The Volatile Composition of Chardonnay Juices: A Study by Flavor Precursor Analysis

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The free volatiles, as well as those released from their glycosidically bound forms by acid or enzyme hydrolysis, have been analyzed for Chardonnay juices from three successive vintages. One hundred eighty compounds were identified. Twenty-eight of these have not been previously reported as grape components. More than seventy percent of the total concentration of volatile secondary metabolites comprised thirteen-carbon norisoprenoids, and these were mainly observed in the acid- and the glycosidase enzyme-released fractions. Benzene derivatives accounted for a further twenty percent of the total volatile concentration, while monoterpenes made up only five percent of the total. Low concentrations of volatiles, apparently derived from primary metabolites or from chlorophyll degradation, were also observed. Volatiles with likely sensory significance were found mainly in the acid hydrolysates. Pathways to these compounds are discussed.

KEY WORDS: Chardonnay, composition, volatiles, norisoprenoids, monoterpenes, volatile phenols, enzyme hydrolysis, acid hydrolysis, precursor, damascenone

The analysis of volatiles released from flavorless glycoconjugates is a strategy being employed increasingly for the investigation of flavor compounds of fruits and wines (52). This strategy is particularly valuable for flavor studies on fruits that contain low concentrations of distinguishing free aroma compounds, *e.g.*, non-floral grape varieties such as Chardonnay (54). Chardonnay juices seldom have a distinct aroma, although the wines of this variety can usually be readily recognized (1). Implicitly then, Chardonnay juices contain constituents which, although odorless, are capable of producing characteristic aroma compounds in the finished wine.

Earlier work on Chardonnay juice used in Australian winemaking indicated that the variety was deficient in monoterpenes (11). This observation derived from quantitative analyses of free and potentially volatile (mono)terpenes, *i.e.*, FVT and PVT. However, the PVT distillate contained a high concentration of norisoprenoid compounds, suggesting that these components might contribute to the varietal character of wines made from Chardonnay.

The possible involvement of norisoprenoids in Chardonnay wine flavor was also suggested from an earlier study of the headspace composition of Chardonnay wines. More than 150 constituents were identified in the wines from six consecutive vintages, and the major fermentation alcohols, esters, and acetic acid were recognized as important aroma compounds. However, the only grape-derived volatile with sensory significance that was observable against the background of fermentation products was the norisoprenoid compound damascenone (43).

This paper reports the results of a study of Chardonnay juices obtained from the same region over three consecutive vintages. The free volatiles of the juices, as well as the acid- and glycosidase-released volatile aglycones of the precursor fraction, were identified and quantified. The data thus allow an assessment of the number and type of volatile constituents that can be generated in Chardonnay wines on hydrolysis of the flavor precursors. This hydrolytic release is responsible, at least in part, for the development of flavor in Chardonnay wines (15,54). Knowledge of the composition of the volatiles released from the grape glycosides could, therefore, help to elucidate varietal aromas of these wines.

Materials and Methods

General procedures: All solvents were of high purity at purchase and were redistilled before use. Details of analyses by GC-MS were as described previously (38), except that the 1989 samples were analyzed with a Finnigan TSQ 70 mass spectrometer coupled to a Varian 3400 gas chromatograph. Reference compounds were either available commercially, donated, or prepared in our laboratory by standard synthetic methods.

Preparation of samples for analysis: The Chardonnay juice samples were obtained from commercial batches of the 1987-1989 vintages from the Southern Vales region of South Australia. These juices were prepared by the same winery and were sampled immediately prior to fermentation. They were a mixture of free-run juice and light pressings and had been clarified by *Pectinex 3XL* enzyme (50 mL/tonne added at crushing and a further 15 mL/1000 L added in the tank). The 1989 juice was 22.8° Brix, and this typified the soluble solids content of the juices from the two earlier vintages.

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Table 1. Norisoprenoid components of 1987, 1988, and 1989 vintage Chardonnay juices and of the glycoside hydrolysates derived from these juices.

			1987[b]			1988[c]			1989[c]				References	
		Kovats[a] indices	Free	Enz	H+	Free	Enz	H+	Free	Enz	H+	Evidence[d]	As grape or wine product	As oak component
1	2,6,6-Trimethylcyclohex-2-en-1-one	n.d.						tr				A (18)	(55)	
2	3,5,5-Trimethylcyclohex-2-en-1-one	1274				tr		tr	tr		1	B	(55)	
3	2,6,6-Trimethylcyclohex-2-ene-1,4-dione	1297		+	+		7.4	20		2.7	3	C	(38)	(37)
4	Vitispirane	1327	+	+	+		4.4	21		5.5	22	C	(38)	(37)
5	2,6,6-Trimethylcyclohexane-1,4-dione	1355			+	tr	tr	tr			1	C	(38)	
6	Riesling acetal	1374			+			7			7.5	C	(58)	
7	1,1,6-Trimethyl-1,2-dihydronaphthalene	1423			+			1.5			11.5	C	(38)	
8	Damascenone	1496	+		+	1.2		2.5	3.8		33	C	(38)	
9	Actinidol - isomer 1	1510			+			15	tr	1.7	14	C	(38)	
10	Actinidol - isomer 2	1515			+			23	tr	2	25	C	(38)	
11	Actinidol - isomer 3	1564						tr	tr			C	(38)	
12	(5R*,9R*)-3,4-Dihydro-3-oxoedulan[e]	1592				tr	1.5	1.5	tr	3.3	3.8	C		(37)
13	(5S*,9R*)-3,4-Dihydro-3-oxoedulan[e]	1614				tr	3	8	1.8	10	11	C		(37)
14	3,4-Dehydro-ß-ionone[e]	1619					4			1.7		A (25)		
15	(7E)-Megastigma-4,7,9-trien-3-one	1632					tr	tr				C	(55)	
16	8,9-Dehydrotheaspirone[e]	1641					tr	1				C	(59)	
17	2-(3-Hydroxybut-1-enyl-2,6,6-trimethylcyclohex-3-en-1-one	1654			+		1	45			17	C	(38)	
18	3-Hydroxytheaspirane - isomer 1[e]	1657		+			14			11	tr	C	(59)	
19	4-(2,3,6-Trimethylphenyl)-but-3-en-2-one	1658			+			1				A (25)		
20	3-Hydroxytheaspirane - isomer 2[e]	1668					5	tr		4.5	tr	C	(59)	
21	3-Hydroxytheaspirane - isomer 3[e]	1684					3	tr		3.2	tr	C	(59)	
22	5,6-Epoxy-3-hydroxymegastigm-7-en-9-one isomer 1	1694		+		tr	5	tr	tr	4		D		
23	*cis*-3-Oxo-ß-damascone	1748					1					D		
24	(*6Z,8E*)-Megastigma-4,6,8-trien-3-one	1750				tr	tr	8		tr	6.5	C	(55)	(37)
25	(*6Z,8Z*)-Megastigma-4,6,8-trien-3-one	1796					tr	tr				C	(55)	
26	Dihydroactinidiolide	1802	+			tr		4	2		5	C	(38)	(37)
27	*tran*-3-Oxo-ß-damascone	1805		+			40			32		C	(38)	
28	3,4-dihydro-3-oxoactinidol isomer 1[e]	1805	+	+	+	tr	16	2	4.7	20	8	C	(59)	(37)
29	(*6E,8E*)-Megastigma-4,6,8-trien-3-one	1810				tr	1	11		3	6	C	(55)	(37)
30	3,4-dihydro-3-oxoactinidol isomer 2[e]	1815	+	+	+	tr	48	7	6.6	37	11	C	(59)	(37)
31	*cis*-3-Oxo-α-damascone	1823		+			3.5			3		D		
32	3,4-Dihydro-3-oxoactinidol isomer 3[e]	1830	+	+	+	tr	20	1.5	1.7	18	2.7	C	(59)	(37
33	(5S*,6R*)-9-Hydroxymegastigm-7-en-3-on	1848	+	+	+	3	117	180	31	108	90	C	(38)	(37)
34	3-Hydroxydamascone	1851	+		+	tr		75	3		obsc	C	(46)	(37)
35	3,4-Dihydro-3-oxoactinidol, isomer 4[e]	1853	+	+	+	tr	5		2	8	1	C	(59)	(37)
36	9-Hydroxymegastigma-5,7-dien-3-one	1863		+			15	1		8		B		
37	Megastigm-5-en-7-yne-3,9-diol	1871		+		tr	2.4		1	4		C	(38)	
38	3,4-Dihydro-3-hydroxyactinidol[e]	1884		+		tr	2	tr	1	3	1	C	(59)	
39	Megastigm-5-ene-3,9-diol	1886				tr	tr			2		C	(39)	
40	*trans*-3-Oxo-α-damascone	1893		+			82	2		50	1.5	C	(38)	(37)
41	3-Oxo-α-ionol isomer 1[f]	1937	+	+	+	4	370	~80	71	260	~60	C	(46)	(37)
42	3-Oxo-α-ionol isomer 2[f]	1938			+			~86			~60	C	(46)	(37)
43	3-Hydroxymegastigma-5,7-dien-9-one	1948				1		6	3		4.5	C	(39)	
44	9-Hydroxymegastigma-4,6,7-trien-3-one	1948		+			22			17	tr	C	(38)	(37)
45	Dehydrololiolide	1959	+	+	+	1.2		12	6.5		10	C	(38)	(37)
46	3-Oxo-α-ionone	1960	+		+	tr	1		3.3		4	C	(38)	(37)
47	5,6-Epoxy-3-hydroxymegastigm-7-en-9-one, isomer 2	1972	+		+	tr	tr	2.5	6		6	C	(39)	
48	9-Hydroxymegastigm-5-en-4-one	1988		+		tr	6	tr		7		C	(59)	(37)
49	9-Hydroxymegastigma-4,6-dien-3-one, isomer 1	2001	+	+	+	1.8	53	18	23	40	20	C	(38)	(37)

continued

Table 1 continued

			1987[b]			1988[c]			1989[c]				References	
		Kovats[a] indices	Free	Enz	H^+	Free	Enz	H^+	Free	Enz	H^+	Evidence	As grape or wine product	As oak component
50	Blumenol C	2002		+		tr	42		4.2	18	5	C	(38)	(37)
51	3-Hydroxymegastigma-5,7-diene-4,9-dione	2039					tr	1			1	A (2)		
52	9-Hydroxymegastigma-4,6-dien-3-one, isomer 2	2081	+	+	+	1.7	57	21	21	46	21	C	(38)	(37)
53	An isomer of grasshopper ketone	2115					15					D		
54	6,9-Dihydroxymegastigm-7-en-3-one	2121	+	+	+	1.7	545	185	60	105	64	C	(40)	(37)
55	Grasshopper ketone	2149		+			110		tr	23		C	(38)	
56	Loliolide, isomer 1	2170				tr	4			6		A (3)		
57	Vomifoliol (Blumenol A)	2180	+	+	+	tr	285	13	14	45	16	C	(46)	(37)
58	Loliolide, isomer 2	2200			+			1				A (3)		
59	Dehydrovomifoliol	2212	+	+	+	tr	44	10	3	17	6.5	C	(38)	(37)
60	Blumenol B	2244		+			17	tr	1	8	2.5	C	(38)	(37)

[a] For GC conditions see Sefton *et al.*, 1990; n.d., not determined. [b] +, constituent observed in this fraction. Free, unconjugated juice volatiles; Enz, volatiles released by hydrolysis of the C_{18} isolate with Rohapect C; H^+, volatiles released by hydrolysis of the C18 R.P. isolate at 50°C, pH 3.0 over 28 days. [c] Concentration in μg/L of juice; tr, trace; obsc, obscured. [d] A, mass spectrum the same as published spectrum; B, the mass spectrum was the same as that of the reference compound and the peak was enhanced by the reference when co-chromatographed; C, previously proven in this laboratory; D, interpretation of mass spectrum. [e] Carotenoid numbering. [f] Assignments interchangeable.

A single juice sample from each vintage was analyzed in duplicate. The acid hydrolysates were prepared as described by Sefton *et al.* (38). The isolation of the free juice volatiles and acid hydrolysis products with Freon F11, as well as the isolation of the grape glycoconjugate fraction with a C-18 reversed-phase (RP) adsorbent, were carried out as described by Wilson *et al.* (56). Enzyme hydrolysates were prepared as follows. A solution of Rohapect C (200 mg) in an aqueous pH 5 buffer [10 mL, prepared by mixing equal volumes of aqueous citric acid solution (0.1 *M*) and aqueous disodium hydrogen phosphate solution (0.2 *M*)], was added to an aqueous solution of the C-18 RP isolate (5 mL, equivalent to 250 mL of juice) in the same buffer (25 mL). The aqueous C-18 RP isolate was stripped of the free volatiles by liquid/liquid extraction with freon overnight, prior to the enzyme addition. The mixture was heated at 37°C for 16 hours, a solution of 1-octanol (20 μg) in ethanol (100 μL) then added, and the hydrolysate isolated by continuous extraction with dichloromethane over 24 hours. The organic extract was dried (magnesium sulfate) and concentrated by fractional distillation through a Vigreux column packed with Fenskes helices prior to analysis by GC-MS.

Results and Discussion

The free volatile components of the Chardonnay juices from the three vintages and the volatile hydrolysis products derived from precursors in these juices fall into four broad categories: norisoprenoids (Table 1), benzene derivatives (Table 2), monoterpenes (Table 3), and aliphatic compounds (Table 4). Some additional components of disparate biogenetic origin are listed in Table 5. Quantitative data are given for samples from the 1988 and 1989 vintages, but only qualitative data were available for those from the 1987 vintage. Only those compounds that have been identified are listed in the tables, and these account for more than 80% of the total concentration of the volatiles observed by GC-MS. Evidence for the assignments, together with references to compounds as previously observed in grape or wine products, are given. Where no such reference is given, the component has not, to the best of our knowledge, been previously reported as a constituent of such products. Because many of the Chardonnay volatiles have been earlier identified as oak wood components (37), these common constituents are also indicated in Tables 1 and 2.

More than 100 minor or trace constituents, the identity of which could not be confidently assigned, were also observed, but these are not included in the tables.

The total concentrations of the free compounds as well as those observed after enzymatic and acid hydrolysis of the precursor fractions are presented in five categories for 1988 and 1989 (Fig. 1). The relative proportion of the acid- and enzyme-released constituents is similar for each category for both years, with norisoprenoids dominating and the benzene derivatives occurring at second highest concentration. The low concentration of monoterpenes in these Chardonnay samples is also illustrated by the data.

The free volatiles in juices from both years were composed largely of aliphatic constituents which can perhaps be regarded as primary rather than secondary metabolites. It should be noted that those compounds which dominated the free volatile fractions, i.e. (Z)-3-hexenol **145**, (E)-2-hexenol **146**, *n*-hexanol **147**, and γ-butyrolactone **150** (Table 4) could have been produced by unwanted partial fermentation taking place during storage of the juices, with the three alcohols **145-147** produced by reduction of the corresponding aldehydes which may be formed from lipoxygenase oxidation of linoleic acid (32). Whether these constituents were present totally as grape metabolites or were contributed partly by post-harvest conditions is, therefore, open to question. However, some of these compounds were also

Table 2. Volatile phenols and other benzene derivatives of 1987, 1988, and 1989 vintage Chardonnay juices and glycoside hydrolysates derived from these juices.

			1987[b]			1988[c]			1989[c]				References	
		Kovats[a] indices	Free	Enz	H^+	Free	Enz	H^+	Free	Enz	H^+	Evidence	As grape or wine product	As oak component
61	Ethylbenzene	n.d.	+			tr			1		tr	E	(34)	
62	1,3 and/or 1,4-Dimethylbenzene	n.d.	+			tr			tr		tr	E	(34)	
63	1,2Dimethylbenzene	n.d.	+			tr			tr		tr	E	(34)	
64	Benzaldehyde	n.d.	+		+	tr	tr		1.2	tr	tr	C	(34)	(26)
65	Benzyl alcohol	n.d.	+	+	+	10	80	7	175	22	8	C	(34)	(26)
66	Phenol	n.d.	+	+	+	1.2		tr	5	5		E	(32)	(26)
67	*o*-Cresol	n.d.	+			tr			tr			E	(32)	(26)
68	*p*-Cresol	n.d.	+			tr			tr			E	(29)	(26)
69	*m*-Cresol	n.d.	+			tr			tr			E	(32)	(26)
70	2-Phenylethanol	1283	+	+	+	4	70	10	95	38	16	C	(34)	(26)
71	Methyl salicylate	1306		+		tr	3.8		1	2.5	tr	E	(29)	(26)
72	Benzothiazole	1353	+		+	tr		tr	1		tr	E	(32)	
73	Quinoline	1376				tr			tr			E	(29)	
73	3-Phenylpropanol	1403	+	+			tr	tr	tr	1	tr	C	(46)	
75	Benzoic acid	1420	+	+	+	10	1.7	20	32	15	9	C	(34)	(26)
76	2,3-Dihydroxybenzoic acid methyl ester	1469	+	+		tr	tr	tr	2	3.2	2	C	(54)	
77	4-Vinylguaiacol	1483	+	+	+	tr	100	25	1.4	16	10	C	(32)	(26)
78	4-Vinylphenol	1493	+	+	+	tr	95	8	4	10	8.5	C	(32)	
79	Eugenol	1505		+		tr	5.6		1	7		C	(32)	(26)
80	Phenylacetic acid	1515	+		+	tr			tr	2	1	C	(34)	(26)
81	A dimethylnaphthalene	1524				tr			tr			A (18)	(29)	(26)
82	A dimethylnaphthalene	1530			+				tr		tr	A (18)	(29)	(26)
83	Indole	1537	+			tr	1	tr	1.6	1		E	(29)	
84	*m*-Hydroxyacetophenone	1543			+		tr	1			tr	D		
85	2-Hydroxymethylbenzoic acid lactone	1589			+	tr	tr	1	1		tr	A (18)		
86	*cis*-Cinnamic acid	1628			+							D		
87	Vanillin	1642	+		+	1.6	1.6	2	9	2.5	4	C	(54)	(26)
88	Coumarin	1672				tr		tr	tr			E		
89	*trans*-Cinnamic acid	1720	+		+	tr	tr	tr		4.3	6	B	(34)	(26)
90	Methyl vanillate	1728	+	+	+	tr	14	6	2.7	6.5	5	C	(54)	
91	Acetovanillone	1731			+	tr	3	5	3	3	5	B	(34)	(7)
92	4-Hydroxybenzaldehyde	1776	+	+	+	tr			1	1	1	C	(54)	g
93	Tyrosol	1781		+		tr	1.4		1	2		C	(54)	
94	3-Methoxytyrosol	1799	+			tr	tr		10	3		D	(46)	g
95	Propiovanillone	1821				tr	4.4	tr				D	(46)	(26)
96	4-Hydroxybenzoic acid methyl ester	1833	+		+	tr		7			1	E	(54)	
97	4-Hydroxyacetophenone	1851	+		+	tr		1	tr	tr	1	C	(54)	
98	Butyrovanillone	1860			+	tr	tr		tr			D		(7)
99	2,5-Dihydroxybenzoic acid methyl ester	1863	+	+	+	tr	3	2	5	1	4	C	(54)	
100	4-Hydroxybenzoic acid ethyl ester	1887	+		+	tr	tr	tr	4		1	E	(16)	
101	Raspberry ketone	1901				tr	tr	tr				C	(54)	
102	Zingerone	1903	+			tr	5	1		4	4	C	(46)	g
103	Vanillic acid	1911			+					7	4	B	(54)	(24)
104	Dihydroconiferyl alcohol	1929		+	+		17	1		11	2	C	(46)	g
105	Syringaldehyde	1950	+	+	+	tr		tr	3	2	3	C	(54)	(26)
106	*cis*-Coniferyl alcohol	1977					tr					D		
107	4-Hydroxybenzoic acid	1979			+					6	5	B	(34)	(24)
108	Methyl syringate	2018	+	+	+	tr	17	13	4	12	8	D	(54)	
109	Acetosyringone	2021			+	tr		tr	1	tr	2	A (18)	(54)	(7)
110	Coniferyl alcohol	2039		+		tr	21	1	1	20	tr	C	(54)	g
111	Propriosyringone	2100		+		tr	10	5	1.3	6	4	E		(7)
112	Butyrosyringone	2107				tr	2	tr	tr			E		(7)
113	*cis*-Ferulic acid	2109			+				tr		3	D		

continued

Table 2 continued

		Kovats[a] indices	1987[b] Free	1987[b] Enz	1987[b] H^+	1988[c] Free	1988[c] Enz	1988[c] H^+	1989[c] Free	1989[c] Enz	1989[c] H^+	Evidence	References: As grape or wine product	References: As oak component
114	Tryptophanol	2119	+			tr	1.5	tr	1	2.5	tr	E	(32)	
115	*cis*-4-Hydroxycinnamic acid	2170			+						1	D		
116	Syringic acid	2183			+					tr		E	(32)	(24)
117	Tryptophanol acetate	2222	+						1.2		tr	E	(16)	
118	*trans*-Ferulic acid	2264			+				1	10	8	C	(32)	
119	*trans*-4-Hydroxycinnamic acid	2289			+					8	3	C	(32)	

For general details see Table 1. E, gas chromatographic retention time and mass spectrum identical to those of a reference sample. [g] Observed in this laboratory.

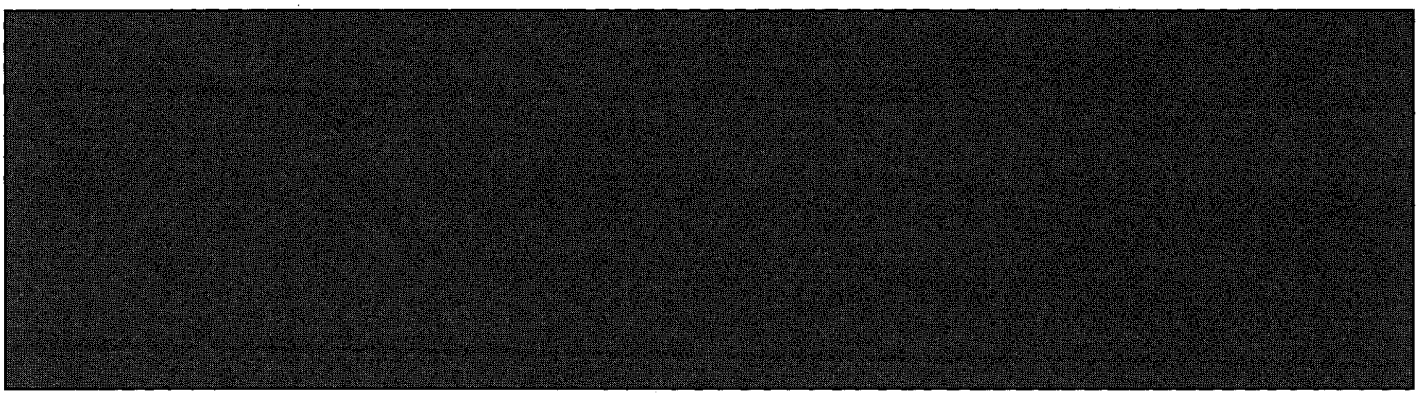

Table 3. Monoterpene components of 1987, 1988, and 1989 vintage Chardonnay juices and glycoside hydrolysates derived from these juices.

		Kovats[a] indices	1987[b] Free	1987[b] Enz	1987[b] H^+	1988[c] Free	1988[c] Enz	1988[c] H^+	1989[c] Free	1989[c] Enz	1989[c] H^+	Evidence	As grape or wine product
120	Limonene	n.d.				tr	tr	tr				C	(48)
121	5-(2-Buten-2-yl)-2,2-dimethyltetrahydrofuran	n.d.						tr			1	C	(48)
122	Furan linalool oxide, isomer 1	n.d.		+	+		10	2.4	tr	5	2.5	C	(48)
123	Furan linalool oxide, isomer 2	n.d.		+	+		6	tr	tr	2.5	1.5	C	(48)
124	Linalool	n.d.	+	+			5		1.6	3.5		C	(48)
125	Hotrienol	n.d.				tr	1.8		tr	tr		C	(48)
126	Myrcenol	n.d.						2.2				C	(48)
127	*cis*-Ociminol	1276						3			2	C	(48)
128	*trans*-Ociminol	1291						1.2			2.5	C	(48)
129	Pyran linalool oxide, isomer 1	1302		+		tr	1.3	tr	1.3		obsc	C	(48)
130	α-Terpineol	1304			tr	tr	1.6	5	1	1.5	2.5	C	(48)
131	Pyran linalool oxide, isomer 2	1310				tr	tr	1	1.8		1.5	C	(48)
132	Nerol	1342		+			2.3		tr	6.5		C	(48)
133	*Z*-2,6-Dimethylocta-3,7-diene-2,6-diol	1358				tr	tr		tr			C	(48)
134	2-Hydroxy-1,8-cineole	1370				tr	1.4					A	(4)
135	*E*-2,6-Dimethylocta-3,7-diene-2,6-diol	1375	+		+	tr	5		1.5	obsc		C	(48)
136	Geraniol	1375	+	+			6		tr	18		C	(48)
137	2,6-Dimethyloct-7-ene-2,6-diol	1416			+		1	14			18	C	(48)
138	*cis*-1,8-Terpin	1497			+		tr	20			8.5	C	(48)
139	*trans*-1,8-Terpin	n.d.			+							C	(48)
140	2,6-Dimethyloct-7-ene-1,6-diol	1539	+	+			2.5	1.5	1.5	6		A	(50)
141	Geranic acid	1552	+	+		tr	3.5	1	1.5	8.5	1.5	C	(34)
142	*Z*-2,6-Dimethylocta-2,7-diene-1,6-diol	1554		+			18	tr	1	22	1	C	(49)
143	*E*-2,6-Dimethylocta-2,7-diene-1,6-diol	1569	+	+		tr	60	1	6	40	4	C	(49)

For general details and references, see Table 1.

Table 4. Aliphatic alcohols, carboxylic acids, lactones, and ethyl esters of 1987, 1988, and 1989 vintage Chardonnay juices and glycoside hydrolysates derived from these juices.

		Kovats[a] indices	1987[b] Free	1987[b] Enz	1987[b] H^+	1988[c] Free	1988[c] Enz	1988[c] H^+	1989[c] Free	1989[c] Enz	1989[c] H^+	Evidence	As grape or wine product
144	Butanoic acid	n.d.	+									E	(34)
145	**Z**-3-Hexenol	n.d.	+	+		4	10	tr	60	tr	tr	E	(34)
146	**E**-2-Hexenol	n.d.	+	+		~110	tr		~150	~14		E	(34)
147	Hexanol	n.d.	+	+		~110	42		~300	~5		E	(34)
148	Ethyl hexanoate	n.d.	+			tr			tr			E	(34)
149	Pentanoic acid	n.d.	+			tr		tr	1.4		1	E	(34)
150	γ-Butyrolactone	n.d.	+			90	tr	tr	200		2.5	E	(32)
151	4-Hydroxypentanoic acid lactone	n.d.				tr			2.2			E	(32)
152	Hexanoic acid	n.d.	+			25	3.3		60	20	15	E	(34)
153	4-Hydroxyhexanoic acid lactone	n.d.	+			tr		tr	3		tr	E	(32)
154	Ethyl octanoate	n.d.	+			tr			tr			E	(34)
155	Heptanoic acid	1279				tr		tr	13	1	3	E	(34)
156	4-Hydroxyheptanoic acid lactone	1360				tr		tr	1		tr	E	(29)
157	Octanoic acid	1369	+	+	+	8	1.6	2	16	3	5	E	(34)
158	Ethyl decanoate	1461	+		+	tr						E	(32)
159	4-Hydroxyoctanoic acid lactone	1469	+		+	tr	tr	tr	2	tr	tr	E	
160	Nonanoic acid	1472	+	+	+	6	2	1	18	2	5	E	(34)
161	Decanoic acid	1561	+	+	+	1.8	tr	1	3.6		1	E	(34)
162	4-Hydroxynonanoic acid lactone	1573	+		+	tr	tr	1	2.7	2	3.5	E	(32)
163	Undecanoic acid	1654	+			tr			tr			E	(34)
164	4-Hydroxydecanoic acid lactone	1688	+		+	tr		tr	1			E	(32)
165	Dodecanoic acid	1760	+		+	1		tr	2.4	2	2	E	(34)
166	Tridecanoic acid	1851	+			tr			tr	1	2	E	(34)
167	Tetradecanoic acid	1945	+		+	1.2			2.5	2	obsc	E	(34)
168	Pentadecanoic acid	2037	+		+	tr			1		3	E	(34)
169	Hexadecanoic acid	2146	+		+	3			8	16	15	E	(34)
170	Heptadecanoic acid	n.d.			+							E	(34)
171	Linoleic acid	2344	+		+				12		2.5	A (18)	(34)
172	Octadecanoic acid	2348	+			tr			tr		3	E	(34)

For general details see Tables 1 and 2.

Table 5. Miscellaneous volatile components of 1987, 1988, and 1989 vintage Chardonnay juices and glycoside hydrolysates derived from these juices.

		Kovats[a] indices	1987[b] Free	1987[b] Enz	1987[b] H^+	1988[c] Free	1988[c] Enz	1988[c] H^+	1989[c] Free	1989[c] Enz	1989[c] H^+	Evidence	As grape or wine product
173	Maltol	1286						1.5	4	1	1	E	
174	2-Ethyl-3-methylmaleic anhydrid	1315				tr	tr	1	tr		1	A (18)	
175	2-Furoic acid	1334	+		+					1.5		E	(32)
176	2-Ethyl-3-methylmaleimide	1462	+		+	tr	1.6	7	4	2	13	A (18)	
177	2-Methyl-3-vinylmaleimide	1497	+		+	tr	tr		tr	1	10	A (18)	
178	5-(2-Hydroxyethyl)-4-methylthiazole	1507	+		+	tr	tr		3.2		1	E	(47)
179	Diethyltartrate	1556				tr	tr		tr			E	(32)
180	2-Ethylidene-3-methylsuccinimide	1623	+		+	tr		1				A (18)	

For general details see Tables 1 and 2.

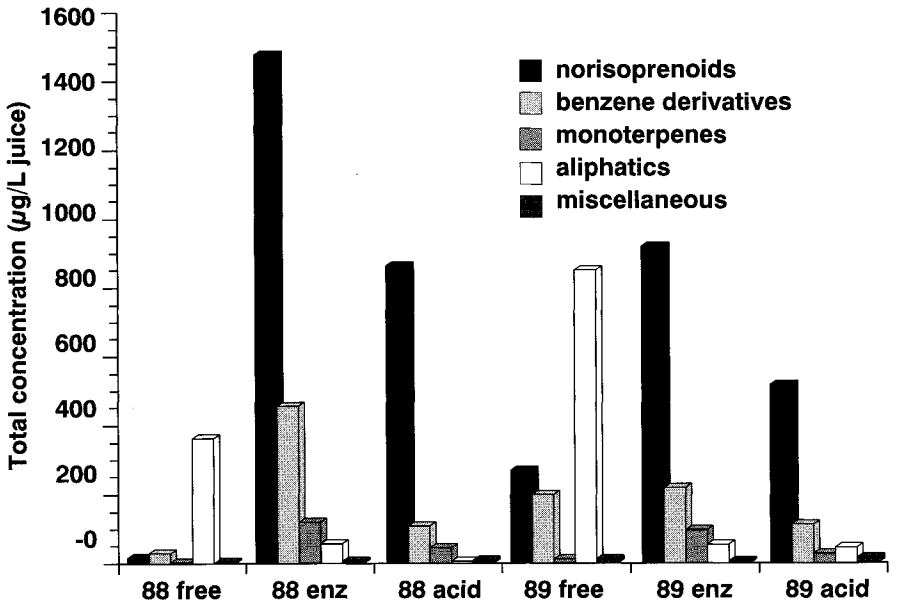

Fig. 1. Concentrations of five categories of volatiles, observed as free compounds (free) or after release by either glycosidase enzyme (enz) or acid hydrolysis (acid) of precursor fractions from the 1988 and 1989 Chardonnay juices.

found in the aglycone fraction, and thus *in vivo* catabolic pathways to **145-147** must be considered [see also Humpf and Schreier, (19)]. The observation in the free fraction of less than 1 µg/L quantities of the major fermentation esters, *e.g.*, ethyl octanoate **154** and ethyl decanoate **158** (Table 4), indicates that virtually no fermentation of the juice had commenced prior to analysis.

The extent of glycoconjugation was in evidence for all but the aliphatic compounds in the juices for both years (Fig. 1). The ratio of the total concentration of the enzyme- or acid-released products to the free components was different for the two years and, in the 1988 juice, this ratio indicated that glycosylation of the secondary metabolites was almost complete.

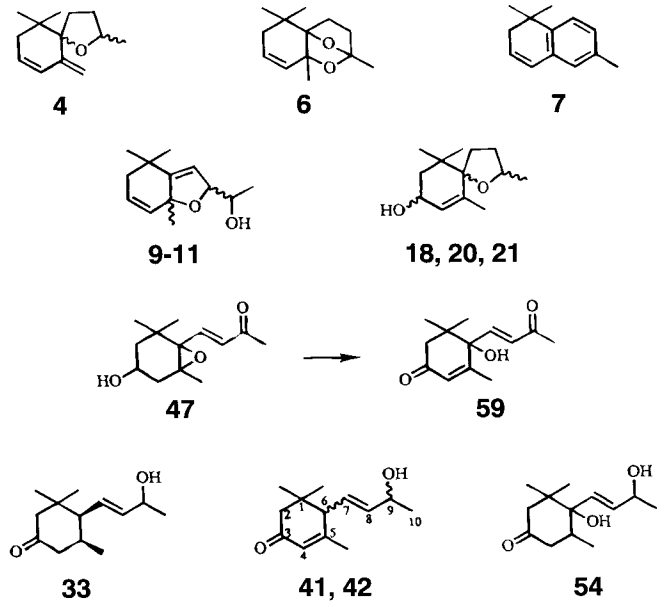

Fig. 2. Some norisoprenoids observed in this study. Names of compounds are indicated in Table 1.

Some norisoprenoids listed as enzyme hydrolysis products are presumably artifacts. These artifacts are derived from two sources. Firstly, extraneous acid hydrolysis of the aglycones, presumably during sample preparation can give, for example, vitispirane 4, the actinidols **9-11** (Fig. 2), the oxoedulans **12** and **13**, and the megastigmatrienones **24**, **25**, and **29** (Fig. 4). Facile formation of acid-generated products from labile precursors during enzyme hydrolysis and work-up has also

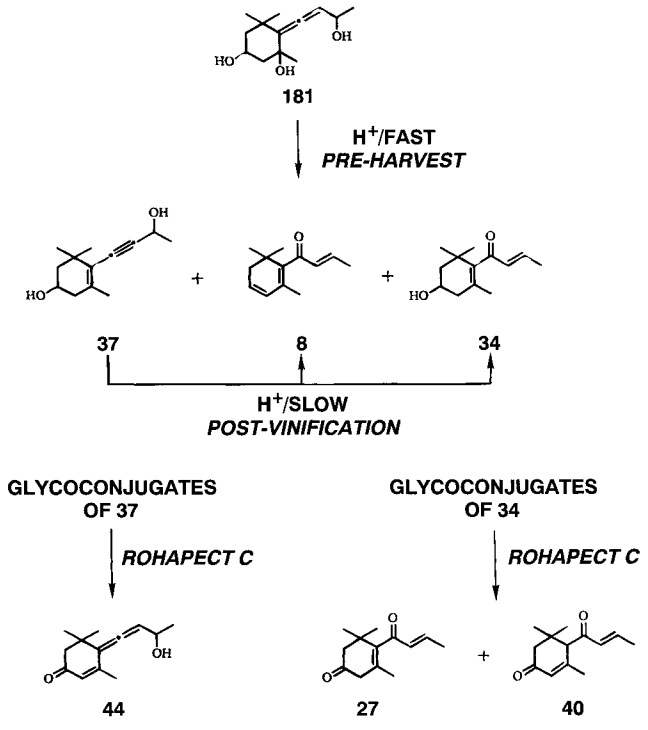

Fig. 3. The generation of damascenone **8** and related norisoprenoids in the free volatile fraction of Chardonnay juices and in the enzyme and acid hydrolysates of Chardonnay precursor fractions. Names of compounds are indicated in Table 1.

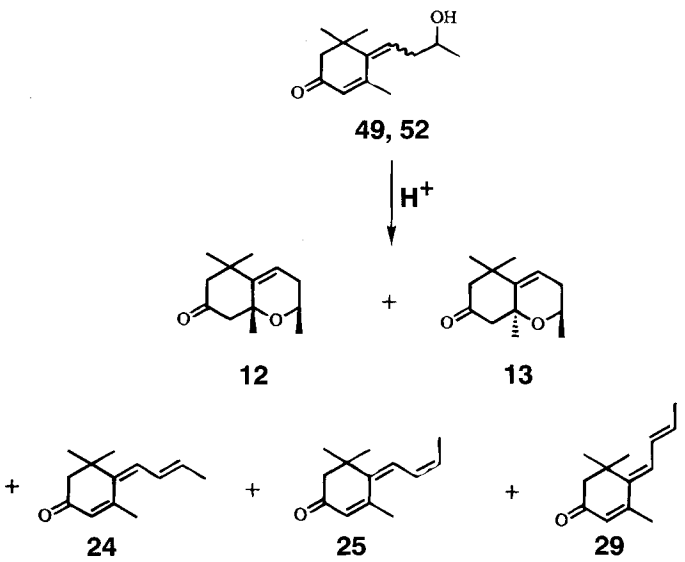

Fig. 4. The formation of acid hydrolysis products from the isomeric 9-hydroxymegastigma-4,6-dien-3-ones **49** and **52**. Names of compounds are indicated in Table 1.

been noted by Humpf and Schreier (19).

The second source of norisoprenoid artifacts was through the use of a high concentration of fungal enzyme which is known to generate some oxidative transformation products from genuine aglycones. High concentrations of these enzymes are necessary to effect near-complete hydrolysis of the glycoconjugate fraction (39). Compounds in this second group of artifacts are the oxodamascones **27** and **40** which are formed from conjugates of 3-hydroxydamascone **34**, the hydroxyketone **44** which is an artifact of oxidation of the enyne diol **37** (Fig. 3), and dehydrovomifoliol **59** which is derived, at least in part, from oxidation and rearrangement of the epoxide **47** (Fig. 2).

Some of the benzene derivatives listed in Table 2 may also be attributed to artifact formation. Thus, the relatively abundant 4-vinylguaiacol (**77**) and 4-vinylphenol (**78**) may be derived from the corresponding cinnamic acids through decarboxylation during gas chromatography (22).

Sensory studies on the 1987 (54) and 1989 (15) juice samples demonstrated that volatiles released by enzyme hydrolysis had no sensory effect on the aroma of wines to which the hydrolysates were added. Nevertheless, the enzyme products, through their relative abundance, aided in the analysis, and helped in rationalizing the formation of both the acid generated products and the minor free volatiles.

In contrast, the volatile fraction released by mild acid hydrolysis, under conditions simulating bottle ageing, had a highly significant sensory effect (15,54). Accordingly, compounds present mainly in the acid hydrolysis fraction are those most likely to contribute to the developed varietal character of Chardonnay wines. Such acid-generated fractions differ from those formed by enzyme hydrolysis in that many acid-catalyzed cyclization, dehydration and rearrangement products are given in the former (37,38). These acid-generated volatiles are the focus of discussion in this study.

Norisoprenoids (Table 1): Of the four biogenetic classes into which these Chardonnay components can be grouped, the norisoprenoids are dominant, both in number and concentration, accounting for about 70% of the hydrolytically-released volatile concentration (Fig. 1). Many of these compounds are key flavor components of foodstuffs and beverages and are also aroma constituents of leaf products and perfumes (33). Sixty norisoprenoids are listed in Table 1 and more than 40 of the still unidentified volatiles in the hydrolysates also appear to belong to this class.

Plausible routes for the biogenesis of the norisoprenoids listed in Table 1 from grape carotenoids have been suggested elsewhere (53). Key biogenetic steps that interrelate the majority of the norisoprenoid components are oxidation of a hydroxyl function at C-3 and reduction of a ketone function at C-9 (carotenoid numbering, shown for 3-oxo-α-ionol **41**, Fig. 2). Also, from the observed accumulation of ketones **33** and **54**, it seems that reduction of the 4,5- double bond of α-ionols is an important biogenetic process in these Chardonnay samples. Reduction of the side chain 7,8- double bond is a significant process, but leads to the formation of less abundant norisoprenoids than those derived from 4,5-double bond reduction. Compounds in which both 4,5- and 7,8- double bonds have been reduced, such as the recently reported oak component 9-hydroxymegastigman-3-one (37), were not observed in the Chardonnay juice samples.

The four 3-oxo compounds **33**, **41**, **42**, and **54** were the major components released by acid hydrolysis. However, because three of these ketones, **33**, **41**, and **54**, were also abundant in the enzyme-liberated aglycone fraction, which had little sensory importance (15,54), their contribution to Chardonnay varietal aroma is presumably limited. The sensory impact of the second isomer of 3-oxo-α-ionol **42**, found only in the acid hydrolysates, needs further investigation.

Several norisoprenoids with known flavor properties were formed at higher concentration during acid hydrolysis than during enzyme hydrolysis and these are discussed below.

*The 3-Oxoedulans **12** and **13** and 3-Oxomegastigmatrienes **24**, **25**, and **29**:* These five acid hydrolysis products are important flavorants of tobacco (23,51) and have recently been observed as constituents of model wine extracts of oak shavings (37). Wahlberg and Enzell (51) have suggested that these compounds may be formed from the isomeric ketones **49** and **52** (Fig. 4), and this is supported by recent data from this laboratory (37). The nexus between products **12**, **13**, **24**, **25**, **29** and their progenitors **49** and **52** was observed in the 1988 and 1989 Chardonnay samples (Table 1) and is also reflected in the 1987 data in which the ketones **49** and **52** were only minor components and their products **12**, **13**, **24**, **25**, and **29** were not detected.

*Vitispirane **4**, Riesling acetal **6**, and TDN **7**:* Recent

studies (57) have shown that the bicyclic norisoprenoids vitispirane **4** and 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN) **7** are formed in relatively high concentrations during acid hydrolysis of Riesling wines. These two volatiles, together with the tricyclic compound Riesling acetal **6**, were also formed in high concentration when glycosidic fractions from that variety were acid-hydrolyzed (57,58). The hydrocarbon TDN **7** has been commonly observed at concentrations in excess of its flavor threshold, and at such levels in wines, it is a contributor to the kerosene-like, bottle-aged character of many Riesling wines (42).

Although the acid hydrolysates from the Chardonnay precursor fractions studied here were particularly rich in norisoprenoids, they were comparatively poor in vitispirane **4**, Riesling acetal **6** and TDN **7**. Vitispirane **4** was produced in the hydrolysates at concentrations well below its threshold in wine (41), while TDN **7** was produced maximally in the 1989 sample at a concentration near to its detection threshold in white wines (41). This presumably accounts for the absence in aged Chardonnay wines of the type of bottle-aged development that characterizes Riesling. Vitispirane **4** was formed in the acid hydrolysates at the concentration expected from hydrolysis of its precursors, *i.e.,* conjugates of the isomeric hydroxytheaspiranes **18**, **20**, and **21** which were observed as aglycones (57).

The reason for the paucity of compounds **4**, **6**, and **7** in the Chardonnay hydrolysates has been attributed to a relative inactivity, in this variety, of enzymes reducing the 7,8- double bond of the megastigmane skeleton which appears to be a prerequisite to the formation of precursors of these three compounds (53).

Damascenone 8: The potent flavorant damascenone **8** was observed both as a free volatile in juice and as a component of the acid hydrolysates. However, the co-occurrence with **8** of various side products from its formation, suggests that the generation of damascenone in these two fractions may come about through different hydrolytic pathways. These pathways to **8** from enyne diol **37** and allenic triol **181** are illustrated in Figure 3.

Hydrolytic studies in our laboratory of synthetic substrates have shown that the allenic triol **181** reacted rapidly at juice pH and at room temperature to give damascenone as a minor product, along with 3-hydroxydamascone **34** and the enyne diol **37** as the major products (44). Furthermore, in the 1988 and 1989 enzyme hydrolysates, the observed ratio of 5:1 for the artifact ketones (**27** + **40**):**44** [*i.e.,* compounds formed from **34** and **37**, (see Fig. 3) (39)] was approximately the same as the ratio of 3-hydroxydamascone **34**:enyne diol **37**, given by the hydrolysis of synthetic allenic triol **181**. This supports the proposition that hydrolysis of the triol **181** was responsible for the formation of the damascenone that is present free in the pre-harvest juices, and simultaneously generated the aglycones **34** and **37**. Although the allenic triol **181** has not yet been identified as a grape component, its presumed progenitor, the so-called Grasshopper ketone **55** (38), was a significant component of the enzyme hydrolysates.

The hydrolytic studies of Skouroumounis *et al.* (44) have also shown that damascenone **8** was generated slowly at pH 3 as a minor product from both the enyne diol **37** and its C-9 glucoside, together with 3-hydroxydamascone **34** as the major product (Fig. 3). Importantly, at pH 3, neither 3-hydroxydamascone **34** nor its 3-glucoside gave any damascenone **8**, even after prolonged heating at 100°C. Both damascenone **8** and 3-hydroxydamascone **34** were formed in the Chardonnay acid hydrolysates (Table 1), and the quantity of damascenone **8** observed in these hydrolysates (*i.e.,* 10% - 15% of the concentration of **37** plus the artifact **44**) was that expected from hydrolysis of the free and conjugated enyne diol. Thus, the formation of damascenone **8** in the acid hydrolysates (analogous to post-harvest formation) can be accounted for entirely by hydrolysis of the free and conjugated enyne diol **37**.

Benzene derivatives (Table 2): After the norisoprenoids, benzene derivatives were the most diverse and abundant group of the hydrolytically released volatiles seen in the samples, accounting for between 10% and 20% of the total volatile concentration in the fractions. The majority of these compounds are presumably shikimate-derived, although several, such as the *ortho*-hydroxymethylbenzoates are probably formed from polyacetate precursors.

More of the individual compounds in this group were found at higher concentration as acid- or enzyme-released aglycones than as free compounds in the juices. However, for the 1989 sample, several components were present at a much higher concentration in the free fraction. These include benzyl alcohol 65, 2-phenylethanol **70**, vanillin **87**, 3-methoxytyrosol **94**, and the phenolic acid esters **99** and **100**. This observation emphasizes the variability of glycosidic binding of individual compounds, both within this constituent category and between years.

Among the compounds listed in Table 2 which have known sensory properties, there were none present at a concentration above threshold (5,10,12). Furthermore, only seven of these benzene derivatives **84**, **85**, **87**, **91**, **96**, **97**, and **109** were present at a greater concentration in the acid hydrolysates than in the enzyme-released fractions of both the 1988 and 1989 juices; even for these, the differences were not great and the absolute concentration of each was low. This indicates that the greater sensory impact of the acid hydrolysates compared to the enzyme hydrolysates (15,54) could have only been marginally contributed to by the group of compounds listed in Table 2. However, several of these volatiles such as vanillin **87**, raspberry ketone **101**, and zingerone **102**, are important aroma compounds in foodstuffs and would be expected to influence the flavor of Chardonnay samples when they occur at higher concentrations than were recorded in this study.

The cinnamic acids **118** and **119** are progenitors of the vinyl phenols **77** and **78**, respectively, through yeast fermentation (8,9) or by the artifact formation of these volatiles during GC analysis. The fermentative generation of volatiles from **118** and **119** means that these

cinnamic acids can possibly be important fruit-derived flavor precursors in wines. However, GC is an inadequate analytical method to quantify the cinnamic acids or to assess their likely contribution to wine flavor through **77** and **78**.

Monoterpenes (Table 3): The concentration of monoterpenes *(ca* 5% of the total volatile concentration) in the Chardonnay samples is the lowest found in any of the white grape varieties that we have examined so far (54), and this observation is consistent with the non-floral character of most Chardonnay wines. The data for the monoterpenes reported in Table 3 are similar to those given by Scienza *et al.* (36) for neutral clones of Chardonnay. The muscat clones of Chardonnay studied by those authors were reported to contain higher concentrations of monoterpenes. The major monoterpenes observed in the present study were the flavorless polyols (**Z**) and (**E**)-2,6-dimethylocta-2,7-diene-1,6-diols (**142**, **143**), and the occurrence of these as ubiquitous constituents of *V. vinifera* has been commented on previously (54).

Those compounds for which sensory threshold data are available (31) were present in both the free and acid-released volatile fractions at concentrations below their threshold values. It is unlikely that the monoterpenes could exert a significant aroma influence individually, either on the juice or on matured wines made from these juices.

One further monoterpene, *i.e.,* 2-hydroxy-1,8-cineole **134**, a volatile previously identified in the variety Sauvignon blanc but not in Chardonnay (4), was tentatively identified as a minor free and bound constituent in the 1988 Chardonnay juice.

Aliphatic and miscellaneous compounds (Tables 4 and 5): The majority of aliphatic constituents reported in Table 4 were found as free compounds in the juices and attention was focused on the free fraction for an assessment of these constituents in Chardonnay.

A range of C-4 to C-18 unbranched aliphatic acids, together with *n*-hexanol (**147**), two isomeric hexenols (**145** and **146**), and γ-butyrolactone (**150**) account for most of the aliphatic fraction of the samples. Of the acids, those with six to ten carbon chains were present at the highest concentration. Most of the aliphatic compounds listed in Table 4 have been reported before as constituents of several grape varieties (34), and they cannot be considered as volatiles specific to Chardonnay. Furthermore, those compounds for which flavor threshold data are available (45) were present in the juice at a concentration below their detection thresholds and accordingly their influence on juice or wine flavor would be limited.

More important are the C-4 to C-10 γ-lactones, of which the C-8 to C-10 homologues are the most potent (13). Although in absolute terms the concentrations of these compounds were low, they were each near their detection threshold, and collectively, these lactones may play a role in Chardonnay flavor. Only the C-8 and C-9 lactones were found as enzyme hydrolysis products, and overall, higher concentrations were released by acid hydrolysis. Presumably, glycosylation plays a minor role in conjugating these compounds or their precursors. Kinsella *et al.* (21) have proposed that thermal hydrolysis of triglycerides containing a hydroxyacid residue leads to the formation of lactones in milk. A similar mechanism may also account for the lactones found in the acid hydrolysates.

Among the compounds of miscellaneous biogenetic origin were several that may be considered as chlorophyll degradation products (6) and which were found mainly in the acid hydrolysates. Three of these, 2-ethyl-3-methylmaleic anhydride (**174**), 2-ethyl-3-methylmaleimide (**176**) and 2-methyl-3-ethylidine succinimide (**180**), have been isolated from various leaf and food products and their flavors have been described (14,17,20,23,28,30,35). 2-Methyl-3-vinylmaleimide (**177**) has not, to our knowledge, been previously reported as a natural product.

Conclusions

Acid hydrolysis of the glycosides gave many products different from those found in the enzyme hydrolysates, both in chemical structure and concentration. Acid hydrolysis of the bound fraction is implicit during cellar maturation of wines, a process of particular importance to non-floral varieties such as Chardonnay (1).

The volatiles of Chardonnay are apparently formed through several secondary metabolic pathways. Compounds from branches of the shikimic acid biosynthetic pathway that give aromatic amino acids, benzoic acids, cinnamic acids, and lignins are present together with mevalonic acid-derived volatiles. The latter compounds arise either directly, *i.e.,* as monoterpenes or, in the case of the norisoprenoids, from the apparent degradation of carotenoid pigments. Additionally, some of the volatiles are possibly derived from chlorophyll pigments while others come from primary metabolites of the vine. Considering the disparate origins of the many compounds found, and the multiplicity of enzymes that are needed for the synthesis and transformation of these volatiles, it is to be expected that great variation could occur in the formation and accumulation of individual constituents.

The observation of individually important food flavorants, *e.g.,* damascenone, 1,1,6-trimethyl-1,2 dihydronaphthalene, several of the benzene derivatives and the γ-lactones, suggests their potential importance to Chardonnay flavor. In the samples studied, most of these compounds were present at concentrations near to or below their individual sensory thresholds. However, in absolute terms only a small increase in concentration, resulting from a stimulation of one of the biosynthetic pathways discussed above, could result in any one of these potential flavorants having a directing aroma influence. It is important to recognize that norisoprenoids and volatile benzene compounds are also flavor components of oak wood (Tables 1 and 2),

Thus, the concentrations of many of these natural

grape compounds could be enhanced during barrel maturation of Chardonnay wines. This could account for the harmony that appropriate wood treatment can bring to Chardonnay wine flavor.

In the case of the specific Chardonnay juices studied here, acid hydrolysis of the glycosides of the 1989 juice gave volatiles exhibiting some of the key varietal aromas that a wine prepared from the juice also possessed, *i.e.,* tea, lime, and honey (15). Thus, the many compounds found in the acid hydrolysate from the 1989 juice (Tables 1 to 5) are, in the combination found, capable of imparting these varietal wine attributes. Although the number of volatiles observed in the acid hydrolysates precludes sensory studies on individual compounds, it is noteworthy that many of the norisoprenoids have been found in leaf products such as tobacco and tea (27). The assignment by the sensory panel of a significant tea aroma to the Chardonnay hydrolysates (15) is an indication of the likely contribution of the norisoprenoids to this varietal wine flavor attribute.

Future investigations on this variety should involve sensory studies on individual constituents within the group of released flavorants, and thus lead to a more complete understanding of flavor development in Chardonnay wines.

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