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Analytical methods for monoterpene glycosides in grape and wine

II. Qualitative and quantitative determination of monoterpene glycosides in grape☆

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ABSTRACT

Free and glycosidically bound terpenes of five *Vitis vinifera* grape cultivars (muscat of Alexandria, muscat of Frontignan, muscat of Hamburg, muscat Ottonel and Gewürztraminer) were investigated. The free and bound fractions were separated by selective retention on Amberlite XAD-2 resin. The glycosidic fractions were analysed by gas chromatography and gas chromatography–mass spectrometry using either enzymic hydrolysis and subsequent analysis of the released aglycones or trimethylsilyl (TMS) and trifluoroacetyl derivatives. The known monoterpenyl, benzyl and 2-phenylethyl β-D-glucopyranosides, β-rutinosides, 6-O-α-L-arabinofuranosyl-β-D-glucopyranosides and 6-O-β-D-apiofuranosyl-β-D-glucopyranosides were determined. A number of other glycosides were detected and the structures of some of them, mainly apiosylglucosides and glucosides with aglycones in higher oxidation state than linalol, were tentatively identified using the mass spectra of their TMS and TFA derivatives and the results obtained from the analysis of their aglycones.

INTRODUCTION

The composition of glycosidically bound volatiles from *Vitis vinifera* grape has been extensively studied. These bound forms consist of β-D-glucopyranosides and, mostly, of 6-O-α-L-arabinofuranosyl-β-D-glycopyranosides, 6-O-α-L-rhamnopyranosyl-β-D-glucopyranosides (rutinosides) [1,2] and 6-O-β-D-apiofuranosyl-β-D-glucopyranosides [3] with monoterpenyl, benzyl and 2-phenylethyl aglycones. Other aglycones have also been identified as monoterpenoids with various oxidation states, carotenoid-related and shikimate-related compounds [4–7]. As many of these aglycones have interesting sensory properties, their flavourless glycosides make up a potential aroma reserve more abundant than the free one [8–10].

Using a procedure involving enzymic hydrolysis, Günata *et al.* [11] showed that grape glycosides are far more abundant in aromatic varieties (muscats and aromatic varieties from the Alsace region) than in non-aromatic varieties. However, as these glycosides have proved difficult to analyse directly [8], no quantitative data have been reported concerning these glycosides individually. The development of a

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method permitting the direct analysis of these glycosides [12] allowed the further investigation of the qualitative and quantitative composition of some *Vitis vinifera* grape cultivars. The results obtained for four muscat cultivars (Alexandria, Frontignan, Hamburg and Ottonel) and Gewürztraminer are reported in this paper.

EXPERIMENTAL

Reagents and reference samples

Analytical-reagent grade solvents (pentane from Labosi and dichloromethane, ethyl acetate and pyridine from Merck) were further purified by redistillation before use. Pektolase 3PA was purchased from Grinsted. All other chemicals were obtained as described in Part I [12].

Plant material

Mature, sound grapes (cultivars Muscat of Frontignan, Muscat of Alexandria, Muscat Ottonel, Muscat of Hamburg and Gewürztraminer) were collected in 1988 at the vineyard of the vine experimental station in Montpellier, France. The grapes were frozen in liquid nitrogen and kept at −18°C until extracted.

Fractionation of free and bound compounds

The clear juice from the grapes was obtained according to the method of Günata *et al.* [11] using 4-nonanol and phenyl β-D-glucopyranoside as internal standards for the free and the bound fractions; 0.28 and 2.5 mg, respectively, were added to 1 kg of berries just before crushing. The juice (50 ml) was fractionated on XAD-2 resin as described in Part I [12]; free compounds were eluted with 50 ml of pentane–dichloromethane (2:1) and glycosides with 50 ml ethyl acetate. The free extract was dried over sodium sulphate and concentrated to a final volume of about 500 μl for gas chromatographic (GC) analysis. The bound extract was concentrated to 1 ml under vacuum at 40°C, then to dryness at 60°C under nitrogen.

GC analysis of the free fractions

The free extracts obtained from 50 ml of grape juice were analysed by GC on a CP-Wax 52 CB fused-silica capillary column (Chrompack) (25 m × 0.32 mm I.D.; 1.2 μm bonded phase) as described in Part I [12].

Enzymic hydrolysis of the bound fractions and GC analysis of the aglycones released

Each enzymic hydrolysis was performed on a glycoside sample obtained from 50 ml of grape juice. The sample was dissolved in 100 μl of 0.2 *M* citrate–phosphate buffer (pH 5.0) and washed four times using 100 μl of pentane–dichloromethane (2:1), 100 μl of Pektolase 3PA solution were added [1.2 mg of Pektolase 3PA in 100 μl of 0.2 *M* citrate–phosphate buffer (pH 5.0)], then the mixure was incubated at 40°C for 12 h. After cooling to room temperature, 28 μg of 4-nonanol were added as internal standard and the mixture was extracted four times with 200 μl of pentane–dichloromethane (2:1). This aglycone extract was concentrated to a final volume of about 50 μl by rectification (Dufton column) at 35°C, then analysed by GC as above for the free fractions.

GC analysis of trimethylsilyl (TMS)-derivatives of the bound fractions

To a glycoside sample obtained from 10 or 15 ml of grape juice were added 20 μl of anhydrous pyridine and 20 μl of trimethylsilylating reagent [N,O-bis(trimethylsilyl)trifluoroacetamide–chlorotrimethylsilane (99:1)]. The mixture was stirred (vortex mixed), heated for 20 min at 60°C and then cooled to room temperature. Injections of about 0.8 μl of these derivatives on to an OV-1 fused-silica capillary column (Delsi Instruments) (50 m × 0.32 mm I.D.; 0.2 μm bonded phase) were made on-column. The equipment consisted of a Varian Model 3300 gas chromatograph fitted with an on-column injector and a flame ionization detector. The injector temperature was programmed at 60°C min^{-1} from 90 to 150°C and then at 10°C min^{-1} to 300°C. The column temperature was programmed at 3°C min^{-1} from 125 to 300°C with hydrogen as carrier gas at 2 ml min^{-1}. The detector temperature was 320°C.

GC analysis of trifluoroacetyl (TFA) derivatives of the bound fractions

A glycoside sample obtained from 15 ml of grape juice as described above was treated as above by using 20 ml of anhydrous pyridine and 20 μl of N-methylbis(trifluoroacetamide) instead of the above trimethylsilylating reagent. Injections of about 0.8 μl of these derivatives on to a CP-Sil 8 CB fused-silica capillary column (Chrompack) (25 m × 0.32

mm I.D.; 1.2 μm bonded phase) were made on-column. The equipment used was the same as above. The injector temperature was programmed at 60°C min^{-1} from 90 to 150°C and then at 10°C min^{-1} to 300°C. The column temperature was programmed at 3°C min^{-1} from 125 to 300°C with hydrogen as carrier gas at 1.3 ml min^{-1}. The detector temperature was 300°C.

Direct GC–MS analysis of the bound fractions

Glycoside samples obtained from 10 or 15 ml of grape juice were subjected to TMS or TFA derivatization, respectively, as reported above, then analysed by gas chromatography–mass spectrometry (GC–MS).

Electron impact mass spectrometry (EI-MS) was applied to the TMS and the TFA derivatives by coupling a Girdel 31 gas chromatograph equipped with the same fused-silica capillary colums as described above to a Nermag R 10-10 mass spectrometer. The transfer line was a platinum capillary tube heated at 260°C. The source temperature was 200°C. Mass spectra were scanned at 70 eV in the range m/z 60–1050 at 2.87-s intervals.

For GC, 2-μl volumes of glycoside derivatives were injected with a splitting ratio of 10:1 into an injector held at 320°C. The helium carrier gas head pressure was 90 kPa for TMS derivatives and 10 kPa for TFA derivatives. For TMS derivatives the column was programmed at 3°C min^{-1} from 130 to 300°C and for TFA derivatives at 4°C min^{-1} from 120 to 280°C.

Chemical ionization mass spectrometry (CI-MS) was applied using the same GC and transfer line conditions as for EI-MS. The source temperature was 90°C and ammonia was used as the reactant gas. Mass spectra were scanned at 70 eV in the range m/z 60–1050 at 2.87-s intervals.

GC–ion trap detection (ITD) of the free fractions and of the aglycones released from the bound fractions

Electron impact mass spectra were recorded for the free fractions and the aglycones enzymatically hydrolysed from the glycosides by coupling the CP-Wax 52 CB fused-silica capillary column (see conditions above) to a Finnigan MAT ITD 700. The transfer line, heated at 240°C, consisted of an open-split GC–ITD interface at atmospheric pressure and a flow restrictor which was a DB-5 fused-silica capillary column (1.2 m × 0.32 mm I.D.; bonded phase). The source temperature was 220°C. Mass spectra were scanned between 50 and 80 eV in the range m/z 31–250 at 2-s intervals.

RESULTS AND DISCUSSION

The technique used in this study involved XAD-2 resin extraction of the grape glycosides, TFA and TMS derivatization followed by GC and GC–MS analysis [12]. Moreover, the aglycones released after enzymic hydrolysis of the five natural glycosidic extracts were analysed by GC and GC–MS in order to obtain further information [11]. Pektolase 3PA, which possessed the glycosidase activity necessary to hydrolyse the four classes of grape glycosides [13,14], was used.

Qualitative analysis

The gas chromatograms of the TMS and TFA derivatives of the grape glycosides studied showed many peaks, most of them arising at retention times higher than that of phenyl β-D-glucopyranoside, as shown for muscat of Alexandria in Figs. 1 and 2. Comparisons with the TFA and TMS derivatives of the synthetic compounds described in Part I [12] and of geranyl 6-O-β-D-apiofuranosyl-β-D-glucopyranoside [3] permitted a positive identification of the corresponding natural glycosides and a tentative identification of the 6-O-β-D-apiofuranosyl-β-D-glucopyranosides with the same aglycones [3] (Table I). However, no glycoside of citronellol was detected in the natural extracts, reinforcing the hypothesis reported by Wilson *et al.* [15] on its enzymic production. Likewise, no 2,6-dimethyl-3-hydroxy-1,7-octadien-6-yl-β-D-glycopyranoside could be positively identified, suggesting that the natural compound might be 3-glycosylated or diglycosylated.

Concerning the absolute configuration of the linalyl glycosides, it was interesting that in the chromatograms of the TFA derivatives of the grape glycoside extracts, only one isomer was found for each glycoside. The synthetic diastereoisomeric (*R*) and (*S*)-linalyl glycosides were shown to be well resolved in Part I [12], which allowed the assignment of the *S* configuration to the linalyl moiety in linalyl β-D-glucopyranoside, linalyl β-rutinoside and linalyl 6-

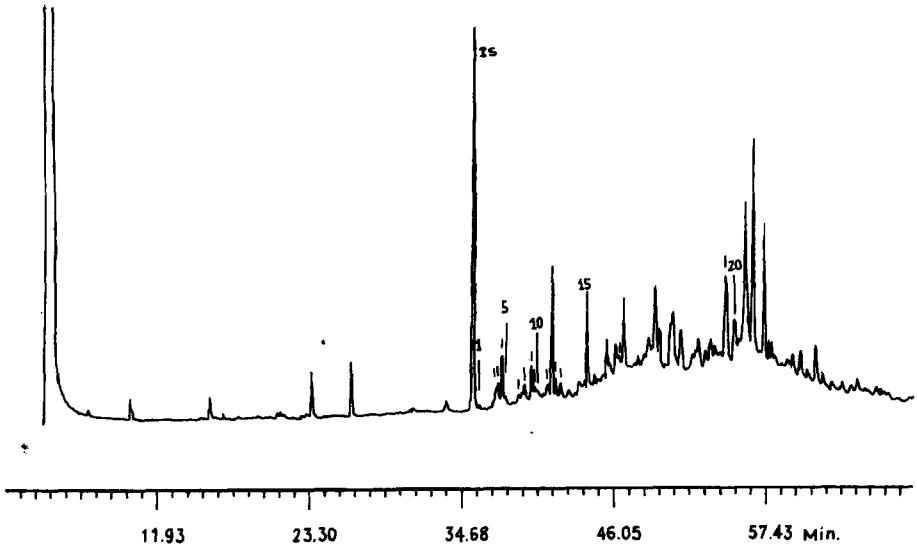

Fig. 1. GC of TMS derivatives of muscat of Alexandria glycosides. For conditions, see Experimental. Dashes are shown over the peaks corresponding to compounds reported in this paper: glycosides of (1) 3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol), (2) benzyl alcohol, (3) (*S*)-linalool, (4,5,6) linalyl oxides, (9) 2-phenylethanol, (10) nerol, (13) geraniol, (7,8,11,12,14–18) unknown monoterpendiols 1–9 reported in Table IV, (19) apiosylglycosides of benzyl alcohol and of linalyl oxides, (20) arabinosylglycoside of nerol, (21) apiosylglycosides of nerol and linalyl oxides, (22) arabinosylglycoside of geraniol and apiosylglycoside of 2-phenylethanol and (23) apiosyl glycoside of geraniol and rutinoside of geraniol.

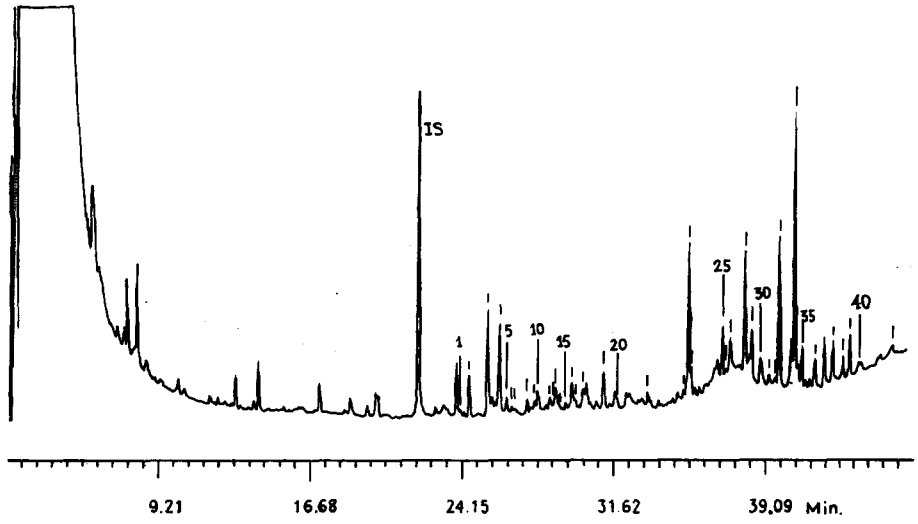

Fig. 2. GC of TFA derivatives of muscat of Alexandria glycosides. For conditions, see Experimental. Dashes are shown over the peaks corresponding to compounds reported in this paper: glycosides of (1) hotrienol, (2) benzyl alcohol, (3,4) furanic linalol oxides, (4) (*S*)-linalool, (8) 2-phenylethanol, (9) nerol, (10) pyranic linalol oxide, (13) geraniol, (19) 3,7-dimethyl-2,6-octadienoic acid, (4–7,11,12,14–21) unknown monoterpendiols 1–14 reported in Table VI, (23) rutinosides of (*S*)-linalool, (28) geraniol, (33) 3,7-dimethyl-2,6-dienoic acid, (25) arabinosylglycosides of (*S*)-linalool, (29) nerol, (33) geraniol, (35,36) 3,7-dimethyl-2,6-octadienoic acids, (26) apiosylglycosides of benzyl alcohol, (27) (*S*)-linalool, (30) nerol, (31) 2-phenylethanol, (32) pyranic linalool oxide, (34) geraniol, (35) α-terpineol, (38,39) 3,7-dimethyl-2,6-octadienoic acids, (37,40,41) apiosylglycosides partially trifluoroacetylated and (22,24) shikimic acid derivatives.

TABLE I

CONCENTRATIONS OF GLYCOSIDES OF TERPENOLS AND AROMATIC ALCOHOLS IN SOME AROMATIC GRAPE VARIETIES (μg PER LITRE OF JUICE)

Glycosides	Muscat of Alexandria	Muscat of Frontignan	Muscat of Hamburg	Muscat Ottonel	Gewürz-traminer
Glucosides					
Geranyl	50	89	–	207	43
Neryl	22	69	–	147	–
(*S*)-Linalyl	[a]	[a]	–	–	–
Terpenic aglycones in higher oxidation state[b]	3424	7427	1208	14 181	1254
Benzyl	360	122	282	417	262
2-Phenylethyl	203	130	–	277	513
Rutinosides					
Geranyl	323	330	257	372	613
Neryl		288[c]	222	227[c]	284[c]
(*S*)-Linalyl	589	478	27	948	–
Terpenic aglycones in higher oxidation state[d]	[d]	–	–	–	–
Benzyl	–	–	[e]	–	–
Arabinosylglycosides					
Geranyl	1315	2169	794	3758	5474
Neryl	461	2448	1110	1577	824
(*S*)-Linalyl	395	297	–	569	–
Terpenic aglycones in higher oxidation state[f]	268	322	342	160	420
Benzyl	–	1555[c]	–	1229[c]	1534[c]
Apiosylglycosides					
Geranyl	2085	1143	625	2554	4136
Neryl	366	1536	786	845	508
(*S*)-Linalyl	406	64	133	–	–
α-Terpineyl	[g]	–	[g]	–	–
Terpenic aglycones in higher oxidation state[h]	257	3843	364	409	343
Benzyl	64	376	241	385	656
2-Phenylethyl	242	–	183	726	–

[a] Minor compound coeluted with unknown glycoside 1 (Table VI).
[b] Glucosides of hotrienol, furanic and pyranic linalol oxides, terpenediols (MW = 170 and 172) and 3,7-dimethyl-2,6-octadienoic acids were tentatively identified. Their total amount was determined without calibration factor.
[c] Co-eluted compounds quantitatively determined using either the calibration factor of the first or that of the second.
[d] A rutinoside of a 3,7-dimethyl-2,6-octadienoic acid was the only compound tentatively identified in this class (as trace compound co-eluted with geranyl arabinosylglucoside in muscat of Alexandria).
[e] Minor compound co-eluted with a ferulic acid glycoside.
[f] Arabinosylglycosides of 3,7-dimethyl-2,6-octadienoic acid were the only compounds tentatively identified in this class. Their total amount was determined without calibration factor.
[g] Minor compound co-eluted with a 3,7-dimethyl-2,6-octadienoyl arabinosylglycoside and a ferulic acid glycoside.
[h] Apiosylglycosides of furanic and pyranic linalol oxides and of 3,7-dimethyl-2,6-octadienoic acids were tentatively identified. Their total amount was determined without calibration factor.

O-α-L-arabinofuranosyl-β-D-glucopyranoside in the grape cultivars studied. This identification was confirmed for the last two linalyl diglycosides [14] by comparison of synthetic (*S*)-linalyl β-D-glucopyranoside with the corresponding compound released by specific hydrolysis of the natural glycoside extracts with either a pure β-L- arabinofuranosidase or a pure α-L-rhamnopyranosidase [10,16,17]; however, as a pure β-D-apiofuranosidase or synthetic (*R*)- or (*S*)-linalyl 6-O-β-D-apiofuranosyl-β-D-glucopyranoside was not available, the configuration of the linalyl apiosylglucoside was not determined.

These results are in good agreement with those obtained by Salles [18] for the linalyl glycosides in muscat of Alexandria grapes.

Many more peaks were detected in the chromatograms of the grape TMS and TFA glycosides; EI-MS showed for most of them osidic fragment ions

TABLE II

CONCENTRATIONS OF BOUND AND FREE TERPENOLS AND AROMATIC ALCOHOLS IN SOME AROMATIC GRAPE VARIETIES (μg PER LITRE OF JUICE)

Compound	Ref.	Muscat of Alexandria		Muscat of Frontignan		Muscat of Hamburg		Muscat Ottonel		Gewürz-traminer	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Geraniol	[a]	606	980	416	928	281	427	425	1780	698	2458
Nerol	[a]	147	284	320	1350	191	452	160	876	133	617
Linalol	[a]	712	201	385	152	451	28	296	231	–	12
α-terpineol	[a]	24	5	27	21	24	14	26	25	10	24
Total		1489	1470	1148	2451	947	921	907	2922	841	3111
Hotrienol	[b]	8	5	5	14	5	–	9	8	–	–
cis + *trans*-Furanic linalol oxides	[b]	20	27	53	40	45	11	191	56	–	–
cis + *trans*-pyranic linalol oxides	[b]	298	29	352	60	198	14	685	61	–	7
2,6-Dimethyl-3,7-octadiene-2,6-diol	[b]	1279	1184	1099	1194	666	54	1777	1250	–	45
2,6-Dimethyl-1,7-octa diene-3,6-diol	[c]	175	54	300	83	160	134	1227	162	–	12
(*E,Z*)-8-Hydroxy linalols + (E)-3,7-dimethyl-2-octene-1,7-diol	[d]	141	454	261	1410	184	251	383	1901	88[j]	439[j]
2,6-Dimethyl-7-octene-2,6-diol	[e]	–	10	14	41	15	6	13	20	–	–
3,7-Dimethyl-1,7-octanediol + 2,6-dimethyl-7-octene-1,6-diol	[f,g]	21	52	76	226	32	55	29	75	33[k]	56[k]
(*Z*)-3,7-Dimethyl-2-octene-1,7-diol	[h]	7	6	23	20	12	9	20	20	32	11
(*E,Z*)3,7-Dimethyl-2,6-octa dienoic acids	[c]	620	429	398	324	937	121	253	125	178	183
Total		2569	2250	2581	3412	2254	655	4587	3678	331	753
Citronellol	[a]	15	24	22	84	30	80	18	42	27	113
Unknown terpene	[i]	–	–	–	–	–	–	–	–	–	221
Benzyl alcohol	[a]	101	184	221	423	130	192	209	464	224	527
2-Phenylethanol	[a]	125	160	106	220	56	54	195	423	141	222

[a] NBS Library.
[b] Rapp and Knipser [24].
[c] Rapp *et al.* [25].
[d] Bock *et al.* [26].
[e] Williams *et al.* [27].
[f] Rapp *et al.* [28].
[g] Versini *et al.* [29].
[h] Ohloff *et al.* [30].
[i] EI-MS: *m/z* (relative intensity, %) 41(100), 69(70), 39(35), 81(33), 67(21), 53(13), 93(13), 95(11), 55(10), 43(10), 121(7), 107(6).
[j] 8-Hydroxylinalols as trace component.
[k] 3,7-Dimethyl-1,7-octanediol only detected.

characteristic of the four classes of grape glycosides. Their tentative identifications relied on the fragmentation rules observed in the EI-MS of synthetic glycosides and on their corresponding CI-MS, as reported previously [3,12]; further, they were facilitated by the qualitative determination of the aglycones released after enzymic hydrolysis of the natural glycoside extracts (Table II).

All the major aglycone compounds analysed have already been positively identified (see references in Table II), except for an unknown compound detected only in Gewürztraminer, the mass spectrum of which showed fragments characteristic of terpene (see footnote *i* in Table II). Those which could not be identified were minor compounds and are not reported in Table II, in addition to C_6 compounds, arising from lipidic precursors [19] and C_{13} norisoprenoids, the results of which will be reported in a future paper.

Among the aglycones released by enzymic hydrolysis, terpenoids with higher oxidation states than linalool (linalool oxides, monoterpendiols, hotrienol, monoterpenic acids) were quantitatively important. As synthetic glycosides of such compounds were not available for most of them, they were tentatively identified in the chromatograms of the grape TMS and TFA glycosides.

Glycosides of linalol oxides and monoterpendiols tentatively identified

Most of the unknown peaks in the chromatograms of the TMS and TFA grape glycosides showed fragment ions characteristic of glycosides with terpenic aglycones. As the structure of the sugar residue was easily deduced by EI-MS [3,12], CI-MS of their TMS derivatives gave for the derivatized aglycones molecular weights of 152, 170, 242 and 244, corresponding respectively to hotrienol, linalol oxides (Table III) and monoterpenediols with two or one double bonds (Table IV).

As shown in Table III for the TMS derivatives of glycosides with aglycones with molecular weights of 152 and 170, one peak corresponding to a glycoside of Ho-trienol and three peaks corresponding to glycosides of linalool oxides were detected in the chromatograms of the grape TMS glycosides together with three peaks corresponding to apiosyl glycosides of linalool oxides reported previously [3]. However, it was not possible by EI-MS to establish further the complete structures of their aglycones among the different possible isomers.

As regards the TFA derivatives, GC–CI-MS coupling gave unsatisfactory results, as reported previously for synthetic glycosides [3,12], but allowed the location of one peak corresponding to a glycoside of Ho-trienol (pseudo-molecular ion at m/z = 716) and one peak corresponding to a glycoside of a linalool oxide (pseudo-molecular ion at m/z = 734). Using EI-MS data, two more peaks corresponding to glycosides of linalool oxides were detected in the chromatograms of the TFA derivatives of the grape extracts together with four peaks corresponding to apiosylglycosides of linalool oxides reported previously [3].

EI-MS (Table V) showed two types of fragmentation for the aglycone residue, as reported previously for furanic (characteristic fragment ions at m/z 111 and 93) and pyranic (m/z 94 and 68) linalol oxide acetates [20] and peracetylated 6-O-α-L-arabinofuranosyl-β-D-glucopyranosides previously isolated from grape [6], thus allowing them to be distinguished.

As regards monoterpenediol glycosides, nine peaks corresponding to monoterpendiol glycosides (molecular weight of derivatized aglycone = 242 or 244) were identified in the chromatograms of the TMS derivatives of the five grape extracts. EI-MS (Table III) exhibited characteristic fragmentations, similar to those obtained from the synthetic diastereoisomeric 2,6-dimethyl-3-hydroxy-1,7-octadien-6-yl-β-D-glucopyranosides [12], and CI-MS provided the expected molecular weights (see above).

Similarly, fourteen unknown peaks exhibiting fragment ions characteristic of glycosides with terpenoid aglycones were located in the chromatograms of the TFA derivatives of the five grape extracts (Table VI). For most of them these fragment ions were similar to those reported for the synthetic diastereoisomeric 2,6-dimethyl-3-hydroxy-1,7-octadien-6-yl-β-D-glucopyranosides [12]. These peaks were thus tentatively identified as monoterpendiols glycosides; their formal identification will be difficult owing to the numerous isomers of these compounds.

At the end of the gas chromatograms of the TMS and TFA glycosides, unknown peaks with low intensities showed EI-MS characteristics of terpenoid

TABLE III

MASS SPECTRA OF TMS DERIVATIVES OF GLYCOSIDES WITH AGLYCONES TENTATIVELY IDENTIFIED AS HOTRIENOL AND LINALOL OXIDES IN *VITIS VINIFERA* GRAPES

Aglycone residue	RRT[a]	EI-MS, characteristic fragment ions [*m/z* (relative intensity, %)] of			MW[b] (MW_s)	MW of aglycone[c] (MW_a)
		Sugar moiety	Aglycone moiety	Sugar and aglycone–moiety		
Glucosides						
Hotrienyl	1.007	217(100), 361(16), 191(8), 204(7), 242(3)	153(33), 71(27), 81(11), 133(4)		601	152
Linalyl oxide	1.067	204(60), 217(35), 361(26), 271(6), 331(5), 191(5), 233(4), 243(3.5), 319(2), 263(2), 305(1.5), 451(0.5)	153(100), 71(44), 81(14), 135(9.7), 154(5.5), 93(5), 69(5)	271(6)	620	170
Linalyl oxide	1.076	204(76), 361(53), 217(48), 271(13), 243(7), 191(7), 331(7), 319(4), 233(4), 263(3), 305(2), 451(0.4)	153(100), 71(53), 81(20), 135(13), 93(8), 69(5)	271(13)	620	170
Linalyl oxide	1.119	204(100), 217(21), 361(20), 271(7), 191(5), 243(3), 305(2), 233(2), 319(2), 263(2), 451(0.5)	81(11), 153(11), 135(9), 71(7), 93(2)	271(7)	620	170
Apiosylglucosides[d]						

[a] Retention time relative to phenyl β-D-glucopyranoside.
[b] From CI-MS with ammonia as reactant gas.
[c] Obtained as $MW_a = MW_s - 451$ (molecular weight of the sugar moiety) + 1.
[d] Data reported previously [3].

TABLE IV

MASS SPECTRA OF TMS DERIVATIVES OF GLUCOPYRANOSIDES WITH UNKNOWN MONOTERPENEDIOL AGLYCONE TENTATIVELY IDENTIFIED IN *VITIS VINIFERA* GRAPES

Aglycone residue	RRT[a]	EI-MS, characteristic fragment ions [*m/z* (relative intensity, %)] of		MW[b] (MW_s)	MW of underivatized aglycon[c] (MW_a)
		Sugar moiety	Aglycon moiety		
Unknown 1	1.128	331(76), 263(38), 204(35), 191(32), 233(27), 217(21), 361(9.2), 243(3.8), 305(3.4), 319(1.7), 271(1.6)	143(100), 82(12)	692	170
Unknown 2	1.143	331(100), 263(46), 204(45), 191(30), 233(27), 217(25), 361(16), 305(4.1), 243(2.7), 271(2.4), 319(1.9)	143(83), 82(8.3), 241(2.5)	692	170
Unknown 3	1.180	204(100), 331(64), 217(54), 191(36), 263(31), 233(27), 361(19), 305(4.6), 243(4)	143(37), 135(36), 225(26), 93(25), 107(24), 75(15), 81(4.5), 131(2.9), 241(1.9)	692	170
Unknown 4	1.190	331(49), 263(27), 191(25), 204(23), 217(21), 233(19), 361(15), 243(2.7), 305(2.5), 271(1.6), 319(0.8)	143(100), 82(15), 135(3.2), 225(2.8), 93(2.6), 81(2.4), 131(2.3), 107(1.5), 141(1.5)	692	170
Unknown 5	1.210	217(100), 204(37), 331(34), 361(20), 263(17), 233(14), 191(9.9), 243(6.3), 271(2.5), 319(1.9)	143(32), 81(20), 69(16), 75(8.3)	694	172
Unknown 6	1.274	204(100), 217(60), 331(36), 361(31), 191(22), 263(17), 233(17), 243(4.1), 305(3.9), 319(3.3), 271(2.9)	135(25), 225(25), 143(21), 93(18), 107(15)	692	170
Unknown 7	1.322	204(74), 331(71), 217(62), 361(35), 263(34), 191(33), 233(30), 243(5.6), 305(4.3), 319(3.6), 271(3.1)	135(47), 225(34), 93(31), 107(30), 143(28), 67 (11), 81(6.3)	692	170
Unknown 8	1.347	331(76), 217(54), 253(46), 204(40), 191 (20)	81(100), 131(76)	694	172
Unknown 9	1.363	217(100), 204(29), 331(22), 361(18), 263(12), 191(7.8), 271(7), 233(6.9), 243(3.7), 319(3.4), 305(2.5)	121(25), 143(18), 75(9.8), 93(8.1)	692	170

[a,b] See footnotes *a* and *b* in Table III.

[c] Obtained as $MW_a = MW_s - 451$ (molecular weight of the glucose moiety) $- 73$ (TMS) $+ 2$ ($2 \times H$).

TABLE V

MASS SPECTRA OF TFA DERIVATIVES OF GLYCOSIDES WITH AGLYCONES TENTATIVELY IDENTIFIED AS HOTRIENOL, LINALOL OXIDES AND MONOTERPENIC ACIDS IN *VITIS VINIFERA* GRAPES

Aglycone residue	RRT[a]	EI-MS, Characteristic fragment ions [*m/z* (relative intensity, %)] of		CI-MS, characteristic fragment ions [*m/z* (relative intensity, %)] of[c]
		Sugar moiety	Aglycone moiety[b]	
Glucosides				
Ho-trienyl	1.081	319(100), 193(17), 205(6), 177(6)	93(82), 134(68), 69(66), 79(63), 135(60), 119(50), 91(47), 107(45), 92(36), 109(29), 81(26), 77(23)	603(20), 716(16), 490(4)
A furanic linalyl oxide	1.160	319(17), 193(2.5), 177(1.5), 205(1.5), 265(0.7), 547(0.3), 291(0.1)	111(100), 93(44), 69(22), 71(21), 153(11), 94(5)	734(13), 508(5)
A furanic linalyl oxide	1.174	319(36), 193(5), 205(4), 177(4), 265(1.3)	111(100), 71(60), 93(54), 69(35), 153(9), 94(6.5)	
A pyranic linalyl oxide	1.279	319(29), 205(2.5), 177(2.5), 193(2), 265(0.7)	68(100), 94(92), 81(46), 71(33), 111(17), 69(11)	
A 3,7-dimethyl-2,6-octadienoic acid	1.427	319(8), 193(1.5), 177(1.5), 205(1.5)	69(100), 123(20), 151(18), 168(12), 82(10)	
Rutinosides				
A 3,7-dimethyl-2,6-octadienoic acid[d]	1.822	193(11), 207(3), 265(1), 206(0.6), 278(0.4), 179(0.4), 177(0.4), 319(0.3), 435(0.3), 292(0.2)	69(100), 123(15.3), 82(7), 151(5), 168(4)	
Arabinosylglycosides				
(*E*) and (*Z*)-3,7-dimethyl-2,6	1.894	193(25), 265(2), 278(1.5), 319(0.8), 177(0.3), 279(0.3)	69(100), 123(24), 151(19), 82(17), 168(10)	
Octadienoic acid	1.921	193(8), 421(3), 279(2.5), 265(0.2), 278(0.1)	69(100), 123(18), 151(18), 82(14), 168(7)	
Apiosylglycosides[e]				

[a] Retention time relative to phenyl β-D-glucopyranoside.
[b] A small portion of *m/z* 69 can be accounted for by CF_3^+.
[c] From CI-MS with ammonia as reactant gas.
[d] Co-eluted with geranyl arabinosylglycoside.
[e] Data reported previously [3].

TABLE VI

MASS SPECTRA OF TFA DERIVATIVES OF GLUCOPYRANOSIDES WITH UNKNOWN MONOTERPENEDIOL AGLYCONE TENTATIVELY IDENTIFIED IN *VITIS VINIFERA*

Aglycone residue	RRT[a]	EI-MS, characteristic fragment ions [*m/z* (relative intensity, %)] of	
		Sugar moiety	Aglycone moiety[b]
Unknown 1	1.182	319(6.5), 193(1.7), 177(0.8), 205(0.7), 265(0.5), 547(0.1)	71(100), 82(75), 83(36), 69(18), 93(11), 81(10)
Unknown 2	1.196	319(100), 193(6.7), 205(6.1), 547(5.4), 177(3.7), 291(0.6)	71(53), 93(21), 69(20), 81(19), 109(12), 107(11)
Unknown 3	1.208	319(8.1), 193(3), 205(2.4), 177(1.6)	71(100), 83(74), 85(66), 82(60), 94(21), 69(19), 109(18), 84(10)
Unknown 4	1.214	319(39.4), 205(2.6), 177(0.5), 193(0.3)	81(100), 69(71), 80(71), 95(58), 68(49), 93(48), 79(41), 121(39), 67(29), 136(18)
Unknown 5	1.296	319(18), 205(2.8), 177(2.1), 193(1.9), 265(0.6), 547(0.2), 291(0.2)	93(100), 71(42), 94(14), 67(12), 79(12), 134(11), 81(11)
Unknown 6	1.302	319(19), 205(2), 177(0.8), 193(0.7)	71(100), 82(19), 93(14), 109(13), 67(11), 72(9), 94(8)
Unknown 7	1.315	205(3), 177(1.9), 319(1.9)	96(100), 93(86), 68(79), 81(78), 95(64), 67(57), 69(42), 110(32), 71(22), 80(21), 109(21), 108(19), 92(18), 135(17)
Unknown 8	1.332	319(62), 193(11), 205(9.1), 177(3.8), 547(3.2), 265(2)	93 (100), 69(93), 71(42), 81(32), 109(22), 91(20), 92(14), 77(12), 70(12) 97(12), 83(12), 121(10), 119(10)
Unknown 9	1.340	319(4.6), 193(2.9), 265(0.3)	69(100), 109(38), 93(28), 121(15)
Unknown 10	1.353	319(17), 177(4.2), 193(2.1), 205(1.6)	93(100), 80(81), 94(41), 71(32), 121(25), 81(25), 79(24), 119(17), 107(17), 97(16), 91(14), 109(14), 105(13), 92(13)
Unknown 11	1.362	319(17), 193(4), 205(2.3), 177(2), 265(0.5), 547(0.5)	71(100), 93(38), 109(31), 69(30), 94(20), 68(19), 84(19), 81(18), 80(16), 82(15), 119(14), 121(12), 95(12), 107(11)
Unknown 12	1.413	319(20), 193(4.5), 205(2.8), 177(2.5), 265(1)	71(100), 69(54), 93(35), 109(29), 80(27), 82(22), 68(19), 81(19), 94(16), 85(13), 107(13), 84(12), 95(11), 97(10)
Unknown 13	1.445	319(16), 193(4.8), 205(4.3), 177(4.3)	80(100), 69(45), 111(44), 147(44), 93(23), 166(22), 165(21), 98(19), 91(18), 97(16), 105(15), 107(15), 119(13), 77(12)
Unknown 14	1.519	319(45), 193(3.6), 205(3.5), 177(3.4), 265(2.1), 547(0.3)	71(100), 93(21), 121(20), 69(20), 114(16), 96(15), 82(13), 97(12), 95(12)

[a,b] See footnotes *a* and *b* in Table V.

diglycosides. CI-MS of their TMS derivatives gave results consistent with diglycosides of monoterpendiols (molecular weights of 242 and 244 for the aglycones), but EI-MS of both TMS and TFA derivatives gave different spectra to those observed above for the glycosides of monoterpendiols. As no synthetic representatives of monoterpendiol diglycosides were available, we could not draw a conclusion, but it was interesting that (*E*)-2,6-dimethyl-6-hydroxy-2,7-octadien-1-yl (unknown configuration of C-6) β-D-glucopyranoside and 6-O-α-L-arabinofuranosyl-β-D-glucopyranoside were previously positively identified in grape [6,7].

Glycosides of monoterpenic acids

In the chromatograms of the TFA derivatives of the five grape extracts, some peaks were tentatively identified from their EI-MS data (Table V) as (*E,Z*)-3,7-dimethyl-2,6-octadienoyl glycosides, rutinosides, arabinosylglycosides and apiosylglycosides. The fragment ions assigned to the aglycones were similar to those reported for (*E,Z*)-3,7-dimethyl-2,6-octadienoic acids [21]; the ions at *m/z* 151 and 123 represented the cleavage of bonds next to C=O and the other diagnostic peaks were found at *m/z* 168, 82 and 69. It is interesting that these glycosides were absent in the gas chromatograms of the corre-

sponding TMS glycoside extracts, as they were probably deglycosylated by the chlorotrimethylsilane present in the silylating reagent, as already reported for other 1-O-acyl glycosides [22].

Other glycosides

The gas chromatograms of the TFA derivatives of the grape glycoside extracts showed few peaks of compounds different from terpenoid derivatives. The more important of them were tentatively identified as glycosides of ferulic, coumaric, vanillic and syringic acids, and have been reported elsewhere [14].

Quantitative analysis

The concentrations reported in Table I were determined using TFA derivatives of the glycoside extracts with phenyl β-D-glucopyranoside as internal standard according to the method developed in Part I [12]; the global calibration factors used were those described previously for the available reference compounds [12], but the concentrations of the tentatively identified apiosylglycosides were determined using the global calibration factors for the 6-O-β-L-arabinofuranosyl-β-D-glucopyranosides with the corresponding aglycone. The other glycosides, tentatively identified as monoglycosides and diglycosides of linalool oxides, monoterpenediols and 3,7-dimethyl-octa-2,6-dienoic acids, could not be determined individually owing to a lack of reference compounds; their total amount, determined using no calibration factor, was reported in Table I as indicative of the relative amounts of glycosides with aglycones in higher oxidation states than linalool in the four different classes of glycosides.

As regards apiosylglycosides, small peaks corresponding to partially trifluoroacetylated apiosylglycosides were found in the range of high retention times, as reported previously [3], and were not taken into the quantitative determination of the apiosylglycosides reported, as they were much smaller than the fully trifluoroacetylated compounds and as no calibration factors were available. Synthesis of these apiosylglycosides is in progress to investigate how to overcome this major drawback for their quantitative determination.

Table I showed a similar general glycoside distribution to that reported previously for muscat of Alexandria and Rhine Riesling grapes [1]. The proportion of disaccharides compared with monoglycosides was generally high, but only for those glycosides with aglycones in the linalol oxidation state. Indeed, glycosides with aglycones in higher oxidation states than linalol were found to be abundant in muscat Ottonel but also in muscat of Frontignan and muscat of Alexandria. In fact, such glycosides were more abundant than those with aglycones in the linalol oxidation state in the five cultivars, but the reverse was true for the diglycosides, except for the apiosylglycosides in muscat of Frontignan. Further, the proportion of rutinosides compared with the two other pentosylglycosides was generally low.

As regards aglycones in the linalol oxidation state, glycosides of geraniol were generally the most abundant (particularly in Gewürztraminer), except for the two pentosylglycosides in muscat of Frontignan and Hamburg; glycosides of α-terpineol appeared as minor components only.

The total amounts of bound aroma components were higher in Gewürztraminer, muscat of Frontignan and Ottonel than in muscat of Alexandria and muscat of Hamburg, the last containing the lowest levels of each class of glycosides, consistent with the results reported by Günata [9] using enzymic hydrolysis.

We then compared the concentrations thus determined for the glycosides with those determined using the known procedure involving enzymic hydrolysis of their aglycones [11]. The volatiles released were analysed by GC and quantitatively determined without calibration factors (Table II).

Comparison of the results in Table I with those in Table II showed that the conclusions deduced from Table I concerning the aglycones (see above) are consistent with thosed which could be obtained from Table II, taking into consideration the properties of the glycosidase activities of Pektolase 3PA [13,23].

Finally the free fractions obtained in these experiments were analysed by GC and the concentrations of the compounds, also reported as bound compounds, were quantitatively determined using the global calibration factors given in the Part I [12] for the available reference compounds. These results are reported in Table II together with those obtained on the bound forms.

CONCLUSIONS

This work showed that direct GC and GC–MS analysis of TFA-derivatized non-volatile glycosides allowed their qualitative and quantitative determinations in some aromatic grape cultivars. This method appeared to be the best suited for the direct analysis of monoterpene glycosides, the most abundant of grape glycosides.

Combination of direct analysis after TMS and TFA derivatization or after enzymic hydrolysis of the glycosidic fractions allowed a breakthrough in the qualitative determination of monoterpene glycosides. It confirmed the complexity and heterogeneous nature of grape glycosides and the high proportions of bound monoterpenes compared with free monoterpenes.

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