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Changes in the Odor-Active Compounds in *Vitis labruscana* Cv. Concord during Growth and Development

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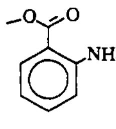
Three compounds important to the characteristic flavor (American character) of *Vitis labruscana* Bailey cv. Concord grapes (*o*-aminoacetophenone, methylfuraneol, and methyl anthranilate) were quantified in both berries and leaves before and after veraison (the onset of color formation in fruit). β-Damascenone, a compound important to the flavor of most grapes, and its hydrolyzable precursors (including glycosides) were also measured. The compounds with American character were detected only after veraison and only in the berries, where they increased rapidly after veraison. However, β-damascenone and its hydrolyzable precursors were detected in the leaf and berry tissue during the entire season. Furthermore, both free and bound β-damascenone increased in the berries after veraison, while only the bound forms increased in the leaves.

INTRODUCTION

Veraison in grapes is that period when growth of the berries increases and final ripening begins. It includes the loss of chlorophyll and in certain varieties the development of color (Winkler, 1974). In grapes, as in most fruit, certain volatile odor-active compounds begin to increase at the onset of ripening. For example, at veraison Concord grapes have shown sharp increases in the level of methyl anthranilate (methyl *o*-aminobenzoate, compound 1) in the fruit (Robinson et al., 1949; Fuleki, 1972). *o*-Aminoacetophenone (compound **2**) (Acree et al., 1990), furaneol [2,5-dimethyl-4-hydroxy-2,3-dihydro-3(2*H*)-furanone, compound **3**] (Rapp et al., 1980; Acree, 1981), and the methoxy derivative of furaneol [2,5-dimethyl-4-methoxy-2,3-dihydro-3(2*H*)-furanone, compound **4**] (Rapp et al., 1980; Schreier and Paroschy, 1981) are three other odor-active compounds that are implicated in the odor character of grapes of the *Vitis labruscana* species. The norisoprenoid β-damascenone (*trans*-2,6,6-trimethyl-cyclohexa-1,3-dienyl-1-but-2-en-1-one, compound **5**) contributes to the odor of both the *V. labruscana* (Braell et al., 1986) and the non-American smelling *Vitis vinifera* species (Strauss et al., 1987b).



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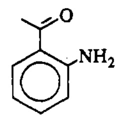

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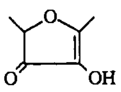

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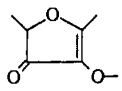

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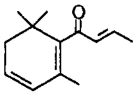

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The flavor in grapes of the *V. vinifera* species has been shown to be affected by the presence of monoterpene and norisoprenoid compounds which occur in both free and nonvolatile conjugated forms (Williams et al., 1982; Strauss et al., 1987a; Sefton et al., 1989). Wilson defined five different phases in the development of free and bound monoterpenes in Muscat of Alexandria grapes in which their concentrations varied throughout the season, culminating in maxima for some of the terpenoids after veraison (Wilson et al., 1984). The monoterpene content of Muscat grapes during development has been shown to rise from the time that the grapes were green (Gunata et al., 1985), and fluctuations of bound monoterpene levels during the growth of Muscat of Alexandria have been related to changes in temperature (Park et al., 1991). Levels of norisoprenoids in Riesling grapes have been correlated with the Brix values during growth and development (Strauss et al., 1987b). Bound monoterpenes in Muscat of Alexandria have also been shown to increase in concentration in the leaf blades as the season progressed, while free compounds decreased (Gunata et al., 1986).

If desirable flavor in *V. vinifera* is due to the development of terpenoid compounds, then the onset of veraison may not play as important a role in *V. vinifera* as it appears to in the development of labrusca character in American grapes. The present study compared the development of three compounds in Concord grapes and leaves considered to be responsible for much of the cultivar's American character with compound **5** and its glycosidic precursor, an odor-active compound common to all grape species. The three American character compounds were compounds **1**, **2**, and **4**.

MATERIALS AND METHODS

Plant Material. Berries and leaves of *V. labruscana* L. cv. Concord were harvested from a local vineyard in Dresden, NY, at 2-week intervals beginning July 15, 1992, until the final harvest on October 7, 1992.

Sample Preparation. In a scheme similar to that of Williams et al. (1981) for the processing of grape material, 2 kg of berries and 500 g of leaves were collected at each harvest date and 0.25 and 4.5 volumes of water were added to the berries and leaves, respectively, prior to blending. During blending, saturated $CaCl_2$ was added to 10% of the final volume as this has been shown to inhibit enzymatic oxidation in tomatoes (Buttery et al., 1987) and in apples (Shure, 1992). The resulting homogenate was digested in a sonic bath for 30 min and then centrifuged at 4 °C for 15 min at 10 000 rpm. The supernatant was decanted, measured for degrees Brix and absorbance at 525 nm (berry samples only) to monitor the physiological development of the grapevine over the season, and saved for analysis at −40 °C. All samples were prepared in duplicate with the exception of the free volatile extract of the berries harvested on the first date.

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Isolation of Free Volatiles. Free volatile extraction utilized a method developed by Braell (1986) for the analysis of Concord grape juice and also used by Marin et al. (1992) to analyze orange juice samples. Samples underwent liquid–liquid extraction with two-thirds volume of Freon 113 for 30 min. At the last harvest date a portion of the berry sample was set aside for extraction with ethyl acetate for analysis of compound **3** as this compound is not soluble in Freon 113. All solvent extracts were concentrated in a Rotovap at 28 °C (15 in. of Hg for Freon, 25 in. of Hg for ethyl acetate).

Isolation of β-Damascenone Precursor. Isolation of β-damascenone precursors utilized a method similar to one previously described for the isolation of monoterpene and norisoprenoid precursors from grape juice and wines (Williams et al., 1982). After Freon 113 extraction, the aqueous samples were placed on a glass column (40 cm × 3.6 cm i.d.) containing 50 g of C_{18} reversed-phase absorbant and washed with several column volumes of water; the precursors eluted in the first 2 of 5 column volumes of methanol. The methanol/water extract was then evaporated to dryness in a Rotovap at 28 °C, 60 mmHg, and resuspended in approximately 4 mL of water. The resuspended sample was hydrolyzed to transform the precursor compounds to free β-damascenone in a method developed by Cox (1991) and utilized by Zhou et al. (1993) with apples. Acid hydrolysis took place in 1-mL batches by adding 0.1 M citric acid (pH 2) at a 3:1 ratio and heating the mixture at 90 °C for 20 min. After rapid cooling in an ice bath, samples were extracted with 2 mL of Freon 113 and combined.

Quantitative Analysis. Gas chromatography mass fragmentometry (SIM, selected ion monitoring) utilized a Hewlett-Packard 5970 mass selective detector equipped with a 0.22 mm × 25 m fused silica capillary column coated with 0.33 μm of cross-linked methyl silicon was utilized for all GCMS analysis. The column was held at 35 °C for 3 min and then programmed at 4 °C/min to 160 °C followed by a 30 °C/min increase to 225 °C which was held for 5 min. The injector temperature was 200° C, the ion source was held at 220° C. Helium was used as a carrier gas with a flow rate of 2 mL/min. The mass fragments (compound **1**, m/z 39, 65, 92, 119, 151; compound **2**, m/z 65, 92, 106, 120, 135; compound **3**, m/z 43, 57, 85, 128; compound **4**, m/z 55, 71, 85, 127, 142; compound **5**, m/z 69, 91, 105, 121, 190) were generated at 70 eV by electron impact. Samples were quantitated using a four-level external standard curve for each of the compounds ranging from 0.05 to 5 μg/mL. Compounds **1–3** were purchased from Aldrich, Milwaukee WI. Compound **5** was from Firmenich, Geneva, Switzerland, and compound **4** was a gift from Prof. Dr. Sponholz of the Justus-Liebig-Universitat Giessen, Germany.

RESULTS AND DISCUSSION

The physiological development of Concord grapes over the 1992 season can be seen in the changes in size, color, and weight (Figure 1). Veraison is indicated by a sharp rise in absorbance at 525 nm after day 58 (September 10, 1992) and is indicated in each figure with a dotted line. Figure 2 shows that compounds **1**, **2**, and **4** were undetected in the berries prior to veraison but underwent rapid increases after veraison. However, these compounds were never detected in the leaves. Figures 3 and 4 shows the level of compound **5** and its hydrolyzable precursors (expressed as amount of compound **5** liberated under acid hydrolysis) in both berries and leaves during the growing season. The precursors were present in the berries before as well as after veraison (Figure 3). However, they underwent a less accelerated increase in concentration after veraison than did compounds **1**, **2**, and **4**, which impart American character. In the berries the free β-damascenone (**5**) increased in concentration at veraison, while the rise in the β-damascenone precursors was delayed 1 week. It is interesting to note that the free compound was always higher than the bound form in the fruit. This is in contrast to what has been reported for β-damascenone in apples (Zhou et al., 1993) and for other terpenes in grapes (Williams et al., 1982; Park et al., 1991).

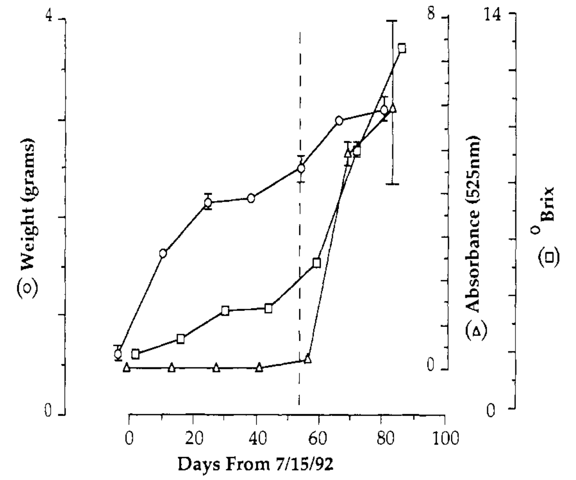

Figure 1. Physical measurements of berry development over the 1992 growing season. Vertical dashed line in *all graphs* represents the point of approximate veraison onset.

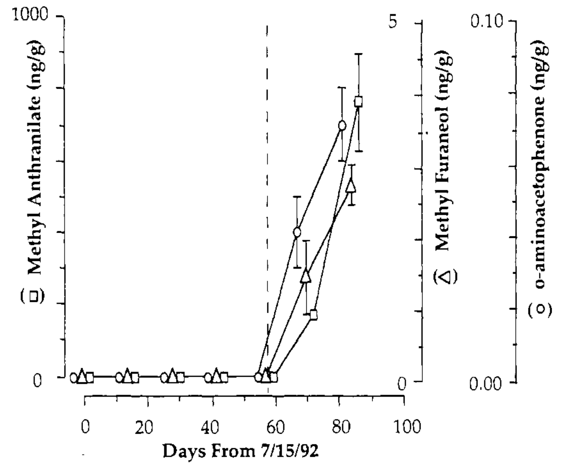

Figure 2. Measurements of American character impact compounds over the 1992 growing season.

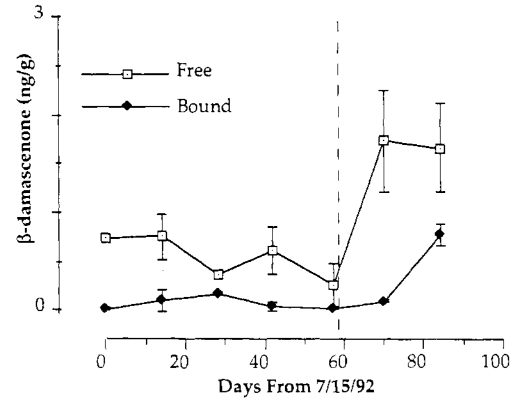

Figure 3. Measurements of free and bound β-damascenone in the berries over the 1992 growing season.

Unlike the American character impact compounds, β-damascenone precursors were present in the leaf tissue throughout the season, and at higher levels than in the berries. However, the β-damascenone precursors increased in the leaves after veraison (Figure 4), similar to the American character compounds in the berries (Figure 2). Monoterpene glycosides have been reported in the leaves of several plants including tea (Kobayasi et al., 1993), the flowering shrub caprifoliaceae (Calis et al., 1993), and

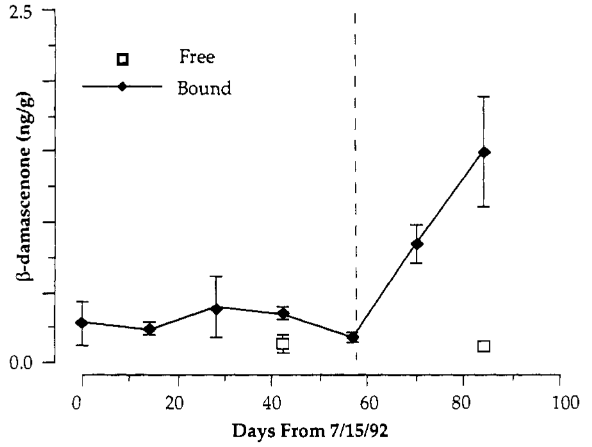

Figure 4. Measurements of free and bound β-damascenone in the leaves over the1992 growing season.

grapevines (Gunata et al., 1986). The present results differ somewhat from the change in monoterpene glycosides observed in the leaves of Muscat of Alexandria, where the rise was more constant throughout the season (Gunata et al., 1986).

The furaneol (**3**) concentration in the mature berries (measured only on the last harvest date) was 355 ± 15 ng/g, much above its reported threshold (Belitz and Grosch, 1987). This was approximately 100-fold higher than the concentration of methylfuraneol (**4**). A similar result was reported for several American species and their hybrids (Huhn, 1992). Although furaneol (**3**) contributes more than methylfuraneol (**4**) to the flavor of these grapes, methylfuraneol (**4**) was measured throughout the season as it could be extracted in the same solvent (Freon 113) as compounds **1**, **2**, and **5**.

The results reported here indicate that the American flavor character of labruscana grapes caused by compounds **1**, **2**, and **4** develops in the plant differently from that of β-damascenone and its precursors. Veraison seemed to have a greater impact on the formation of the American character compounds, whereas β-damascenone and its precursors, being present in the leaves and preveraison berries, behaved more like the isoprenoid and norisoprenoid compounds reported in other species of grapes.

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LITERATURE CITED

Acree, T. E. The Odor Quality of Labrusca Grapes. In *Quality of Selected Fruits and Vegetables of North America*; Teranishi, R., Barrera-Benitez, H., Eds.; American Chemical Society: Washington, DC, 1981; pp 11–19.

Acree, T. E., Lavin, E. H., Nishida, R.; Watanabe, S. o-aminoacetophenone the Foxy Smelling Component of Labruscana Grapes. In *Flavour Science And Technology, 6th Weurman Symposium, Geneva, Switzerland;* Bessiere, Y., Thomas, A. F., Eds.; Wiley: New York, 1990; pp 49–52.

Belitz, H. D.; Grosch, W. Aroma Substances. In *Food Chemistry;* Springer Verlag: New York, 1987; pp 257–304.

Braell, P. A. Characterization of the Flavor of *Vitis labruscana* Using Charm Analysis. Ph.D. Dissertation, Cornell University, Ithaca, NY, 1986.

Braell, P. A.; Acree, T. E.; Butts, R. M.; Zhou, P. G. Isolation of Nonvolatile Precursors of β-Damascenone from Grapes Using Charm Analysis. In *Biogeneration of Aromas*; Parliment, T. H., Croteau, R., Eds.; American Chemical Society: Washington, DC, 1986; pp 75–84.

Buttery, R. G.; Teranishi, R.; Ling, L. C. Fresh tomato aroma volatiles: A quantitative study. *J. Agric. Food Chem.* **1987**, *35*, 540–544.

Calis, I.; Yuruker, A.; Ruegger, H.; Wright, A. D.; Sticher, O. Anatoliosides five novel acyclic monoterpene glycosides from viburnum-orientale. *Helv. Chim. Acta* **1993**, *76* (1), 416–424.

Cox, J. A. Partial Purification of β-damascenone Precurors in Apples. M.S. Thesis, Cornell University, Ithaca, NY, 1991.

Fuleki, T. Changes in the chemical composition of Concord grapes grown in Ontario during ripening in the 1970 season. *Can. J. Plant Sci.* **1972**, *52*, 863–868.

Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components in cultivar muscat during first development and maturation. *J. Sci. Food Agric.* **1985**, *36* (9), 857–862.

Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. Changes in free and bound fractions of aromatic components in vine leaves during development of Muscat grapes. *Phytochemistry* **1986**, *25* (4), 943–946.

Huhn, T. Investigations of Specific Aroma Compounds in the Crossing Partners of Interspecific Grapevines. Diplomarbeit, Justus-Liebig-Universitat Geissen, 1992.

Kobayasi, A.; Winterhalter, P.; Morita, K.; Kubota, K. Glycosides in fresh tea leaves—Precursors of black tea flavor. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 257–260.

Marin, A. B.; Acree, T. E.; Hotchkiss, J. H.; Nagy, S. Gas chromatography-olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *J. Agric. Food Chem.* **1992**, *40*, 650–654.

Park, S. K.; Morrison, J. C.; Adams, D. O.; Noble, A. C. Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. *J. Agric. Food Chem.* **1991**, *39* (3), 514–518.

Rapp, A.; Knipser, W.; Engel, L.; Ullemeyer, H.; Heimann, W. [Atypical aroma compounds in grapes and wines from interspecies hybrid vines. I. Strawberry note.] Fremdkomponenten im Aroma von Trauben und Weinen interspezifischer Rebsorten. I. Die Erdbeernote. *Vitis* **1980**, *19* (1), 13–23.

Robinson, W. B.; Shaulis, N. J.; Pederson, C. S. Ripening studies of grapes grown in 1948 for juice manufacture. *Fruit Prod. J. Am. Food Manuf.* **1949**, *29*, 36–37, 54, 62.

Schreier, P.; Paroschy, J. H. Volatile constituents from Concord, Niagara (Vitis labrusca) and Elvira (V. labrusca × V. riparia) grapes. *Can. Inst. Food Sci. Technol. J.* **1981**, *14* (2), 112–118.

Sefton, M. A.; Skouroumounis, G. K.; Massy, W. R. A.; Williams, P. J. Norisoprenoids in Vitis vinifera white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust. J. Chem.* **1989**, *42* (12), 2071–2084.

Shure, K. B. Changes in the Odor of Apple Homogenates Due to Enzymatic Oxidation. M.S. Thesis, Cornell University, Ithaca, NY, 1992.

Strauss, C. R.; Gooley, P. R.; Wilson, B.; Williams, P. J. Application of droplet countercurrent chromatography [DCCC] to the analysis of conjugated forms of terpenoids, phenols, and other constituents of grape juice. *J. Agric. Food Chem.* **1987a**, *35* (4), 519–524.

Strauss, C. R.; Wilson, B.; Anderson, R.; Williams, P. J. Development of precursors of C13 nor-isoprenoid flavorants in Riesling grapes. *Am. J. Enol. Vitic.* **1987b**, *38* (1), 23–27.

Williams, P. J.; Strauss, C. R.; Wilson, B. Classification of the monoterpenoid composition of Muscat grapes. *Am. J. Enol. Vitic.* **1981**, *32* (3), 230–235.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy, W. R. A. Use of C18 reversed-phase liquid chromatography for the isolation of monoterpene glycosides and norisoprenoid precursors from grape juice and wines. *J. Chromatogr.* **1982**, *235* (2), 471–480.

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* (4), 919–924.

Winkler, A. J. Devlopment and Composition of Grapes. In *General Viticulture.* University of California Press: Berkeley, CA, 1974; pp 138–196.

Zhou, P. G.; Cox, J. A.; Roberts, D. D.; Acree, T. E. β-damascenone Precursors in Apples. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 275–278.

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