Fractionation of glycoside precursors of aroma in grapes and wine

J.J. Mateo[b,*], N. Gentilini[a], T. Huerta[b], M. Jiménez[b], R. Di Stefano[a]

[a]Istituto Sperimentale per l'Enologia, Via Pietro Micca 35, I-14100-Asti, Italy
[b]Dpto. Microbiología, Facultad CC. Biológicas, Dr. Moliner 50, E-46100-BurjasotValencia. Spain

Abstract

The glycosides in mono-, di- and trihydroxylated terpene and norisoprenoid alcohols and also those in the related shikimate pathway have been isolated on C_{18} reversed-phase cartridges and then fractionated into classes of different polarity at increasing percentages of methanol. The benzyl alcohol glycosides are the most polar, while those of terpene monohydroxylated alcohols and geranic acid are the least polar. The terpene diols, linalool furanoid and pyranoid oxides and also norisoprenoid precursors show intermediate polarity and separate into well defined fractions according to their polarity. © 1997 Elsevier Science B.V.

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1. Introduction

The occurrence of glycosidic forms of monoterpenols in Muscat grapes and other cultivars has been established [1–3]. These forms occur mainly as monoglucosides or disaccharide glycosides: 6-O-α-L-arabinofuranosyl-β-D-glucopyranosides, 6-O-α-L-rhamnopyranosyl-β-D-glucopyranosides [3] or 6-O-β-D-apiofuranosyl-β-D-glucopyranosides [4].

In the aromatic varieties of grapes, Williams et al. [5] discovered two classes of precursors of C_{10} and C_{13} components with different polarity, separable by chromatography on C_{18} reversed-phase (RP) cartridges. The method these authors described requires the absorption of dealcoholized wine on C_{18} RP cartridges of a size appropriate to the volume of wine used. It also requires the elimination of hydrophilic components with water, the elution of more polar precursors with 30% acetic acid and the elution of monohydroxylated terpenic alcohols with methanol. The components in both fractions were recovered by

evaporation of solvent and subjected to further purification or hydrolysis.

The drawbacks with this method are that volatile compounds are not completely eliminated during the evaporation of wine alcohol under vacuum and that the precursors are only partially recuperated with an acidic solvent, with the possibility of hydrolytic adulterations and chemical transformations during the solvent evaporation.

More recently, Strauss et al. [6] fractionated the C_{10}, C_{13} and aromatic ring precursors in must by droplet counter-current chromatography (DCCC) after isolation on a C_{18} RP column, and Bitteur et al. [7] described an analytical HPLC method with a C_{18} column to fractionate aroma precursors, using acetonitrile and water as solvents.

Developments in chromatographic techniques in totally liquid phase [8] permitted the fractionation of the precursors in leaves of Renan grape variety after isolation on XAD-2 resin column.

The aim of this study was to develop fractionation techniques for use in laboratories which do not have instruments for totally liquid chromatography by

*Corresponding author.

using commercial C_{18} RP columns and inert solvents.

2. Experimental

2.1. Must and wine

Must and wine from the 'Moscato bianco' variety was obtained from the vineyard of the Istituto Sperimentale per l'Enologia (Asti, Italy) in 1995. Wine must be previously diluted to obtain an alcoholic content lower than 4%.

2.2. Isolation of glycosides

Must or wine (250 ml) or a proportional volume of an extract of skin, grape berries or other vine tissue are eluted through a 10-g C_{18} RP column (Waters, Milford, MA, USA), previously activated with 30 ml of methanol (Merck, Darmstadt, Germany) followed by 50 ml of water. The cartridge was washed with 100 ml of water and free components were eluted with 50 ml of dichloromethane (Merck). Glycosides were recovered with 50 ml of methanol.

2.3. Fractionation of glycosides

The methanolic extract is reduced under vacuum, recovered with 5–10 ml of water and passed through a 5-g C_{18} cartridge (Waters) (previously activated with 15 ml of methanol followed by 30 ml of water), washed with 50 ml of water and eluted by the following solvents:
1. methanol–water (20:80 v/v), 50 ml;
2. methanol–water (30:70 v/v), 50 ml;
3. methanol–water (40:60 v/v), 50 ml, and
4. methanol, 25 ml,
collecting 25-ml fractions.

2.4. Enzymatic hydrolysis of glycosides

Each fraction is evaporated under vacuum, recovered with 3 ml of citric–phosphoric acid buffer 0.1 M (pH 5.0). 0.1 ml of a commercial pectolytic enzyme (Rohapect 7104, Rohm, Germany) was added and hydrolysis was carried out at 40°C for 24

h to liberate the volatile aglycone moiety. Then, 0.25 ml of 1-heptanol (30 mg/l) was added as internal standard and each hydrolyzed fraction was eluted through a 300-mg C_{18} RP cartridge (Waters) previously activated with 3 ml of methanol and 5 ml of water. It was washed with 3 ml of water and the components produced by enzymatic hydrolysis were eluted with 4 ml of CH_2Cl_2. This extract was dried with anhydrous Na_2SO_4, transferred into a 100-ml distillation flask, concentrated and analyzed by GC and GC–MS.

2.5. Chemical hydrolysis of glycosides

After GC analysis, extracts were evaporated under vacuum, recovered with 10 ml of tartaric buffer, pH 3.0 (tartaric acid 5 g/l, NaCl 10% and adjusted to pH 3.0 with 1 M NaOH) and heated for 60 min in a boiling water bath. Then, each flask was cooled and the extract eluted through a 300-mg C_{18} RP cartridge (Waters) as described above. This procedure can simulate wine transformations in terpenic and norisoprenoidic components during storage and ageing. Extracts originating from chemical hydrolysis were analyzed by GC and GC–MS.

2.6. Gas chromatography

Analytical chromatography was performed on a Carlo Erba HRGC 5300 Mega series instrument (Milan, Italy) equipped with a flame ionization detector and a 30 m×0.25 mm I.D. FFAP fused-silica capillary column (J&W Scientific, Folsom, CA, USA). Hydrogen was used as carrier gas (1.5 ml/min) and the flame ionization detection temperature was 250°C. Injections were made with a 1:30 split at an injector temperature of 250°C. The oven was held isothermally at 60°C for 2 min, then programmed from 60–190°C at 2°C/min and then to 230°C at 15°C/min.

2.7. Gas chromatography–mass spectrometry

Analysis was performed on a HP 5890 gas chromatograph–HP 5970 mass detector system (Hewlett–Packard, Wilmington, DE, USA). The chromatograph was equipped with a FFAP fused-silica capillary column (30 m×0.2 mm I.D.). The

splitless injector was maintained at 250°C. Helium was used as carrier gas (inlet pressure 10.5 p.s.i., 1 p.s.i.=6894.76 Pa) and the interface temperature was 230°C. The oven was held isothermally at 30°C for 1 min and then taken to 60°C at 30°C/min. Then, the oven temperature was programmed from 60–160°C at 2°C/min and then to 220°C at 3°C/min.

3. Results

Fig. 1 shows a typical GC–MS chromatogram showing all the components identified in this study.

Table 1 shows the concentration of components originating from the enzymatic hydrolysis of glycosidic precursors. Among non-terpenic compounds, 1-hexanol is distributed between fractions 4 and 5, while cis-3-hexen-1-ol and trans-2-hexen-1-ol are found in fractions 3 and 4. Benzyl alcohol is present in fractions 1–3 (with the maximum in fraction 2), 2-phenethyl alcohol is distributed among fractions 3–5 (with the maximum in fraction 3), 4-vinyl-guaiacol and 4-vinyl-phenol (components originating from the decarboxylation of cinnamic

acids into the GC injector) are found in fraction 1 and 2 and eugenol in fraction 5.

Among terpenic components with an oxidation state higher than linalool, trans and cis-furan-linalool oxides show the two highest values: one between fractions 2 and 3 and another in fraction 5. 2,6-Dimethyl-7-octen-1,6-diol (8-hydroxydihydrolinalool) and 2,6-dimethyl-7-octen-2,3,6-triol (triol) elutes in fractions 2–3 while terpines 1 and 2 show a maximum in fraction 3. Many other compounds elute mainly in fractions 4 and 5: cis-pyran-linalool oxide, 3,7-dimethyl-1,7-octadien-3,6-diol (diol 2), trans-pyran-linalool oxide, 2,6-dimethyl-1,7-octadien-2,6-diol (diol 1), 2,6-dimethyl-7-octen-2,6-diol (endiol), E-2,6-dimethyl-2,7-octadien-1,6-diol (trans-8-hydroxylinalool) and Z-3,7-dimethyl-2-octen-1,7-diol (hydroxynerol). The first two components have the maximum in fraction 4 and the rest in fraction 5. The ratio between trans and cis pyranlinalool oxide is lower than one in fraction 4 and higher than one in fraction 5. Z-2,6-Dimethyl-2,7-octadien-1,6-diol (hydroxygeraniol), together with cis-8-hydroxylinalool are eluted in fractions 5–6, with a maximum in fraction 5, while 3,7-dimethyl-1,7-octanediol (hy-
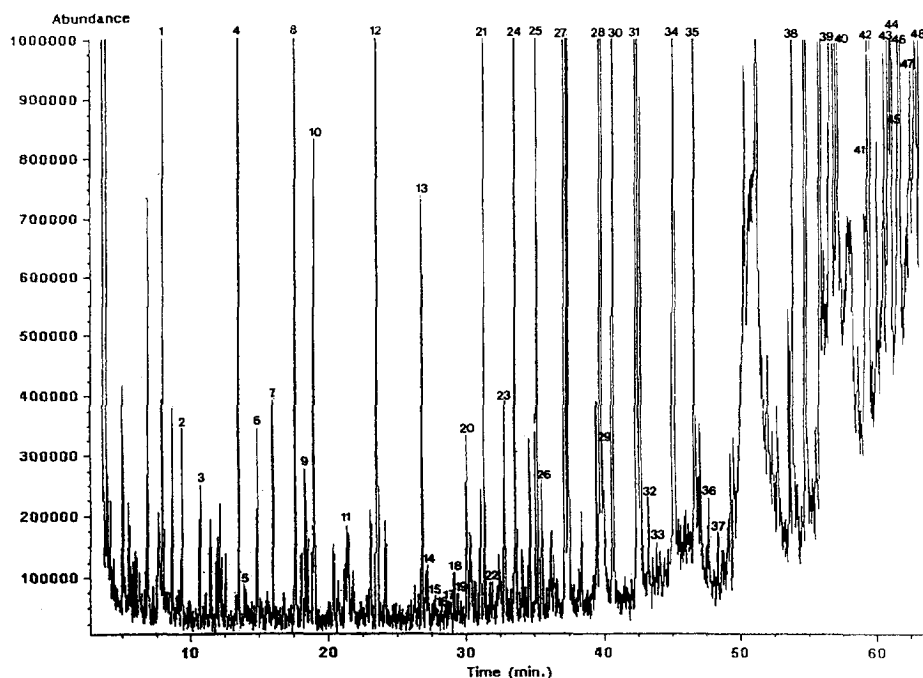

Fig. 1. Typical GC–MS chromatogram of an extract showing all components listed in Tables 1 and 2. Component numbers as in tables.

Table 1
Concentration (μg/l) of terpenes and isoprenoids obtained form enzymatic hydrolysis of glycosides

No.	Precursors	Methanol–water (20:60 v/v)		Methanol–water (30:70 v/v)		Methanol–water (40:60 v/v)		Methanol Fraction
		Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	7
4	1-Hexanol	-	-	-	17.3	190.3	-	-
5	trans-3-Hexen-1-ol	-	-	2.3	-	-	-	-
6	cis-3-Hexen-1-ol	-	-	40.5	11.0	-	-	-
7	trans-2-Hexen-1-ol	-	-	33.4	71.9	6.8	-	-
8	trans-Furanlinalool oxide	-	44.8	45.0	141.4	776.5	-	-
9	cis-Furanlinalool oxide	-	25.2	24.3	168.1	355.2	-	-
10	Nerol oxide	-	-	-	13.1	-	-	-
12	Linalool	-	-	-	-	-	13.8	773.3
13	Ho-trienol	-	-	-	5.2	-	-	-
20	Neral	-	-	-	-	-	-	142.0
21	α-Terpineol	-	-	-	-	-	5.4	120.9
23	Geranial	-	-	-	-	-	-	116.9
24	trans-Pyranlinalool oxide	-	-	2.7	190.3	546.0	-	-
25	cis-Pyranlinalool oxide	-	3.5	104.2	363.7	11.7	-	-
26	Citronellol	-	-	-	-	-	-	43.5
27	Nerol	-	-	-	-	-	30.2	1586.8
28	Geraniol	-	-	-	-	-	42.6	2170.9
29	2-Hydroxy-1,8-cineol	-	-	69.0	-	-	-	-
30	Benzyl alcohol	32.7	742.3	354.5	-	-	-	-
31	2-Phenethyl alcohol	-	-	671.6	86.0	10.9	-	-
24	E-2,6-Dimethyl-3,7-octadien-2,6-diol	-	-	16.6	648.6	302.5	-	-
25	2,6-Dimethyl-7-octen-2,6-diol	-	-	-	56.0	113.9	-	-
36	Terpina 1	-	-	31.3	6.3	-	-	-
37	Terpina 2	-	-	17.0	-	-	-	-
38	2,6-Dimethyl-1,7-octadien-3,6 diol	-	-	-	122.8	152.5	-	-
39	3,7-Dimethyl-1,7-octanediol	-	-	-	-	82.3	224.6	-
40	2,6-Dimethyl-7-octen-1,6-diol	-	-	-	-	190.9	-	-
41	Z-3,7-Dimethyl-2-octen-1,7-diol	-	-	-	80.0	253.9	-	-
42	E-2,6-Dimethyl-2,7-octadien-1,6-diol	-	-	-	245.4	1082.8	-	-
43	Z-2,6-Dimethyl-2,7-octadien-1,6-diol	-	-	51.7	1227.5	843.7	-	-
44	E-3,7-Dimethyl-2-octen-1,7-diol	-	-	-	-	356.2	79.0	-
45	Compound X	-	-	-	-	-	50.4	-
46	trans-Geranic acid	-	-	-	-	-	-	1734.9
47	Mentendiol	-	-	106.2	52.2	-	-	-
48	Triol	-	162.3	76.6	-	-	-	-

droxycitronellol), the most hydrophobic compound together with compound X (an unknown diol), are distributed between fractions 5–6 with a maximum in fraction 6. All other terpenic compounds, i.e. monohydroxylated alcohols and geranic acid are found mainly in fraction 7, but also in fraction 6.

Isoprenoids are eluted in fractions 2–6 and their identification is MS-based: epoxy-β-ionone, 3,4-di-dehydro-7,8-dihydro-β-ionone, 3-oxo-α-ionol, 3,9-dihydromegastigma-5-ene, 3-hydroxymegastigma-5,8-dien-7-one and vomifoliol; other compounds, with PM 190, 194 and 208, are still unknown.

Identified compounds after acid hydrolysis of components originating from enzymatic hydrolysis of glycosides are listed in Table 2. Two vitispyrane can be seen in fractions 2–5 with an uncertain maximum; the Riesling acetal, 1,1,6-trimethyl-1,2-dihydronaphtalene (TDN) and hydroxy-TDN (in

Table 2

Concentration (μg/l) of terpenes and isoprenoids obtained from chemical hydrolysis of precursors

No.	Precursors	Methanol–water (20:60 v/v)		Methanol–water (30:70 v/v)		Methanol–water (40:60 v/v)		Methanol Fraction
		Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	7
1	2,6,6-Trimethyl-2-vinyl-tetrahydropyrane	-	-	-	22.2	164.0	79.5	65.0
2	trans-Anhydrofuranlinalool oxide	-	-	-	64.4	87.1	-	-
3	cis-Anhydrofuranlinalool oxide	-	-	-	72.6	194.8	-	-
4	1-Hexanol	-	-	-	20.3	203.9	-	-
6	cis-3-Hexen-1-ol	-	-	36.1	10.9	-	-	-
7	trans-2-Hexen-1-ol	-	-	30.9	68.3	7.8	-	-
8	trans-Furanlinalool oxide	136.4	455.9	718.3	1162.7	883.5	19.7	-
9	cis-Furanlinalool oxide	9.8	215.4	307.5	52.9	436.0	15.1	-
10	Nerol oxide	-	-	-	156.2	137.1	-	-
11	Vitispyrane	-	2.3	16.3	171.9	15.5	-	-
12	Linalool	-	-	-	-	-	10.8	566.3
13	Ho-trienol	-	2.6	7.9	230.9	112.4	7.0	-
14	Myrcenol	-	8.0	12.1	18.8	112.0	3.6	53.7
15	Riesling acetal	-	-	4.8	3.2	-	-	-
16	Ocimenol 1	-	16.2	23.4	34.4	235.8	5.4	94.9
17	Compound Y	-	8.4	18.4	94.5	152.8	-	-
18	Ocimenol 2	-	17.5	28.1	53.5	322.6	8.9	153.6
19	Compound Z	-	-	-	-	43.5	77.8	61.0
21	α-Terpineol	-	-	9.7	5.4	30.1	23.8	854.1
22	TDN	-	-	1.6	-	-	-	-
24	trans-Pyranlinalool oxide	-	-	9.5	128.1	351.3	-	-
25	cis-Pyranlinalool oxide	-	-	88.1	282.4	14.4	-	-
26	Citronellol	-	-	-	-	-	36.8	18.5
27	Nerol	-	-	-	-	-	-	224.1
28	Geraniol	-	-	-	-	-	-	313.9
29	2-Hydroxy-1,8-cineol	-	-	76.1	20.2	-	-	-
30	Benzyl alcohol	36.2	566.9	253.8	-	-	-	-
31	2-Phenethyl alcohol	-	-	689.2	95.8	-	-	-
32	Actinidol 1	-	-	14.9	-	-	-	-
33	Actinidol 2	-	-	60.3	-	-	-	-
34	E-2,6-Dimethyl-3,7-octadien-2,6-diol	-	-	-	55.0	37.1	-	-
35	2,6-Dimethyl-7-octen-2,6-diol	-	-	-	-	104.0	105.3	173.6
36	Terpina 1	-	-	-	-	-	-	429.9
37	Terpina 2	-	-	-	-	-	-	128.1
39	3,7-Dimethyl-1,7-octanediol	-	-	-	-	82.0	199.3	-
40	2,6-Dimethyl-7-octen-1,6-diol	-	-	-	-	29.9	-	-
46	trans-Geranic acid	-	-	-	-	-	-	661.4
47	Menten-1-ene-7,8-diol	-	-	99.4	56.9	-	-	-

trace amounts) in fractions 3 and 4, damascenone in fractions 4–5 (also in trace amounts) and actinidols in fraction 3.

Unlike other cultivars, 'Moscato Bianco' shows a low content of norisoprenoidic compounds which, after enzymatic and chemical hydrolysis, give rise to vitispyrane, TDN, damascenone and actinidols. This variety belongs to a group of cultivars whose metabolism is firstly directed to the production of actinidols and vitispyrane precursors. This fact seems to be very important because, as it is possible to separate the different vine cultivars into groups which share similar biosynthetic activities towards terpenics compounds, it also seems to be possible to

put them in groups which share the same biosynthetic activities towards norisoprenoidic and aromatic volatile compounds.

The described fractionation permits a more efficient study of the chemical transformation of each terpenic diol originated by enzymatic hydrolysis of the related glycosidic precursor. It is especially interesting the study of the two 8-hydroxylinalool isomers: according to Strauss et al. [6], their transformation gives rise to 2,6-dimethyl-2,6-octen-1,8-diol as principal component. It is impossible to identify this compound using the proposed method although a group of numerous components with an intense mass, in MS, in 79 and 94, i.e. *p*-ment-1-ene-9-ale (tentatively identified on the basis of its mass spectrum) can be seen. Since the retention time is between that of the unidentified components with mass 79 and 94 and that of α-terpineol, it is possible to find the main transformation product of compound X eluted in fraction 6, with a retention time similar to that of hydroxygeraniol. This compound has been tentatively identified as *p*-ment-3-ene-9-ol on the basis of its mass spectrum.

4. Conclusions

The results of this study confirm that it is possible to fractionate the glycosides of terpenes and norisoprenoidic compounds into two main groups: one containing glycosides of di- and trihydroxylated compounds and other containing glycosides of monohydroxylated compounds. It is possible to obtain further fractions in the first group by decreasing the polarity of the solvents: from glycosides of benzyl alcohol, the component with highest polarity, to glycosides of hydroxycitronellol, with lowest polarity. Furthermore chemical hydrolysis could be used after the enzymatic treatment for a more in depth study of components originating from terpenic and norisoprenoidic precursors during wine ageing.

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