

Structural identification and presence of some p-menth-1-enediols in grape products

by

G. VERSINI¹⁾, A. RAPP²⁾, F. RENIERO¹⁾ and H. MANDERY³⁾

1) Istituto Agrario Provinciale, Laboratorio di Analisi e di Ricerca, San Michele all'Adige, Italia

2) Bundesanstalt für Züchtungsforschung im Wein- und Gartenbau, Institut für Rebenzüchtung, Geilweilerhof, Siebeldingen, BR Deutschland

3) BASF AG, Ludwigshafen/Rh., BR Deutschland

Aufklärung der Struktur und Vorkommen von p-Menth-1-endiolen in Traubenerzeugnissen

Zusammenfassung: In der vorliegenden Arbeit wird die Stukturaufklärung einiger p-Menthendiole gegeben, die bereits als Glycoside in Traubenmosten und Weinen nachgewiesen und als Isomere 1, 2 und 3 bezeichnet wurden. Es zeigte sich, daß *cis*-p-Menth-1-en-6,8-diol (*cis*-Sobrerol) und p-Menth-1-en-7,8-diol mit den Isomeren 2 und 3 übereinstimmen und *trans*-p-menth-1-en-6,8-diol (*trans*-Sobrerol), das nur in geringen Spuren gefunden wurde, vermutlich mit dem Isomer 1 identisch ist. In Traubenmosten verschiedener Rebsorten wurden die Gehalte dieser Verbindungen sowohl in der freien als auch in der gebundenen Form ermittelt und mit den Gehalten von α -Terpineol und 2-Oxo-hydroxy-1,8-cineol verglichen. Die freien Komponenten lagen im allgemeinen nur in geringer Konzentration vor. Bei den gebundenen Formen war p-Menth-1-en-7,8-diol vorherrschend, gefolgt von *cis*-p-Menth-1-en-6,8-diol. Die identifizierten Verbindungen erlauben eine bessere Sortencharakterisierung, darüber hinaus läßt das Vorkommen dieser Komponenten auf eine Allylhydroxylase-Aktivität in Weinbeeren schließen.

Key words: must, flavour, terpene, free and bound constituents, analysis, variety of vine, classification, allylic hydroxylase.

Introduction

Recent research showed the presence of some p-menthenediols in must and/or wines either as α -terpineol metabolites of *Botrytis cinerea* (MANDERY 1986; RAPP and MANDERY 1988) or as typical grape compounds either in free form, such as two isomers of p-menth-8-ene-1,2-diol (BAUMES *et al.* 1986) or as glycosides. The latter include the p-menth-1-ene-8,9-diol (WINTERHALTER *et al.* 1990) and the so called p-menthenediols — isomers 1 and 2 (spectral data in STRAUSS *et al.* 1987) or isomers 1, 2 and 3 (WILLIAMS *et al.* 1989; WINTERHALTER *et al.* 1990). No spectral data are reported for WILLIAMS' isomer 1, whereas isomers 2 and 3 correspond to 1 and 2 reported by STRAUSS, from the same working group. These are not yet defined as concerns the position of the double bond as well as the position of at least one of the two alcoholic functions, the other one being likely in the position C 8 because of the relevant MS fragment at m/z 59. These compounds or some of them have been found in Rhine Riesling musts and wines as aglycons after enzymatic hydrolysis or in other varieties after chemical hydrolysis.

In this study the undefined structures of the two most relevant above mentioned isomers are cleared, a probable structure of the third one, present only in traces, is presented, and the concentrations of these compounds in some varietal musts are exemplified.

Materials and methods

Materials

Must samples of different grape varieties from the vine collection of the Istituto Agrario Provinciale or from vineyards of the same area were analyzed.

Reference compounds

1. *trans* p-menth-1-ene-6,8-diol (*trans* sobrerol) was purchased from Aldrich.
2. *cis* p-menth-1-ene-6,8-diol (*cis* sobrerol) was synthesized from (-)-carveol (isomer mixture from Aldrich) by hydroxylation in water at pH 1, under stirring for 15 h at room temperature and subsequent extraction of the raw reaction products with pentane : methylene chloride, 2:1 in vol., and purification by preparative TLC silica gel (ethyl acetate : toluene, 9:1 in vol.). In this way, it was also possible to separate partially (80 %) the *cis* isomer from the *trans* form (yield of ca. 8 %).
Mass spectral data (m/z (%)): 152 (4), 137 (21), 119 (3), 109 (62), 94 (57), 79 (86), 69 (30), 59 (100), 55 (25), 43 (79), 41 (38), 31 (28), similar to those already reported by MANDERY (1986).

¹H and ¹³C NMR spectra data (δ , ppm): ¹H NMR (300 MHz, (CD₃)₂CO): 1.17 (s, 6,8-CH₃), 1.34 (m, 1,4-H), 1.74 (m, 3,7-H), 1.85 (m, 1,5-H), 2.24 (m, 3-H), 3.28 (s, 1,8-OH), 3.72 (d, 1, J = 6.1 Hz, 6-OH), 4.11 (m, 1,6-H), 5.43 (m, 1,2-H); ¹³C NMR (75 MHz, (CD₃)₂CO): 20.92 (C-7), 26.92 (C-3), 27.79 (C-9), 28.11 (C-10), 35.69 (C-5), 45.66 (C-4), 68.77 (C-6), 71.58 (C-8), 123.53 (C-2), 136.0 (C-1).

3. p-menth-1-ene-7,8-diol was synthesized from (S) (-)-perillyl alcohol (Aldrich) by using the same cited reaction and purification processes (yield of ca. 5 %).

Mass spectral data: (m/z (%)): 152 (11), 137 (2), 121 (12), 109 (24), 94 (20), 93 (25), 91 (24), 81 (18), 79 (94), 67 (16), 59 (100), 55 (19), 43 (64), 41 (30), 31 (32).

¹H and ¹³C NMR (δ , ppm, CDCl₃) spectral data accord with those partially published by BLUTHE *et al.* (1980), except for 3.93 (s, 2,7-H).

Methods

100 ml of must, after addition of 2-octanol as internal standard (23 μ g), clarification with bentonite (700 mg/l) and centrifugation, were subjected to liquid chromatography on Amberlite XAD-2 adsorbent (0.1–0.2 mm; Serva) using a glass column (15 \times 120 mm) (GUNATA *et al.* 1985). The free and bound compounds were separately eluted as previously reported (VERSINI *et al.* 1988). The bound fraction was enzymatically hydrolyzed (Rohaspect C (Röhm), buffer citrate at pH 5, 15 h at 40 °C); after addition of the same quantity of internal standard, it was extracted three times by hand with pentane : methylene chloride, 2:1 in vol., concentrated and quantified by capillary gas chromatography.

Two C.Erba HRGC 5300 gas chromatographs with FID were used. One was equipped with two fused silica WCOT capillary columns: Hewlett-Packard HP-20 M (30 m; 0.32 mm i.d.; d.f. = 0.5 μ m) and J and W DB-WAX (30 m; 0.32 mm i.d.; d.f. = 0.3 μ m). The other gas chromatograph had a fused silica Supelco SPBtm-5 (60 m; 0.32 mm i.d.; d.f. = 0.25 μ m) WCOT capillary column.

The respective temperature programmes were:

5 min at 55 °C, 2.5 °C/min up to 193 °C, 50 min at 193 °C; carrier gas (H₂)-flow: 25 ml/min; t.inj = t.det = 250 °C; split injection.

5 min at 80 °C, 2 °C/min up to 260 °C, 50 min at 260 °C; carrier gas (H₂)-flow: 1.5 ml/min; t.inj = t.det = 280 °C; split injection.

Electron impact mass spectra were recorded at 70 eV with a 5979 Hewlett-Packard mass detector connected to a 5980 Hewlett-Packard gas chromatograph equipped with a HP-20M fused silica WCOT capillary column (25 m; 0.2 mm i.d.; d.f. = 0.2 μ m); He as carrier gas, splitless injection; t_{inj} = 220 °C, t_{source} = 200 °C, and programmed temperature: 1 min at 60 °C; 10 °C/min up to 80 °C, 0.5 min at 80 °C, 2.5 °C/min up to 190 °C, 30 min at 190 °C.

The ^1H and ^{13}C NMR spectra were recorded with a Bruker AC 300 instrument.

Results and discussion

1. Identification of p-menth-1-ene-7,8-diol and of *cis* and *trans* p-menth-1-ene-6,8-diols

An allylic hydroxylase activity on monoterpene compounds is known to be present in several plants (KARP and CROTEAU 1988). Thus, the synthesis of *trans* 2,6-dimethylocta-2,7-diene-1,6-diols from linalool by suspension cells of *Nicotiana tabacum* (SUGA *et al.* 1976) and — with some *cis* isomer, too — by cultured *Botrytis cinerea* (RAPP *et al.* 1986) can be explained. Both the same compounds were found as free and bound forms and in different ratios in grapes (RAPP *et al.* 1986; STRAUSS *et al.* 1988; VERSINI *et al.* 1988).

When starting from α -terpineol, some p-menthenediols were produced by *Botrytis cinerea* (MANDERY 1986; RAPP and MANDERY 1988) and p-menth-1-ene-7,8-diol, *cis* and *trans* p-menth-1-ene-6,8-diols by *Nicotiana tabacum* callus suspension cells (SUGA *et al.* 1980). These compounds have been searched for also in grapes or in grape products, assuming that a similar biotransformation of α -terpineol takes place. The three compounds identified on tobacco were found mostly as glycosides (Fig. 2); their retention

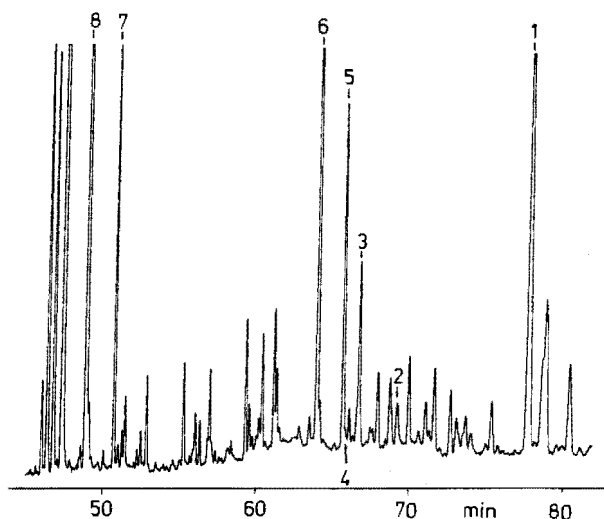
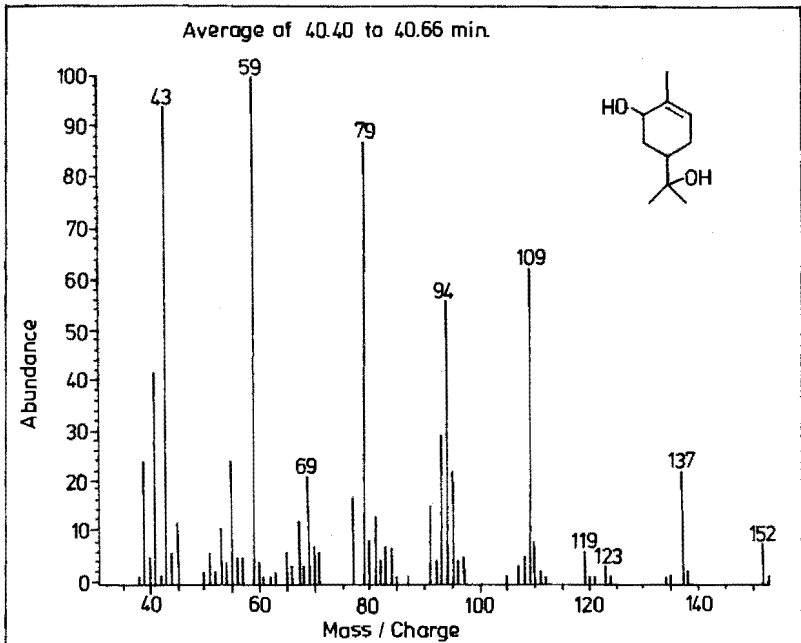
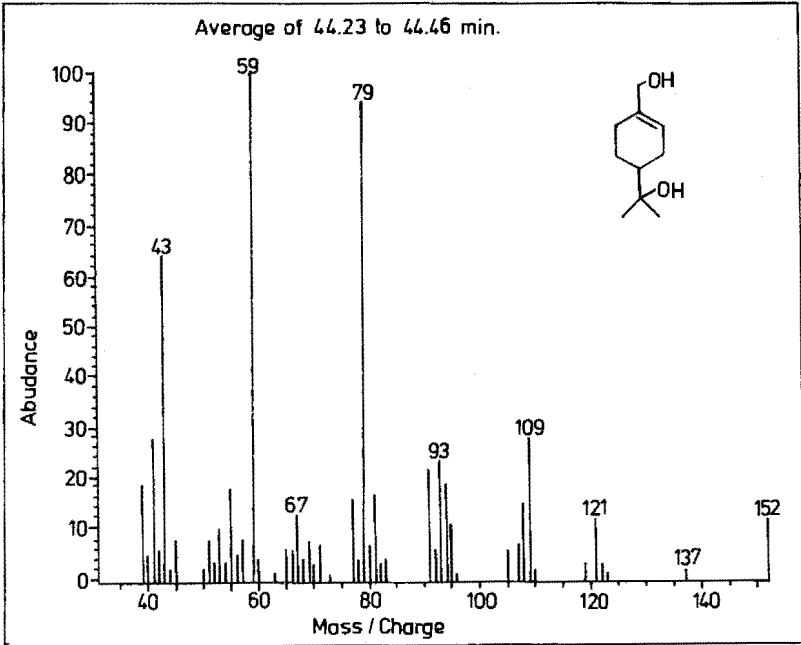


Fig. 1: Part of a FID-chromatogram (DB-Wax) of an extract of Silvaner must including p-menth-1-ene-diols aglycons. — 1 = p-menth-1-ene-7,8-diol, 2 = *cis* sobrerol, 3 = *trans* geranic acid, 4 = *trans* sobrerol, 5 = *cis* 8-hydroxy linalool + hydroxy geraniol, 6 = *trans* 8-hydroxy linalool, 7 = 3,7-dimethyl-1,5-diene-3,7-diol (= terpendiol I), 8 = 2-phenylethanol.

Ausschnitt aus dem FID-Chromatogramm (DB-Wax) eines Extraktes aus Silvanermost mit dem Bereich der p-Menth-1-en-diol-Aglykone.



times on capillary columns with different polarity (HP-20M, DB-WAX and SPBtm-5) and mass spectral data were either the same as with the reference compounds or similar for the most relevant fragments i.e. for *trans* p-menth-1-ene-6,8-diol.

Fig. 1 shows a short section of a chromatogram of an extract from Silvaner must. The identified compounds (p-menth-1-ene-7,8-diol, *cis* and *trans* p-menth-1-ene-6,8-diol (*cis* and *trans* *sobrerol*)) together with few other aroma compounds (i.e. 2-phenylethanol, *trans* geranic acid, terpendiol I) are marked. The mass spectra of p-menth-1-ene-diols identified in grape must are shown in Fig. 2 (p-menth-1-ene-7,8-diol) and Fig. 3 (*cis* p-menth-1-ene-6,8-diol). These spectra correspond with those of the reference substances. So it was possible to ascertain that the already mentioned p-menthenediol isomers 1 and 2 of STRAUSS *et al.* (1987) or 2 and 3 of WILLIAMS *et al.* (1989) correspond respectively to the *cis* p-menth-1-ene-6,8-diol and to the p-menth-1-ene-7,8-diol, whereas the third or isomer 1, reported by WILLIAMS *et al.* (1989), probably corresponds to *trans* p-menth-1-ene-6,8-diol. The p-menth-1-ene-7,8-diol was also reported as an unidentified compound of α -terpineol metabolite of *Botrytis cinerea* (MANDERY 1986; RAPP and MANDERY 1988).

2. Presence of identified compounds in musts of some varieties

In the table the concentrations of free and bound forms of the above components as well as those of α -terpineol and of 2-*exo*-hydroxy-1,8-cineole, another probable α -terpineol derivative recently identified (BITTEUR *et al.* 1990), are reported for musts from different varieties.

Contents (ppb) of free and bound α -terpineol and its derivatives in different varietal musts

Gehalte an freiem und gebundenem α -Terpineol und daraus resultierenden Derivaten (ppb) in Mosten verschiedener Rebsorten

Varieties	α -Terpineol		2- <i>exo</i> -OH-1,8-Cineole		<i>trans</i> Sobrerol		<i>cis</i> Sobrerol		p-Menth-1-ene-7,8-diol	
	f	b	f	b	f	b	f	b	f	b
Chardonnay	+	0.4	-	+	-	-	-	-	-	+
Teroldego*	0.4	+	+	+	-	-	-	+	+	0.4
Pinot blanc	+	0.9	+	0.5	-	+	-	1	+	9
Pinot noir*	+	3.3	+	1.3	+	0.4	+	1.5	1	17
Sauvignon blanc	+	13	1.1	3.5	-	+	+	2	0.6	30
Sylvaner	+	56	+	20	-	0.4	+	8	2.7	102
Rhine Riesling	3.0	51	1.7	10.5	+	0.5	0.4	12.6	2.8	89
Gewürztraminer	+	16.5	+	7	+	+	+	6.5	+	22
Yellow Muscat of Trentino	32.5	59	3.1	4.8	0.6	0.9	+	2.5	3.5	25
Rose Muscat*	5.5	47.5	1.1	10	0.4	0.8	0.7	20	8	200

f = Free form.

- : < 0.1 ppb.

b = Bound form.

+ : 0.1 - 0.3 ppb.

* = Red grape variety.

Generally, they are present as glycosides and in varieties with a content approximately higher than 1 ppb of bound α -terpineol. Among the other p-menthenediols, p-menth-1-ene-7,8-diol prevails in all cases, similar as with the free compounds metabolized by tobacco cells, but in grape must the ratio between *cis* and *trans* p-menth-1-ene-6,8-diol concentrations is inverted, i.e. the *cis* isomer is much more abundant than the *trans* one, which is only present in traces or not detectable.

A higher level of bound *cis* p-menth-1-ene-6,8-diol has recently been found as typical for grapes and its derivatives from the Traminer family (Traminer, Gewürztraminer and Savagnin) (VERSINI *et al.* 1990).

Summary

The present work aims at clearing the structure of some p-menthenediols, which are already known to be present in musts and wines as glycosides, referred to as isomers 1, 2 and 3. Isomers 2 and 3 correspond to *cis* p-menth-1-ene-6,8-diol and p-menth-1-ene-7,8-diol; isomer 1, which is only found in traces, is probably identical with *trans* p-menth-1-ene-6,8-diol. Their concentration is reported for both free and bound forms in musts from some varieties and compared with the α -terpineol and 2-exo-hydroxy-1,8-cineole concentrations.

In general, the free forms were scarcely present; among the bound forms p-menth-1-ene-7,8-diol first and then *cis* p-menth-1-ene-6,8-diol prevailed. Besides improvement of varietal classification, the compounds identified give evidence of allylic hydroxylase activity in grape berries.

Financial support of Progetto Strategico: „Innovazione produttiva nelle piccole e medie imprese“, sottoprogetto: „Qualità e tipicità degli alimenti: metodologie innovative d'indagine“ - C.N.R. (Rome) is acknowledged.

Literature

- BAUMES, R.; CORDONNIER, R.; NITZ, S.; DRAWERT, F.; 1986: Identification and determination of volatile constituents in wines from different wine cultivars. *J. Sci. Food Agric.* **37**, 927—943.
- BITTEUR, S. M.; BAUMES, R. L.; BAYONOVE, C. L.; VERSINI, G.; MARTIN, C. A.; DALLA SERRA, A.; 1990: 2-exo-hydroxyl-1,8-cineole: a new component from grape var. Sauvignon. *J. Agric. Food Chem.* **38**, 1210—1213.
- BLUTHE, N.; ECOTO, J.; FETIZON, M.; LAZARE, S.; 1980: Cyclobutane ring opening of pin-2(10)-ene with mercury (II) salts. A new, high-yield synthesis of p-mentha-1,8-diene-7-ol. *J. Chem. Soc. Perkin I*, 1747—1751.
- GUNATA, Y. Z.; BAYONOVE, C. L.; BAUMES, R. L.; CORDONNIER, R. E.; 1985: The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **331**, 83—90.
- KARP, F.; CROTEAU, R.; 1988: Role of hydroxylases in monoterpene biosynthesis. In: SCHREIER, P. (Ed.): *Bioflavour 87*, 173—198. de Gruyter, Berlin, New York.
- MANDERY, H.; 1986: Einfluß von *Botrytis cinerea* auf die Monoterpenfraktion des Weinaromas. Diplomarbeit, Univ. Karlsruhe.
- RAPP, A.; MANDERY, H.; 1988: Influence of *Botrytis cinerea* on the monoterpene fraction of wine aroma. In: SCHREIER, P. (Ed.): *Bioflavour 87*, 445—452. de Gruyter, Berlin, New York.
- — —; — — —; NIEBERGALL, H.; 1986: Neue Monoterpendiole in Traubenmost und Wein sowie in Kulturen von *Botrytis cinerea*. *Vitis* **25**, 79—84.
- STRAUSS, C. R.; GOOLEY, P. R.; WILSON, B.; WILLIAMS, P. J.; 1987: Application of droplet countercurrent chromatography to the analysis of conjugated forms of terpenoids, phenols, and other constituents of grape juice. *J. Agric. Food Chem.* **35**, 519—524.

- — ; — — ; WILSON, B.; WILLIAMS, P. J.; 1988: Novel monoterpene diols and diol glycosides in *Vitis vinifera* grapes. *J. Agricult. Food Chem.* **36**, 569—573.
- SUGA, T.; AOKI, T.; HIRATA, T.; LEE, Y. S.; NISHIMURA, O.; UTSUMI, M.; 1980: Biotransformation of foreign substrates with callus tissues. Transformation of terpineols with tobacco suspension cells. *Chem. Lett.* **15**, 229—230.
- — ; HIRATA, T.; HIRANO, Y.; ITO, T.; 1976: Biotransformation of monoterpenes by tobacco tissue cultures. Selective hydroxylation of *trans*-methyl group in isopropylidene group. *Chem. Lett.* **11**, 1245—1248.
- VERSINI, G.; DALLA SERRA, A.; DELL'ÉVA, M.; SCIENZA, A.; RAPP, A.; 1988: Evidence of some glycosidically bound new monoterpenes and norisoprenoids in grapes. In: SCHREIER, P. (Ed.): *Bioflavour 87*, 161—170. de Gruyter, Berlin, New York.
- — ; — — ; SCIENZA, A.; BARCHETTI, P.; 1990: Particolarità compositive dell'uva e del vino Traminer aromatico: confronto fra cultivar e variazioni a livello terpenico in fermentazione e nell'invecchiamento. *Proc. Symp. Gewürztraminer*, Bolzano, May 22nd (in press).
- WILLIAMS, P. J.; SEFTON, M. A.; WILSON, B.; 1989: Nonvolatile conjugates of secondary metabolites as precursors of varietal grape flavor components. In: TERANISHI, R.; BUTTERY, R. G.; SHAHIDI, F. (Eds.): *Flavor Chemistry: Trends and Developments*, 35—48. ACS Symp. Ser. No. 388. American Chemical Society, Washington, DC.
- WINTERHALTER, P.; SEFTON, M. A.; WILLIAMS, P. J.; 1990: Two-dimensional GC-DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoids, and shikimate-derived metabolites from Riesling wine. *J. Agricult. Food Chem.* **38**, 1041—1048.

Received, 21. 3. 1991

Dr. G. VERSINI
Istituto Agrario Provinciale
Laboratorio di Analisi e di Ricerca
I-38010 San Michele all'Adige (Trento)
Italia
Prof. Dr. A. RAPP

Bundesanstalt für Züchtungsforschung
im Wein- und Gartenbau
Institut für Rebenzüchtung Geilweilerhof
D-6741 Siebeldingen
BR Deutschland