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Glycosidically Bound Volatiles and Flavor Precursors in *Laurus nobilis* L.

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Glycosidically bound volatile compounds in different parts (leaves and buds) of *Laurus nobilis* L. were investigated. After isolation of extracts obtained by Amberlite XAD-2 adsorption and methanol elution, glycosides were analyzed after enzymatic hydrolysis by GC-MS or directly after trifluoroacetyl (TFA) derivatization by GC-MS in EI and NCI mode. In the leaves most of the glycosidically bound volatiles occur as β-D-glucopyranosides. Among the disaccharides, primeverosides are predominant; smaller amounts of α-L-arabinofuranosyl-β-D-glucopyranosides, rutinosides, and vicianocides could also be identified. Major aglycons comprised benzyl alcohol, some linalool-diols, 2-hydroxy-1,8-cineole and its derivatives such as 2,3-dehydro-1,8-cineole, sobrerols, and menthadien-8-ols. Among the identified nor-carotenoids, 3-oxo-α-ionol, the corresponding 7,8-dihydro derivative, and vomifoliol are predominant in leaves. 3-Hydroxy-β-damascone and 3-hydroxy-7,8-didehydro-β-ionol, precursors of the sensorially active damascenone, were identified only in the buds.

KEYWORDS: Laurel; *Laurus nobilis* L.; aglycons; glycosides; glycosidically bound volatiles

INTRODUCTION

The presence of glycosidically bound volatile compounds in plants has been well established. These compounds are able to release free aroma compounds by enzymatic or chemical cleavage during plant maturation, industrial pretreatments, or processings and can be considered as aroma precursors (*1*).

Analyses showed that glycosidically bound volatiles are more common in plants than was originally assumed. In almost 170 plants (e.g., grapes and wine, hops, fruits, tea, spices, and herbs) many glycosidically bound volatiles were detected. Glycosides were found not only in the green parts (leaves) but also in other organs such as rhizomes, petals, fruits, and flowers. Seasonal variation is another factor in the distribution of glycosidically bound volatiles (*2*, *3*).

Laurus nobilis L., a symbol of Apollo in Greek mythology, is a characteristic evergreen Mediterranean tree. It is used extensively in the food industry, as well as in drugs and cosmetics (*4*, *5*). In 2000 Turkey exported 3600 tons of *L. nobilis* leaves with U.S. $7 500 000 income (*6*, *7*). Recently we reported the volatile constituents and key odorants in *L. nobilis* L. (*8*). To our knowledge there has not been any study on the glycosidically bound volatiles in laurel; therefore, we aimed to determine whether glycosides of volatiles and flavor precursors in leaves and buds might be present in this spice.

In this paper the identification of enzymatically liberated aglycons by GC-MS and on-line identification of glycosidically bound volatiles and flavor precursors by GC-MS of their TFA derivatives are reported.

MATERIALS AND METHODS

Reagents and Reference Samples. Amberlite XAD-2 resin was purchased from Supelco, Bellefonte, PA. Methanol, pyridine (water-free), *N*-methyl-bis(trifluoroacetamide) (MBTFA), phenyl-β-D-glucopyranoside, β-glucosidase from almonds (6.3 units/mg), and hesperidinase from *Aspergillus niger* (7 units/g) were purchased from Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany. Polyclar AT was obtained from Serva Electrophoresis GmbH, Heidelberg, Germany.

Plant Material. Fresh leaves and buds of *L. nobilis* L. were collected from the Black Sea region of Turkey (Zonguldak) in 2000 and were sent to Germany. They were frozen until analyses. Fresh leaves were sampled both in March (61 g) and in August (86 g). Buds were taken in March (77 g).

Isolation of Glycosides. Laurel samples were ground in liquid nitrogen, and 1 mL of an aqueous solution of phenyl-β-D-glucopyranoside (62.5 mg/mL) was added as an internal standard. Samples were extracted twice with distillated hot (90 °C) water (350 and 250 mL) for 25 min each, as described by Wang et al. (*9*).

The slurry was filtered through glass wool and rinsed with 100 mL of distilled water. To remove polyphenols, 12 g of Polyclar AT was added by stirring. The resulting filtrate was concentrated to 70 mL under reduced pressure (<50 °C, <80 mbar). To precipitate proteins, 210 mL of methanol was added and then filtered. Methanol was removed completely on a rotary evaporator. Twenty milliliters of resulting aqueous residue was introduced into a glass column filled with 100 g of Amberlite XAD-2, conditioned as described (*9*, *10*). The column was eluted with 1 L of distilled water (flow rate = 6 mL/min) to remove sugars, 500 mL of pentane/dichloromethane (2:1 v/v; flow rate = 7 mL/min) to remove volatile compounds, and 500 mL of

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methanol (flow rate = 5 mL/min) to elute the glycosides. This eluate was named glycoside extract (GE). The GE of fresh leaves was divided into two parts (GE_LI and GE_LII) and the GE of buds into four parts (GE_BI, GE_BII, GE_BIII, and GE_BIV) for determination of aglycons and glycosides.

Trifluoroacetylation of GE. MBTFA was used as TFA reagent. GE_LII was concentrated to dryness; 100 μL of pyridine (water free) and 100 μL of MBTFA were added for trifluoroacetylation. The closed vial was maintained at 60 °C for 1 h and analyzed directly by GC-MS (*11*, *12*) and NCI-GC-MS (*13*).

Enzymatic Hydrolysis of GE. GE_LI was concentrated to dryness and dissolved in 50 mL of phosphate−citrate buffer (pH 5.0) and then divided into two parts for incubation. A 25 mL portion was incubated with 25 mg of β-D-glucosidase at 40 °C for 20 h. The other 25 mL was used as a control sample (incubated without enzyme).

After concentration to dryness GE_BI, GE_BII, and GE_BIII were each dissolved in 25 mL of phosphate−citrate buffer (pH 5.0) and GE_BIV was dissolved in 370 mL of water. GE_BI was incubated at 40 °C with 25 mg of β-D-glucosidase for 20 h, GE_BII at 40 °C with 250 mg of hesperidinase for 120 h, and GE_BIII without enzyme at 40 °C for 120 h. Hydrolysis with hesperidinase for 5 days should liberate high yields of nor-carotenoids such as vomifoliol (*14*). After incubation, each sample was extracted four times with 50 mL of ether. The organic extracts were concentrated on a Vigreux column (40 °C) to a final volume of 1 mL and analyzed by GC-MS to determine the aglycons. GE_BIV was extracted first with ether to remove free volatiles and then subjected to a simultaneous distillation extraction (SDE) for 2 h.

GC-MS Analyses of TFA Derivatives and Aglycons. A Hewlett-Packard 5890A gas chromatograph coupled to a Finnigan MAT 8200 mass spectrometer equipped with a DB-5 (J&W, 30 m × 0.25 mm i.d., 0.25 μm) fused silica capillary column was used for the analyses of TFA derivatives. The temperature program was 120 °C raised at 2 °C/min to 260 °C, the injector temperature was 250 °C, the carrier gas was helium at 1.15 mL/min, the split ratio was 1:10, the transfer line temperature was 230 °C, and the ionization chamber temperature was 210 °C. Mass spectra were recorded at 70 eV in electron impact (EI) mode and at 150 eV in negative chemical ionization (NCI) mode; the mass range was from 400 to 1200 amu. NCI mass spectrometry was applied using CH_4 as reagent gas. The injected sample amount was 1 μL.

For the analyses of aglycons a Siemens SiChromat II GC directly coupled to a Finnigan MAT 8222 mass spectrometer equipped with an SPB 5 (Supelco, 30 m × 0.53 mm i.d., 1.5 μm) fused silica capillary column was used. The temperature program was 100 °C raised at 5 °C/min to 250 °C, the split ratio was 1:10, the carrier gas was helium at 3 mL/min, the injector temperature was 250 °C, the transfer line temperature was 200 °C, the ionization chamber temperature was 200 °C, and the ionization energy was 70 eV. The injected sample amount was 2 μL.

Identification of compounds detected by GC-MS analyses was done by comparing mass spectra and retention indices (RI) with our own MS/RI library created from commercially available (Merck, Fluka, Sigma-Aldrich Chemie GmbH) reference substances and compounds isolated from natural sources (*3*, *8*, *15*).

Ratio data (**Table 1**) were based on the integration of characteristic fragment ions, which were normalized with respect to phenol liberated from the internal standard phenyl-β-D-glycopyranoside.

RESULTS AND DISCUSSION

Aglycons obtained from different laurel extracts after enzymatic cleavage with glucosidase and also hesperidinase are summarized in **Table 1**.

As can be seen from **Table 1**, with the exception of eugenol, glycosidically bound aliphatic [e.g., 3(*Z*)-hexenol] and aromatic (e.g., benzyl alcohol) alcohols predominate in leaves from August when compared to those from March. Compared to leaves, higher amounts of some of these aglycons (e.g., methylbutanols, benzyl alcohol, phenylethanol, and eugenol) were liberated from buds. On the contrary, the peak areas and consequently the amounts of 3(*Z*)-hexenol and hexanol as well as phenolic compounds (e.g., vanillin, *E*-isoeugenol, tyrosol, and its methoxy derivatives) were found to be much lower or negligible in buds. In general, better yields of aglycons from buds were obtained with hesperidinase, perhaps due to the different activities and longer incubation time.

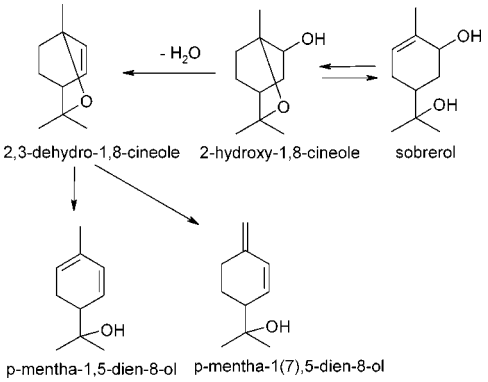

Figure 1. Proposed transformation pathway of 2-hydroxy-1,8-cineole to its derivatives.

Only small amounts of glycosidically bound linalool, geraniol, and α-terpineol could be identified in the extracts from leaves and buds. Major monoterpenes such as 2,3-dehydro-1,8-cineole and the 2-hydroxy-1,8-cineoles as well as the structurally related *p*-menthadien-8-oles (**Figure 1**) were also found in higher amounts in August, whereas the contents of the sobrerols, linalool-diols (such as 3,7-dimethylocta-1,5-diene-3,7-diol, 3,7-dimethylocta-1,7-diene-3,6-diol, and 3,7-dimethylocta-1,6-diene-3,8-diol) and hotrienol showed to be higher in March. In buds, lower or similar amounts of monoterpenic glycosides were observed compared to that in leaves. In the experiments with hesperidinase 2-hydroxy-1,8-cineole I could not be detected; at the same time an increase of the compounds shown in **Figure 1** was observed. This might be attributed to a chemical transformation of 2-hydroxy-1,8-cineole I during incubation (see **Figure 1** and **Table 1**). Linalool oxides were found in only the glycosidic extract of buds.

Nor-carotenoids such as 3-oxodamascenone as well as 3-hydroxy-β-damascenone and 3-hydroxy-7,8-didehydro-β-ionol, which are known to be precursors of β-damascenone, were observed only in the buds. The contents of 3-oxo-α-ionol and 3-oxo-7,8-dihydro-α-ionol were also increased in buds in March, but these compounds obviously rise until August in the leaves. As expected, nor-carotenoid aglycons such as vomifoliol were liberated in a higher yield after 5 days of incubation with hesperidinase (*14*). It cannot be excluded that during that prolonged incubation time artifact formation takes place, as indicated by formation of free β-damascenone. Surprisingly, no free volatile isoprenoids have been identified in laurel leaves and buds (*8*).

After simultaneous distillation extraction (SDE) of GE_BIV, β-damascenone and megastigma-3,5-dien-7-yn-9-one were found to be the major compounds, liberated by hydrolytic cleavage. Additionally, 3-hydroxy-β-damascone, 3-oxodamascone, the isomeric linalool oxides, hotrienol, α-terpineol, *p*-cymen-8-ol, *p*-cymene, eugenol, benzyl alcohol, and an unidentified compound with a mass spectrum similar to that of β-damascenone have been formed.

The acetylenic diol 3-hydroxy-7,8-didehydro-β-ionol is of particular interest due to its important role as flavor precursor. It has been described to give 3-hydroxy-β-damascone and β-damascenone (**Figure 2**) under acidic conditions (*16*). It is

Table 1. Comparison of the Ratio of Peak Areas (Normalized to Phenol) of Selected Fragment Ions from Aglycons Liberated by Enzymatic Treatment of Glycosidic Extracts from *L. nobilis* L. (Leaves and Buds)

RI SPB5	aglycon	leaves, Aug/March, glucosidase	buds/leaves, March, glucosidase	buds, hesperidinase/ glucosidase	ID[a]
734	3-methylbutan-1-ol	1.5	1.5	1.2	r
738	2-methylbutan-1ol	2.0	2.0	1.0	r
764	pentanol	2.0	1.0	2.0	r
775	3-methybut-2-en-1-ol	1.4	1.2	1.5	r
862	3(*Z*)-hexenol	4.5	0.1	2.5	r
869	hexanol	1.3	0.5	2.0	r
1006	2,3-dehydro-1,8-cineole	2.0	0.2	2.3	r
1054	benzyl alcohol	4.0	1.6	1.5	r
1082	linalool oxide furanoside I	*b*	*b*	24.0	r
1097	linalool oxide furanoside II	*b*	*b*	26.7	r
1106	linalool	2.0	2.0	1.0	r
1112	hotrienol	0.3	0.3	5.0	r
1137	2-phenylethanol	2.6	2.2	1.1	r
1163	nerol oxide	0.3	0.3	5.0	r
1183	linalool oxide pyranoside	*b*	*b*	1.1	r
1187	*p*-menth-1,5-dien-8-ol	2.7	0.3	3.5	r
1190	borneol	0.5	0.3	11.0	r
1198	3,7-dimethylocta-1,5-diene-3,7-diol	0.5	1.2	1.0	r
1210	α-terpineol	2.5	15	5.0	r
1215	p-menth-1(7),5-dien-8-ol	2.9	*c*	*c*	r
1239	2-hydroxy-1,8-cineole I	19.5	2.0	0.0	r
1247	2-hydroxy-1,8-cineole II	8.2	1.6	1.1	r
1258	3-hydroxy-1,8-cineole	*b*	*b,c*	*c*	r
1263	geraniol ?	1.5	0.5	1.0	r
1286	3,7-dimethylocta-1,7-diene-3,6-diol	0.2	0.8	1.0	r
1310	menthadien-8-ol, isomer 1	3.6	0.6	0.7	t
1331	menthadien-8-ol, isomer 2	4.4	0.8	0.5	t
1373	menthadien-8-ol, isomer 3	0.3	0.2	1.2	t
1380	eugenol	0.7	1.7	2.2	r
1402	β-damascenone	*b*	*b*	9.0	r
1403	*trans*-sobrerol	0.4	0.7	0.9	r
1423	sobrerol isomer	0.2	0.1	2.6	t
1439	vanillin	2.2	0.2	2.0	r
1474	*E*-isoeugenol	4.7	*c*	*c*	r
1478	tyrosol	2.1	*d*	*d*	r
1502	p-menth-1-ene-7,8-diol	2.0	0.3	1.4	r
1565	4-hydroxy-3-methoxyphenylpropan-2-one	14.5	*d*	*d*	t
1571	methoxytyrosol	1.7	0.2	1.5	t
1640	3-hydroxy-β-damascon	*b*	*b*	5.3	r
1651	3-hydroxy-7,8-dehydro-β-ionol	*b*	*b*	1.9	l
1659	hydroxycineyl acetate	0.2	0.4	0.6	t
1664	3-oxodamascone	*b*	*b*	1.0	r
1682	3-oxo-α-ionol	26.0	3.6	1.2	r
1685	4-hydroxy-3-methoxyphenylacetic acid	6.4	0.6	4.6	t
1715	3-hydroxy-5,6-epoxy-β-ionone	0.7	0.7	1.4	r
1719	3-hydroxy-β-ionone	0.3	0.3	1.0	r
1745	3-oxo-7,8-dihydro-α-ionol	10.0	3.6	1.1	r
1839	vomifoliol	1.0	0.7	2.1	r

[a] Identification: r, comparison of mass spectra and retention index with a library created from authentic reference substances; t, tentative identification only by MS data; l, mass spectra and retention index according to literature data (*20*, *21*). [b] Not detected in leaves. [c] Detectable only with hesperidinase in buds. [d] Not detected in buds.

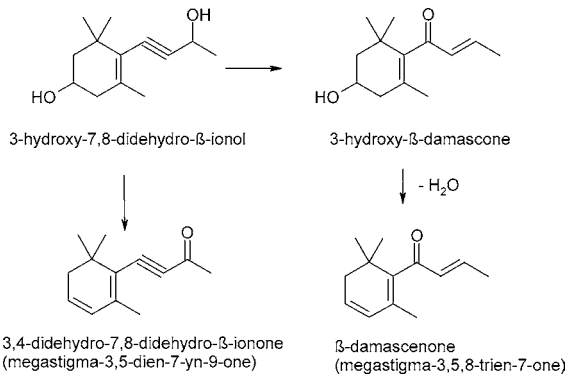

Figure 2. 3-Hydroxy-7,8-didehydro-β-ionol and its derivatives according to ref *16*.

striking that these glycosides and aglycons are present in only laurel buds and not in leaves. Additionally, other aglycons such as linalool, eugenol, isoeugenol, and vanillin were found to be free volatile key odorants in extracts of laurel leaves (*8*).

Intact glycosides of laurel leaves were analyzed directly after derivatization with TFA by GC-MS and GC-MS-NCI. Trifluoroacetylation is more suitable than trimethylsililation (*11*, *12*), because no interferences between the fragment ions from the sugar moiety and those resulting from the aglycon moiety are observed in the EI mode (*1*). Retention indices (RI), molecular weights (MW), and characteristic fragment ions of β-D-glucopyranosides and disaccharide glycosides are presented in **Tables 2** and **3**.

In EI mode the aglycon moiety usually shows an intense fragment ion at $M^+ - OH$ [e.g., benzyl alcohol, 108 − 17 = 91; phenylethanol, 122 − 17 = 105; (*Z*)-3-hexenol, 100 − 17 = 83; hydroxy-1,8-cineoles, 170 − 17 = 153]. After the loss of the alcoholic group, the rest of the aglycon molecule gives a fragmentation pattern similar to those observed for the corresponding free alcoholic compound [e.g., *m*/*e* 67 and 82

Table 2. β-D-Glucopyranosides in TFA-Derivatized Extracts of *L. nobilis* L. Leaves

			m/e NCI		*m/e* EI	
RI	aglycon	MW[a] aglycon	aglycon	sugar moiety	aglycon	sugar moiety
1311	propyl-	60	606, 493, 719	563, 451, 547		319, 177, 193
1401	2-methylpropyl-	74	620, 507, 733	451, 563		319
1410	2-methyl-3-buten-2-yl-	86	632, 745, 519	451, 544, 564	71, 86	319, 177, 193
1478	3-methylbutyl-	88	634, 521, 747	451, 563	71	319, 177, 193
1505	3-methyl-2-buten-1-yl-	86	632, 745, 519	451, 544, 564	86	319, 193
1511	pentyl-	88	634, 747, 521	563, 451		319,193
1575	methylpentyl-?[b]	102	648, 535	563		319
1595	3(*Z*)-hexenyl-	100	646, 759, 533	451, 563		319, 193
1598	hexyl-	102	648, 535	563, 451	85	319
1655	phenyl- (STA)	94	640, 753, 527	547, 451	94	319, 177
1726	benzyl-	108	654, 541, 767	563, 451, 544	91, 92, 108, 107	193, 319
1768	linalyl-	154	700, 813	451, 563		319
1809	2-phenylethyl-	122	668, 781, 555	563, 451	105, 106, 104, 91	319
1831	terpenyl oxide-?	170	716, 602, 603, 829	451, 563, 544	95, 93, 153, 137	319, 177, 193
1859	1,8-cineole-2-hydroxyl-	170	716, 829, 603	451, 563	126, 71, 108, 153	319, 177, 193
1866	terpenyl oxide-?	170	716, 603, 829	451	93, 112, 83, 111, 153	319, 193
1871	1,8-cineole-2-hydroxyl	170	716, 603, 829	563, 451, 544	126, 93, 108, 153	319, 177
1884	α-terpineyl-	154			93, 136, 81	319
1910	1,8-cineole-?-hydroxyl-	170	716, 603, 829	451, 564, 544	126, 108, 93, 153	319, 177, 193
2053	nor-carotenoid-?		868, 754, 755, 641	563, 451	207, 191, 165	319
2115	3-oxo-α-ionol-	208	754, 867, 641	563, 451	108, 135, 91	319, 193
2150	nor-carotenoid-?		754, 641, 870	563		
2169	3-oxodihydro-α-ionol-	210	756, 868, 869, 643	451, 563	108, 123, 95, 81, 207	319, 93, 177
2190	nor-carotenoid-?		870, 757, 642	563, 451	109, 149, 192, 209	319, 193
2217	vomifoliol	224	770, 657	563, 451	124, 150	319, 177, 193
2240	nor-carotenoid-?		870, 757, 983, 642	563, 451	109, 192, 149, 209	319, 193

[a] Molecular weight. [b] ?, tentative identification.

Table 3. Disaccharidic Glycosides in TFA-Derivatized Extracts of *L. nobilis* L. Leaves

RI DB5	glycoside[a]	MW[b]	*m/e* NCI	*m/e* NCI, sugar moiety	*m/e* EI, aglycon	*m/e* EI, sugar moiety
1726	2-methylpropyl-X	74	944, 831, 1057			
1906	2-methylpropyl-P	74	944, 831	563, 451		193, 319, 265, 278, 421
1915	2-methylpropyl-C	74	944, 831	563, 451		193
1791	3-methylbutyl-X	88	958, 845, 1071	635, 451		
1796	2?-methylbutyl-X	88	958, 845, 1071	635, 451		
1969	3-methylbutyl-P	88	958, 845, 1071	649, 563	71	193, 278, 307, 265, 421
1974	3-methylbutyl-C	88	958, 845	563, 451, 649		
1997	methylbutenyl- ?	86	956, 843	775, 66		
1876	3(*Z*)-hexenyl-X	100	970, 1083, 857	563, 451		
2067	3(*Z*)-hexenyl-P	100	970, 857	563, 451	83, 82	193, 421, 265, 278, 307
2069	hexyl-P	102	972, 859	563	85	193, 278, 421, 307
2070	benzyl-R	108	992, 879, 1105	901, 882	91, 107, 92, 108	193, 207
1990	benzyl-X	108	978, 865, 1091	563		
2126	benzyl-A	108	978, 865, 1091	868, 887, 437	91, 107, 92, 108	193, 165, 279, 319, 278
2186	benzyl-P	108	978, 865	669, 887	91, 107, 92	193, 165, 278, 307, 265
2195	benzyl-C	108	978, 865	563, 669, 887	91, 92, 108	193, 319, 265, 278, 421
2250	phenylethyl-P	122	992, 879	451	105, 104, 91	193, 165, 278, 265, 307
2171	2-hydroxycineole-R	170	1054, 941, 1167	901, 451	108, 126	207
2180	2-hydroxycineole-R	170	1054, 941, 1167	882, 901, 451	108, 126, 153	207, 193, 319, 435
2227	?-hydroxycineole-R	170	1054, 941, 1167	563, 451	108, 126, 153	207, 193, 319, 435
2234	?-hydroxycineole-X	170	1040, 731, 927, 1153	563, 887, 660, 437	126, 108, 153	193
2244	?-hydroxycineole-X	170	1040, 927, 1153	563, 451, 775	126, 108	193, 319
2299	?-hydroxycineole-X	170	1040, 731, 927, 1153	563, 660, 775	108, 126, 153	193
2201	terpenyl- ?	154	1024, 911	563, 451	95, 110, 81	193, 165, 279, 319
2310	α-terpineyl-P	154	1024, 772, 911, 1138	563, 451, 775, 660	81, 136–137	193, 278, 265
2328	*p*-cymen-8-yl-P	150	1020, 907	887, 563, 775, 660	91, 117, 133, 150	193, 319, 278, 279, 307
2784	ni		1068, 955		233	193
2817	ni		1098, 985		263	193, 165, 279
2824	ni		1098, 985		263	193, 279, 165

[a] X, disaccharidic glycoside (sugar structure not identified); P, β-D-xylopyranosyl-β-D-glucopyranoside (primeveroside); C, tentatively α-L-arabinopyranosyl-β-D-glucopyranoside (vicianoside); A, α-L-arabinofuranosyl-β-D-glucopyranoside; R, α-L-rhamnopyranosyl-β-D-glucopyranoside (rutinoside); ?, tentative identification; ni, not identified. [b] Molecular weight.

for 3(*Z*)-hexenol; *m/e* 126 and 108 for hydroxy-1,8-cineole; *m/e* 93, 81, and 136 for α-terpineol].

According to literature data, the sugar moiety of the glycosides is characterized in EI mode by typical fragments at *m/e* 319, 193, 177, 205, 265, and 547 for β-D-glucosides (*9*, *11*, *15*), *m/e* 193, 165, 265, 278, 279, 319, and 421 for α-L-arabinofuranosyl-β-D-glucosides (*11*), *m/e* 193, 165, 265, 278, 177, 307, 319, and 421 for α-L-arabinopyranosyl-β-D-glucosides

(vicianosides) (*9*), and *m*/*e* 193, 165, 265, 278, 177, 307, 421, and 319 for β-D-xylopranosyl-β-D-glucosides (primeverosides) (*9*). α-L-Rhamnopyranosyl-β-D-glucopyranosides (rutinosides) showed intense fragments at *m*/*e* 207 and 435 as reported by Voirin et al. (*11*). Arabinofuranosyl-β-D-glucosides can be distinguished from apiofuranosyl-β-D-glucosides by the intensities of the fragment ions *m*/*e* 278, 279, and 265 and by their retention times (*11*, *17*). Rutinosides have a shorter retention time than arabinofuranosyl-glucosides (*11*), which elute before the primeverosides, and the latter elute just before the vicianosides on a DB-5 stationary phase (*9*).

The EI mass spectra and retention indices for 3-oxo-α-ionol-β-D-glucoside and vomifoliol-β-D-glucoside (*13*, *15*), for benzyl-, phenylethyl-, linalyl-, α-terpinyl-β-D-glucosides, benzyl-rutinoside, and benzyl-α-l-arabinofuranosyl-β-D-glucoside (*11*), and for 3(*Z*)-hexenyl-β-D-glucoside and 3(*Z*)-hexenyl-, benzyl-, and phenylethyl-primeverosides (*9*), as well as for 3-methylbutyl-β-D-glucoside (*18*), are in agreement with published data.

Additional information about the structure of the glycosides is obtained by NCI-MS. Characteristic fragment ions of the derivatized glycosides are $[M^+ + 113]$, and $[M^+ - 113]$, resulting from cleavage or addition of a trifluoroacetyl group (*19*). Typical mass fragments also characterize the sugar moiety. β-D-Glucosides show fragments at *m*/*e* 563 (TFA-substituted glucosyl fragment), 451, 544, and 677 (*1*); apiosyl- and arabinosyl-glucosides show fragments at *m*/*e* 887 (TFA-substituted pentosyl-glucosyl fragment), 868, 775, 660, and 437; rutinosides show fragments at *m*/*e* 901 (TFA-substituted rhamnosyl-glucosyl fragment), 882, 789, 674, and 451 (*1*, *18*). Typical NCI mass spectra for hexyl-, benzyl-, linalyl-, and phenylethyl-β-D-glucosides and for benzyl-α-L-arabinofuranosyl-β-D-glucopyranoside and benzyl-rutinoside were published by Chassagne et al. (*13*, *19*).

Although quantification of the TFA-derivatized glycosides was not performed due to lack of authentic reference compounds, it should be mentioned that highest peak areas were found for benzyl-β-D-glycopyranoside, followed by isomeric 2-hydroxy-1,8-cineole β-D-glucopyranosides and benzyl-primeveroside. Most of the glycosides occur as β-D-glucopyranosides. Among the disaccharides, primeverosides are predominant.

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