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CHAVICOL β-D-GLUCOSIDE, A PHENYLPROPANOID HETEROSIDE, BENZYL-β-D-GLUCOSIDE AND GLYCOSIDICALLY BOUND VOLATILES FROM SUBSPECIES OF *CEDRONELLA CANARIENSIS*

MATTHIAS COEN, RALF ENGEL and ADOLF NAHRSTEDT*

Institut für Pharmazeutische Biologie und Phytochemie, Westfälische Wilhelms-Universität Münster, Hittorfstr, 56, D-48149 Münster, Germany

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Key Word Index—*Cedronella canariensis*; ssp. *anisata*; ssp. *canariensis*; Lamiaceae; glycosidically bound volatiles; chavicol glucoside; benzyl alcohol glucoside; database SeKoMS.

Abstract—The volatile substances obtained after enzymatic hydrolysis of purified polar extracts from the aerial parts of the two subspecies of *Cedronella canariensis* were shown to be, among others, benzyl alcohol (29.4%), chavicol (11.6%), *cis*-3-hexenol (9.3%), 2-phenylethanol (8.6%), *cis*-pinocarveol (5.1%), myrtenol (2.2%), 1-phenylethanol (2.0%, tentatively), 1-octen-3-ol (1.8%), 1-hexanol (1.1%) for ssp. *canariensis* and chavicol (85.1%), benzyl alcohol (2.5%), *cis*-3-hexenol (2.5%), 1-octen-3-ol (2.3%); 1-hexanol (0.8%) for ssp. *anisata*. Sugars detected in the polar fraction after hydrolysis were glucose, rhamnose and fructose. The main glycosides obtained from the polar fraction before hydrolysis were chavicol glucoside (6 ppm, ssp. *anisata*) and benzyl alcohol glucoside (2 ppm, ssp. *canariensis*).

INTRODUCTION

Cedronella canariensis (L.) Webb & Berth., Lamiaceae (syn. *C. triphylla* Moench, *Dracocephalum canariensis*) is endemic to Makaronesia [1] and is traditionally used to reduce swelling of the mucous membranes of the respiratory tract, as a carminative, capillary tonic, hypoglucemiant and to reduce high blood pressure [2]. The plant showed analgesic, antipyretic and anti-inflammatory activity in experiments with rats and mice [3]. Crossing experiments and chemical data presented in [4] allow *C. canariensis* to be divided into two subspecies, ssp. *canariensis* and ssp. *anisata*. Ssp. *canariensis* contains 0.59% (m/v) essential oil, in which 210 compounds have been detected [4, 5], the main constituents being pinocarvone (52.9%), β-pinene (19.6), β-myrcene (9.3), sabinene (3.7), myrtenyl methyl ether (2.9), β-caryophyllene (2.2), myrtenol (1.7), β-phellandrene (1.6) and limonene (1.1). Ssp. *anisata* contains 0.37% essential oil, with 257 compounds [4, 5]; the main constituents are methylchavicol (82.2%), β-pinene (10.2), β-caryophyllene (2.8), pinocarvone (0.9), β-myrcene (0.8), trans-β-bergamotene (0.6, tentatively), α-pinene (0.6), oct-1-en-3-ol (0.4), sabinene (0.4) and β-phellandrene (0.15). Methylchavicol was not detected in ssp. *canariensis*. Plants that produce essential oils often contain heterosides with volatile aglycones; their physiological role in essential oil plants, however, is not fully understood [6]. We describe here the composition of volatile aglycones obtained after hydrolysis of the polar glycosidic fraction of both subspecies of the title plant and the main heteroside in each subspecies.

RESULTS AND DISCUSSION

The lyophilized aerial parts of the two subspecies of *C. canariensis* were extracted with MeOH and the heteroside fraction was prepared mainly by the procedure of Mulkens *et al.* [7], which includes petrol extraction to remove lipophilic compounds, column chromatography on Al_2O_3 to remove phenolic substances and MPLC on RP-18 to separate free sugars. The fraction obtained was 0.08% of the fresh plant material of ssp. *canariensis* and 0.06% of ssp. *anisata*. An aliquot of the heteroside fraction was incubated with β-glucosidase and partitioned with pentane. In the residual aqueous phase of ssp. *canariensis* glucose (67%), rhamnose (27%) and fructose (9%) could be detected by gas chromatography [8]; the aqueous fraction of ssp. *anisata* contained the same carbohydrates in slightly different amounts (64%, 17%, 12%, respectively). The pentane fraction containing the aglycones was analysed by GC-mass spectrometry. Identification was performed by the use of 'SeKoMS' (Search-Kóvats-Index-Mass-Spectra), a database for volatiles with more than 10 000 entries based on retention indices and mass fragment data [4, 9]. The results for the pentane fraction obtained from ssp. *canariensis* are presented in Table 1 and for that of ssp. *anisata* in Table 2.

Many aglycones of the glycosidically bound volatiles in both subspecies are aliphatic alcohols, phenylpropane

*Author to whom correspondence should be addressed.

Table 1. Volatiles obtained by enzymatic hydrolysis of the glycosidic fraction from *Cedronella canariensis* ssp. *canariensis* f. *pubescens*

No.	Compound	R_t ap.	R_t po.*	%†	M_r	m/z‡	Ident.
1	n. i.	776		0.84	?	57, 41, 56, 70, 84	
2	3-Methyl-2-buten-1-ol	798	1330	0.58	86	41, 71, 53, 67, 68, 59, 86	a
3	n. i.	807		4.05	?	41, 63, 69, 55, 59, 84	
4	3-Methyl-2-pentanol (t.)			0.18	102	45, 69, 56, 41, 87	b
5	n. i.	831		1.58	?	41, 69, 43, 71, 53, 84	
6	*cis*-3-Hexenol	853	1396	9.25	100	41, 67, 55, 82	a
7	*cis*-2-Hexenol	856		0.14	100	57, 41, 43, 67, 82	b
8	1-Hexanol	859	1367	1.09	102	56, 41, 55, 43, 69, 84	a
9	2-Methylcyclopentanol	876		0.29	100	57, 44, 82, 67, 41, 71, 99	b
10	1-Butoxy-2-propanol	928		0.21	132	57, 45, 41, 87	b
11	Benzaldehyde	932	1532	0.11	106	77, 51, 105, 106, 50	a
12	1-Heptanol	954		0.11	116	70, 55, 56, 43, 41, 69	b
13	Phenol	959		0.07	94	94, 66, 65	a
14	1-Octen-3-ol	960	1464	1.75	128	57, 43, 55, 67, 72, 110, 99	a
15	Benzyl alcohol	1007	1885	29.40	108	77, 79, 51, 108, 107, 91	a
16	2-Ethyl-1-hexanol	1020		0.63	130	57, 69, 43, 55, 70, 83	b
17	1-Phenylethanol (t.)	1035	1822	2.02	122	77, 79, 51, 107, 43, 104, 105	a
18	*p*- or *m*-Cresol	1056	2081	0.03	108	108, 107, 77, 79, 51, 90	b
19	n. i.	1081		0.11	154	43, 79, 91, 55, 59, 119, 85, 134	
20	2-Phenylethanol	1086	1924	8.55	122	91, 92, 65, 51, 122, 77	a
21	n. i. (isomer 1)	1105		0.66	122	91, 107, 77, 79, 122, 119, 120	
22	n. i. (isomer 2)	1112		0.28	122	91, 107, 77, 79, 122, 119, 120	
23	ar-Ethyl-benzenemethanol (t.)	1129		1.31	136	79, 107, 77, 51, 105, 117, 91, 118	b
24	Dimethylbenzylalcohol (t.)	1134		0.44	136	91, 93, 43, 65, 77, 117, 118, 121	b
25	n. i.	1137		0.16	?	91, 43, 67, 77, 93, 117, 121, 136	
27	Pinocarvone	1139	1574	3.52	150	41, 53, 81, 108, 135	a
28	n. i.	1157		0.41	?	43, 55, 70, 71, 88, 99	
29	*p*-Cymen-8-ol	1159		0.08	150	43, 132, 117, 135	b
30	Terpinen-4-ol	1163	1621	0.16	154	71, 93, 43, 111, 119, 86	a
31	*cis*-Pinocarveol	1176		5.12	152	41, 91, 92, 55, 70, 69, 119, 134	b
32	Methylsalicylate	1180	1787	0.62	152	120, 92, 152, 65, 121	a
33	Myrtenol	1183	1813	2.18	152	79, 93, 108, 135, 67, 121	a
34	*p*-Vinyl phenol or 2, 3 Dihydrobenzofuran	1188		0.17	120	91, 120, 65, 119, 81	b
35	n. i.	1200		0.36	?	67, 53, 139, 68, 96, 124	
37	n. i.	1202		0.19	?	69, 41, 83, 55, 95	
38	n. i.	1209		0.11	?	79, 115, 116, 134	
39	*p*-Chavicol	1234	2345	11.60	134	134, 133, 77, 107, 51, 91, 115	a
40	Geraniol	1238	1860	0.44	154	41, 69, 93, 77, 136	a
41	n. i.	1271		0.19	152	55, 81, 41, 69, 83, 124, 137	
42	Perilla alcohol	1290	2021	0.48	152	79, 91, 67, 68, 119, 134	a
43	Dihydroedulan-isomer	1297		0.13	194	179, 69, 43, 41, 107, 95	
43	n. i.	1318		0.13	?	73, 43, 91, 119, 134	
44	Eugenol	1331	2177	0.23	164	164, 77, 149, 55, 91, 103, 137	a
45	n. i.	1537		0.21	?	127, 55, 99, 82	
46	Dihydroactinidiolide	1460		0.19	180	43, 111, 67, 109, 137, 180, 152	b
47	Neophytadiene	1842		0.62	278	68, 43, 82, 95, 123	b
48	Benzyl salicylate	1846		0.10	228	91, 65, 228, 77, 51	b
				Σ 91.08			

n. i. Not identified.

? Not known.

t. Tentative.

*Retention index on apolar or polar column resp.

†Quantification according to the Area Percent Method without consideration of calibration factors (f), F = 1.0 for all compounds on apolar column.

‡Fragmentation ions, base peak and characteristic ions in decreasing order of relative abundance.

[a]Identification of compounds is based on comparison of their mass spectra with those of authentic samples in combination with their retention indices (SeKoMS).

[b]Identification of compounds is based on comparison of their mass spectra in combination with retention indices reported in the literature (SeKoMS).

Table 2. Volatiles obtained by enzymatic hydrolysis of the glycosidic fraction from *Cedronella canariensis* ssp. *anisata* f. *glabra*

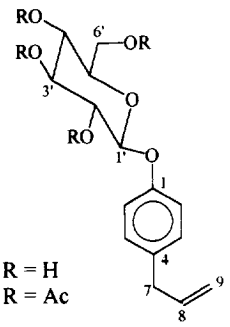
No.	Compound	R_I ap.	R_I po.*	%†	M_r	m/z‡	Ident.
1	n.i.	776		0.29	?	57, 41, 56, 70, 84	
2	2-Hexanol	784		0.13	102	45, 43, 41, 69, 84, 87	b
3	n. i.	807		0.21	?	41, 43, 69, 55, 59, 84	
4	*cis*-3-Hexenol	853	1395	2.45	100	41, 67, 55, 82	a
5	*cis*-2-Hexenol	856		0.13	100	57, 41, 43, 67, 82	b
6	1-Hexanol	859	1364	0.82	102	56, 41, 55, 43, 69, 84	a
7	4-Heptanol	881		0.05	116	55, 43, 73, 41, 56	b
8	3-Heptanol	884		0.03	116	59, 69, 41, 55, 53, 87, 98	b
9	2-Heptanol	897		0.02	116	45, 43, 44, 55, 69, 70, 83, 98	b
10	4-Methylcyclohexanol	929		0.01	114	57, 41, 55, 81, 70, 96	b
11	Benzaldehyde	932	1533	0.07	106	77, 51, 105, 106, 50	a
12	Phenol	957	2010	0.02	94	94, 66, 65	a
11	1-Octen-3-ol	960	1462	2.26	128	57, 43, 55, 67, 72, 110, 99	a
11	3-Octanol	979	1404	0.06	130	59, 55, 83, 41, 101	a
12	Benzyl alcohol	1007	1885	2.53	108	77, 79, 51, 108, 107, 91	a
13	2-Ethyl-1-hexanol	1020		0.05	130	87, 41, 43, 70, 83, 98	b
14	*o*-Cresol	1032		0.05	108	108, 107, 77, 79, 51, 90	b
15	1-Phenylethanol (t.)	1035	1822	0.44	122	77, 79, 51, 107, 43, 104, 105	a
16	*m*- or *p*-Cresol	1054	2083	0.15	108	107, 108, 79, 77, 51, 90	
17	2, 6-Dimethylphenol	1080	1918	0.06	122	107, 122, 77, 91, 79, 121	a
18	2-Phenylethanol	1086	1923	0.65	122	91, 92, 65, 51, 122, 77	a
19	*o*-Ethylphenol	1108		0.02	122	107, 77, 122, 79, 51, 91	a
20	n. i.	1131		0.15	?	77, 79, 107, 51, 117, 91, 134, 136	
21	n. i.	1151		0.13	?	91, 93, 43, 118, 117, 77, 136	
22	Methylsalicylate	1177	1787	0.63	152	120, 92, 152, 65, 121	a
23	Myrtenol	1185	1813	0.01	152	79, 91, 119, 108, 67, 134	a
24	*p*-Chavicol	1241	2345	85.05	134	134, 133, 77, 107, 51, 91, 115	a
25	Dihydroxymethyl-benzoate (isomer I)	1303		0.04	168	136, 52, 168, 80, 108	b
26	*trans*-*p*-Anol	1321		0.03	134	134, 133, 105, 77, 107, 91, 115	b
27	Eugenol	1344	2176	0.05	164	164, 77, 55, 149, 103, 137	a
28	Chavibetol	1350		0.06	164	164, 77, 149, 91, 103, 137	b
29	(isomer II of cmpd. 25)	1356		0.07	168	136, 52, 168, 80, 108	
30	*trans*-β-Damascenone	1373		0.01	190	69, 41, 91, 121, 77, 105	b
31	3-Hydroxy-β-damascone (t.)	1591		0.04	208	69, 43, 71, 175, 121, 208, 193, 190	b
32	n. i.	1738		0.68	252	105, 77, 84, 122, 188	
33	Benzyl salicylate	1846		0.01	228	91, 65, 228, 77, 51	b
				Σ 97.46			

For abbreviations see footnote to Table 1.

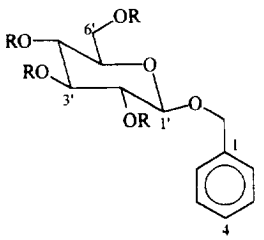
derivatives and phenylpropane-related compounds. Apart from 1-octen-3-ol (0.21% in the essential oil of ssp. *canariensis*, 0.43% in that of ssp. *anisata*) the volatile aglycones found here occurred in the respective essential oil only in traces or not at all [5]. Aglycones such as *cis*-3-hexenol, hexan-1-ol, 1-octen-3-ol, 3-octanol, benzyl alcohol, 2-phenylethanol and eugenol have been found in varying combinations in all plant species of which the total glycosidic bound volatiles pattern has been analysed, including plants without essential oils [6]. The ubiquity of eugenol glycosides in the Lamiaceae has led to the hypothesis that eugenol may play a role in lignin biosynthesis [10].

Some of the detected aglycones lack a hydroxyl group for the glycosidic bond. 3-Hydroxy-β-damascone in the aglycone fraction of apple fruits [11] may be the precursor of β-damascenone [12]. Aldehydes such as benzaldehyde may bind to sugars in the form of acetals and/or hemiacetals, which are labile in an acidic environment [13]. The monoterpene ketone pinocarvone in the aglycone fraction of ssp. *canariensis* may be formed from a corresponding enol glycoside. Glycosidically bound *cis*-pinocarveol (5.12% in the aglycone fraction of ssp. *canariensis*) may indicate a link to the essential oil as the monoterpene ketone pinocarvone was 52.9% of the steam-volatile fraction of ssp. *canariensis* [5].

From the heteroside fraction of ssp. *anisata*, **1** was obtained by chromatography on silica gel and was finally purified by preparative HPLC. Compound **1** gave glucose (GC) and chavicol (GC) on hydrolysis with β-glucosidase. The ^1H and ^{13}C NMR data (Table 3) are in full agreement with the proposed chavicol β-D-glucoside. The allylic side chain of the *p*-disubstituted aromatic ring system (AA'BB' at δ = 6.92 and 7.11 ppm) is indicated by the multiplet of the terminal olefinic protons (C-9) at δ5.01 and the multiplet of the single olefinic proton (C-8)



1 R = H
2 R = Ac



3 R = H
4 R = Ac

at δ5.94. The anomeric glucose proton at δ4.90 with its coupling constant of 7.25 Hz reveals β-configuration of the glycosidic linkage. Acetylation of **1** yielded the tetraacetate **2**, with NMR data as shown in Table 3. From the heterosidic fraction of ssp. *canariensis* **3** was purified by the same methods as used for **1**. The ^{1}H and ^{13}C NMR data of **3** (Table 3) were in agreement with those published in [14] and [15] for benzyl alcohol β-D-glucoside; acetylation led to the peracetate **4**. Synthesis of **1** and **3** was performed with established methods using acetobromoglucose and chavicol [16, 17] and benzyl alcohol [18, 19], respectively, yielding the peracetates **2** and **4**. Deacetylation was performed with NaOMe [17, 20] to give **1** and **3**. The spectroscopic data for synthesized **1** and **3** were in full agreement with **1** and **3** isolated from the natural sources.

To our knowledge, chavicol β-D-glucoside is isolated and described here for the first time [21]. Other glucosides of chavicol, however, *are* known: chavicol rutinoside (lusitanicoside) has been isolated from *Cerasus lusitanica* Lois. (Rosaceae) [22], its β-D-apiofuranosyl-(1 → 6)-β-D-glucoside (furcatin) from *Viburnum furcatum* (Caprifoliaceae) [23] and its β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (myaginin) from *Lespedeza thunbergii* (Leguminosae) [24]. Compound **3** was recently isolated from *Epimedium grandiflorum* var. *thunbergianum* (Berberidaceae) [15] and was found in *Vitis vinifera* (Vitaceae) [25] and *Camellia sinensis* var. *yabukita* (Theaceae) [26]. Compound **3** was also detected as a bitter substance in aqueous extracts of apricot kernels [27], as a major metabolite of benzyl alcohol in barley resistant to green bug [28] and in the growth inhibiting fraction of male flowers of *Cucurbita pepo* (Cucurbitaceae) [29].

A good correlation is observed between the level of chavicol glucoside in the polar fraction and methylchavicol in the essential oil of ssp. *anisata*. Chavicol represents 85% of the aglycone fraction, whereas methylchavicol reaches 82% in the essential oil [5]. Although methylchavicol and chavicol were not detected in the essential oil of ssp. *canariensis* [4, 5], chavicol comprises 11% of the aglycone fraction of ssp. *canariensis* after enzymatic hydrolysis. One may argue that there is a biogenetic link between chavicol β-D-glucoside and methylchavicol in the ssp. *anisata*. If this is true it seems obvious that only ssp. *anisata* contains the enzymes necessary to metabolize **1** and chavicol [4] to methylchavicol. Investigations to confirm this hypothesis are in progress.

EXPERIMENTAL

General. Mps were measured with a Leitz Laborlux 12 apparatus. Optical rotation was recorded at 21°C with

Table 3. ^{1}H and ^{13}C NMR data of compounds **1–3**

	^{1}H NMR data in δ-scale			^{13}C NMR data	
Position	**1**	**2***	**3**	**1**	**3**
1				157.5	139.0
2	7.11, *d*, *J* = 8	7.11, *d*, *J* = 8.7	7.35 m	117.8	128.6
3	6.92, *d*, *J* = 8.68	6.92, *d*, *J* = 8.68	7.35 *m*	130.5	129.2
4			7.35 *m*	135.3	129.1
5	6.92, *d*, *J* = 8.68	6.92, *d*, *J* = 8.68	7.35 *m*	130.5	129.2
6	7.11, *d*, *J* = 8	7.11, *d*, *J* = 8.7	7.35 *m*	117.8	128.6
7	3.34, *d*, *J* = 6.57	3.34, *d*, *J* = 6.57	A 4.66, *d*, *J* = 11.79	40.3	71.6
			B 4.93, *d*, *J* = 11.78		
8	5.93, *m*	5.94, *m*		139.2	
9	5.00, *m*	5.01, *m*		115.6	
1′	4.90, *d*, *J* = 7.25	5.04, *d*, *J* = 8.23	4.37, *d*, *J* = 7.36	102.5	103.2
2′				75.0	75.0
3′				78.0	78.0
4′				71.4	71.6
5′		3.84, *ddd*		78.1	77.9
6′	A 3.68, *dd*, *J* = 4.6, 11.9	A 4.16, *dd*, *J* = 2.52, 14.84	A 3.70, *dd*, *J* = 5.33, 17.18	62.5	62.8
	B 3.86, *dd*, *J* = 1.8, 11.9	B 4.30, *dd*, *J* = 5.22, 17.56	B 3.91, *dd*, *J* = 1.98, 13.66		

*Four signals due to acetyl groups at δ2.04, 2.05, 2.06, 2.08 (each 3H, *s*).

a Perkin-Elmer 241 polarimeter and UV spectra on a Shimadzu UV-260. ^1H and ^{13}C NMR spectra were recorded for **1** in acetone-d_6, **2** and **4** in $CDCl_3$ and **3** in CD_3OD on a Varian Gemini 200 at 200 (^1H) and 50 (^{13}C) MHz; chemical shifts are in ppm relative to TMS.

Plant material. The two subspecies of *Cedronella* were grown in the experimental garden of this Institute and harvested at the flowering stage. The plants were obtained by vegetative propagation of two mother plants from Teneriffe to give two clones (C1, collected in the Anaga mountains above the small town of El Bailadero, ca. 800 m above sea level, 1983; A1 collected southeast of the Ladera de Güimar, ca. 1000 m above sea level, 1983). Voucher specimens are deposited under PBMS70a (ssp. *canariensis* forma *pubescens*, clone C1) and PBMS71a (ssp. *anisata* forma *glabra*, clone A1).

Isolation. Fresh aerial parts (1200 g ssp. *anisata*/2000 g ssp. *canariensis*) were chopped under liquid N_2, lyophilized and extracted (dry wt 312/518 g) three times with 2 l MeOH at room temp.; the extract was taken to dryness under reduced pressure and the residue (18.4/31.5 g) suspended in 100 ml H_2O. The suspension was kept for 10 hr at 4° and then centrifuged (5 min, 4000 rpm) to remove chlorophyll. The supernatant was extracted six times with petrol until no volatiles could be detected by GC in the organic phase. The red–brown aqueous phase was concd under red. press. and chromatographed on a column of neutral alumina (Merck 1077, 50 × 2.5 cm) with 2 l H_2O. The heterosidic fraction eluted between 80 and 400 ml as indicated by TLC on silica gel (detection: UV and anisaldehyde–H_2SO_4). The residue (5.3/9.7 g) obtained after evapn was chromatographed using MPLC (Büchi, Flawil, CH; 26 × 2.6 cm) on RP 18 (Europrep 60–30 C18, 60 A 20–45 μ, Besta Technik) and 2 l H_2O (5 ml min^{-1}) followed by 1 l MeOH as the mobile phase. The heterosides eluted between 2200 and 2420 ml as indicated by UV detection (235 nm, LKB Bromma 2151, variable wavelength monitor) and TLC on silica gel. This fraction was taken to dryness and lyophilized to a light yellow powder (0.8/1.6 g). Aliquots of this were hydrolysed (see below). The rest of the heterosidic fraction was chromatographed on silica gel (30 × 2 cm) with a step gradient of 50 ml H_2O–satd EtOAc, then H_2O–satd EtOAc with 1, 2 and 3% MeOH as eluent (50 ml each, 2 ml min^{-1}). Compound **1** eluted between 110 and 140 ml, **3** between 170 and 185 ml. They were finally purified by preparative HPLC (**1**: system A; **2**: system B; see below).

Compound **1**. Yield 7 mg. $[\alpha]_D^{20} = -53.01°$ (MeOH; *c* 0.2), mp 142°, UV λ_{max}^{MeOH} nm (log ε): 229 (2.1), 273 (0.9), 280 (0.8), 320 (0.31).

Compound **2**. Yield 5 mg. $[\alpha]_D^{20} = -54.5°$ (MeOH; *c* 0.2), mp 124°, UV λ_{max}^{MeOH} nm (log ε): 213.6 (0.9), 252.2 (0.056), 257.8 (0.06).

Enzymatic hydrolysis [30]. A 10 mg aliquot of the heterosidic fraction was dissolved in 5 ml citrate buffer (pH 5.5). The solution was extracted with *n*-pentane (Nanograde®) until no volatiles could be detected in the organic phase by GC, and incubated with 5 mg β-glucosidase (Serva, No. A2830) for 24 hr at 37° under pentane (1 ml). The aglycones were extracted with *n*-pentane and analysed by GC-MS. Hydrolysis of **1** and **3** was carried out by incubation with β-glucosidase in the same way.

Preparation of sugars for GC [8]. To 0.5 ml of the aqueous phase 0.5 ml of 1% $NaBH_4$ in water was added. The solution was kept for 30 min at room temp. and the excess borohydride was destroyed by addition of 0.5 ml of Amberlite IR-120 (H^+, 300–1000 μm, analytical grade, Serva). The resin was removed by filtration and the filtrate evapd to dryness. 1 ml MeOH was added and evapd to dryness to remove borate as methyl borate; this was repeated three times. The sample was then dried *in vacuo*, dissolved in 20 μl pyridine and treated with 60 μl BSTFA and 20 μl TMCS. After standing 1 hr at room temp. the mixture was used for GC and compared with sugar standards. To separate glucose and fructose preparation was done without reduction.

Synthesis of chavicol from methyl chavicol [31]. To an ice-cooled solution of methyl iodide (7.2 g) in dry ether (20 ml) magnesium ribbon (1.3 g) was added while stirring. The solvent was removed from the reaction mixture by heating to 160° to give a solid. At ambient temp. methyl chavicol (6.0 g, 4-allylanisole, Aldrich) was added. The reaction mixture was kept for 3 hr at 160–170° under reflux. After cooling ice water was added, followed by water and 1 N HCl (10 ml). The mixture was extracted with EtOEt and the EtOEt layer further extracted with 10% NaOH (50 ml). The alkaline solution was acidified with HCl to pH 1 and extracted with EtOEt. The latter was washed with water, dried over anhydrous Na_2SO_4 and evapd to give a light red solid. The crude product was purified by vacuum distillation (15 mm Hg, 110°) to give chavicol (2.2 g) as a colourless oil.

Synthesis of **2** [16, 17]. α-Acetobromoglucose (Merck, 4.12 g) was dissolved at room temp. in 16 ml Me_2CO and added to a solution of 0.68 g chavicol in 4 ml Me_2CO and 7 ml 0.725 N KOH. After ca. 20 min the pH of the resulting solution dropped below 8.0. Then 7 ml 0.725 N KOH was added dropwise, at a rate sufficient to maintain the pH above 8.0. The reaction mixture was then set aside for 2 hr until the pH was approximately 7.0. The solution was extracted with PhMe and the organic layer evaporated. The residue was purified by CC on silica gel with PhMe–Me_2CO (4:1) and crystallized from Me_2CO–H_2O to give **2**.

Synthesis of **4** [18, 19]. To a solution of benzyl alcohol (109 mg; Merck) in 25 ml dry ether, Ag_2O (2 g; Merck) and a solution of α-acetobromoglucose (2.05 g) in 25 ml dry EtOEt were added. The mixture was stirred for 3 days under N_2 atmosphere, filtered and the solvent evapd to give a colourless syrup, which was purified as described for **2**.

Deacetylation [17, 20]. Compound **2** or **4** was dissolved in 5 ml 0.5% NaOMe in dry MeOH and kept at room temp. with stirring for 20 min. The alkaline solution was neutralized with Dowex 50 WX4 (H^+-form, 20–50 mesh, Fluka); the solution was then evapd. The

crude product was purified on BondElut® C8 (Analytichem International) using a step gradient from 100% H_2O to 100% MeOH. Compound **1** was eluted with 50% MeOH, **3** with 30% MeOH.

Acetylation. Compound **1** (4 mg) was dissolved in 100 μl pyridine, and 100 μl Ac_2O was added. After 24 hr at ambient temp. 10 ml ice water was added; the resulting precipitate was sepd by filtration through a glass filter (G4) and redissolved in Me_2CO. The solvent was evaporated to give **2**.

TLC systems. The heterosides were chromatographed on silica gel plates (Merck) with EtOAc–$CHCl_3$–H_2O–HCOOH (82:8:5:5) [32]; detection was with UV and anisaldehyde–H_2SO_4; R_f of **1** 0.39; R_f of **3** 0.29. Compounds **2** and **4** were chromatographed on silica gel with PhMe–Me_2CO (25:3); detection with UV and anisaldehyde–H_2SO_4; R_f of **2** 0.36; R_f of **4** 0.31.

GC systems. The volatile aglycones were chromatographed on a Carlo Erba GC 6000 equipped with a FID. A DB-wax capillary column (J&W Scientific), 30 m × 0.32 mm, film thickness 0.5 μm was used for sepn with a temp. program increasing from 50° at 2° min^{-1} to 230°, then isothermally; He 39 cm s^{-1}, cold on-column injection: 0.6 μl, 20 sec secondary cooling, injector temp. < 50°, detector temp. 250°. The silylated sugars were chromatographed on a Satochrom GC with a FID. A cross-linked methylsilicon capillary column (DB-1, J&W Scientific), 30 m × 0.32 mm, film thickness 0.25 μm was used with a temp. program from 150° increasing 3° min^{-1} to 280°, then isothermally, N_2 32.3 cm s^{-1}. Split 1:20; injection volume 1 μl; injection temp. 250°, detect. temp. 290°.

GC-MS systems. The volatile aglycones were investigated by GC-MS on a non-polar methyl silicone column using a Varian 3700 equipped with 50 m × 0.32 mm i.d. (film thickness 0.52 μm) HP-1 (Hewlett-Packard) column; 50–290°, 4° min^{-1}, then isothermally, He (2 ml min^{-1}), (5 sec. splitless injection: 1 μl, 230°), detector temp. 250°, the GC was coupled with a Finnigan MAT 44S (open split coupling – 200°), ion source 200°, EI = 70 eV, mass range 41–360. Linear retention indices were determined externally with a series of *n*-alkanes (C_7–C_{27}) [33] and were calculated with a basic program included in the SeKoMS database.

HPLC systems. HPLC was carried out with a Waters 600 pump, a Waters 490 multiwavelength detector and a Rheodyne 7125 injector; the column was from Knauer (250 × 20 mm) Hyperosil ODS 5 μm. System A: MeOH/H_2O (1:1), isocratic, flow 8 ml min^{-1}, detection at 225 and 280 nm; System B: MeCN/H_2O (1:4), isocratic, flow 8 ml min^{-1}, detection at 210 and 257 nm.

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