Volatile Compounds Released by Enzymatic Hydrolysis of Glycoconjugates of Leaves and Grape Berries from *Vitis vinifera* Muscat of Alexandria and Shiraz Cultivars

Jérémie Wirth, Wenfei Guo, Raymond Baumes, and Ziya Günata*

Unité de recherches Biopolymères et Arômes, IPV-ENSAM-INRA, 2 place viala, 34060 Montpellier Cedex 1, France

Glycoconjugates from Muscat of Alexandria and Shiraz leaves and grape berries were isolated by adsorption on Amberlite XAD-2 resin, and enzymatically released aglycons were analyzed by GC-FID and GC-MS. About 120 aglycons were fully or tentatively identified. Compositional differences were observed between leaves and berries of the two varieties in five aglycon chemical groups: C6 alcohols, aliphatic alcohols, monoterpenes, shikimates, and C_{13}-norisoprenoids, which were much more abundant in the leaves than in the berries. The differences observed for C_{13}-norisoprenoids were in agreement with their hypothetical independent biosynthesis in leaves and berries. Thus, 3-hydroxy-β-damascone, an important norisoprenoid aglycon of grape berries, was not detected in leaves, whereas its oxidized derivative, 3-oxo-α-damascone, was absent in berries. Compositional differences were also observed between Muscat and Shiraz leaves. 3-Oxo-α-ionol was not detected in Shiraz leaves, and its retro derivatives were less abundant than in Muscat of Alexandria leaves. Conversely, in Shiraz leaves the levels of 7,8-dihydroionone derivatives, such as megastigman-3,9-diol and 3-oxo-7,8-dihydro-α-ionol, were higher than in Muscat of Alexandria leaves.

Keywords: *Vitis vinifera; Shiraz; Muscat; leaves; berries; aglycons; norisoprenoids*

INTRODUCTION

During the past two decades, considerable research on the structural characterization of the aglycon moiety of glycoconjugates involved in the formation of odorants has been published and reviewed (*1*−*5*). Glycoconjugates contribute to wine flavor through the release of volatiles during the biotechnological sequence leading from grape to aged wine (*6*, *7*). Flavorless glycoconjugates, mainly glycosides of monoterpenes, C_{13}-norisoprenoids, and shikimic compounds, accumulate in grape berries during maturation. Presumably, volatiles are produced before glycosylation, which is consistent with the view that glycosylation is a terminal step in any biosynthetic pathway (*8*). The hydrolysis of glycoconjugates can yield odor-active aglycons or aglycons that are themselves precursors of flavor compounds as observed for most C_{13}-norisoprenoidic aglycons (*3*, *6*, *7*).

Although the qualitative and quantitative glycoconjugate composition of the berries from different cultivars has been previously studied (*7*, *9*, *10*), knowledge is quite limited with regard to that of grapevine leaves, particularly the quantitative composition, with the exception of the Riesling cultivar (*5*). The purpose of the present work was to study the aglycon composition of vine leaves from two cultivars, Muscat of Alexandria, a monoterpene-dependent variety, and Shiraz, widely used in wine-making, and to compare it to that of grape berries. This should permit the flavor potential of the grape plant to be assessed and provide some insight into the biochemical pathways involved.

* Corresponding author [telephone (33) 467149321; fax (33) 467144292; e-mail gunata@arpb.univ-montp2.fr].

MATERIALS AND METHODS

Solvents. High-purity solvents (Merck, Carlo-Erba) were distilled before use.

Vine Leaves and Berries. Leaves and berries from two cultivars (*Vitis vinifera* cv. Shiraz and Muscat of Alexandria) were harvested at grape maturity in 1996 from the Montpellier region (INRA vineyard, Domaine de Villeneuve-les-Maguelones, France). Vine leaves were stored at −20 °C before use, and grape berries were analyzed just after their harvest.

Extraction of Glycosidic Extract. For each sample a triplicate extraction was performed. Twenty-five grams of vine leaves was crushed in liquid nitrogen. The resulting leaf powder was stirred with 80 mL of methanol overnight at room temperature. After centrifugation, the supernatant was concentrated to dryness under vacuum and then dissolved in 10 mL of distilled water. The aqueous solution was washed with 2 × 30 mL of pentane and then subjected to Amberlite XAD-2 column chromatography (10 × 300 mm) (*11*).

For grape berries 100 mL of juice obtained by crushing 2 kg of fresh berries was directly subjected to XAD-2 column chromatography.

The column was rinsed with water (100 mL) and then by 100 mL of pentane/dichloromethane (2:1, v/v), and the glycosidic fraction was eluted with 70% aqueous methanol (100 mL). After concentration of the methanolic fraction to 4 mL under vacuum, 150 μL of the leaf extract and the whole fraction of berry extract were dried under nitrogen and then dissolved in 100 μL of 0.2 M phosphate−citrate buffer, pH 5.0.

Enzymatic Hydrolysis. Glycosidic extracts were washed with pentane/dichloromethane (2:1, v/v) (5 × 0.5 mL), and 100 μL of an enzyme solution containing 20 mg/mL pektolase 3PA (Grinsted) and 10 mg/mL hemicellulase (Gist-Brocades) in 0.2 M phosphate−citrate buffer, pH 5.0, was added; the mixture was incubated at 40 °C for 16 h (*11*). The released aglycons were extracted with pentane/dichloromethane (2:1, v/v) (5 × 1 mL). The organic layer was dried over anhydrous Na_2SO_4, and 32 μg of 4-nonanol was added as standard (3.22 mg/mL in ethanol). Finally, the extract was concentrated at 35 °C to

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Table 1. Levels (Micrograms per Kilogram Equivalents of 4-Nonanol) of Glycosidically Bound C-6 Alcohols in Shiraz and Muscat of Alexandria Leaves and Berries

compound	RI[a]	identity assignment[b]	berries		leaves	
			Muscat	Shiraz	Muscat	Shiraz
hexan-1-ol	1358	A	82	148	1765	1384
(*Z*)-hex-3-en-1-ol	1383	A	13	79	10475	10057
(*E*)-hex-2-en-1-ol	1406	A	27	64	833	390
total			122	291	13072	11832
percentage of the total level of all the aglycons			1.2	6.4	3.0	3.3

[a] RI, linear retention index based on a series of *n*-hydrocarbons. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards; B, identities tentatively assigned by comparing mass spectra with those obtained from literature numbered and italicized within parentheses; C, tentatively identified.

Table 2. Levels (Micrograms per Kilogram Equivalents of 4-Nonanol) of Glycosidically Bound Aliphatic Alcohols in Shiraz and Muscat of Alexandria Leaves and Berries

compound	RI[a]	identity assignment[b]	berries		leaves	
			Muscat	Shiraz	Muscat	Shiraz
3-methylbut-3-en-1-ol	1240	A	–[c]	–	1149	926
pentanol	1249	A	–	–	865	902
3-methylbut-3-en-1-ol + pentan-1-ol	1250	A	85	105	–	–
2-methylbut-2-en-1-ol	1320	A	72	54	3128	1972
heptan-1-ol	1457	A	–	4	–	–
furan linalool oxide + 6-methylhept-5-en-2-ol	1468	A	–	–	464	571
e-methylheptanol	1507	C	–	–	3735	320
total			158	163	9341	4693
percentage of the total level of all the aglycons			1.6	3.6	2.1	1.3

[a] RI, linear retention index based on a series of *n*-hydrocarbons. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards; B, identities tentatively assigned by comparing mass spectra with those obtained from literature numbered and italicized within parentheses; C, tentatively identified. [c] –, without signification or insufficient level for quantification.

Table 3. Levels (Micrograms per Kilogram Equivalents of 4-Nonanol) of Glycosidically Bound Monoterpenes in Shiraz and Muscat of Alexandria Leaves and Berries

compound	RI[a]	identity assignment[b]	berries		leaves	
			Muscat	Shiraz	Muscat	Shiraz
trans-furan linalool oxide	1439	A	114	10	884	1089
cis-furan linalool oxide + 6-methylhept-5-en-2-ol	1468	A	–[c]	5	464	571
nerol oxide + *cis*-furan linalool oxide	1465	A	56	–	–	nd
linalool	1549	A	73	–	–	nd
α-terpineol	1677	A	84	–	344	nd
trans-pyran linalool oxide	1731	A	149	12	499	1330
cis-pyran linalool oxide	1758	A	56	13	387	281
citronellol + *cis*-pyran linalool oxide	1760	A	106	–	–	–
nerol + 1-phenylethanol	1795	A	–	8	538	996
nerol	1800	A	1426	–	–	nd
geraniol	1846	A	1108	43	6898	1209
3,7-dimethylocta-1,5-diene-3,7-diol	1969	A	698	10	–	nd
3,7-dimethylocta-1,7-diene-3,6-diol	2128	A	122	–	–	nd
6,7-dihydro-7-hydroxylinalool+ citronellol hydrate	2219	A	307	29	–	–
p-menthan-7-ol	2234	A	–	–	2489	300
nerol hydrate + (*E*)-8-hydroxylinalool	2275	A	936	78	4137	1512
geraniol hydrate + (*Z*)-8-hydroxylinalool	2344	A	906	183	13244	3965
geranic acid	2353	A	1493	34	237	48
p-menth-1-ene-7,8-diol	2528	A	44	13	1943	448
terpenic unknown 1	2542	C	43	–	–	nd
terpenic unknown 2	2584	C	116	–	–	nd
(*E*) 8-hydroxynerol	2613	A	–	–	251	120
(*Z*) 8-hydroxygeraniol	2632	A	–	20	902	778
(*E*) 8-hydroxygeraniol	2651	A	157	–	2500	1100
geranic acid hydrate	2836	C	–	–	1036	338
total			7995	458	36753	14084
percentage of the total level of all the aglycons			80.5	10.1	8.4	3.9

[a] RI, linear retention index based on a series of *n*-hydrocarbons. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards; B, identities tentatively assigned by comparing mass spectra with those obtained from literature numbered and italicized within parentheses; C, tentatively identified. [c] –, without signification or insufficient level for quantification.

~500 μL by distillation through a Vigreux and then a Dufton column. The samples were stored at −20 °C until analyzed.

GC-FID and GC-MS of the Aglycon Extracts. The aglycons were analyzed by GC-FID with a Varian 6500 chromatograph equipped with an on-column injector. The flow of hydrogen carrier gas was 1.2 mL/min. The oven was kept at 60 °C for 3 min and then programmed to 245 °C at 3 °C/min and kept at 245 °C for 20 min. The flame ionization detector was kept at 250 °C, and the injector was programmed from 20 to 250 °C at 180 °C/min. One microliter of each sample was injected on a DB-Wax (J&W Scientific, Folsom, CA) capillary column (30 m, 0.32 mm i.d., 0.5 μm film thickness).

Table 4. Concentration (Micrograms per Kilogram Equivalents of 4-Nonanol) of Glycosidically Bound Shikimates in Shiraz and Muscat Alexandria Leaves and Berries

compound	RI[a]	identity assignment[b]	QI[c] (%)	berries		leaves	
				Muscat	Shiraz	Muscat	Shiraz
methyl salicylate	1742	A		–[d]	56	365	190
nerol + 1-phenylethanol	1795	A		–	8	538	996
guaiacol	1840	A		–	17	–	–
benzyl alcohol	1864	A		444	1690	102228	55042
2-phenylethanol	1902	A		244	303	12903	37412
alcohol methyl benzylic	1969	C		–	9	–	–
phenol	1987	A		–	10	1101	1286
eugenol	2154	A		–	4	–	–
4-vinylguaiacol	2180	A		–	21	–	–
4-methoxybenzene methanol	2268	C		–	7	–	–
4-vinylphenol	2379	A		–	6	–	–
acetovanillone	2460	A		42	–	7261	2460
vanillin	2551	A		40	31	261	259
methyl vanillate	2580	A		154	25	–	–
3,4-dimethoxyphenol	2750	A		–	16	–	–
ethyl homovanilate	2759	A		–	11	–	–
benzyle salicylate	2771	A		–	2	–	–
vanillol	2787	A		–	31	–	–
zingerone	2790	A		–	23	619	1523
vanilloyl methyl ketone	2800	A		15	15	–	–
tyrosol isomer	2817	C		–	35	3255	2975
2-(4-guaiacyl)-ethanol	2830	B (15)	68	–	30	929	496
3,5-dimethoxyphenol	2848	A		–	7	–	–
phenolic unknown	2899	C		–	95	2421	1875
zingerol	2908	A		–	6	–	–
zingerol + norisoprenoidic unknown	2910	A + C		–	–	1080	702
4-hydroxybenzaldehyde	2930	A		–	3	–	–
4-hydroxybenzaldehyde + methyl 4-hydroxybenzoate	2932	A		40	–	–	–
syringaldehyde + methyl 4-hydroxybenzoate	2934	A		–	21	805	606
methyl syringoate	2957	A		–	27	–	–
3-(4-guaiacyl)propanol	2969	B (16)	73	18	27	–	–
raspberry ketone	2982	A		–	–	6536	14538
methyl 2,6-dihydroxybenzoate	2993	A		–	54	1330	173
tyrosol	2999	A		–	–	2530	1993
3,4,5-trimethoxyphenol	3049	A		33	66	1624	1639
4-(4-hydroxyphenyl)butan-2-ol	3076	A		–	–	422	216
total				1031	2656	146206	124381
percentage of the total level of all the aglycons				10.4	58.6	33.6	34.9

[a] RI, linear retention index based on a series of *n*-hydrocarbons. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards; B, identities tentatively assigned by comparing mass spectra with those obtained from literature numbered and italicized between brackets; C, tentatively identified. [c] QI, quality index of the MS. [d] –, without signification or insufficient level for quantification.

Acquisition and integration of chromatograms were performed with APEX software (Autochrom Inc.). All analyses were made in triplicate.

Identification of the compounds was performed by GC-MS by comparing linear retention index and electronic mass spectra with published data or with authentic compounds. The tentatively identified volatiles are listed in Tables 1−5.

A Hewlett-Packard 5890 series II chromatograph equipped with an on-column injector was used with the same DB-Wax capillary column as above. The flow of helium N60 (Linde Gaz, Marseille, France) carrier gas was 1.35 mL/min. The oven and injector temperature programs were as above. Injection volume was 1 μL. A Hewlett-Packard 5989A mass spectrometer with a quadrupole mass filter was coupled to the GC. Mass spectra were recorded in electronic impact ionization mode at 70 eV with a source temperature of 250 °C and in positive chemical ionization mode at 230 eV. Both transfer line and source temperatures were 250 °C. Mass spectra were scanned in the range *m*/*e* 29−350 amu at 1 s intervals.

RESULTS AND DISCUSSION

Glycosides from Muscat of Alexandria and Shiraz leaves and berries were obtained by the XAD-2 method (*11*) and submitted to enzymatic hydrolysis to release aglycons. The enzyme preparation used contains glycosidase activities (β-D-glucosidase, α-L-arabinofuranosidase, α-L-rhamnopyranosidase, and β-D-apiofuranosidase) involved in the hydrolysis of vine glycosides (*12*, *13*).

GC-FID and GC-MS analyses of aglycons liberated from glycosidic extracts are reported in Tables 1−5. The coefficients of variation ranging from 2 to 21% were similar to those observed in a previous work (*14*).

About 120 aglycons are detected in the enzymatic hydrolysates of the glycosidic extracts of Shiraz and Muscat of Alexandria leaves, and the total levels are 356 and 435 mg/kg, respectively. These total levels are much higher than the total levels of those found in Shiraz and Muscat of Alexandria berries (4.5 and 9.9 mg/kg, respectively). This difference between the levels in leaves and berries is also observed for each chemical class studied (Tables 1−5). About half the total level of the aglycons in the leaves of the two cultivars are C_{13}-norisoprenoids. The decreasing orders of abundance of the other chemical classes, namely, shikimate-derived compounds, monoterpenes, aliphatic alcohols, and six-carbon alcohols, are the same in the leaves of the two varieties. However, monoterpenes are more abundant in Muscat of Alexandria than in Shiraz leaves (8.4 versus 3.9%, respectively). The monoterpene group represents ~80% of the total level of aglycons in the

Table 5. Concentration (Micrograms per Kilogram Equivalents of 4-Nonanol) of Glycosidically Bound C_{13}-Norisoprenoids in Shiraz and Muscat Alexandria Leaves and Berries

compound	RI[a]	identity assignment[b]	QI[c] (%)	berries		leaves	
				Muscat	Shiraz	Muscat	Shiraz
4-oxoisophorone	1677	A		–[d]	–	447	559
3,4-dihydro-3-oxoactinidol I	2416	B (17)	69	–	37	740	931
3-oxo-α-damascone 1	2438	B (18)	77	–	–	1651	901
3,4-dihydro-3-oxoactinidol II	2456	B (17)	71	–	3	1067	130
3,4-dihydro-3-oxoactinidol III	2476	B (17)	65	–	7	2477	2713
3-hydroxy-β-damascone (**4**)	2537	A		28	70	–	–
3,4-dihydro-3-oxoactinidol IV	2543	B (17)	61	–	–	329	250
3-hydroxymegastigman-9-one (**6**)	2559	C		48	–	77	12418
3-hydroxy-7,8-dihydro-β-ionone	2567	B (19)	82	48	–	5761	11563
megastigm-7-ene-3,9-diol	2589	C		–	8	24214	3866
norisoprenoidic unknown	2600	C		42	–	6879	3907
3-oxo-α-ionol (**3**)	2651	A		118	177	48453	–
megastigman-3,9-diol (**7**)	2651	A		–	–	–	33876
3-hydroxy-7,8-dihydro-β-ionol	2676	B (20)	83	83	53	8923	3690
4-oxo-7,8-dihydro-β-ionol	2694	B (21)	74	–	14	5833	7498
3-hydroxy-β-ionone	2700	B (22)	89	31	–	1798	722
3,4-dihydro-3-hydroxyactinidol I	2713	B (23)	64	–	–	2091	1324
3-oxo-7,8-dihydro-α-ionol (**8**)	2726	B (21)	90	–	–	1283	17834
3-hydroxy-5,6-epoxy-β-ionone	2741	B (19)	91	–	–	16822	13015
3-oxo-α-retroionol I (**10**)	2756	A		–	–	5365	1268
3-hydroxy-7,8-dehydro-β-ionol (**5**)	2775	A		32	28	–	–
3,4-dihydro-3-hydroxyactinidol II	2779	B (23)	74	–	–	1522	522
3-oxo-α-retroionol II 11	2894	A		–	–	3997	1082
zingerol + norisoprenoidic unknown	2910	A + C		–	–	1080	702
norisoprenoidic unknown	2946	C		–	–	1516	491
4,5-dihydrovomifoliol (**9**)	3024	B (24)	66	28	–	8606	14416
dehydrovomifoliol + norisoprenoidic unknown	3065	A + C		–	–	2210	2427
3,6-dihydroxy-megastigm-7-en-9-ol (**14**)	3100	B (25)	69	–	61	4174	697
3,6-dihydroxy-megastigm-7-en-9-one (**13**)	3118	B (25)	67	–	13	3550	28928
vomifoliol (**2**)	3167	A		161	476	22685	15912
grasshopper ketone (**12**) + norisoprenoidic unknown	3170	A + C		–	–	29222	6140
isololiolide	3228	B (26)	71	–	–	10516	8006
7,8-dihydrovomifoliol	3262	A		–	12	5957	5308
total				620	959	229232	201096
percentage of the total level of all the aglycons				6.2	21.2	52.7	56.5

[a] RI, linear retention index based on a series of *n*-hydrocarbons. [b] A, identities confirmed by comparing mass spectra and retention time with those of authentic standards; B, identities tentatively assigned by comparing mass spectra with those obtained from literature numbered and italicized within parentheses; C, tentatively identified. [c] QI, quality index of the MS. [d] –, without signification or insufficient level for quantification.

monoterpene-dependent variety berries, Muscat of Alexandria, as previously reported (*1*, *27*) versus only 10% in Shiraz berries, in which shikimates derivatives are predominant, followed by C_{13}-norisoprenoids (60 and 20%, respectively).

Further differences were observed between the two cultivars and between grape berries and leaves when the different compounds of each class were compared individually. The leaves of both varieties of *V. vinifera* show similar qualitative composition and distribution in C-6 alcohols (~3% of the total level) with (*Z*)-3-hexen-1-ol as the major compound (Table 1). Conversely, Shiraz berries exhibit a higher herbaceous flavor potential than Muscat of Alexandria (6.4 versus 1.2%, respectively), but the levels in the leaves of the two varieties are intrinsically much higher than in the berries, 40 and 100 times, respectively. With similar low relative levels (2.1 versus 1.3%), the aliphatic alcohols (Table 2) are twice as abundant in Muscat of Alexandria as in Shiraz leaves, whereas much lower levels are similarly found in the berries of both varieties (60 and 30 times, respectively).

Muscat of Alexandria leaves and berries are known to be quite rich in monoterpenes (*1*, *13*, *28*, *29*). Monoterpenes are twice as abundant in Muscat of Alexandria as in Shiraz leaves (Table 3), and this difference is much more pronounced in the berries (nearly 20 times more abundant in Muscat of Alexandria). Indeed, the monoterpene proportion is much higher in the Muscat of Alexandria berries, in which it reaches a peak at 80%, than in Shiraz berries (10%). Conversely, the proportions in leaves of both cultivars are quite similar. Moreover, the more odorant monoterpenols analyzed (linalool, nerol, and geraniol) occur in higher levels in Muscat of Alexandria berries than in leaves (33 versus 20%) or than in Shiraz berries and leaves (10 and 16%, respectively).

Similar total levels of shikimate derivatives are observed in leaves of Shiraz and Muscat of Alexandria cultivars (35 and 34% of the total level, respectively) with an identical distribution of compounds for both cultivars (Table 4), but individual differences are found. Thus, benzyl alcohol is twice as important in Muscat of Alexandria, contrary to Shiraz, which is richer in 2-phenylethanol and raspberry ketone. On the other hand, the berries of the Shiraz variety show higher total levels of shikimate compounds than Muscat of Alexandria berries, but the levels of these compounds in the berries of both varieties are ~2 orders of magnitude lower than in leaves. However, the relative importance of shikimate derivatives in Shiraz berries reaches a peak of 60% against 10% in Muscat of Alexandria leaves.

The C_{13}-norisoprenoidic aglycon composition is shown in Table 5, and the structures of the main compounds differing significantly in both cultivars are shown in Figure 1. Oxidative artifacts can be formed from

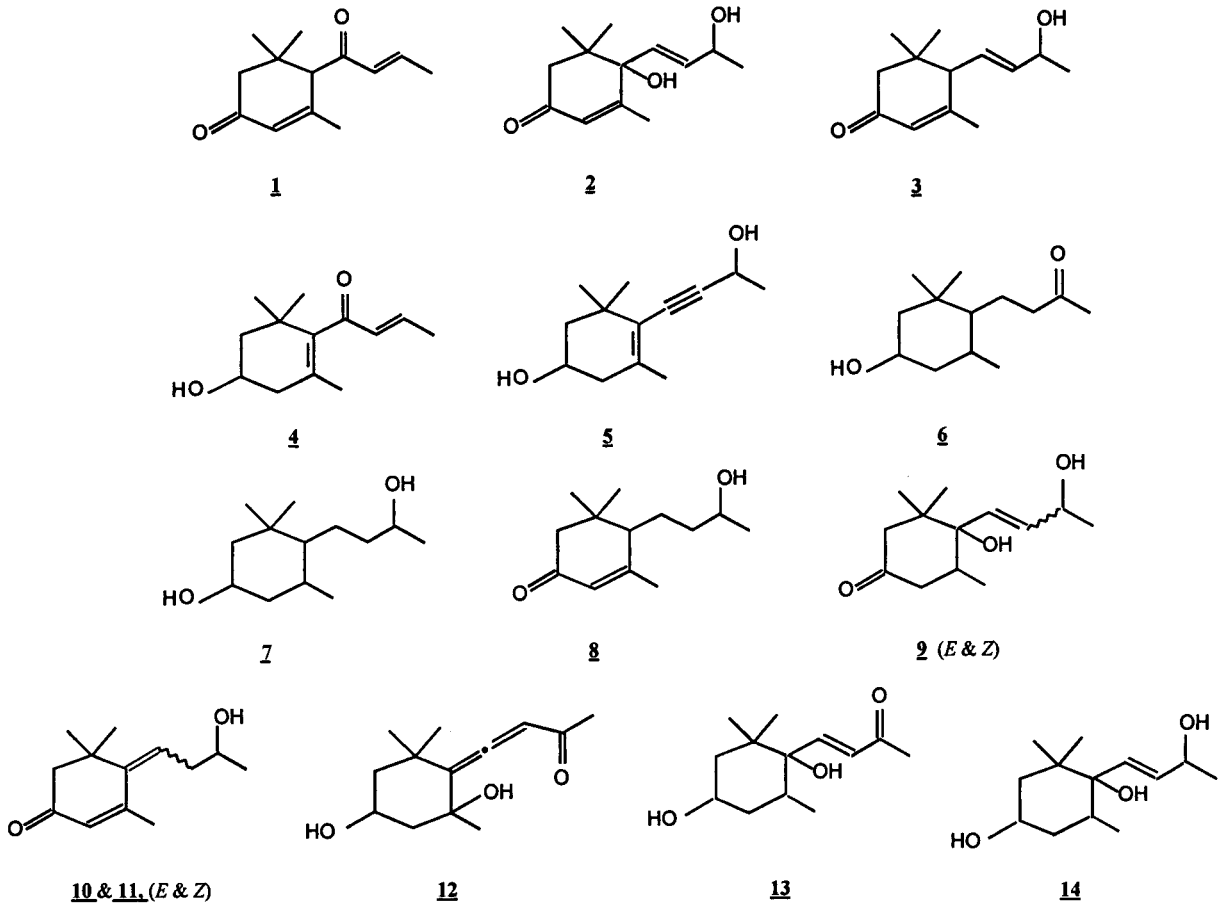

Figure 1. Structures of the main C_{13}-norisoprenoids differing significantly in both cultivars: **1**, 3-oxo-α-damascone; **2**, vomifoliol; **3**, 3-oxo-α-ionol; **4**, 3-hydroxy-β-damascone; **5**, 3-hydroxy-7,8-dehydro-β-ionol; **6**, 3-hydroxymegastigman-9-one; **7**, megastigman-3,9-diol; **8**, 3-oxo-7,8-dihydro-α-ionol; **9**, 4,5-dihydrovomifoliol; **10**, 3-oxo-retro-α-ionol I; **11**, 3-oxoretro-α-ionol II; **12**, grasshopper ketone; **13**, 3,6-dihydroxymegastigm-7-en-9-one; **14**, 3,6-dihydroxymegastigm-7-en-9-ol.

C_{13}-norisoprenoids when high concentrations of enzyme preparations are used to liberate aglycons (*31*). This phenomenon is quite limited in our study because oxidation products such as 3-oxo-α-damascone (**1**) and vomifoliol (**2**) and isomerization products such as 3-hydroxy-7,8-dihydro-α-ionol are absent or at low levels in our samples. The C_{13}-norisoprenoidic aglycons are very abundant in the leaves of both varieties (201 and 230 mg/kg for Shiraz and Muscat of Alexandria, respectively), whereas relatively low amounts are detected in the berries (0.9 and 0.6 mg/kg for Shiraz and Muscat of Alexandria, respectively, i.e., <0.5% of the total level found in the leaves). Similar results were reported for Riesling leaves (*5*, *30*). These high levels in leaves could be attributed to the abundance of their hypothetical precursors, carotenoids, pigments associated with proteins and chlorophylls in the light-harvesting complexes of leaves. Furthermore, the relative contributions of the C_{13}-norisoprenoids are more than half the total levels of the aglycons of leaves but only 6 and 20% of the Muscat of Alexandria and Shiraz berries, respectively. Qualitatively, the C_{13}-norisoprenoid compositions of the berries of both varieties are quite similar. The major aglycons are 3-hydroxy-β-damascone (**4**), 3-oxo-α-ionol (**3**), and vomifoliol (**2**), in accordance with data reported for other cultivars (*9*, *10*) (Table 5). In contrast, leaves and berries possess different patterns of C_{13}-norisoprenoid aglycons. Thus, 3-hydroxy-β-damascone is not detected in either Shiraz or Muscat of Alexandria leaves, as reported previously for Riesling leaves (*5*), although its plausible carotenoid progenitor, neoxanthin, is detected in vine leaves in our laboratory. The absence of 3-hydroxy-β-damascone in leaves is correlated to the presence in low levels only (<1% of the norisoprenoid level) of its oxidized 3-oxo-α-derivative, 3-oxo-α-damascone (**1**) (*31*) and to the presence as trace compound only of one of its known precursors, 3-hydroxy-7,8-dehydro-β-ionol (**5**) (*2*, *7*). In contrast, the levels of 3-hydroxy-7,8-dehydro-β-ionol (**5**) in berries of both cultivars studied are in the range of those of other norisoprenoid aglycons. This suggests the occurrence of different metabolic pathways of neoxanthin in leaves and berries.

Compositional differences are clearly observed between Muscat of Alexandria and Shiraz leaves. 3-Oxo- and 3-hydroxy-7,8-dihydroionone derivatives **6**−**8** are far more abundant in Shiraz leaves than in Muscat of Alexandria leaves, and the level of 4,5-dihydrovomifoliol (**9**) is twice as high in Shiraz leaves. Megastigmane-3,9-diol (**7**), a fully saturated compound identified recently in our laboratory (*32*), is one of the major norisoprenoid aglycons (33.9 mg/kg) in Shiraz leaves. Neither berries of both varieties nor Muscat of Alexandria leaves contain this compound, which could lead by dehydration to less polar C_{13} derivatives potentially odorant. It occurs in Shiraz leaves mainly as C-3 and C-9 monoglucosides.

A high reductase activity in Shiraz leaves is suggested by the abundance of 7,8-dihydroionone derivatives compared to Muscat of Alexandria leaves. Indeed, one

of the major aglycons (48 mg/kg) in Muscat of Alexandria leaves, 3-oxo-α-ionol (**3**), is not detected in Shiraz leaves in which more reduced derivatives of this compound occur, for example, 3-oxo-7,8-dihydro-α-ionol (**8**). Furthermore, Muscat of Alexandria leaves contain higher levels of the retro isomers of 3-oxo-α-ionol **10** and **11** than Shiraz leaves. Additionally, grasshopper ketone (**12**), the oxidized derivative of a precursor of β-damascenone, previously reported in Riesling leaves, is detected in Muscat of Alexandria leaves but not in Shiraz leaves, where it may be reduced to other trioxygenated C_{13}-norisoprenoids (*5*).

Among the trioxygenated C_{13}-norisoprenoidic compounds, the levels of 4,5-dihydrovomifoliol (**9**) and 3,6-dihydroxy-megastigm-7-en-9-one (**13**) are much higher in Shiraz leaves than in Muscat of Alexandria leaves. The latter compound, as well as 3,6-dihydroxymegastigm-7-en-9-ol (**14**), was reported previously in starfruit (*25*). Contrary to the dioxygenated derivatives, the double bond of the side chain of the major trioxygenated C_{13} compounds in Shiraz leaves is not saturated.

This study of the glycoconjugated volatiles found in the berries and leaves of a monoterpene-dependent and a neutral vine cultivar shows that in both cultivars these compounds are much more abundant in the leaves than in grape berries. This difference, in the range of 2 orders of magnitude, is observed in all groups of aglycons biogenetically related, except for the monoterpenes. In this case, the total level of the monoterpenic glycoconjugates found in the grape berries of the monoterpene-dependent variety is ~5 times lower than that in the leaves and 2 times lower than that in the leaves of the neutral variety. Strikingly, more than half the total level of aglycons in leaves are norisoprenoidic compounds in both varieties. These levels are >500 times those found in the grape berries; similar differences in the levels of carotenoids, pigments associated to the photosynthetic systems, between berries (*33*) and leaves are observed (unpublished results). That supports an apo-carotenoid pathway in the biogeneration of C_{13}-norisoprenoidic derivatives, although nothing is known about the influence of photosynthesis on these degradative pathways.

Furthermore, the occurrence of different patterns of C_{13}-norisoprenoid aglycons in grape berries and leaves of the same varieties is in support of the independence of their biosynthesis in grapes and leaves (*34*). This suggests that pathways for their formation from the initial bio-oxidative cleavage reactions of carotenoids would involve a series of enzymatic transformations. This work shows that the enzymes involved would be different in grape berries and leaves and also in the leaves of the two cultivars, because the same difference in their structural composition in C_{13}-norisoprenoid derivatives is observed.

Finally, the high levels in vine leaves of glycoconjugates, known to be flavor precursors, show that vine leaves are a potential natural source of aroma substances.

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