1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) Formation in Wine. 1. Studies on the Hydrolysis of 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol Rationalizing the Origin of TDN and Related C_{13} Norisoprenoids in Riesling Wine

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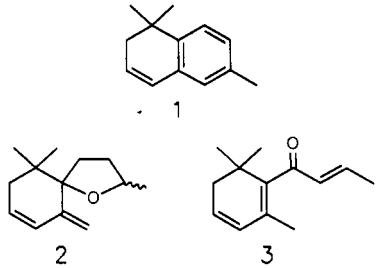
In a glycosidic fraction of Riesling wine, obtained by retention of the glycosides on C_{18} reversed-phase adsorbent followed by enzymatic hydrolysis and trimethylsilylation of the liberated aglycons, capillary gas chromatography (HRGC) and capillary gas chromatography–mass spectrometry (HRGC–MS) revealed the occurrence of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol. The identification was verified by comparison of HRGC and MS data of TMS derivatives of the new natural product with those of an authentic reference compound. Heating a diastereoisomeric mixture of synthetic 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diols under simultaneous distillation/extraction (SDE) conditions at pH 3.2 gave a number of volatile C_{13} norisoprenoid degradation products, including the important off-flavor-causing wine constituent 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). Additional volatile degradation products, which have also been found in grape products, included 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.$0^{1,6}$]undec-4-ene (Riesling acetal), 4-(2,3,6-trimethylphenyl)-2-butanone, 2,10,10-trimethyl-6-methylene-1-oxaspiro[4.5]dec-7-en-2-ol, and, tentatively, 6-hydroxy-1,1,6-trimethyl-1,2,5,6-tetrahydronaphthalene.

INTRODUCTION

The bouquet of a wine, formed by the action of several hundred different flavor compounds on the sensory organs, is easily influenced by the generation of undesirable aroma compounds during the production and storage of wine. In many cases the resulting wine fault is of a complex nature and, as such, extremely difficult to analyze. However, there are some cases in which single aroma substances have been identified as off-flavor-causing wine constituents. Examples are the 2-acetyltetrahydropyridines, responsible for a "mousy" taint in wine (Strauss and Hersztyn, 1984), and 2,5-dimethyl-4-hydroxy-2,3-dihydro-3-furanone (furaneol), a potent odorant, causing a pronounced "strawberry-like" note in some new grape hybrid cultivars (Rapp et al., 1980).

A further undesired aroma substance is the C_{13} hydrocarbon 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN, 1). This compound is generally absent in grapes and young



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wines but develops during the maturation or aging of wine. High concentrations of 1 have been found in the Riesling variety with maximum concentrations reaching almost 200 ppb (Simpson and Miller, 1983; Strauss et al., 1987a; Winterhalter et al., 1990b). In such high concentrations, i.e., 5–10 times above the flavor threshold of 1, which is approximately 20 ppb in wine (Simpson, 1978), TDN produces a pronounced off-flavor, and the wines are generally described as "kerosene or petrol like".

Initial research into the origin of 1 showed that this compound is obviously formed by a hydrolytic degradation of nonvolatile precursor substances (Williams et al., 1982). For Riesling wine, the precursors of TDN, which are thought to be acid-labile glycoconjugates, are mainly located in the juice of the grape and appear to develop along with sugar accumulation (Strauss et al., 1987a). Although easily isolated from grape juice or wine by selective retention on C_{18} reversed-phase adsorbent (Williams et al., 1982) or on Amberlite XAD-2 resin (Günata et al., 1985), the progenitors are present in a highly complex mixture of polar substances. For the analysis of these mixtures a variety of enzymatic as well as chromatographic techniques has been applied (Bitteur et al., 1989; Günata et al., 1988; Schwab and Schreier, 1988). However, due to the structural diversity of the numerous bound constituents present in wine, progress in the separation and purification of individual precursor conjugates has been slow.

In recent years, the all-liquid chromatographic technique of countercurrent chromatography has been found to be of paramount importance in the fractionation of these mixtures. Especially, droplet countercurrent chromatography (DCCC) has been employed for the preparative separation of precursor isolates from grape juice and wine (Strauss et al., 1987b; Winterhalter et al., 1990a). In combination with HRGC–MS analysis, this technique revealed the presence of almost 100 variously glycosylated aglycons in Riesling wine. The same technique has also been employed for the separation of precursors of the target compound 1 and the related C_{13} norisoprenoids, isomeric vitispiranes 2, and damascenone 3 (Winterhalter et al., 1990b). As a result of DCCC prefractionation and subsequent heat treatment of separated DCCC fractions, it was concluded that multiple precursors are responsible for the formation of these important C_{13} flavor compounds in wine. However, contrary to vitispiranes 2 and damascenone 3, for which the principal progenitors are known today (Winterhalter and Schreier, 1988; Sefton et al., 1989;

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Winterhalter et al., 1990b, 1991; Näf et al., 1990), the question of TDN formation remained unsolved. This study reports for the first time the identification of glycosidically bound forms of 2,6,10,10-tetramethyl-1-oxaspiro-[4.5]dec-6-ene-2,8-diol (**4a**) as natural precursors of **1** in Riesling wine and investigates the degradation of **4a** at pH conditions of wine.

EXPERIMENTAL PROCEDURES

Chemicals. All commercial chemicals used were of analytical grade quality. Triphenyltin hydride was obtained from Aldrich, Steinheim. All solvents used were of the highest purity obtainable commercially and were redistilled before use.

Preparation of 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol (4a). Regiospecific reduction of the side-chain double bond of 3,4-didehydro-β-ionone (7 g) with triphenyltin hydride (25 g) according to the method of Wolf and Zink (1973) and subsequent LC purification on silica gel (pentane/Et_2O 95:5) gave 3,4-didehydro-7,8-dihydro-β-ionone (5g). R_i (DB-Wax) 1841; MS *m/z* 192 (M^+, 12), 177 (2), 159 (3), 134 (13), 121 (31), 119 (100), 105 (13), 91 (18), 77 (9), 65 (5), 53 (4), 43 (39); FTIR (vapor phase; ν, cm^{-1}) 3039, 2966, 2878, 2818, 1729, 1466, 1359, 1166, 724. Purified ketone (3 g) was added to a solution of 80% *m*-chloroperoxybenzoic acid (3.5 g) in Et_2O (100 mL) at room temperature. After 2 h, 3% aqueous $Na_2S_2O_5$ solution (100 mL) was added and the mixture stirred for 30 min. The aqueous phase was basified with 1 N NaOH. The Et_2O phase was separated, and the remaining aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). Drying (Na_2SO_4) and careful concentration gave an oily residue, which was taken up in Et_2O (100 mL). During storage at −25 °C crystals were obtained, which after recrystallization from Et_2O/pentane (4:1) afforded pure **4a** (1.2 g, white crystals, mp 131 °C): ^1H NMR (400 MHz, CD_3OD) δ 1.10* and 1.11* (6 H, 2 s, $(CH_3)_2C1O$), 1.72* (3 H, s, CH_3C2), 1.73 (1 H, dd, *J* = 13.0, 9.6 Hz, $H_{ax}C9$), 1.89 (1 H, ddd, *J* = 13.0, 6.5, 1.7 Hz, $H_{eq}C9$), 2.02* (3 H, dd, *J* = 2.2, 1.4 Hz, CH_3C6), 2.06–2.35 (4 H, m, H_2C3 and H_2C4), 4.31 (1, H, dddq, *J* = 9.6, 6.5, 2.3, 2.2 Hz, H-C8), 5.55* (1 H, ddq, *J* = 2.3, 1.7, 1.4 Hz, H-C7) (*splitting of the signal indicating the occurrence of diastereoisomers, ratio 3:1); ^{13}C NMR (50 MHz, CD_3OD) major diastereoisomer δ 19.9, 22.6, 26.2, 27.6 (CH_3 at C2, C6, C10, C10), 33.6, 39.8, 45.6 (H_2C3, H_2C4, H_2C9), 41.2 (C10), 66.4 (HC8), 92.3 (C5), 107.0 (C2), 126.8 (HC7), 143.0 (C6); minor isomer δ 19.9, 23.6, 26.2, 28.0, 34.2, 40.3, 40.4, 45.9, 66.4, 92.3, 107.0, 126.3, 143.0 (chemical shifts were assigned on the basis of a DEPT experiment); IR (KBr, ν, cm^{-1}) 3300, 2980, 2940, 2890, 1450, 1380, 1220, 1100, 785. As **4a** was not amenable to HRGC analysis—even under cold on-column injection conditions a dehydration was observed—a sample of **4a** was trimethylsilylated. R_i (DB-5) of TMS derivative 1528; MS *m/z* 224 [M^+ − 2 $Si(CH_3)_3$, 19], 209 (24), 207 (30), 195 (100), 190 (22), 175 (35), 157 (9), 147 (19), 133 (15), 131 (16), 119 (13), 105 (19), 91 (14), 75 (41), 73 (79), 55 (6), 43 (46).

Degradation of 4a under Simultaneous Distillation Extraction (SDE) Conditions at pH 3.2. (*a*) *Non Steam Distillable Products.* Hemiacetals **4a** (500 mg) were dissolved in 0.2 M citric acid/phosphate buffer, pH 3.2 (60 mL), and this mixture was heated for 1 h in the SDE apparatus described by Schultz et al. (1977). From this treatment resulted 270 mg of steam volatile degradation products. For the recovery of less volatile products, the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), and this extract (230 mg), after drying and concentration, was subjected to flash chromatography (FC) on silica gel using an Et_2O/CH_2Cl_2 gradient. Besides 10 mg of nonspecified trace components, a mixture of diastereoisomeric rearranged products **10** (220 mg) was obtained. Spectral data of 3,4-dihydroxy-7,8-dihydro-β-ionones **10**: R_i (DB-1701) 2190 and 2193; MS *m/z* 226 (M^+, 0.5), 208 (13), 190 (22), 175 (20), 168 (14), 151 (18), 147 (13), 135 (48), 131 (27), 123 (25), 119 (15), 109 (40), 91 (20), 79 (16), 67 (13), 55 (19), 43 (100), 41 (24); FTIR (vapor phase, ν, cm^{-1}) 3635, 2968, 2936, 2878, 1732, 1471, 1365, 1224, 1160, 1064, 1018, 942; first eluting isomer ^1H NMR (200 MHz, $CDCl_3$) δ 1.00 and 1.04 (6 H, 2 s, H_3C11/H_3C12), 1.74 (3 H, s, H_3C13), 2.14 (3 H, s, H_3C10), 2.2–2.6 (4 H, m, H_2C7/H_2C8), 3.65–3.75 (1 H, m, HC3), 3.85 (1 H, br s, HC4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.9, 27.1, 29.1, and 29.8 (C10/C11/C12/C13), 22.0 (C7), 37.7 (C1), 41.1 (C2), 43.5 (C8), 66.7 (C3), 71.6 (C4), 127.3 (C5), 142.0 (C6), 208.3 (C9). Second eluting isomer ^1H NMR δ 1.02 and 1.05 (6 H, 2 s, H_3C11/H_3C12), 1.67 (3 H, s, H_3C13), 2.14 (3 H, s, H_3C10), 2.15–2.55 (4 H, m, H_2C7/H_2C8), 3.65–3.75 (1 H, m, HC3), 3.76 (1 H, br s, HC4); ^{13}C NMR δ 14.3, 27.7, 29.6, and 29.8 (C10/C11/C12/C13), 22.2 (C7), 37.6 (C1), 43.6 and 44.4 (C2/C8), 71.0 (C3), 77.4 (C4), 127.9 (C5), 139.2 (C6), 208.4 (C9).

(*b*) *Volatile Degradation Products of Hemiacetals* **4a.** FC separation on a silica gel column (30 cm × 1.5 cm i.d.) with pentane/Et_2O (95:5) afforded compounds **1** (40,5 mg), **5** (131 mg), and **6** (4 mg), showing the following spectral data. 1,1,6-Trimethyl-1,2-dihydronaphthalene **1**: R_i (DB-Wax): 1712, R_i (DB-1701) 1435; MS *m/z* 172 (M^+, 27), 157 (100), 142 (57), 141 (25), 128 (8), 115 (11), 91 (2), 77 (9), 71 (3), 63 (4), 51 (4); FTIR (vapor phase, ν, cm^{-1}) 3042, 2987, 2945, 2878, 2833, 1609, 1493, 1367, 882, 813, 697; ^1H NMR (200 MHz, $CDCl_3$) δ 1.25 [6 H, s, $(CH_3)_2C1$], 2.22 (2 H, dd, *J* = 4.4, 1.7 Hz, H_2C2), 2.29 (3 H, s, CH_3C6), 5.92 (1 H, dt, *J* = 9.6, 4.4 Hz, HC3), 6.41 (1 H, dt, *J* = 9.6, 1.7 Hz, HC4), 6.86 (1 H, d, *J* = 1.2 Hz, HC5), 6.99 (1 H, dd, *J* = 7.8, 1.2 Hz, HC7), 7.18 (1 H, d, *J* = 7.8 Hz, HC8); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.9, 28.5, 33.1, 39.0, 123.7, 127.2, 127.3, 127.6, 128.1, 132.8, 135.5, 141.2. These data are identical with those obtained for an authentic specimen of **1**, synthesized according to the method of Strauss et al. (1987a).

2,2,6,8-Tetramethyl-7,11-dioxatricyclo[6.2.1.0^{1,6}]undec-4-ene (Riesling Acetal) (5): R_i (DB-Wax) 1612; R_i (DB-1701) 1368; MS *m/z* 208 (M^+, 0.5), 193 (0.5), 180 (0.5), 166 (2), 165 (2), 148 (17), 138 (38), 133 (13), 125 (34), 123 (30), 119 (10), 109 (9), 95 (10), 91 (9), 77 (7), 67 (6), 55 (8), 43 (100), 41 (19); FTIR (vapor phase, ν, cm^{-1}) 3032, 2985, 2951, 2911, 1471, 1392, 1167, 1059, 927, 868, 727; ^1H and ^{13}C NMR data have been published recently (Winterhalter et al., 1990c; Näf et al., 1991). An initial sample of acetal **5**, obtained by flash chromatography, showed a fruity, ionone-like odor. After thorough purification by HPLC (LiChrospher 100, 5-μm column; 250 × 16 mm; Knauer, Berlin; pentane/Et_2O gradient), acetal **5** was characterized by a less fruity, mainly camphoraceous note.

4-(2,3,6-Trimethylphenyl)-2-butanone (6): R_i (DB-Wax) 2193; R_i (DB-1701) 1774; MS *m/z* 190 (M^+, 17), 172 (66), 157 (74), 147 (23), 133 (91), 132 (100), 119 (26), 117 (39), 115 (21), 105 (20), 91 (28), 77 (16), 65 (9), 51 (8), 43 (60); FTIR (vapor phase, ν, cm^{-1}) 3020, 2936, 1730, 1600, 1468, 1361, 1272, 1159, 803; ^1H NMR (200 MHz, $CDCl_3$) δ 2.18, 2.19, 2.25, 2.27 (4 × 3 H, 4 s, CH_3 at C2′ C3′ C6′, and H_3C1), 2.5–3.0 (4 H, m, H_2C3, H_2C4), 6.92 (2 H, s, HC4′, HC5′). ^1H NMR data are in good agreement with those published by Stevens et al. (1975). A minor compound eluted together with **6** (ratio 9:1). On the basis of MS and FTIR data this isomeric ketone could be assigned as 4-(2,3,4-trimethylphenyl)-2-butanone: R_i(DB-Wax) 2258; MS *m/z* 190 (M^+, 24), 175 (10), 172 (45), 157 (57), 147 (35), 133 (100), 132 (55), 120 (12), 117 (22), 115 (16), 105 (18), 91 (24), 77 (12), 65 (7), 55 (6), 43 (46); FTIR (vapor phase, ν, cm^{-1}) 3020, 2937, 1731, 1600, 1481, 1362, 1158, 804 (1,2,3,4-tetrasubstituted benzene derivative).

Rinsing of the flash column with Et_2O (200 mL) led to the elution of further degradation products of hemiacetals **4a**. Besides a number of not further investigated trace components, including 3,4-didehydro-β-ionone as well as still unknown isomers with m/z 208 (M^+, 8), 180 (32), 147 (15), 138 (11), 122 (23), 107 (44), 95 (80), 43 (100), HRGC and HRGC–MS analysis revealed the presence of two major products, **8** and **9**. 2,10,10-Trimethyl-6-methylene-1-oxaspiro[4.5]deca-2,7-diene (**8**): R_i (DB-Wax) 1504; R_i (DB-1701) 1307; MS *m/z* 190 (17), 175 (11), 157 (6), 147 (20), 142 (5), 131 (100), 120 (8), 119 (8), 117 (6), 115 (6), 105 (26), 91 (20), 79 (9), 77 (11), 65 (5), 53 (7), 43 (33); FTIR (vapor phase, ν, cm^{-1}) 3103 (vinylidene), 3038, 2980, 2934, 1682 (enol ether), 1640, 1605, 1433, 1386, 1273, 1043, 897 (vinylidene), 779 (trisubstituted), 718 (cis). Spiroether **8** is obviously an artifact formed under HRGC conditions from isomeric hemiacetals **7**, which could be isolated from the Et_2O eluate by repetitive FC, using a pentane/Et_2O gradient. 2,10,10-Trimethyl-6-methylene-1-oxaspiro[4.5]-dec-7-en-2-ol **7** was completely degraded to **8** under HRGC conditions used for study of DCCC fractions (Winterhalter et al., 1990b); however, in the case of cold on-column injection a small amount of **7** survived HRGC conditions. R_i (DB-Wax) 1860; MS *m/z* (%) 208 (M^+, 3), 190 (25), 175 (28), 157 (12), 147 (25), 133

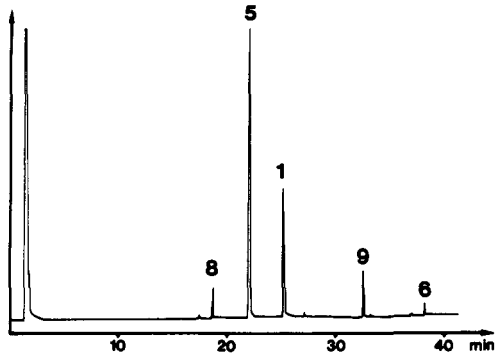

Figure 1. HRGC separation (J&W DB-Wax, 30 m × 0.25 mm i.d., film thickness 0.25 μm) of thermal degradation products (SDE, pH 3.2) of hemiacetals 4a.

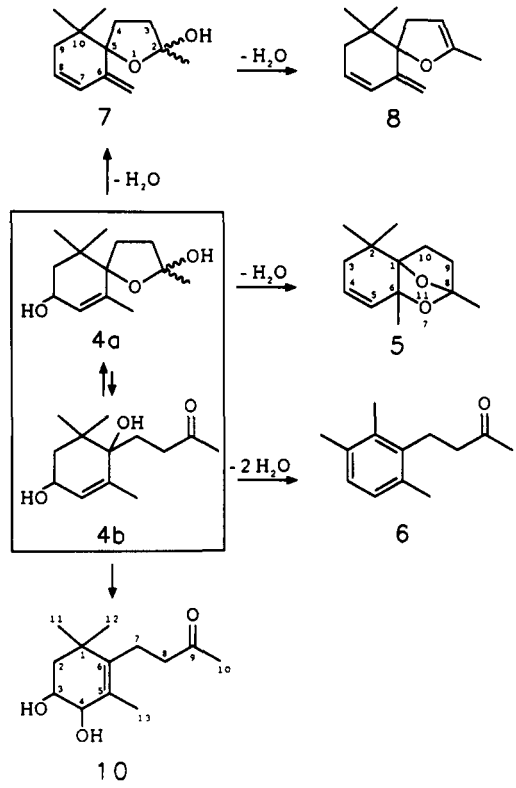

Figure 2. Structures and formation of thermal degradation products 5–8, as well as of rearranged ketone 10.

(28), 131 (100), 119 (18), 105 (43), 91 (33), 77 (16), 65 (10), 55 (15), 43 (73); FTIR, not available, due to dehydration to 8; ^{1}H NMR (200 MHz, $CDCl_3$) 0.86* and 1.06* [6 H, 2 s, $(CH_3)_2C10$], 1.56* (3 H, s, CH_3C2), 1.4–2.4 (6 H, m, H_2C3, H_2C4, H_2C9), 4.87 and 5.15 (isomer 1), 4.99 and 5.05 (isomer 2) (2 H, 4 br s, $H_2C{=}C6$), 5.54–5.66 (1 H, m, HC8), 6.00–6.08 (1 H, m, HC7) (*splitting of signals, indicating the occurrence of diastereoisomers, ratio 3:1).

6-Hydroxy-1,1,6-trimethyl-1,2,5,6-tetrahydronaphthalene (9) was unstable and degraded completely within 5 days at −18 °C. R_i (DB-Wax) 1973; R_i (DB-1701) 1553; MS m/z 190 (M^+, 14), 172 (19), 170 (5), 158 (9), 157 (100), 156 (13), 142 (28), 141 (9), 132 (8), 131 (8), 129 (6), 117 (8), 115 (11), 105 (6), 91 (8), 77 (8), 65 (4), 51 (3), 43 (22); FTIR (vapor phase) cf. Figure 4.

Identification of Bound Hemiacetal 4a in Riesling Wine. A C_{18} reversed-phase isolate (Williams et al., 1982) of 1 L of wine (1988 Riesling from McLaren Vale, South Australia) was enzymatically hydrolyzed (24 h, 37 °C) with 100 μL of a nonselective pectinase (Rohapect D5L, Röhm, Darmstadt, FRG). The liberated aglycons were extracted with CH_2Cl_2, dried over Na_2SO_4, and carefully concentrated in vacuo. The solvent-free residue was trimethylsilylated with Silyl 21 (Macherey-Nagel, Düren, FRG) and analyzed by using HRGC and HRGC-MS. The TMS derivative of synthetic **4a** symmetrically enhanced the peak

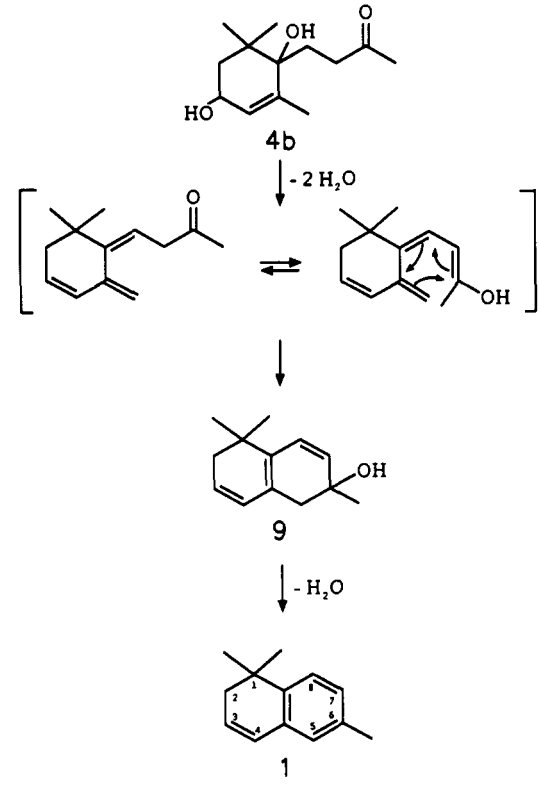

Figure 3. Proposed pathway for the formation of TDN 1 via hydroxy derivative 9.

assigned to natural 4a TMS derivative when cochromatographed on a DB-5 column and also gave an identical electron impact MS.

Capillary Gas Chromatography (HRGC). Carlo Erba Fractovap 4160 gas chromatographs equipped with FID were used. Three types of fused silica capillary columns were employed: (a) J&W DB-Wax (30 m, 0.25 mm i.d., film thickness 0.25 μm); (b) J&W DB-1701 (30 m, 0.25 mm i.d., film thickness 0.25 μm); (c) for silylated samples; J&W DB-5 (30 m, 0.25 mm i.d., film thickness 0.25 μm). Split injection (1:20) as well as on-column injection (DB-Wax column) was used. The temperature programs were (a) 3 min isothermal at 50 °C increased at 4 °C/min to 240 °C, (b) 1 min isothermal at 60 °C increased at 4 °C/min to 260 °C, and (c) from 60 to 300 °C at 5 °C/min. The flow rates for the carrier gas were 2.0 mL/min of He, for the makeup gas 30 mL/min N_2, and for the detector gases 30 mL/min of H_2 and 300 mL/min of air. The injector temperature was kept at 220 (split) and 25 °C (on-column) and the detector temperature at 300 °C. Linear retention indices (R_i) are based on a series of hydrocarbons.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with both split and on-column injectors was combined by direct coupling to a Finnigan MAT 44 mass spectrometer with PCDS data system. The same types of columns as mentioned above for HRGC analysis were used. The conditions were as follows: temperature programs, (a) from 50 to 240 °C at 4 °C/min, 10 min isothermal at 240 °C, (b) 3 min isothermal at 50 °C increased at 4 °C/min to 260 °C, 10 min isothermal at 260 °C, (c) from 60 to 300 °C at 5 °C/min; carrier gas flow rate, 2.5 mL/min of He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.7 mA.

Capillary Gas Chromatography–Fourier Transform Infrared Spectroscopy (HRGC–FTIR). A HP-IRD system (5965B with a wide band MCT detector) interfaced by a HP 5890 Series II gas chromatograph equipped with FID was used. A HP-5 fused silica capillary column (25 m × 0.32 mm i.d.; film thickness 0.52 μm) and a J&W DB-Wax fused silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm) were employed. On-column injection was used. The temperature program was 60 to 250 °C at 4 °C/min. Light pipe and transfer line were held at 250 °C. He (2.5 mL/min) was used as carrier

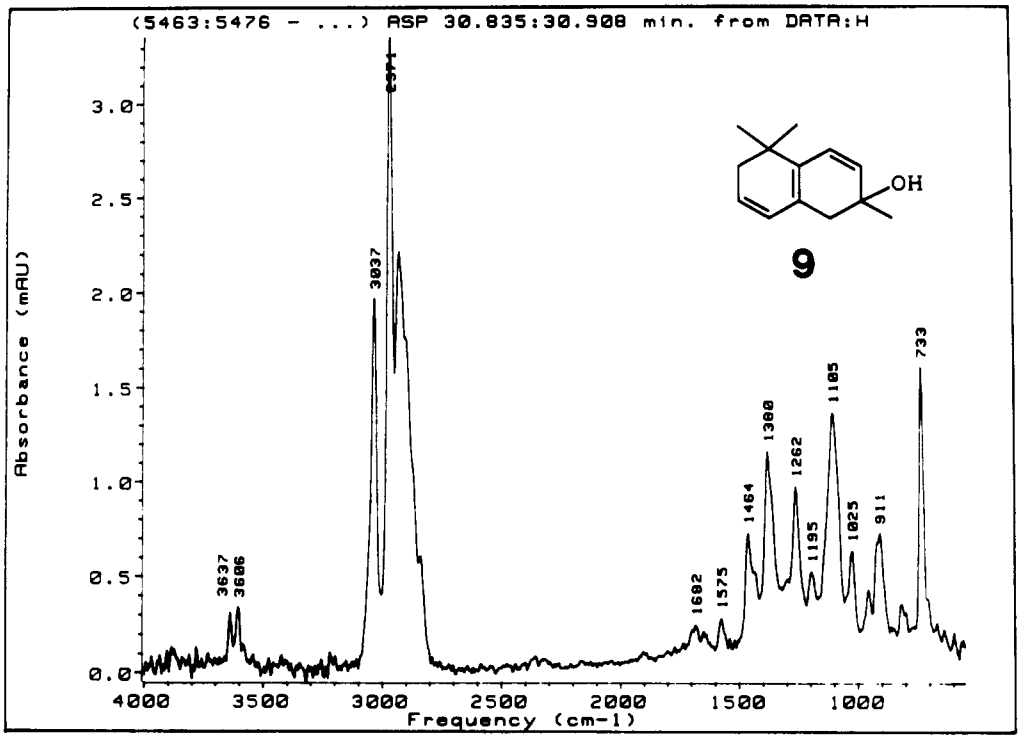

Figure 4. Vapor-phase FTIR spectrum of tentatively assigned 6-hydroxy-1,1,6-trimethyl-1,2,5,6-tetrahydronaphthalene (**9**).

gas. Vapor-phase spectra were recorded from 550-4000 cm^{-1} with a resolution of 8 cm^{-1}.

RESULTS AND DISCUSSION

In a glycosidic fraction of an Australian Riesling wine, obtained by retention of the glycosides on C_{18} reversed-phase absorbent (Williams et al., 1982) followed by enzymatic hydrolysis (Rohapect D5L) and trimethylsilylation of the liberated aglycons, capillary gas chromatography (HRGC) and capillary gas chromatography–mass spectrometry (HRGC–MS) revealed the occurrence of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol (**4a**). The identification was verified by comparison of HRGC and MS data of TMS derivatives of the new natural product with those of a synthetic specimen. Heating a diastereoisomeric mixture of synthetic hemiacetal **4a** under simultaneous distillation/extraction (SDE) conditions at the natural pH of wine (pH 3.2) gave a number of volatile degradation products, including the important off-flavor-causing wine constituent 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN, **1**). For the identification of further degradation products of **4a** (cf. Figure 1) a large-scale degradation of synthetic hemiacetals **4a** was carried out.

Degradation of 2,6,10,10-Tetramethyl-1-oxaspiro-[4.5]dec-6-ene-2,8-diol (4a) at pH 3.2 (cf. Figures 2 and 3). Volatile products formed upon SDE treatment (pH 3.2) were separated with the aid of flash chromatography (Still et al., 1978). Besides TDN (**1**), which eluted first from the flash column (pentane/Et_2O 95:5), the most recently identified intramolecular acetal **5** (Winterhalter et al., 1990c; Näf et al., 1991) was obtained as the major volatile degradation product of **4a**. The formation of **5** is a classical example of a "prototropic dehydration of an allyl-1,7-diol" giving rise to the formation of tetrahydropyran derivatives. Precedent for this type of reaction can be found in earlier work on the dehydration of monoterpene diols (Ohloff et al., 1964). As a further degradation product of **4a**, ketone **6** was obtained, which has been previously identified in C_{18} reversed-phase hydrolysates from grape juice and wine (Williams et al., 1982). Additionally, a minor component isomeric with ketone **6** was detectable, exhibiting an almost identical MS fragmentation pattern. The FTIR spectrum revealed an aromatic structure, a nonconjugated C=O band at 1731 cm^{-1}, and a C—H out-of-plane bending at 804 cm^{-1}, the latter being characteristic of 1,2,3,4-tetrasubstituted benzene derivatives. From these data the isomer was decided to be 4-(2,3,4-trimethylphenyl)-2-butanone.

Rinsing of the flash column with Et_2O led to the elution of compounds **7** and **9**. Diastereoisomeric hemiacetals **7**, purified by repetitive flash chromatography, were completely dehydrated under HRGC conditions (injector temperature 220 °C), giving rise to formation of enol ether **8**. The tentative structure of artifact **8** is mainly deduced from the vapor-phase FTIR spectrum, showing a strong enol ether band (1682 cm^{-1}) as well as three different types of double bonds (897 cm^{-1}, vinylidene; 779 cm^{-1}, trisubstituted; 718 cm^{-1}, cis). The remaining degradation product **9** was unstable and dehydrated completely within a few days of storage at −18 °C. Compound **9** has been frequently seen in grape and wine products, including aged Riesling wine, where its structure has been tentatively assigned as 1,1,6-trimethyl-4-hydroxy-1,2,3,4-tetrahydronaphthalene (Di Stefano, 1985). However, from the vapor-phase FTIR spectrum it is apparent that compound **9** possesses a nonaromatic structure (cf. Figure 4). Furthermore, FTIR data reveal the presence of a tertiary OH group (3637 cm^{-1}), and a further band at 3606 cm^{-1} indicates an intramolecular H-bonding (Nyquist, 1984). But most important are the strong absorption bands at 733 and 3037 cm^{-1}, which, together with the lack of additional signals for trisubstituted double bonds, led to the conclusion that structure **9** possesses two cis-configurated double bonds. The hydroxylated TDN derivative was therefore assigned as 6-hydroxy-1,1,6-trimethyl-1,2,5,6-tetrahydronaphthalene (**9**). Final confirmation, however, must await the chemical synthesis of **9**, which is presently in progress. A proposed pathway for TDN formation via the hydroxy derivative **9** is outlined in Figure 3.

In addition to the steam-volatile degradation products 1 and 5–9, a mixture of diastereoisomeric 3,4-dihydroxy-7,8-dihydro-β-ionones 10 was recovered from the aqueous phase as major hydrolytic products (46%) of 4a. The formation of 10 can be explained by an allylic rearrangement of the open-chain equilibrium product 4b of hemiacetals 4a (cf. Figure 2). Compound 10 was also observed as aglycon in the Riesling wine under investigation.

Evidence for the Presence of Multiple Bound Forms of Hemiacetal 4a in Riesling Wine. In a recent investigation (Winterhalter et al., 1990b) droplet countercurrent chromatography (DCCC) was employed for a preparative fractionation of a glycosidic extract, obtained from the same Riesling wine used in the present study. Evidence gathered from DCCC separation and subsequent heat treatment of DCCC fractions indicated that the naphthalene derivative 1 is generated from multiple precursor forms. Importantly, an identical pattern of volatile degradation products as outlined in Figure 1 has been found in three clearly resolved regions of the DCCC separation, i.e., in DCCC fractions 60–90, 100–120, and 190–220, respectively. This finding implies that the TDN precursor 4a exists in different glycosidically bound forms in Riesling wine. The structure elucidation of the different glycoconjugates of 4a will remain the subject for continuing research.

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