

Figure 2. Mass fragmentogram of Tosa-buntan oil. Magnetic mass was  $m/z$  131.0 from PFK. Peak 1 was limonene ( $R_t = 3.33$  min), and peak 2 was 1,8-cineole ( $R_t = 3.35$  min).

#### ACKNOWLEDGMENT

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**Registry No.**  $\alpha$ -Pinene, 80-56-8;  $\beta$ -pinene, 127-91-3; myrcene, 123-35-3; limonene, 138-86-3; 1,8-cineole, 470-82-6; ocimene, 29714-87-2;  $\gamma$ -terpinene, 99-85-4; *p*-cymene, 99-87-6; terpinolene, 586-62-9; 2-carene, 554-61-0; isooctyl acetate, 31565-19-2; nonanal, 124-19-6; citronellal, 106-23-0; *n*-octyl acetate, 112-14-1; decanal, 112-31-2; linalool, 78-70-6; terpinen-4-ol, 562-74-3;  $\beta$ -caryophyllene, 87-44-5;  $\beta$ -terpineol, 138-87-4;  $\alpha$ -terpineol, 98-55-5;  $\beta$ -cubebene, 13744-15-5; geranial, 141-27-5; carvone, 99-49-0;  $\Delta$ -cadinene, 483-76-1; neryl acetate, 141-12-8; perillaldehyde, 2111-75-3; carveol, 99-48-9; nootkatone, 4674-50-4.

#### LITERATURE CITED

- Demole, E.; Enggist, P.; Ohloff, G. "1-*p*-Menthene-8-thiol: A Powerful Flavor Impact Constituent of Grapefruit Juice (*Citrus paradisi* Macfadyen)". *Helv. Chim. Acta* 1982, 65, 1785-1794.
- Hashimoto, H. "On the Special Diagnosis and the History of Tosa-buntan (*Citrus grandis* Osbeck forma *Tosa-buntan*)". *Bull. Kochi Fruit Tree Exp. Sta.* 1976, No. 1, 1-10.
- Hodgson, R. W. "Hortical Varieties of Citrus: Pummelos and Grapefruits". In *The Citrus Industry Vol. I*; Reuther, W., Webber, H. J., Batchelor, L. D., Eds.; University of California: Berkeley, CA, 1967.
- Kirchner, J. G.; Miller, J. M. "Preparation of Terpeneless Essential Oils". *Ind. Eng. Chem.* 1952, 44, 318-321.
- Kusunose, H.; Sawamura, M. "Aroma Constituents of Some Sour Citrus Oils". *Nippon Shokuhin Kogyo Gakkaishi* 1980, 27, 517-521.
- MacLeod, W. D., Jr.; Buigues, N. M. "Sesquiterpenes. I. Nootkatone, A New Grapefruit Flavor Constituent". *J. Food. Sci.* 1964, 29, 565-568.
- Middleditch, B. S.; Desiderio, D. M. "Comparison of Selective Monitoring and Repetitive Scanning during Gas Chromatography-Mass Spectrometry". *Anal. Chem.* 1973, 45, 806-808.
- Moshonas, M. G. "Analysis of Carbonyl Flavor Constituents from Grapefruit Oil". *J. Agric. Food Chem.* 1971, 19, 769-770.
- Nursten, H. E. "Why Flavour Research? How Far Have We Come Since 1975 and Where Now?". In *Progress in Flavour Research*; Land, D. G., Nursten, H. E., Eds.; Applied Science: London, 1979.
- Shaw, P. E. "Review of Quantitative Analyses of Citrus Essential Oils". *J. Agric. Food Chem.* 1979, 27, 246-257.
- Wilson, C. W. III; Shaw, P. E. "Glass Capillary Gas Chromatography for Quantitative Determination of Volatile Constituents in Cold-Pressed Grapefruit Oil". *J. Agric. Food Chem.* 1980, 28, 919-922.

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## Novel Monoterpene Diols and Diol Glycosides in *Vitis vinifera* Grapes

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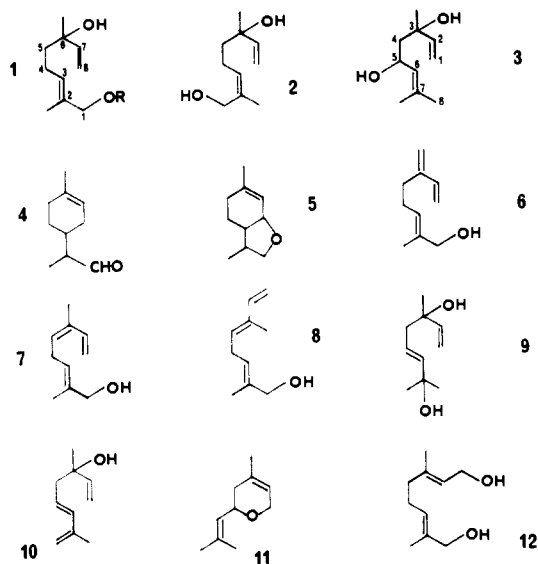
The  $\beta$ -D-glucopyranoside and the 6-O- $\alpha$ -arabinofuranosyl- $\beta$ -D-glucopyranoside of (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol were isolated from *Vitis vinifera* cv. Riesling grape juice and their structures determined by spectral methods. Additionally, the diastereoisomeric 3,7-dimethylocta-1,6-diene-3,5-diols and (*E,E*)-2,6-dimethylocta-2,6-diene-1,8-diol were identified for the first time in grapes, and these three diols together with (*E*)- and (*Z*)-2,6-dimethylocta-2,7-diene-1,6-diol were quantified both as free monoterpenes and as glycosides in the juice of eight grape varieties. Model experiments were carried out on (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol and the 3,7-dimethylocta-1,6-diene-3,5-diols to assess their roles as potential precursors of flavorants in juice and wine. The first compound, although largely resistant to acid hydrolysis, yielded (*E,E*)-2,6-dimethylocta-2,6-diene-1,8-diol as the major product and *p*-menth-1-en-9-al and 3,9-epoxy-*p*-menth-1-ene as trace products. The mixture of 3,7-dimethylocta-1,6-diene-3,5-diols decomposed to give (*E*)-2,6-dimethylocta-3,7-diene-2,6-diol, hotrienol, and nerol oxide, all of which are known wine components.

The significance of volatile monoterpenes to the flavor and varietal character of some cultivars of *Vitis vinifera* grapes is well documented (Strauss et al., 1986). In addition to those monoterpenes occurring free in the fruit,

further volatiles are formed in juices by acid hydrolysis of free monoterpene polyols and glycosides that are also present. Enzymic studies have indicated that the monoterpene polyols can themselves be glycosylated in the fruit (Wilson et al., 1984), although the nature of these glycosides and the particular oxygens of the polyols that are glycosylated have, until lately, been uncertain. These points were recently clarified with the report, from this laboratory, of the occurrence in grape juice of two glycosides 1d and 1e (Figure 1) of (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol (1a) (Strauss et al., 1987).

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a, R = H e, R = 6-O- $\alpha$ -arabinofuranosyl- $\beta$ -D-glucopyranosyl  
 b, R = 6-O- $\alpha$ -arabinofuranosyl- $\beta$ -D-glucopyranosylhexaacetate  
 d, R =  $\beta$ -D-glucopyranosyl c, R =  $\beta$ -D-glucopyranosyltetraacetate  
**Figure 1.** Compounds referred to in this work.

This paper gives details of the identification of glycosides **1d** and **1e** and additionally reports the positive identification of three diols new to *V. vinifera* grape juice, viz diastereoisomeric 3,7-dimethylocta-1,6-diene-3,5-diols (**3**) and (*E,E*)-2,6-dimethylocta-2,6-diene-1,8-diol (**12**). The roles of **1a**, **3**, and **12** as potential progenitors of volatile monoterpene flavorants in grape juice and wine are also investigated.

#### EXPERIMENTAL SECTION

**General Procedures.** All solvents were of high purity at purchase and were redistilled before use. Details of gas chromatography-mass spectrometry (GC-MS),  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy, preparation of isolates from grape juice, droplet countercurrent chromatography (DCCC) of grape concentrates, enzymic hydrolysis of DCCC fractions, and isolation of compounds from DCCC fractions were given previously (Strauss et al., 1987).

**Isolation and Quantification of Diols.** Analyses were made on juice samples of the *V. vinifera* varieties Muscat of Alexandria, Riesling (syn. White Riesling), Traminer (syn. Gewürztraminer), Chardonnay, Sauvignon Blanc, Semillon, and Shiraz (syn. Syrah), obtained from the 1987 vintage. Earlier analyses were made on samples of the first four of the above varieties from the 1985 vintage. White Frontignan (syn. Muscat à petits grains blanc) were from the 1986 vintage.

For the 1987 samples the following method was employed: Juice samples (200 mL) were continuously liquid-liquid extracted with Freon F11 (trichlorofluoromethane) after adjustment to pH 7 with KOH and addition of 1-octanol (0.077 mg) and *n*-octyl  $\beta$ -D-glucopyranoside (0.354 mg) (Sigma Chemical Co.) as internal standards. The extraction proceeded over 72 h with daily changes of solvent. Pooled solvent extracts were stored at  $-20^\circ\text{C}$  until ready for use, concentrated as previously described (Wilson et al., 1984), and analyzed for free diols by GC-MS.

The solvent-stripped juice was next passed through a  $\text{C}_{18}$  reversed-phase column and the adsorbed material eluted with MeOH. After solvent removal the residue was

**Table I.** Concentrations of Free and Glycosidically Bound Monoterpene Diols in Grape Juices

variety <sup>b</sup>	fraction <sup>c</sup>	diol, <sup>a</sup> ppb				
		1a	2	3 <sup>d</sup>	3 <sup>e</sup>	12
Muscat of Alexandria	F	nd <sup>f,g</sup>	nd <sup>h</sup>	nd	32	nd
	B	46	92	86	126	29
White Frontignan	F	i	20	4	2	2
	B	160	330	9	4	9
Riesling	F	6	1	nd	nd	nd
	B	186	37	nd	nd	nd <sup>j</sup>
Traminer	F	nd	nd	nd	nd	nd <sup>h</sup>
	B	12	7	nd	nd	nd <sup>i</sup>
Sauvignon Blanc	F	7	2	nd	nd	nd
	B	54	22	nd	nd	nd
Semillon	F	nd	nd	nd	nd	nd
	B	18	nd	nd	nd	nd

<sup>a</sup> For structures see Figure 1. <sup>b</sup> Additionally, two samples of Chardonnay (1987, 1985) and one of Shiraz were analyzed; however, none of the diols **1a**, **2**, **3**, or **12** were detected either as a free compound or as a glycoside in the juices of these cultivars. <sup>c</sup> Key: F, free in the juice; B, glycosidically bound. <sup>d</sup> First eluting isomer. <sup>e</sup> Second eluting isomer. <sup>f</sup> nd, not detected by selected ion monitoring in the 1987 sample. <sup>g</sup> Found at 9 ppb in a 1985 sample. <sup>h</sup> Found at 12 ppb in a 1985 sample. <sup>i</sup> Peak obscured. <sup>j</sup> Detected but not quantified in a 1985 sample. <sup>k</sup> Found at 5 ppb in a 1985 sample. <sup>l</sup> Found at 4 ppb in a 1985 sample.

taken up in buffer (pH 5.0) and treated with a crude glycosidase enzyme (Rohpect C, Rohm), and the enzyme-liberated aglycons were extracted with Freon F11, stored, concentrated, and analyzed for bound diols under the same conditions as above.

The concentration of each diol was estimated by comparing its GC-MS peak height with that of the added free 1-octanol internal standard for free diols and with 1-octanol released from *n*-octyl  $\beta$ -D-glucopyranoside internal standard for bound diols.

For the 1985 and 1986 samples a similar workup was employed except that only 1-octanol was added as an internal standard for estimation of free diols and a second portion of 1-octanol was added, after enzyme treatment and before Freon F11 extraction, for estimation of bound forms.

The concentrations of free and glycosidically bound forms of diols **1a**, **2**, **3**, and **12** in the juice samples of the eight varieties screened in this survey are given in Table I.

**Reference Compounds.** (*E*)-2,6-Dimethylocta-2,7-diene-1,6-diol (**1a**) was prepared by oxidation of linalool with  $\text{SeO}_2$  according to the method of Hase et al. (1982). The crude reaction mixture contained a low concentration of the *Z* isomer **2**. The product was purified by flash chromatography on Si gel 60 (230–400 mesh) with pentane-Et<sub>2</sub>O (1:1) as solvent. Spectral data obtained for both diols were as follows.

EIMS of **1a** (70 eV) *m/z* (relative intensity): 152 (0.2), 137 (2), 134 (0.5), 119 (3), 110 (3), 109 (2), 96 (4), 93 (5), 79 (7), 71 (34), 68 (14), 67 (28), 55 (26), 53 (10), 43 (100), 41 (43).

EIMS of **2** (70 eV) *m/z* (relative intensity): 137 (2), 134 (0.5), 119 (4), 110 (2), 109 (2), 96 (4), 93 (4), 82 (8), 81 (7), 79 (7), 71 (37), 68 (13), 67 (31), 55 (27), 43 (100), 41 (43).

For  $^{13}\text{C}$  NMR of diols **1a** and **2**, see Table II.

A mixture of the diastereoisomeric 3,7-dimethylocta-1,6-diene-3,5-diols (**3**) (Kitahara et al., 1980) was donated to us. Both isomers were well resolved on the GC column, and their EIMS [*m/z* (relative intensity)] at 70 eV were identical: 137 (1), 109 (2), 96 (3), 85 (33), 83 (20), 82 (13), 71 (19), 68 (57), 67 (37), 55 (33), 43 (100), 41 (77).

(*E,E*)-2,6-Dimethylocta-2,6-diene-1,8-diol (**12**) was prepared by the method of Inouye et al. (1978), involving  $\text{SeO}_2$

Table II.  $^{13}\text{C}$  NMR Spectral Data

C	chemical shift of each compd <sup>a</sup>			
	1a <sup>b,c</sup>	1b	1c <sup>c</sup>	2 <sup>c</sup>
1	68.2	72.5*	72.8**	62.9
2	134.7	130.8	130.9	134.2
CH <sub>3</sub> -C2	13.5	13.6	13.5	20.0
3	125.6	129.7	129.7	128.1
4	22.2	22.5	22.5	22.2
5	41.6	41.7	41.7	43.0
6	73.2	74.9*	75.2 (obscured)**	72.6
CH <sub>3</sub> -C6	27.4	27.9	28.0	27.4
7	144.7	144.9	144.9	144.7
8	111.7	112.0	112.0	112.0
G1		98.5	98.8	
G2		71.4	71.4***	
G3		73.2	73.0	
G4		69.2	68.6	
G5		73.2	71.8***	
G6		66.7	62.1	
A1		106.0		
A2		80.2		
A3		76.2		
A4		83.9		
A5		63.2		
CH <sub>3</sub> CO		20.6, 21.1	20.6	
CH <sub>3</sub> CO		169.2, 169.4, 169.7, 170.0, 170.2, 170.6	169.3–170.3	

<sup>a</sup>For structures see Figure 1. \*, \*\*, and \*\*\* indicate assignments may be reversed. <sup>b</sup>Compare with data of Behr et al. (1978). <sup>c</sup>Compare with data of Tschesche et al. (1977).

oxidation of geranyl acetate, followed by reduction. The deacetylation was completed with 0.1 M NaOH in MeOH. Purification of 12 was achieved by flash chromatography on Si gel 60 (230–400 mesh) using CHCl<sub>3</sub>-MeOH (95:5) as eluent. The product, *R<sub>f</sub>* 0.36 (CHCl<sub>3</sub>-MeOH, 9:1), had the following spectral properties: <sup>1</sup>H NMR (80 MHz, CDCl<sub>3</sub>)  $\delta$  1.61 (6 H, br s, 2 CH<sub>3</sub>C=C), 2.05 (4 H, br, CH<sub>2</sub>CH<sub>2</sub>), 2.44 (2 H, br s, 2 OH), 3.93 (2 H, s, C=C(Me)CH<sub>2</sub>OH), 4.08 (2 H, d, *J* = 6.6 Hz, CHCH<sub>2</sub>OH), 5.34 (2 H, br t, 2 HC=C); EIMS (70 eV) [*m/z* (relative intensity)] 137 (1), 121 (2), 109 (2), 95 (2), 94 (3), 93 (3), 84 (13), 83 (4), 81 (6), 79 (4), 69 (7), 68 (40), 67 (21), 57 (12), 55 (17), 53 (11), 43 (100), 41 (72); cf. data of Inouye et al. (1978) and Williams et al. (1985).

**Purification of Glycosides of (*E*)-2,6-Dimethylocta-2,7-diene-1,6-diol (1a).** The disaccharide derivative from pooled DCCC fractions 58–74 obtained from an isolate of 11 L of Riesling juice (Strauss et al., 1987) was purified by acetylation, followed after workup by flash chromatography using CHCl<sub>3</sub>-pentane (1:1) as solvent. The isolated acetate derivative 1b (11.0 mg), *R<sub>f</sub>* 0.27 (Et<sub>2</sub>O solvent), had the following spectral properties: EIMS (probe at 70 eV) [*m/z* (relative intensity)] 547 (0.2), 331 (3), 317 (0.2), 289 (0.6), 273 (2), 260 (8), 259 (45), 169 (17), 152 (29), 139 (57), 135 (20), 134 (16), 129 (13), 127 (16), 110 (24), 109 (37), 107 (19), 97 (37), 96 (29), 95 (15), 93 (29), 85 (22), 82 (28), 81 (35), 71 (34), 69 (29), 68 (29), 67 (32), 55 (34), 43 (100), 41 (28); <sup>1</sup>H NMR (90 MHz, CDCl<sub>3</sub>)  $\delta$  1.27 (3 H, s, CH<sub>3</sub>-monoterpenyl C6, i.e. M6), 1.56 (3 H, br s, CH<sub>3</sub>-M2), 1.6–2.3 including singlets at 1.97, 2.00, 2.05, 2.08 (23 H, m, H-M4, M5, OH, 6 CH<sub>3</sub>CO), 3.5–3.7 (3 H, m, H-glucosyl C5, C6, i.e. G5, G6), 3.7–4.3 (5 H, m, H-arabinosyl C4, C5 (i.e. A4, A5), M1), 4.45 (1 H, d, *J* = 7.8 Hz, H-G1), 4.64 (1 H, d, *J* = 8.1, H-A1), 4.7–5.6 (8 H, m, H-A3, A4, G2, G3, G4, M3, M8), 5.9 (1 H, dd, H-M7); <sup>13</sup>C NMR, see Table II.

The glucoside of (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol (1a) was also obtained from the above Riesling isolate, in DCCC fractions 104–140, which were pooled, concentrated,

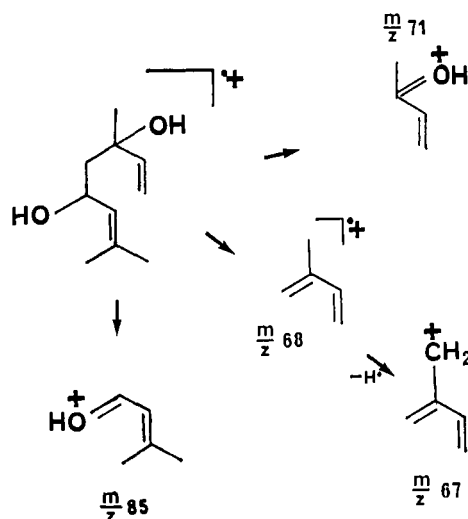


Figure 2. Postulated mass spectral fragmentation of diols 3.

and acetylated. Flash chromatography of the crude acetates afforded 15.2 mg of pure product 1c, *R<sub>f</sub>* 0.38 (Et<sub>2</sub>O solvent). The <sup>13</sup>C NMR (see Table II) and the <sup>1</sup>H NMR spectra for the isolated compound 1c were in agreement with published data of Tschesche et al. (1977) (see Results and Discussion). The EIMS (probe at 70 eV) was as follows [*m/z* (relative intensity)]: 331 (3), 169 (34), 152 (18), 139 (15), 135 (10), 134 (8), 127 (18), 115 (17), 110 (24), 109 (43), 107 (16), 103 (11), 97 (24), 96 (27), 95 (15), 93 (30), 82 (31), 81 (35), 71 (38), 69 (34), 68 (30), 67 (41), 55 (44), 43 (100), 41 (38); cf. the EIMS reported by Tschesche et al. (1977), which did not include ions lower than *m/z* 71.

**Acid Hydrolysis of Monoterpene Diols 1a and 3.** Each diol (2.3 mg) was taken up in 13 mL of tartrate buffer solution, pH 3.0, and heated on a boiling water bath for 15 min. The reaction mixture was cooled in ice and extracted with redistilled Et<sub>2</sub>O (1 × 15 mL, 1 × 10 mL, 1 × 5 mL), and the pooled organic phase was dried over MgSO<sub>4</sub>, filtered, and concentrated by distillation through a small column packed with Fenske's helices.

In addition, a preparative-scale acid hydrolysis of (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol (1a) was carried out. The major transformation product of this reaction was diol 12, the structure of which was confirmed by comparison with authentic synthetic material. Also isolated from the reaction were diastereoisomeric *p*-menth-1-en-9-als (4) and 3,9-epoxy-*p*-menth-1-ene (5). The aldehydes gave EIMS and <sup>1</sup>H NMR spectra in close agreement with those in the literature (Ohloff et al., 1969), and the ether 5 gave an EIMS consistent with those published by Ohloff et al. (1966) and Schreier et al. (1981).

Three compounds tentatively identified as trienols 6–8 were also isolated as a mixture from the preparative-scale hydrolysis of diol 1a. These minor transformation products had the following EIMS [*m/z* (relative intensity)]. 6: 134 (2), 119 (3), 93 (22), 91 (8), 79 (13), 67 (8), 55 (10), 53 (12), 43 (100), 41 (75). 7: 121 (3), 119 (5), 105 (6), 94 (25), 93 (37), 92 (21), 91 (28), 79 (57), 77 (26), 67 (19), 65 (10), 55 (30), 53 (28), 51 (11), 43 (62), 41 (100). 8: 152 (*M*<sup>+</sup> <1), 134 (2), 121 (8), 119 (8), 93 (32), 91 (26), 80 (25), 79 (57), 77 (26), 67 (20), 55 (33), 53 (13), 43 (67), 41 (100).

## RESULTS AND DISCUSSION

**Structural Assignment of Diols 3 and 12 and of Glycosides 1d and 1e.** GC-MS analysis of extracts from Muscat juices showed a pair of compounds with identical EIMS, characterized by prominent ions *m/z* 85, 68, 67, and 71. The structures of diols 3 were deduced from these spectra according to the postulated fragmentation shown

in Figure 2. Proof of identity was obtained by cochromatography and spectral comparison with a sample of authentic diols 3.

Diol 12 was recognized in this work, both as a hydrolytic product from diol 1a (see below) and as a natural product in grapes (see Table I). In several samples of those juices in which diol 12 was found there also occurred three other compounds giving EIMS similar to that of 12. These other three compounds are believed to be the *E,Z*, *Z,E*, and *Z,Z* isomers of 2,6-dimethylocta-2,6-diene-1,8-diol, but these have not yet been proven by comparison with authentic materials.

With regard to the glycosides, the less polar of these was identified as (*E*)-6-hydroxy-2,6-dimethylocta-2,7-dien-1-yl  $\beta$ -D-glucopyranoside (1d) after spectral interpretation, including comparison with literature data for the acetate derivative 1c (Tschesche et al., 1977).

Although the  $^{13}\text{C}$  NMR spectrum of 1c agreed closely with that published for this compound, two of the signals, viz. for C1 and glucose C4, have been interchanged in our assignment (see Table II). That for C1 of the monoterpene moiety is ascribed here to the peak at  $\delta$  72.8. Such an assignment would be consistent with a downfield shift as a result of glycoside formation, compared with the corresponding signal in the parent alcohol (Williams et al., 1982). The value  $\delta$  68.6, assigned here for the C4 of the acetylated glucosyl unit, is also in accord with published data for monoterpene tetra-*O*-acetyl- $\beta$ -D-glucopyranoside (Williams et al., 1982). The  $^{13}\text{C}$  NMR spectral data thus indicated that the primary, and not the tertiary, hydroxyl function of diol 1a was the site of glycosylation, a conclusion supported by the observation of only four acetyl units in the  $^1\text{H}$  NMR spectrum.

The more polar component isolated from the juice was found to be (*E*)-6-hydroxy-2,6-dimethylocta-2,7-dien-1-yl 6-*O*- $\alpha$ -arabinofuranosyl- $\beta$ -D-glucopyranoside (1e). Its acetate derivative 1b by EIMS showed the significant ions  $m/z$  547 (arabinoglucosyl hexaacetate), 259 (arabinosyl triacetate), 139 (arabinosyl triacetate - 2  $\text{CH}_3\text{COOH}$ ), and 97 (139 -  $\text{CH}_2\text{CO}$ ), which indicated that the glycoside was a disaccharide of glucose and arabinose, with the pentose as the terminal residue (Williams et al., 1982). A prominent ion  $m/z$  71 ( $\text{C}_7\text{H}_7\text{O}^+$ ), attributable to fragmentation of the monoterpene 5,6 bond (also seen in the EIMS of 1c), suggested that diol 1a was glycosidically linked via the monoterpene C1.

The carbohydrate signals in the  $^{13}\text{C}$  NMR spectrum of the acetate derivative of the more polar glycoside 1b were in close agreement with those previously reported for other acetylated monoterpene 6-*O*- $\alpha$ -L-arabinofuranosyl- $\beta$ -D-glucopyranosides (Williams et al., 1982). The  $^1\text{H}$  NMR spectrum was also consistent with the assignment. Both sets of NMR data showed six acetate groups in the molecule. In the  $^{13}\text{C}$  NMR spectrum, the signal at  $\delta$  72.5 was assigned to the C1 of the monoterpene unit, analogous to the signal at  $\delta$  72.8 in the spectrum for 1c.

Enzymic hydrolysis of the more polar glycoside, followed by GC-MS analysis and coinjection with authentic diol 1a, confirmed the identity of the liberated aglycon.

**Occurrence of Diols 1a, 2, 3, and 12 in Grapes.** Diols 3 and 12 are newly reported here as grape constituents whereas 1a and 2 have been previously identified in Morio Muscat juice and wine (Rapp et al., 1986).

Table I gives the grape varieties surveyed and the incidence of occurrence of the four hydroxylated monoterpenes, both as free compounds in the juices and after enzymic hydrolysis of  $\text{C}_{18}$  reversed-phase isolates from the juices (Strauss et al., 1987). It can be seen from the data

that, in most cases where the diols 1a, 2, 3, and 12 were found, the glycosidically bound form was more abundant than the free compound.

Of the four diols, isomers 1a and 2 were observed most commonly in the varieties surveyed. Furthermore, the finding of 1a and 2 in Sauvignon Blanc and 1a in Semillon is noteworthy because juices of these varieties contain very low concentrations of total monoterpenes. However 1a and 2 were not observed in the extracts of Chardonnay and Shiraz juices, indicating that these monoterpenes are not general *V. vinifera* grape products.

In contrast to the above, diols 3 and 12 were confined to the highly flavored varieties, particularly the Muscats in which concentrations of all monoterpenes are high (Strauss et al., 1986).

To confirm that the absence of the diols in some juices was not due to limited solubility in Freon, recovery experiments from model juice medium were made on the most polar of the compounds surveyed here, i.e. diol 12. From these experiments the efficiency of the Freon extraction was demonstrated by the absence of diol 12 in 20-h  $\text{CH}_2\text{Cl}_2$  extracts made subsequent to the 72-h Freon extraction procedure.

**Role of Diols 1a, 3, and 12 as Potential Flavor Precursors.** In previous work it was demonstrated that certain monoterpene diols and a triol of grapes can play an important role as precursors of flavorants in juices (Williams et al., 1980a). Under the acidic conditions of the juice, rearrangements of the polyols occur to give a number of volatile components found in wine. Accordingly, experiments were undertaken in which diols 1a and 3 were heated at pH 3 (an acidity appropriate to grape juice) in aqueous solution.

After 15 min on a boiling water bath, diols 3 had almost totally decomposed to yield the allylic rearrangement product diol 9 and its dehydrated derivative, hotrienol 10, as major components along with lesser quantities of nerol oxide 11. Compounds 10 and 11 are readily formed from diol 9, itself a major natural product in juices of many grape varieties (Rapp and Knipser, 1979; Williams et al., 1980b). Both hotrienol and nerol oxide are recognized as potent odorants in wine (Simpson, 1979), highlighting the potential value of diols 3 as flavor precursors in juice. This experiment also demonstrated that diols 3 were more labile to acid than diol 9 and indicated that they could be precursors of 9 in grapes.

In contrast to the reactivity of 3, the 1,6-diol system in 1a was relatively stable to acid treatment at pH 3. Although 1a remained largely intact, it did yield traces of volatiles 4, 5, and the tentatively identified alcohols 6-8. A preparative-scale hydrolysis of 1a had an aroma of dill, attributable to ether 5 (Schreier et al., 1981), while aldehydes 4 and the isolated mixture of 6-8 had little odor. The major transformation product of 1a at pH 3 was the odorless diol 12, formed by allylic rearrangement. The diol 12 is obviously stable to acid treatment, implying that, like 1a, it too would have only a limited role as a flavor precursor in the fruit.

## CONCLUSION

In an earlier listing of grape monoterpene polyols (Strauss et al., 1986) only products with additional oxygens at positions 6 and/or 7 (numbering as for diols 3) of the acyclic monoterpene skeleton were recorded. The identification of diols 1a, 2, 3, and 12 now extends this list and demonstrates that products with hydroxyls at position 5, as well as at the chain terminus, are formed, presumably by allylic oxidation of parent monoterpenes such as linalool and geraniol.

Also, the finding of glycosides **1d** and **1e** suggests that glycosylation occurred after the second hydroxyl was introduced into the linalool skeleton. This is consistent with the view that polyol formation precedes glycosylation and that the latter process is a late step in monoterpene biosynthesis in the grape.

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**Registry No.** **1a**, 51724-50-6; **1b**, 113378-79-3; **1c**, 113531-02-5; **1d**, 113349-31-8; **1e**, 113471-54-8; **2**, 103619-06-3; **3**, 75654-19-2; **4**, 29548-14-9; **5**, 70786-44-6; **6**, 17015-29-1; **7**, 113378-77-1; **8**, 113378-78-2; **9**, 51276-34-7; **10**, 20053-88-7; **11**, 1786-08-9; **12**, 26488-97-1; linalool, 78-70-6; geranyl acetate, 105-87-3.

## LITERATURE CITED

- Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R. "Tobacco Chemistry. 45. (2E,6S)-2,6-Dimethyl-2,7-octadiene-1,6-diol, a New Monoterpenoid from Greek Tobacco". *Acta Chem. Scand., Ser. B* 1978, *B32*, 228-234.
- Hase, T.; Iwagawa, T.; Munesada, K. "A Bitter Monoterpene Glucoside from *Viburnum phlebotrimum*". *Phytochemistry* 1982, *21*, 1435-1437.
- Inouye, H.; Ueda, S.; Uesato, S.; Kobayashi, K. "Studies on Monoterpene Glucosides and Related Natural Products. XXXVII. Biosynthesis of the Iridoid Glucosides in *Lamium amplexicaule*, *Deutzia crenata* and *Galium spurium* var *echinospermon*". *Chem. Pharm. Bull.* 1978, *26*, 3384-3394.
- Kitahara, T.; Takagi, Y.; Matsui, M. "Structure and the Synthesis of Novel Constituents of Yuzuru Peel Oil and Their Conversion to Related Monoterpenes". *Agric. Biol. Chem.* 1980, *44*, 897-901.
- Ohloff, G.; Schulte-Elte, K. H.; Willhalm, B. "251. Zum Bildungsmechanismus bicyclischer Äther aus 1,6- und 1,7-Allyldiolen durch protonenkatalytische Dehydratation in der Allylstellung. Darstellung von 2,9- und 3,9-Epoxy-p-menthenen aus (+)- $\Delta^1$ -p-Menthen-9-ol und Stereochemie der 8 Tetrahydromenthofurane". *Helv. Chim. Acta* 1966, *49*, 2135-2150.
- Ohloff, G.; Giersch, W.; Schulte-Elte, K. H.; Kovats, E. S. " $\Delta^1$ -p-Menthen-9-ol, ein Bestandteil des bulgarischen Rosenöls". *Helv. Chim. Acta* 1969, *52*, 1531-1536.
- Rapp, A.; Knipser, W. "3,7-Dimethyl-okta-1,5-dien-3,7-diol-eine neue terpenoid Verbindung des Trauben- und Weinaromas". *Vitis* 1979, *18*, 229-233.
- Rapp, A.; Mandery, H.; Niebergall, H. "Neue Monoterpene Diole in Traubenmost und Wein sowie in Kulturen von *Botrytis cinerea*". *Vitis* 1986, *25*, 79-84.
- Schreier, P.; Drawert, F.; Heindze, I. "The Quantitative Composition of Natural and Technologically Changed Aromas of Plants VIII. Volatile Constituents of Fresh Dill Herb, *Anethum graveolens*, L. (Umbelliferae)". *Lebensm.-Wiss. Technol.* 1981, *14*, 150-152.
- Simpson, R. F. "Some Important Aroma Components of White Wine". *Food Technol. Aust.* 1979, *31*, 516-522.
- Strauss, C. R.; Wilson, B.; Gooley, P. R.; Williams, P. J. *Role of Monoterpenes in Grape and Wine Flavor*; Parliment, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC, 1986; pp 222-242.
- Strauss, C. R.; Gooley, P. R.; Wilson, B.; Williams, P. J. "Application of Droplet Countercurrent Chromatography to the Analysis of Conjugated Forms of Terpenoids, Phenols, and Other Constituents of Grape Juice". *J. Agric. Food Chem.* 1987, *35*, 519-524.
- Tschesche, R.; Ciper, F.; Breitmaier, E. "Monoterpen-Glucoside aus den Blättern von *Betula alba* und den Früchten von *Chaenomeles japonica*". *Chem. Ber.* 1977, *110*, 3111-3117.
- Williams, P. J.; Strauss, C. R.; Wilson, B. "Hydroxylated Linalool Derivatives as Precursors of Volatile Monoterpenes of Muscat Grapes". *J. Agric. Food Chem.* 1980a, *28*, 766-771.
- Williams, P. J.; Strauss, C. R.; Wilson, B. "New Linalool Derivatives in Muscat of Alexandria Grapes and Wines". *Phytochemistry* 1980b, *19*, 1137-1139.
- Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. "Novel Monoterpene Disaccharide Glycosides of *Vitis vinifera* Grapes and Wines". *Phytochemistry* 1982, *21*, 2013-2020.
- Williams, J. R.; Lin, C.; Chodos, D. F. "Synthesis of 1,5-Dienes via [2+2] Photocycloaddition Between 2,5-Dihydrothiophene 1,1-Dioxides (Sulfolenes) and  $\alpha,\beta$ -Unsaturated Cyclic Ketones and Anhydrides. Synthesis of 10-Hydroxygeraniol". *J. Org. Chem.* 1985, *50*, 5815-5822.
- Wilson, B.; Strauss, C. R.; Williams, P. J. "Changes in Free and Glycosidically Bound Monoterpenes in Developing Muscat Grapes" *J. Agric. Food Chem.* 1984, *32*, 919-924.

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