8 1.35										

† No. 1 corresponds to the glucosinolate carbon atom. No. 2 is the benzylic atom except for sinigrin and 2-hydroxy-2-methylpropylglucosinolate where Nos. 2, 3 and 4 designate the atoms in the aglucone part, for the other Nos., see Fig. 1.

‡ From ref. [20].

§ ^{13}C chemical shifts from ref. [15].

‖ From ref. [21].

¶ A complex pattern at 3.2–3.9 characteristic of the glucose part.

** A complex pattern at 3.3–4.2 characteristic of the rhamnose part; $\Delta J = 6$ Hz for the doublet from the three protons at C-6′.

†† A peak with five protons at 7.6.

‡‡ A complex pattern at 7–7.4; H-4 and H-6 in a multiplet, H-5 in a triplet, H-3 in a doublet, and with *o*-coupling constants $\Delta J = 7$ Hz, *m*-coupling constants $\Delta J = 2$ Hz.

technique on Whatman No. 1 paper or Schleicher and Schüll 2043 b paper [34]. HVE was carried out on Whatman 3MM 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 $AgNO_3$ [34] after drying at room temp. Prep HVE papers (buffer system 1 pH 1.9) were dried at room temp. in an NH_3 atmosphere.

Isolation of glucosinolates. Freeze-dried inflorescences (*ca* 30 g) were added to 200 ml boiling 70% MeOH and homogenized with an Ultra-Turrax homogenizer. The homogenate was boiled for 2–3 min, cooled and filtered. The residue was washed twice with 50 ml 70% MeOH. The combined filtrates were concd (to *ca* 5 ml), diluted with H_2O to 25 ml, extracted with 3 × 50 ml $CHCl_3$ and transferred to a column of Amberlite IR 120 (H^+, 2.5 × 90 cm). On elution with H_2O (fractions of 21 ml were collected at 150 ml/hr) the glucosinolate-containing fractions (5–20) were pooled, neutralized with M KOH, concd to small vol. (*ca* 10 ml), and applied to a weakly basic ion-exchange column (Ecteola-Cellulose, AcO^-, 2.5 × 80 cm). Fractions (14 ml) were collected at 80 ml/hr. After washing with H_2O (fractions 1–30), the column was eluted with M Py. The glucosinolates usually appeared in fractions 35–50. These fractions were taken to dryness and further purified by prep HVE in buffer pH 1.9 and prep PC in solvent b, followed by chromatography on Ecteola-Cellulose (AcO^-, 0.7 × 10 cm), leaving chromatographically pure glucosinolates. For PC and electrophoretic behaviour of **1** isolated from *R. odorata*, **8** isolated from *R. alba* and **9** isolated from *R. luteola*, see Table 1, and for ^{13}C and ^1H chemical shifts see Table 3. UV spectra of **1** in H_2O, M HCl and M NaOH were similar to those obtained for **10** [11], λ_{max} nm (log ε): 270 (3.04) and 276 (3.0).

Hydrolysis of **1** *with myrosinase catalysis* and $EtOH/NH_3$ treatment were performed by the previously described method [35] with the exception that the thiourea derivative **6** remained in the H_2O phase. **6** and glucose were isolated by prep PC in solvent 1. For TLC behaviour and ^1H chemical shifts of **6**, see Tables 2 and 3. UV spectra of **6** in H_2O and M NaOH showed peaks at λ_{max} nm: 248, 270, and 276. The glucose isolated from the hydrolysis mixture gave a positive response in the glucose oxidase assay and co-chromatographed with commercial D-glucose in PC using solvents 1, 2 and 3.

Hydrolysis of **6**. Reflux in 4 M NaOH (15 ml, 4 hr) of **6** (10 mg) yielded **7** which was isolated from the hydrolysis mixture by prep PC in solvent 1. The isolated compound was identical with authentic *o*-hydroxybenzylamine [11] as shown by co-chromatography on paper in solvents 1, 2 and 3, and HVE in the buffer systems 1, 2 and 3. UV spectra showed λ_{max} nm (log ε): 274 (3.30) in M HCl and λ_{max} nm (log ε): 293 (3.48) in M NaOH.

Hydrolysis of **1**. Heating in 6 M HCl (2.5 ml 60°, 4 hr followed by 18 hr at 25°) of **1** (25 mg) gave **2**, **3** and **4** (Fig. 1). The hydrolysis mixture was evapd to dryness, stored in a desiccator containing $CaCl_2$ for 24 hr at 0.02 mm Hg. The residue was then mixed with 300 μl DMF and 500 μl BSTFA (*N,O*-bis-(trimethylsilyl)-trifluoracetamide) and left at 60° for 30 min. Silylation of reference compounds was performed similarly using 2 mg carboxylic acid (or carbohydrate) and 1 mg methylstearate in 200 μl DMF and 200 μl BSTFA. MS of the TMSi derivative of **4** *m/e* (%): M^+ 452 (0.1), 437 (0.2), 393 (11.5), 370 (0.6), 369 (0.6), 362 (1.2), 355 (1.6), 347 (2.6), 341 (2.0), 355 (0.6), 333 (0.7), 319 (9.4), 305 (16.1), 291 (11.7), 268 (43.7), 245 (7.7), 231 (9.4), 217 (88.5), 203 (98.9), 191 (97.7), 189 (78.2), 147 (98.9), 130 (83.9), 117 (53.1), 75 (77.6), 74 (79.9), 73 (100). The MS of the TMSi derivatives of **2**, **3** and **4** were identical with lit. values [17, 19]. Compounds **2**, **3** and **4** isolated from an acid hydrolysis mixture of **1** by prep PC in solvent 1 were identical with authentic compounds as shown by co-chromatography on paper (solvents 1, 2 and 3) and for **2** by HVE (buffer systems 2 and 3).

Isolation and semiquantitative determination of **1** were performed similarly to the above method with homogenization of 2 g (freeze-dried inflorescences, leaves, or roots) in 50 ml boiling MeOH (twice). The conc filtrate was purified by prep HVE (buffer system 1), prep PC (solvent b), and chromatography on Ecteola-Cellulose (AcO^-, 0.7 × 10 cm). The amount of **1** was determined by UV using log $\varepsilon_{276} = 3.0$.

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REFERENCES

1. Kjær, A. and Larsen, P. O. (1973) *Biosynthesis* **2**, 71.
2. Swain, T. (1977) *Annu. Rev. Plant Physiol.* **28**, 479.
3. Kjær, A. (1977) *Pure Appl. Chem.* **49**, 137.
4. Kjær, A. and Larsen, P. O. (1976) *Biosynthesis* **4**, 179.
5. Ettlinger, M. G. and Kjær, A. (1968) in *Recent Advances in Phytochemistry* (Mabry, T. J., Alston, R. E. and Runeckles, V. C., eds.) Vol. 1, p. 58. Appleton-Century-Crafts, New York.
6. Vose, J. R. (1972) *Phytochemistry* **11**, 1649.
7. Benn, M. (1977) *Pure Appl. Chem.* **49**, 197.
8. Cole, R. A. (1976) *Phytochemistry* **15**, 759.
9. Kjær, A., Madsen, J. Ø. and Maeda, Y. (1978) *Phytochemistry* **17**, 1285.
10. Migirab, S. El., Berger, Y. and Jadot, J. (1977) *Phytochemistry* **16**, 1719.
11. Sørensen, H. (1970) *Phytochemistry* **9**, 865.
12. Dalgaard, L., Nawaz, R. and Sørensen, H. (1977) *Phytochemistry* **16**, 931.
13. Nielsen, J. K., Dalgaard, L., Larsen. L. M. and Sørensen, H. (1979) *Entomol. Exp. Appl.* (in press).
14. Ettlinger, M. G. and Lundeen, A. J. (1956) *J. Am. Chem. Soc.* **78**, 4172.
15. Friis, P., Larsen, P. O. and Olsen, C. E. (1977) *J. Chem. Soc. Perkin Trans.* 1, 661.
16. Daxenbichler, M. E., Van Etten, C. H. and Spencer, G. F. (1977) *J. Agric. Food Chem.* **25**, 121.
17. DeJongh, D. C., Radford, T., Hribar, J. D., Hanessian, S., Bieber, M., Dawson, G. and Sweeley, C. C. (1969) *J. Am. Chem. Soc.* **91**, 1728.
18. Karoum, F. and Sandler, M. (1971) *Clin. Chim. Acta* **32**, 391.
19. Mamer, O. A., Crawhall, J. C. and San Tjoa, S. (1971) *Clin. Chim. Acta* **32**, 171.
20. Olsson, K., Theander, O. and Åman, P. (1977) *Carbohydr. Res.* **58**, 1.
21. Voelter, W., Bilik, V. and Breitmaier, E. (1973) *Collect. Czech. Chem. Commun.* **38**, 2054.
22. Gorin, P. A. J. and Mazurek, M. (1975) *Can. J. Chem.* **53**, 1212.
23. Abdallah, M. S. (1967) *Meded. Landbouwhogesch. Wageningen* **67–8**, 1.
24. Delaveau, P. (1957) *Bull. Soc. Bot. Fr.* **104**, 148.
25. Kjær, A. and Gmelin, R. (1958) *Acta Chem. Scand.* **12**, 1693.

26. Underhill, E. W. and Kirkland, D. F. (1972) *Phytochemistry* **11**, 1973.
27. Gmelin, R. and Kjær, A. (1970) *Phytochemistry* **9**, 599.
28. Schraudolf, H. (1965) *Experientia* **21**, 520.
29. Gmelin, R. and Virtanen, A. I. (1961) *Ann. Acad. Sci. Fenn. Ser. A* II **107**, 3.
30. Saarivirta, M. (1973) *Planta Med.* **24**, 112.
31. Lüthy, J. and Benn, M. H. (1977) *Can. J. Biochem.* **55**, 1028.
32. Tookey, H. L. (1973) *Can. J. Biochem.* **51**, 1654.
33. Cole, R. A. (1978) *Phytochemistry* **17**, 1563.
34. Von Schultz, O.-E. and Wagner, W. (1956) *Z. Naturforsch. Teil B* **11**, 73.
35. Appelquist, L.-Å. and Josefsson, E. (1967) *J. Sci. Food Agric.* **18**, 510.