Table I. Effects of Different Media on Callus and Shoot Formation[a]

	A	B	C	D	E	F	G
callus	0.80 ± 0.23	0.84 ± 0.15	0.94 ± 0.10	0.02 ± 0.04	0.05 ± 0.10	0.00 ± 0.00	0.90 ± 0.22
shoot	0.09 ± 0.11	0.09 ± 0.14	0.18 ± 0.17	0.51 ± 0.22	0.64 ± 0.42	0.12 ± 0.13	0.05 ± 0.11
root							
IAA, mg/L	4.0	4.0	1.0				
kinetin, mg/L	2.56	2.56	0.04	0.1	0.1	0.1	
2,4-D, mg/L							0.5
pCPA, mg/L							2.0
TEA derivative, mg/L					10	1	
basal media	MS	MS	MS	MS	MS	MS	SH
pH	5.7	4.9	5.7	5.7	5.7	5.7	5.7
sucrose, g	30	15	30	30	30	30	30

[a] Each value represents the mean average of at least 3 separate observations with each observation comprised of a group of 2-40 slants containing stem tissue. The standard deviation is included. The group of slants were randomly placed in a growth area illuminated on a light/dark ratio of 16:8. The temperature and humidity varied during this cycle between 29 °C and 31% humidity during light cycles and 25 °C and 35% humidity in the dark cycles as measured by a Foxboro temperature and relative humidity meter. The slants were scored after a minimum of 14-days incubation and the fraction of tubes containing callus and/or shoots was recorded.

For quantification of the effect of the TEA derivative on callus formation and organogenesis, a matrix experiment was conducted to test the action and interaction of kinetin and TEA over a broad range of concentrations. The F test revealed a confidence level of 5% for the data produced in the entire matrix. In an effort to identify the location of differences with this limited data, a contour plot of the data was made. Figure 2 shows the results of this plotting.

The most significant result of this plotting is shown by comparing callus and shoot formation as a function of TEA derivative concentration. Shoot formation was greatest at 10 mg/L TEA derivative and is somewhat independent of the kinetin levels used. At this level callus formation was strongly inhibited. This derivative compound thus produces a concentration-dependent inhibition on callus formation that concomitantly produces an increase in shoot formation. This result provides possible evidence for an unique hormonal action.

Further experiments to better define the effect of the TEA derivative on guayule tissue in vitro are needed. No further experiments have yet been conducted to test the effect of TEA derivative in synergism with any other growth hormones.

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The Presence of Damascenone in Cultivars of *Vitis vinifera* (Linneaus), *rotundifolia* (Michaux), and *labruscana* (Baily)

Damascenone, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, was identified in several cultivars of three species of grapes (*Vitis*). Because damascenone has a pleasant floral odor and a very low threshold in water, its concentration in extracts of juice and wine of several cultivars of *Vitis* was determined. The highest concentration was found in Concord, a cultivar of *Vitis labruscana*.

The odor of *Vitis* species *vinifera*, *labruscana*, and *rotundifolia* is very different and easily recognizable. Although hundreds of odorous compounds have been found in grapes (Schreier, 1979), only methyl anthranilate seems unique to one specie, *labruscana*. However, methyl anthranilate has been found in only a very few of the cultivars of *labruscana* grapes (Sale and Wilson, 1926; Nelson et al., 1977a). Damascenone, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one, was identified in wines of a *Vitis vinifera* cultivar, Riesling, (Schreier and Drawert, 1974) and quantified in the grapes and wines of two cultivars of *labruscana* grapes (Masuda and Nishimura, 1980).

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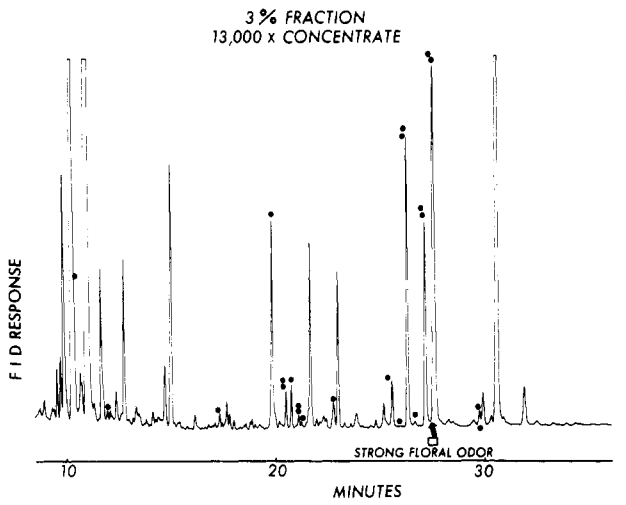

Figure 1. A portion of the chromatogram of the 3% Florisil fraction of Concord grape juice extract. The dots indicate the times when odors were detected and the arrow indicates the retention time of strongest odor. The column was a 0.3 mm by 30 m Carbowax 20M programmed at 6 °C/min from 40 to 190 °C.

In this communication we report the presence of this compound in three other cultivars of *V. labruscana* and two of *V. rotundifolia*. We also report the concentrations of this compound in extracts of juices and wines of these different species.

EXPERIMENTAL SECTION

Extraction and Concentration. Juice samples prepared from grapes grown in the Experiment Station vineyard were stemmed, crushed, and pressed in 5-kg lots, and 1200 mL of the resulting juice was treated with 120 mL of 95% ethanol and extracted with 800 mL of Freon 113 (1,1,2-trichlorotrifluoroethane). These extracts were concentrated 25-fold in a rotary evaporator at 0.5 atm and 30 °C and stored under nitrogen at −10 °C. Wine samples were extracted directly since they already contained ~11% ethanol. The wines were prepared according to the methods reported by Nelson et al. (1977b). We did not have enough damascenone to test the efficiency of the extraction procedure. However, this procedure has been shown (Nelson et al., 1976) to extract greater than 95% of the methyl anthranilate, and damascenone is less polar than methyl anthranilate, i.e., it elutes before methyl anthranilate from Florisil.

Gas Chromatography. Preparative gas chromatography, odor verification, mass spectroscopy, and selected ion monitoring were performed by using 0.3 mm by 30 m soft glass columns coated with Carbowax 20M according to the procedures of Grob and Grob (1976). The gas chromatographs were modified with a multiple purge injector (Acree and Butts, 1980).

Mass Spectrometry (GC–MS). GC–MS identifications and selected ion monitoring for quantitations were performed on a Hewlett-Packard 5985 GC–MS–data system modified with a multiple purge injector and a platinum–iridium direct interface.

Florisil Chromatography. For minimization of interferences from the many Freon-extractable compounds in grapes, the extracts were fractionated by using 1 g of Florisil (deactivated with 6% water) per kg of juice equivalent and 5 g/kg of wine equivalent. The extracts were concentrated to 10 mL applied to the columns, and eluted with 10-mL aliquots of 0, 1, 3, 10, 30, and 100% diethyl ether in pentane. Greater than 90% of the damascenone eluted in the 3% ether fraction.

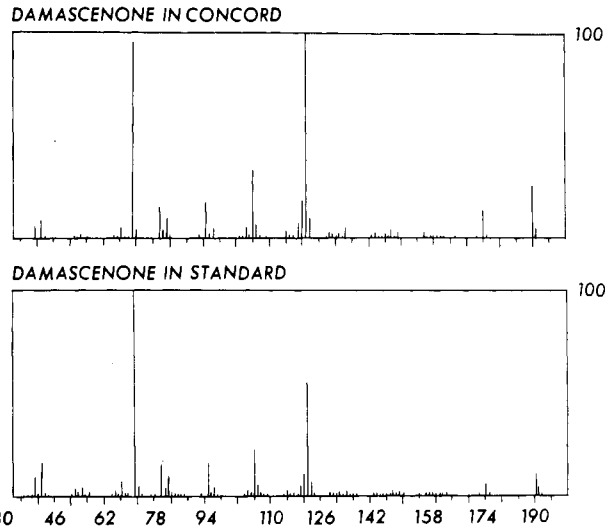

Figure 2. A comparison between the mass spectra obtained for the sweet floral compound isolated from grape juice and that obtained for damascenone.

Quantitation of Damascenone. As an internal standard 0.1 mL of Freon 113 containing 250 ng of 1-chloronaphthalene was added to the 3% ether–pentane fractions. The molecular ions, 162 for 1-chloronaphthalene and 190 for damascenone, were scanned selectively, and a response factor was calculated from a solution containing authentic damascenone.

RESULTS AND DISCUSSION

labruscana grapes and to some extent grapes of the species *rotundifolia* characteristically have a sweet perfumy aroma which is generally stronger than the odor of most vinifera cultivars. Methyl anthranilate, found only in the *labruscana* grapes, was for years associated with this species difference. However, most of the *labruscana* species and all of the *rotundifola* contain little or none of this compound (Sale and Wilson, 1926; Nelson et al., 1976). In an attempt to identify the compounds responsible for the sweet floral aromas characteristic of *labruscana* grapes, Freon 113 extracts of Concord grape juice were fractionated on water-deactivated Florisil. Stepwise elution of the Florisil with pentane–ether mixtures, whose solvent strengths (Snyder and Kirkland, 1974) approximately doubled with each step, yielded six fractions, none of which retained the total Concord-like aroma. However, a combination of all six Florisil fractions recreated a mixture with an odor very similar to the original Freon extract of Concord grape juice.

Among the six Florisil fractions only the 3% ether fraction has a strong perfumy aroma. Certainly the other fractions contain compounds essential to the total Concord odor, but the basic floral fruity character seems to come mostly from the 3% fraction. A small amount (less than 5%) of the methyl anthranilate was found in this fraction; the bulk of it eluted in the 10% ether fraction.

Figure 1 shows the result of sniffing (Acree et al., 1976) the effluent of a gas chromatographic separation of the 3% Florisil fraction. By far, the strongest odor was observed at 27.5 min. Figure 2 shows that the mass spectrum obtained for a compound trapped in this region of the chromatogram is superposable on the spectrum of authentic damascenone. The isobutane CI mass spectra (Munson, 1977) were also identical, both showing a protonated molecular ion at 191. Further, the retention time of a 190-mass peak observed by using selected ion monitoring had an identical retention time to that produced

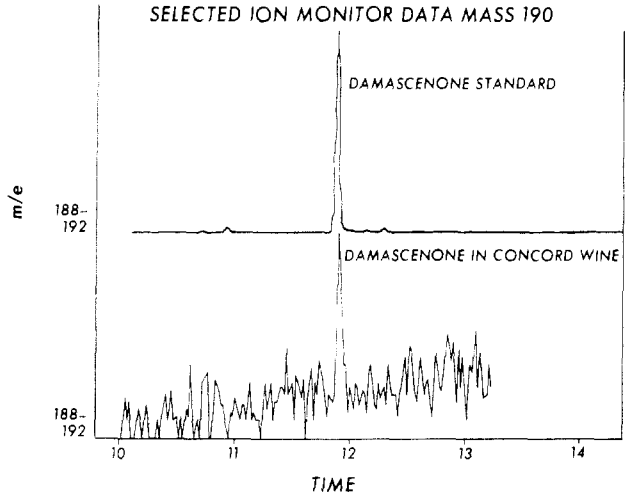

Figure 3. A simultaneous plot of the selected ion chromatograms obtained for the damascenone in Concord extract and authentic damascenone.

by authentic damascenone (Figure 3). Similar verification for the presence of damascenone in all the cultivars listed in Table I was obtained by this method.

Damascenone was found in all 11 cultivars of the three grape species shown in Table I. Clearly, the presence of this compound is not indicative of a particular species or cultivar. However, the concentrations found for damascenone were in every case well above the threshold reported for this compound in water (Ohloff, 1978) and indicate that it may contribute something to the odor of all grapes. The fact that the concentration of damascenone was highest in Concord juice (almost 40 times the amount found in Pinot Chardonnay and 5 times that found in Riesling) implies that it may be a very important odor component of this cultivar.

Table I. Concentration of Damascenone in Several Cultivars of *V. labruscana, vinifera,* and *rotundifola*

cultivar	species	damascenone, ng/g
Concord, juice	*labruscana*	4.92
Catawba, juice	*labruscana*	1.54
Concord, wine	*labruscana*	1.58
Riesling, wine 1974	*vinifera*	0.85
Riesling, wine 1972	*vinifera*	0.72
Delaware, juice	*labruscana*	0.53
Ives, juice	*labruscana*	0.43
Noble, wine 1977	*rotundifolia*	0.38
Niagara, juice	*labruscana*	0.17
Fry, wine 1974	*rotundifolia*	0.14
Pinot Chardonnay	*vinifera*	0.13

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CORRECTIONS

ANALYSIS OF CARROT VOLATILES COLLECTED ON POROUS POLYMER TRAPS, by Philipp W. Simon,* Robert C. Lindsay, and Clinton E. Peterson, *J. Agric. Food Chem.* **1980**, *28*, 549.

On p 550, under Sample Preparation, the fifth sentence should read as follows: Traps were prepared with ±0.01 g of Tenax GC between glass and plugs in a small-bore pipet as described by Steinke (1978).

FLAVOR PROTEIN INTERACTIONS. BINDING OF CARBONYLS TO BOVINE SERUM ALBUMIN: THERMODYNAMIC AND CONFORMATIONAL EFFECTS, by Srinivasan Damodaran and John E. Kinsella,* *J. Agric. Food Chem.* **1980**, *28*, 567.

On p 570, the fourth sentence in the legend for Figure 6 should read as follows: (□—□) BSA treated with 2-nonanone ($\bar{\nu}$ = 5); (●—●) native BSA.