Volatile Norisoprenoid Compounds as Constituents of Oak Woods Used in Wine and Spirit Maturation

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Thirty-one norisoprenoid compounds have been identified for the first time in model wine extracts of American and Vosges oak woods. Only one compound of this type, β-ionone, had been previously reported as an oak constituent. The four isomeric 3,4-dihydro-3-oxoactinidols [i.e., isomers of 2,2,6-trimethyl-8-(1-hydroxyethyl)-7-oxabicyclo[4.3.0]non-9-en-4-one], (*E*)- and (*Z*)-9-hydroxymegastigma-4,6-dien-3-one, 9-hydroxymegastigm-4-en-3-one (blumenol C), and two diastereomers of each of the two oxoedulan derivatives 2,3,5,6,8,8a-hexahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one and octahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one were found at a collective concentration of 11.6 mg/kg of wood in the American sample but were either absent or found at low concentrations only in the French timber. The Vosges oak yielded 9-hydroxymegastigm-5-en-4-one and two diastereomers of 2,6,10,10-tetramethyl-1-oxaspiro[4,5]decan-7-one as significant constituents. Most of the newly recognized oak norisoprenoids have been previously identified in grape products, thus further linking the compositions of oak and wines. The work reports, for the first time, 5*S**,6*R**-9-hydroxymegastigm-7-en-3-one and (tentatively) 9-hydroxymegastigman-3-one as natural products.

INTRODUCTION

The contribution of oak extractives to the flavor of barrel-aged alcoholic beverages has been the subject of much research. Over 200 volatile components of oak wood have so far been identified, and the aroma and flavor properties of some of these have been investigated (Masuda and Nishimura, 1971; Nishimura et al., 1983; Maga, 1985; Nykänen et al., 1985; Puech and Moutounet, 1988; Boidron et al., 1988). While many of these volatile compounds are presumably components of untreated oak, others appear to be generated by thermal degradation of oak macromolecules during barrel-making or by hydrolytic or oxidative pathways during extraction.

The majority of known volatile oak extractives can be categorized according to their biogenetic or chemical origin. Thus, volatile phenols based on the 4-hydroxy-3-methoxyphenyl (guaiacyl) or 4-hydroxy-3,5-dimethoxyphenyl (syringyl) nucleus are generally regarded as products of lignin degradation, while substituted furans and pyrans are presumed to be formed by thermolysis of cellulose and hemicellulose during coopering. Other groups of volatile oak-wood extractives include γ-lactones, mono- and sesquiterpenes, hydrocarbons, and fatty acids (Nishimura et al., 1983). Pyrazines and pyridines have been observed as components of charred oak (Maga, 1985).

This paper reports the identification, for the first time, of a group of 13-, 11-, and 9-carbon norisoprenoids as significant components of model wine extracts of American and French oak shavings. Previously, only one member of this class, β-ionone (**4**) (Figure 1), had been reported from oak-wood sources (Nishimura et al., 1983). Such compounds, which are thought to be degraded carotenoids (Wahlberg and Enzell, 1987), are important to the flavor of tobacco, tea, and some fruits (Schreier, 1984) and also occur, mainly as glycoconjugates, in both black and white grape varieties and in wines (Abbott et al., 1990; Sefton et al., 1989; Winterhalter et al., 1990).

EXPERIMENTAL PROCEDURES

General procedures, including the isolation of volatiles by Freon Extraction and analysis of these extracts by gas chromatography–mass spectrometry (GC–MS), were as described previously (Sefton et al., 1989), except that mass spectra were acquired with a Finnigan TSQ 70 mass spectrometer. Concentrations of norisoprenoid components in the oak samples were determined by comparing the intensities of the component peaks with that of added 1-octanol standard in the total ion chromatograms of both the oak extracts and standard solutions of available reference compounds.

Preparation of Oak Extracts. Staves and headboards of green oak, harvested from the Vosges region in France and from a single 40-acre area in Ohio, were seasoned in the open air for 6 months in Adelaide, Australia. A sample of oak from each source, comprising 28 randomly selected staves and headboards (1% of the total), was taken. A 25-cm piece was cut from the center of each stave and a 12-cm piece from the center of each headboard. The exterior of the oak pieces was trimmed to a depth of 2 mm with an electric plane, and the shavings were discarded. A further 2 cm was trimmed from the end of each piece of oak with the electric plane set to cut to a depth of 1 mm. These end-shavings were combined, and a portion (200 g) was soaked at 50 °C for 7 days, under a nitrogen atmosphere, in a model wine solution (3600 mL), prepared by saturating aqueous ethanol (12% v/v) with potassium hydrogen tartrate and adjusting the pH to 3.2 by the addition of a 10% aqueous solution of tartaric acid. The extracts were cooled and the oak shavings removed by filtration through sintered glass. Volatile components of these model wine extracts were isolated with Freon, prior to analysis by GC–MS. Extraction and analyses of the model wine extracts were carried out in duplicate.

Reference Samples. Compounds **1**, **2**, **13**, **16**, **17**, **19**, **21–28** and a mass spectrum of **20** were obtained as described previously (Sefton et al., 1989; Winterhalter et al., 1990), while samples of **3**, **5–8**, **11**, and **12** were donated. β-Ionone (**4**) was commercially available. The isomeric megastigmatrienones **10** and **14** were prepared as described by Aasen et al. (1972). A sample of the ketone **15**, which chromatographed as a single peak on GC, was obtained by flash chromatography as a byproduct of the synthesis of blumenol C, described earlier (Sefton et al., 1989). An additional byproduct of this reaction was tentatively identified as the ketone **18**.

Compounds **3**, **5**, **7**, **9**, **11**, **15**, and **18** had the following spectral data, which were not available in the literature. **3**: EIMS [*m*/*z* (relative intensity)] 208 (25), 193 (100), 164 (10), 151 (50), 149 (10), 124 (35), 123 (25), 122 (10), 109 (65), 107 (55), 105 (15), 93 (15), 91 (20), 81 (20), 79 (20), 77 (20), 57 (20), 51 (25), 49 (25), 43 (65), 42 (20), and 41 (20). **5**: EIMS [*m*/*z* (relative intensity)] 208 (10), 193 (100), 166 (10), 164 (2), 151 (15), 149 (7), 124 (35), 123 (10), 109 (90), 107 (30), 105 (10), 93 (10), 91 (10), 86 (7),

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Table I. Norisoprenoids Observed in Oak-Wood Extracts

compd	Kovats indices[a]	concn[b] American	concn[b] Vosges	evidence for assignment[c]	as grape products
(1) 2,6,6-trimethylcyclohex-2-ene-1,4-dione	1297	180	nd	A	*e*
(2) vitispirane	1327	tr	70	A	*e*
(3) (5*R**,9*R**)-3,4-dihydro-3-oxoedulan [i.e., (2*R**,8a*R**)-2,3,5,6,8,8a-hexahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one]	1592	450	nd	A	
(4) β-ionone	1614	nd	tr	B	*f*
(5) (5*S**,9*R**)-3,4-dihydro-3-oxoedulan [i.e., (2*R**,8a*S**)-2,3,5,6,8,8a-hexahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one]	1616	1450	tr	A	
(6) (5*R**,6*R**,9*R**)-4,5-dihydro-4-oxotheaspirane [i.e., (2*R**,5*R**,6*R**)-2,6,10,10-tetramethyl-1-oxaspiro[4,5]decan-7-one]	1646	nd	15	A	
(7) (5ξ,6*R**,9*S**)-tetrahydro-3-oxoedulan [i.e., (2*S**,4a*R**,8aξ)-octahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one]	1660	270	nd	A	
(8) (5*S**,6*R**,9*R**)-4,5-dihydro-4-oxotheaspirane [i.e., (2*R**,5*R**,6*S**)-2,6,10,10-tetramethyl-1-oxaspiro[4,5]decan-7-one]	1718	nd	75	A	
(9) isomer of tetrahydro-3-oxoedulan (i.e., octahydro-2,5,5,8a-tetramethyl-7*H*-1-benzopyran-7-one)	1722	60	nd	B	
(10) (6*Z*,8*E*)-megastigma-4,6,8-trien-3-one	1750	60	nd	A	*g*
(11) (6*S**,9*S**)-theaspirone	1752	tr	nd	A	*h*
(12) dihydroactinidiolide	1802	70	nd	A	*f*
(13) (isomer 1) 3,4-dihydro-3-oxoactinidol	1805	150	tr	A	*e*
(14) (6*E*,8*E*)-megastigma-4,6,8-trien-3-one	1810	40	nd	A	*g*
(13) (isomer 2) 3,4-dihydro-3-oxoactinidol	1815	270	tr	A	*e*
(13) (isomer 3) 3,4-dihydro-3-oxoactinidol	1830	200	20	A	*e*
(15) (5*S**,6*R**)-9-hydroxymegastigm-7-en-3-one	1848	70	120	A	*e*
(16) 3-hydroxy-β-damascone	1851	20	tr	A	*e*
(13) (isomer 4) 3,4-dihydro-3-oxoactinidol	1853	300	30	A	*e*
(17) 3-oxo-α-damascone	1893	tr	nd	A	*e*
(18) (5*S**,6*S**)-9-hydroxymegastigman-3-one	1902	100	120	B	
(19) 3-oxo-α-ionol	1937	450	900	A	*e*
(20) 9-hydroxymegastigma-4,6,7-trien-3-one	1948	tr	nd	C	*e*
(21) 3,4-dihydro-3-oxoactinidiolide	1959	tr	nd	A	*e*
(22) 3-oxo-α-ionone	1960	50	130	A	*e*
(23) 9-hydroxymegastigm-5-en-4-one	1988	nd	800	A	*i*
(24) (isomer 1) 9-hydroxymegastigma-4,6-dien-3-one	2001	2900	obsc	A	*e*
(25) blumenol C	2002	3100	430	A	*e*
(24) (isomer 2) 9-hydroxymegastigma-4,6-dien-3-one	2081	2500	30	A	*e*
unknown[d]	2121	80	260	nd	*d*
(26) vomifoliol (blumenol A)	2180	230	250	A	*e*
(27) dehydrovomifoliol	2212	50	30	A	*e*
(28) 7,8-dihydrovomifoliol (blumenol B)	2244	tr	nd	A	*e*

[a] For a 30-m J & W DB1701 fused silica column, 0.25-mm i.d. and 0.25-μm film thickness, with helium carrier gas at a linear velocity of 40 cm/s. The column was held at 60 °C for 1 min, programmed at 4 °C/min to 250 °C, and held at this temperature for 20 min. [b] As micrograms per kilogram of oak shavings; tr, trace; obsc, obscured; nd, not detected. [c] A, the mass spectrum was the same as that of the reference, and the peak was enhanced by the reference compound when cochromatographed. B, see Experimental Procedures and Results. C, mass spectrum the same as that of a donated reference spectrum. [d] This is the same as unknown 17 in Strauss et al. (1987a) and was also seen in Riesling wine by Winterhalter et al. (1990). Several other norisoprenoid unknowns, not seen previously in wine grapes, have not been included in the table. [e] Sefton et al. (1989). [f] Schreier (1976). [g] Williams et al. (1982). [h] Seen in this laboratory in Riesling wine. [i] Winterhalter et al. (1990).

84 (8), 81 (80), 80 (12), 79 (15), 77 (10), 57 (10), 55 (10), 51 (10), 49 (8), 43 (35), and 41 (25). **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77 (s, 3, 1-CH_3), 1.03 (s, 3, 1-CH_3), 1.10 (d, 3, J = 6.0 Hz, 9-CH_3), 1.19 (d, 3, J = 0.9 Hz, 5-CH_3), 1.31 [m, 1, J = 45.6 Hz, 8_{ax}-H (decoupled on irradiation at δ 3.74)], 1.49 (m, 1, J = 45.2 Hz, 7_{ax}-H), 1.77 (m, 3, 6-, 7_{eq}-, and 8_{eq}-H), 2.16 (A part of ABX, 1, J_{AB} = 13.6, J_{AX} = 2.1 Hz, 2_{eq}-H), 2.30 (B part of ABX, 1, J_{AB} = 13.6 Hz, 2_{ax}-H), 2.46 (A part of $ABMX_3$, 1, J_{AB} = 12.3, J_{AM} = 2.1 Hz, 4_{eq}-H), 2.52 (B part of $ABMX_3$, 1, J_{AB} = 12.3, J_{BX} = 0.9 Hz, 4_{ax}-H), and 3.74 (ddq, 1, J_d = 11.7, J_d = 2.1, J_q = 6.0 Hz, 9-H); EIMS [m/z (relative intensity)] 210 (5), 195 (85), 153 (100), 139 (10), 138 (18), 137 (8), 123 (18), 111 (50), 110 (30), 96 (25), 95 (35), 85 (10), 83 (30), 82 (20), 81 (10), 69 (55), 67 (25), 55 (30), 43 (65), 41 (45), and 39 (15). **9**: EIMS [m/z (relative intensity)] 195 (100), 153 (55), 125 (10), 123 (18), 112 (20), 111 (60), 96 (30), 95 (35), 93 (20), 83 (50), 81 (25), 69 (40), 55 (35), 51 (55), 49 (60), 43 (50), and 41 (30). **11**: EIMS [m/z (relative intensity)] 208 (<1), 193 (1), 166 (1), 165 (1), 152 (100), 124 (7), 123 (6), 111 (14), 110 (60), 96 (10), 82 (12), 69 (10), 68 (8), 55 (7), and 43 (8). **15**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 and 0.80 (each br s, 1_{ax}-CH_3), 0.90 and 0.93 (each d, J = 5.8 Hz, 5-CH_3), 0.93 and 0.95 (each s, 1_{eq}-CH_3), 1.28 (d, 3, J = 6.4 Hz, 10-H_3), 1.84 [br d, 1, J = 9.3 Hz, 6-H (decoupled on irradiation at δ 5.35)], 1.99 (br, A part of ABMX, 1, J_{AB} = 13.4 Hz, 4_{ax}-H), 2.09 (br, A part of ABX, 1, J_{AB} = 13.2, J_{AX} = 2.2 Hz, 2_{eq}-H), 2.26 (br, B part of ABX, 1, J_{AB} = 13.2 Hz, 2_{ax}-H), 2.36 (B part of ABMX, 1, J_{AB} = 13.4, J_{BM} = 3.8, J_{BX} = 2.2 Hz, 4_{eq}-H), 4.33 (dq, 1, J_d = 6.3, J_q = 6.4 Hz, 9-H), 5.35 (br, A part of ABMX, 1, J_{AB} = 15.4, J_{AM} = 9.3 Hz, 7-H), 5.60 (B part of ABMX, 1, J_{AB} = 15.4, J_{BX} = 6.3 Hz, 8-H). The signal for 5-H was obscured. **18**: EIMS [m/z (relative intensity)] 212 (2), 197 (5), 194 (8), 179 (30), 161 (8), 151 (10), 138 (45), 137 (30), 110 (30), 109 (20), 96 (95), 95 (60), 83 (75), 81 (100), 69 (60), 68 (35), 67 (30), 55 (70), 45 (35), 43 (60), 41 (70), and 39 (20). Additional spectral data for these compounds and for all other compounds listed in Table I are recorded in the literature (Aasen et al., 1972; Kaiser et al., 1978; Sefton et al., 1989; Winterhalter et al., 1990).

RESULTS

The norisoprenoids identified in the model wine extracts of the American and Vosges oak samples are listed in Table I, together with the evidence for the assignments and retention indices. Additionally, the occurrences of these compounds in grape products is also recorded. The structural elucidation of many of these compounds as grape or wine components has already been discussed (Sefton et al., 1989; Winterhalter et al., 1990). Additional structural information was obtained as follows.

The reference compound **7**, which was a donated synthetic sample, comprised one main isomer (purity

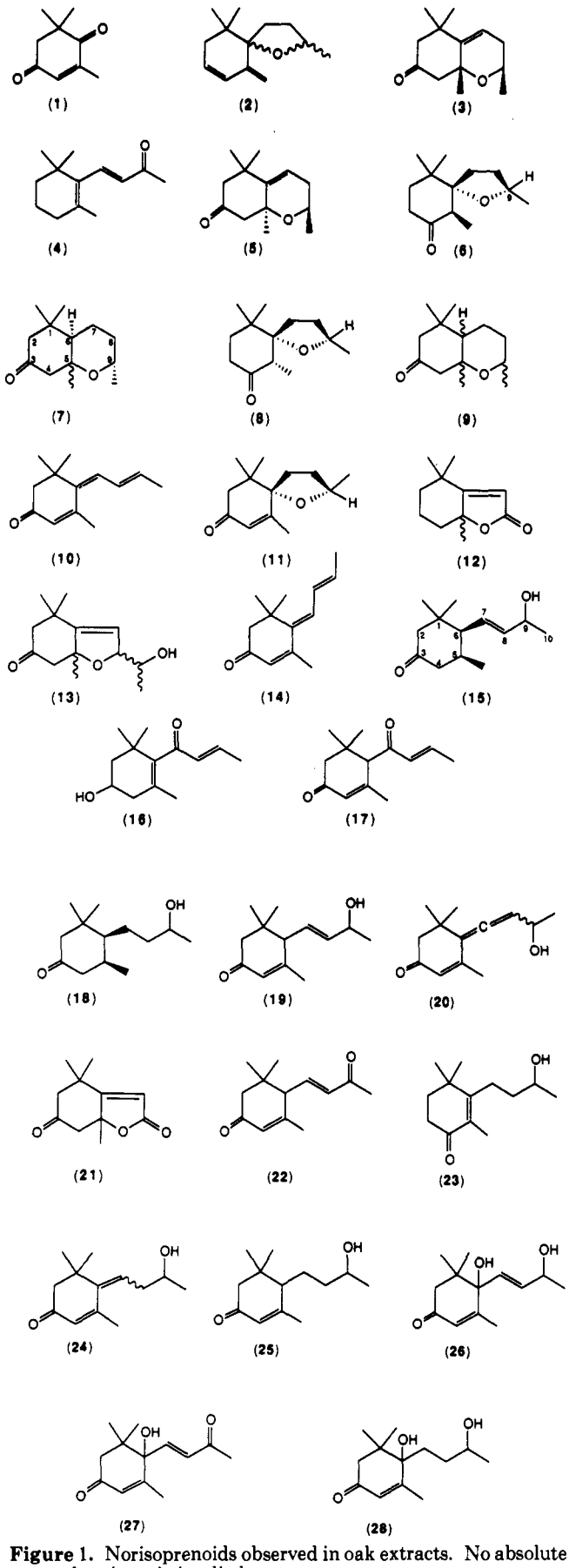

Figure 1. Norisoprenoids observed in oak extracts. No absolute stereochemistry is implied.

>99% by GC–MS). The high-field ^{1}H NMR spectrum showed diaxial coupling between H-9 and H-8_{ax} (carotenoid numbering, see Figure 1), and the sum of the couplings to H-7_{ax} (45.2 Hz) indicated that H-6 was also axial. The oxoedulan **7** therefore has 6*R**,9*S** relative stereochemistry, with the stereochemistry at C-5 as yet undefined.

The reference sample **7** also contained two trace components with similar mass spectra to **7** but longer GC retention times. These were tentatively identified as diastereoisomers of **7**. The last eluting component **9** had a mass spectrum and retention time identical with those of a component of the American oak extract, and the latter is therefore also tentatively assigned as a diastereoisomer of **7**.

The reference sample of the ketoalcohol **15** was obtained as a byproduct of the hydrogenation of 3-oxo-α-ionol (**19**) to blumenol C (**25**) and was identified by its mass spectrum (Sefton et al., 1989) and high-field ^{1}H NMR spectrum. In the latter, the absence of a large diaxial coupling between either of the C-4 protons and the C-5 proton indicated that the secondary methyl group at C-5 was axial. A consideration of models suggests that the side chain at C-6 is cis to the C-5 methyl group since both groups should adopt equatorial stereochemistry when they are trans disposed. Pairs of signals for the methyl groups at C-1_{ax}, C-1_{eq}, and C-5 indicated that the synthetic sample was, as expected, a mixture of diastereoisomers with differing relative configurations between C-6 and C-9. Another byproduct of the reduction of **19** to **25**, observed only by GC–MS, had a mass spectrum identical with but a longer retention time than **15** and was presumably the C-5 epimer.

The 5*S**,6*R** isomer **15** was observed in model wine extracts of both the American and Vosges oak. Comparison with the reference compounds also showed the presence of this isomer **15** in grape extracts, confirming an earlier, tentative assignment (Sefton et al., 1989). These are the first reported occurrences of **15** as a natural product.

The oak component **18** had the same mass spectrum and retention time as an additional byproduct observed by GC–MS of the reduction of **19** to **25**. Yields of the byproduct **18**, which had absorbed 2 equiv of hydrogen (M^+ 212), increased with prolonged reaction times, and this compound was therefore tentatively assigned the structure shown. The norisoprenoid **18** has not been previously reported as a natural product.

β-Ionone (**4**) was earlier observed as an oak-wood component by Nishimura et al. (1983). Both single stage and daughter ion (*m*/*z* 177) mass spectra of a component with retention time identical with that of a reference sample of β-ionone indicated the presence of this compound in the Vosges oak extract. However, the spectra were extremely weak, and this assignment is only tentative.

DISCUSSION

Initial examination of known oak-wood constituents in the extracts of the American and Vosges oak samples, each seasoned in Australia, showed little variation between these two samples in the composition of lignin-derived compounds or in the concentration of the oak lactones. Some workers have reported that oak lactone levels vary widely with origin and species of oak (Guymon and Crowell, 1972; Otsuka et al., 1974). However, experiments by Maga (1989) and in our laboratories (Sefton et al., 1990) have shown that oak lactone concentrations can be strongly influenced by seasoning conditions and that oak lactone levels may depend more on this factor than on oak type.

More than half of the volatile components of the two extracts were compounds not previously reported as oak-wood constituents. Among these previously unrecognized oak volatiles, the norisoprenoid compounds given in Table

I were present in significant concentration. Importantly also, the norisoprenoids showed the greatest variation between the two oak extracts, with a higher overall concentration and greater variety of these compounds in the American than in the Vosges oak (Table I). The American oak extract included the four isomeric 3,4-dihydro-3-oxoactinidols (**13**), both *E* and *Z* isomers of the hydroxydienone **24**, and blumenol C (**25**), together with the bicyclic ethers **3**, **5**, **7**, and **9** as major components, whereas these compounds were either absent from or present in small amounts only in the Vosges oak extract. Conversely, 4-oxo-7,8-dihydro-β-ionol (**23**) was a major norisoprenoid of the French oak but was not observed in the American oak extract. Similarly, the spiro compounds **6** and **8** were only found in the Vosges oak extract.

These differences were also observed between samples of oak from the same sources but seasoned in the country of origin (data not shown). Whether these differences are typical of American and French oak as a whole or only of the two forests from which the samples were obtained has yet to be determined.

Several of these norisoprenoid oak constituents may well contribute to oak-derived flavor in wines and spirits. Compounds **3**–**10** and **14** have all been patented as flavor additives in the food, tobacco, and perfume industries. The (*Z*,*E*)- and (*E*,*E*)-megastigmatrienone isomers **10** and **14** are key flavor constituents of Burley tobacco (Ohloff, 1978) and have a spicy, peppery note (Lloyd et al., 1976; Rowland, 1965; Hasagawa, 1981). The oxoedulan **5** and the saturated analogue **7**, with undefined stereochemistry, are also components of Burley tobacco (Demole and Berthet, 1972; Schumacher, 1968; Roberts and Schumacher, 1965), and the former also occurs as a volatile constituent of passionfruit (Winter et al., 1979). A synthetic mixture of isomers of the saturated oxoedulan **7** has a camphor-like odor, while a mixture of isomers of the unsaturated analogue **5** has an oriental tobacco flavor (Lloyd et al., 1976). The oxaspiro compounds **6** and **8**, together with their corresponding C-9 epimers, are constituents of *Osmanthus* absolute (Kaiser et al., 1978), and the mixture of all four diastereoisomers has been described as having a woody, dried fruit and tobacco-like odor (Kaiser and Naegeli, 1978, 1980). β-Ionone (**4**) has a low sensory threshold and a pleasant violet-like aroma (Ohloff, 1978).

In addition to the compounds listed in the table, there are several unknown compounds in the oak extracts that also appear to belong to the norisoprenoid class, and these may also include important flavorants.

The co-occurrence of the unsaturated oxoedulans **3** and **5** with the ketoalcohols **24**, of the saturated oxoedulans **7** and **9** with blumenol C (**25**), and of the oxaspiro compounds **6** and **8** with the ketoalcohol **23** suggests product–precursor relationships within each of these groups of compounds. The formation of these oxoedulans and oxaspiro compounds by intramolecular acid-catalyzed conjugate addition may well take place only during the extraction of the oak components into the acidic medium of the model wine. Such reactions have already been utilized in the synthesis of the oxoedulans **5** and **7** (with undefined stereochemistry) as flavor additives (Schumacher, 1968; Roberts and Schumacher, 1965).

Dehydration of the isomers of the hydroxydienone **24** could also account for the formation of the megastigmatrienones **10** and **14**. Although these compounds, **10** and **14**, have been shown to be formed as major products of dehydration of 3-oxo-α-ionol (**19**) with strong acid (Aasen et al., 1972; Strauss et al., 1987b), they were presumably not formed from this precursor under the conditions of model wine extraction used here as the Vosges oak extract, which contained the larger proportion of 3-oxo-α-ionol (**19**), yielded no detectable trienones **10** and **14**.

CONCLUSION

Our research on the composition of volatiles derived from grapes and from oak wood is increasingly showing a link between the two. Some of the lignin-derived volatiles from oak wood have also been isolated from grape sources and can be formed by acid hydrolysis of involatile precursors from grapes under conditions simulating bottle aging (Abbott et al., 1990; Williams et al., 1989). Similarly, the majority of the oak-wood norisoprenoids reported here are, in conjugated form, significant components of white and black grape varieties (Sefton et al., 1989; Abbott et al., 1990) and also of Riesling wine (Winterhalter et al., 1990). Presumably it is this conjugation that prevents the intramolecular cyclizations of the alcohols **23**–**25** to give the bicyclic compounds **3** and **5**–**9**, and hence this latter group of compounds has not yet been identified in these grape sources.

Winemakers have traditionally distinguished between flavors derived from oak wood of different origins. Variation in norisoprenoid composition could account, at least in part, for some of these observed flavor differences.

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