Glycosidically Bound Norisoprenoids from *Vitis vinifera* Cv. Riesling Leaves

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HRGC and HRGC–MS identifications of glycosidically bound Riesling leaf constituents were achieved after extraction with MeOH, Amberlite XAD-2 adsorption, and subsequent enzymatic hydrolysis of the glycoconjugates. Among the enzymatically liberated aglycons 23 bound C_{13} norisoprenoids were identified. With the aid of multilayer coil countercurrent chromatography (MLCCC) five major C_{13} glycosides were purified, and their structures were elucidated by high-field NMR, i.e., the β-D-glucopyranosides of vomifoliol, 4,5-dihydrovomifoliol, 3-hydroxy-5,6-epoxy-β-ionone, grasshopper ketone, and 3-oxo-megastigman-9-ol. The latter glucoside with a saturated megastigmane skeleton is reported here for the first time. In addition, the β-D-glucopyranosides of benzyl alcohol and raspberry ketone have been isolated.

INTRODUCTION

Thirteen-carbon (C_{13}) norisoprenoids are important aroma constituents of Riesling wine (Strauss et al., 1987a; Winterhalter et al., 1990a). Recent studies have revealed that virtually all volatile norisoprenoids in grape juices and wines are derived from nonvolatile precursor forms (Williams et al., 1992, 1993; Winterhalter et al., 1990b). With the structural elucidation of the aglycon moiety of glycoconjugates involved in the formation of odoriferous C_{13} constituents, major pathways leading to C_{13} volatiles could be elucidated in recent years (Sefton et al., 1989; Skouroumounis et al., 1992; Winterhalter, 1992; Winterhalter and Schreier, 1994). The identification of multiple conjugating moieties (Williams et al., 1982; Voirin et al., 1990) and the discovery that the site of glycoconjugation influences the reactivity of the aglycons (Skouroumounis et al., 1993) have made the isolation of the intact glycosidic progenitors a necessity.

Vitis vinifera grapes and wines contain C_{13} glycosides only at low levels; however, these are often the major constituents in leaf products. The strategy undertaken for the structural elucidation of trace amounts of wine glycosides started by the isolation of reference glycosides from Riesling grapevine leaves and by employing multilayer coil countercurrent chromatography (MLCCC) (Ito, 1986; Ito et al., 1982) as an efficient preparative separation and purification technique (Winterhalter, 1993). After their successful isolation and characterization, the leaf glycosides can then be used for subsequent mass spectrometric analyses to identify the respective glycosidic trace constituents in wine.

MLCCC applied to Riesling grapevine leaves has already allowed the structural elucidation of several important C_{13} glucosides, including a major glucosidic precursor, 3,4-dihydroxy-7,8-dihydro-β-ionone 3-*O*-β-D-glucopyranoside, involved in the formation of a "kerosene" off-flavor in Riesling wine (Roscher and Winterhalter, 1993). In this study, we report the identification of additional C_{13} glycosides in Riesling leaves.

EXPERIMENTAL PROCEDURES

General Procedures. Details of ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, preparation of glycosidic extracts from Riesling vine leaves, and preparative MLCCC separations using $CHCl_3$/MeOH/H_2O (7:13:8) as solvent system were given previously (Roscher and Winterhalter, 1993). Eight combined glycosidic fractions (MLCCC fractions I–VIII) had been collected. With the solvent system used, the most polar constituents eluted early, and less polar compounds were found in the later-eluting fractions. After deacetylation and enzymatic hydrolysis (sweet almond emulsin), circular dichroism (CD) spectra of the liberated aglycons were recorded in MeOH (20 °C) using an ISA Jobin Yvon Dichrograph CD 6 system. Fourier transform infrared spectra (FTIR) were taken in the NMR solvent used with a Nicolet 20 SXB system. For thermospray–mass spectrometry (TS–MS) a MAT 4500 mass spectrometer equipped with a thermospray bypass interface jet 220 was utilized.

Enzymatic Hydrolysis and Quantification of the Aglycons. An aliquot of the total glycosidic isolate was incubated overnight with Rohapect D5L (Röhm, Darmstadt) in citrate–phosphate buffer (pH 5). Phenyl-β-D-glucose was used as internal standard. The liberated aglycons were extracted with Et_2O and analyzed by HRGC and HRGC–MS.

Capillary Gas Chromatography (HRGC) and Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). For aglycon analyses HRGC and HRGC–MS was undertaken with a J&W DB-5 fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The same analytical systems as described previously have been used (Winterhalter, 1991).

Purification of Glucoside 24. Combined preparative MLCCC fractions VI and VII (60 mg) obtained by MLCCC preseparation of Riesling leaf glycosides (Roscher and Winterhalter, 1993) were rechromatographed on the "analytical" coil (160 m × 1,6 mm i.d. PTFE tubing) using EtOAc/BuOH/H_2O (3:2:5) as solvent system (mobile phase, upper layer; mode, tail to head; flow rate, 1 mL/min; rotational speed, 800 rpm). Fifty-four 10-mL fractions were collected. Screening by TLC revealed a major compound in fractions 29–33. These fractions were combined, acetylated, and finally purified by flash chromatography using a pentane/EtOAc gradient (Still et al., 1978). Spectral data for *3-oxomegastigman-9-yl tetra-O-acetyl-β-D-glucopyranoside* (**24a**) (29.5 mg): TS–MS (70 eV) pseudomolecular ion at m/z 560 $[M + NH_4]^+$; molecular mass 542; FTIR (ν cm^{-1}) 1048, 1240, 1368, 1445, 1702, 1750, 2874, 2963; ^1H NMR (400 MHz, $CDCl_3$) δ (coupling constant J in Hz) 0.73 and 1.00 (2 × 3H, 2s, 2 × CH_3-1), 1.02 (1H, m, H-6), 1.03 (3H, d, J = 6.3, CH_3-5), 1.10 (3H, d, J = 6.2, CH_3-9), 1.53 (1H, m, H-5), 1.55–1.80 (4H, m, H_2-7 and H_2-8), 1.95–2.05 (14H, 4s, 4 acetates and H_a-2/H_a-4, multiplicity and coupling constants were obscure due to partial overlapping), 2.22 (1H, br d, J = 12.8, H_b-2), 2.26 (1H, dxdxd, J = 13.9, 4.4, 2.5, H_b-4), 3.64 (1H, dxdxd, J = 9.9, 4.7, 2.5, H-5′), 3.70 (1H, m, H-9), 4.10 (1H, dxd, J = 12.2, 2.5, H_a-6′), 4.20 (1H, dxd, J = 12.2, 4.7, H_b-6′), 4.52 (1H, d, J = 7.9, H-1′), 4.92 (1H, dxd, J = 9.6, 7.9, H-2′), 5.06 (1H, dxd, J = 9.9, 9.5, H-4′), 5.18 (1H, dxd, J = 9.6, 9.5, H-3′); ^{13}C NMR (100 MHz) δ 19.6 (CH_3-

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C9), 20.7 (CH_3-C1), 21.0 (CH_3-C5), 24.6 (C7), 29.9 (CH_3-C1), 36.1 (C8), 38.9 (C5), 39.3 (C1), 50.2 (C4), 52.5 (C6), 56.4 (C2), 62.1 (C6′), 68.6 (C4′), 71.6 (C2′ and C5′), 72.9 (C3′), 76.1 (C9), 99.2 (C1′), 211.2 (C3), 20.6 and 169.2–170.5 (4 acetates). Signals were assigned on the basis of two-dimensional $^1H,^{13}C$ COSY experiments. NOE experiments for clarifying the relative stereochemistry of **24** failed due to partial overlapping of the relevant signals with the acetate signals.

Separation of MLCCC Fraction III. MLCCC fraction III (2.16 g) was rechromatographed on the "preparative" coil (75 m × 2.6 mm i.d. PTFE tubing) using EtOAc/BuOH/H_2O (3:2:5) as solvent system (mobile phase, upper layer; mode, tail to head; flow rate, 2 mL/min; rotational speed, 800 rpm). Seventy fractions were collected. Screening of the fractions was undertaken by TLC as well as enzymatic hydrolyses. Major products were detected in fractions 25–34 (phenolic structures) and fractions 35–48 (C_{13} norisoprenoid structures). Combined fractions 35–48 (688 mg) were further separated on the analytical coil using EtOAc/BuOH/H_2O (4:1:5) as solvent system (flow rate, 1 mL/min; mode, tail to head; rotational speed, 800 rpm). Separated MLCCC fractions were acetylated (Ac_2O/pyridine) and after usual workup subjected to flash chromatography. Final purifications were achieved by preparative HPLC (Eurospher 100-Si column, 5 μm, 250 × 16 mm, Knauer Säulentechnik, Berlin; eluent Et_2O).

Spectral Data for Isolated C_{13} Glucosides. *Vomifoliol 9-O-β-D-glucopyranoside* (**25**) isolated as its tetraacetate **25a**: 44.5 mg. All spectral data were in good agreement with previously published data (Achenbach et al., 1981).

4,5-Dihydrovomifoliol 9-O-β-D-glucopyranoside (**26**) isolated as its tetraacetate **26a**: 28.6 mg; TS-MS (70 eV) pseudomolecular ion at m/z 574 $[M + NH_4]^+$; molecular mass 556; FTIR (ν cm^{-1}) 980, 1038, 1065, 1220, 1368, 1445, 1712, 1742, 2876, 2974, 3612; ^1H NMR (400 MHz, $CDCl_3$) δ 0.84 (3H, d, J = 6.4, CH_3-5), 0.92 and 0.94 (2 × 3H, 2s, 2 × CH_3-1), 1.25 (3H, d, J = 6.4, CH_3-9), 1.88 (1H, dxd, J = 13.5, 2, H_a-2), 1.98–2.06 (12H, 4s, 4 acetates), 2.20 (2H, m, H_a-4 and H-5), 2.37 (1H, br t, J = 13.5, H_b-4), 2.82 (1H, d, J = 13.5, H_b-2), 3.60 (1H, dxdxd, J = 9.8, 4.1, 2.6, H-5′), 4.12 (1H, dxd, J = 12.3, 4.1, H_a-6′), 4.18 (1H, dxd, J = 12.3, 2.6, H_b-6′), 4.27 (1H, dxq, J = 6.4, 6.1, H-9), 4.56 (1H, d, J = 7.9, H-1′), 4.96 (1H, dxd, J = 9.5, 7.9, H-2′), 5.07 (1H, dxd, J = 9.8, 9.5, H-4′), 5.17 (1H, t, J = 9.5, H-3′), 5.65 (1H, br d, J = 15.9, H-7), 5.81 (1H, dxd, J = 15.9, 6.1, H-8); ^{13}C NMR (100 MHz) δ 15.8 (CH_3-C5), 21.2 (CH_3-C9), 24.4 and 24.6 (2 × CH_3 at C1), 36.3 (C5), 42.6 (C1), 45.1 and 51.4 (C2/C4), 61.7 (C6′), 68.3 (C4′), 71.6 and 71.8 (C2′/C5′), 72.8 (C3′), 76.6 (C9), 76.8 (C6), 99.8 (C1′), 132.8 and 133.1 (C7/C8), 211.2 (C3), 20.6–20.9 and 169.2–170.7 (4 acetates). The detection of only four acetate groups—the sterically hindered hydroxy group at C-6 remained underivatized—indicated that the sugar moiety had to be placed at C-9.

3-Hydroxy-5,6-epoxy-β-ionone 3-O-β-D-glucopyranoside (**27**) isolated as its tetraacetate **27a**: 48 mg; TS-MS (70 eV) pseudomolecular ion at m/z 572 $[M + NH_4]^+$; molecular mass 554; FTIR (ν cm^{-1}) 990, 1042, 1065, 1240, 1367, 1677, 1752, 2964; ^1H NMR (200 MHz, $CDCl_3$) δ 0.96 and 1.16 (2 × 3H, 2s, 2 × CH_3-1), 1.18 (3H, s, CH_3-5), 1.37 (1H, dxd, J = 13.2, 9.9, H_a-2), 1.66 (1H, dxd, J = 14.5, 8.2, H_a-4), 1.72 (1H, dxdxd, J = 13.2, 3.4, 1.2, H_b-2), 2.00–2.07 (12H, 4s, 4 acetates), 2.28 (3H, s, CH_3-9), 2.29 (1H, dxdxd, 14.5, 5.2, 1.2, H_b-4), 3.68 (1H, dxdxd, J = 9.8, 4.1, 2.6, H-5′), 3.82 (1H, dxdxdxd, J = 9.9, 8.2, 5.2, 3.4), 4.12 (1H, dxd, J = 12.2, 2.5, H_a-6′), 4.23 (1H, dxd, J = 12.2, 5.2, H_b-6′), 4.54 (1H, d, J = 7.9, H-1′), 4.93 (1H, dxd, J = 9.4, 7.9, H-2′), 5.04 (1H, dxd, J = 9.7, 9.4, H-4′), 5.19 (1H, t, J = 9.4, H-3′), 6.26 (1H, d, J = 15.6, H-8), 7.02 (1H, d, J = 15.6, H-7); ^{13}C NMR (100 MHz) δ 20.0 (CH_3-C5), 25.2, 28.1, and 28.9 (CH_3 at C1 and C9), 34.9 (C1), 37.5 (C4), 43.8 (C2), 62.2 (C6′), 66.5 (C5), 68.6 (C4′), 69.8 (C6), 71.5 (C2′), 71.8 (C5′), 72.8 (C3 and C3′), 99.9 (C1′), 132.8 (C8), 141.9 (C7), 197.2 (C9), 20.6 and 169.1–170.5 (4 acetates). CD data of the aglycon moiety were in close agreement with the previously published data (Mori, 1974), thus revealing 3*S*,5*R*,6*S* configuration.

Grasshopper ketone 3-O-β-D-glucopyranoside (**28**) isolated as its tetraacetate **28a**: 32 mg; TS-MS (70 eV) pseudomolecular ion at m/z 572 $[M + NH_4]^+$; molecular mass 554; FTIR (ν cm^{-1}) 1040, 1240, 1367, 1456, 1673, 1750, 1938, 2887, 2929, 2964, 3600; ^1H NMR (400 MHz, $CDCl_3$) δ 1.14 and 1.36 (2 × 3H, 2s, 2 × CH_3-1), 1.39 (1H, Ha-2)*, 1.41 (3H, s, CH_3-5), 1.47 (1H, br t, J = 12.1, H_a-4), 2.00–2.08 (12H, 4s, 4 acetates), 2.05 (1H, H_b-2)*, 2.16 (3H, s, CH_3-9), 2.22 (1H, dxdxd, J = 13.0, 4.0, 2.0, H_b-4), 3.72 (1H, dxdxd, J = 9.9, 5.4, 2.5, H-5′), 4.14 (1H, dxd, J = 12.2, 2.5, H_a-6′), 4.22 (1H, m, H-3)*, 4.24 (1H, dxd, J = 12.2, 5.4, H_b-6′), 4.64 (1H, d, J = 8.0, H-1′), 4.96 (1H, dxd, J = 9.6, 8.0, H-2′), 5.05 (1H, dxd, J = 9.8, 9.6, H-4′), 5.20 (1H, t, J = 9.6, H-3′), 5.85 (1H, s, H-8) (Asterisks indicate that both multiplicity and coupling constants were obscure due to partial overlapping.); ^{13}C NMR (100 MHz) δ 26.4 (CH_3-C9), 29.1 (CH_3-C5), 30.9 and 31.6 (2 × CH_3 at C1), 36.0 (C1), 45.6 and 46.9 (C2/C4), 62.2 (C6′), 68.7 (C4′), 71.5 and 71.8 (C2′/C5′), 72.1 (C5), 72.9 (C3′), 73.1 (C3), 100.2 (C1′), 100.9 (C8), 118.6 (C6), 197.9 (C7), 209.4 (C9), 20.5–20.7 and 169.2–170.6 (4 acetates). Comparison of the ^{13}C NMR data of **28** with those of grasshopper ketone revealed a downfield shift of the C-3 signal by 9.2 ppm and an upfield shift of the C-2/C-4 signals by 3.2 and 2.1 ppm, thus indicating glycosylation at C-3. CD data of the aglycon correspond to those published by Shiraga et al. (1988), showing 3*S*,5*R*,8*R* as the absolute configuration.

In addition to the above-mentioned C_{13} glucosides, acetylation and subsequent flash chromatography of combined MLCCC fractions 25–34 yielded *benzyl tetra-O-acetyl-β-D-glucopyranoside* (**29**), 40 mg (Williams et al., 1983), and *raspberry ketone tetra-O-acetyl-β-D-glucopyranoside* (**30**): 64 mg; TS-MS (70 eV) pseudomolecular ion at m/z 512 $[M + NH_4]^+$; molecular mass 494; ^1H NMR (400 MHz, $CDCl_3$) δ 1.99, 2.00, 2.02, 2.04 (acetates), 2.09 (3H, s, H_3-1), 2.69 (2H, t, J = 7.4, H_2-3), 2.81 (2H, J = 7.4, H_2-4), 3.81 (1H, dxdxd, J = 9.7, 5.3, 2.5, H-5′), 4.13 (1H, dxd, J = 12.2, 2.4, H_a-6′), 4.23 (1H, dxd, J = 12.2, 5.3, H_b-6′), 5.00 (1H, d, J = 7.5, H-1′), 5.12 (1H, dxd, J = 9.7, 9.4, H-4′), 5.21 (1H, dxd, J = 9.4, 7.5 H-2′), 5.23 (1H, t, J = 9.3, H-3′), 6.87 (2H, d, J = 8.6, H-7/H-9), 7.06 (2H, d, J = 8.6, H-6/H-10); ^{13}C NMR (100 MHz) δ 29.1 (C1), 30.3 (C4), 45.4 (C3), 62.2 (C6′), 68.6 (C4′), 71.4 and 72.2 (C2′/C5′), 72.9 (C3′), 99.6 (C1′), 117.4 (C7/C9), 129.6 (C6/C10), 136.3 (C5), 155.5 (C8), 207.9 (C2), 20.8–20.9 and 169.5–170.8 (4 acetates).

Aglycon and Sugar Analysis. After deacetylation with 0.02 M NaOMe in MeOH, 2 mg of each of the glucosides in 1 mL of H_2O was acidified with 1 drop of diluted acetic acid and incubated overnight (37 °C) with 5 mg of β-glucosidase (sweet almond emulsin, Serva). The liberated aglycon was extracted with Et_2O (2 × 2 mL), and the aqueous layer was passed through an ultrafilter (Ultrafree-MC 5000 NMGG, Millipore); 20 μL of the enzyme-free filtrate was then injected into the HPLC system (Shandon Hypersil APS 5-μm column, 125 × 4.6 mm; eluent; acetonitrile/H_2O 80:20; flow, 0.5 mL/min). The presence of D-glucose was verified by on-line coupled refractive index (RI detector, Knauer, Berlin) and polarimetric detection (Chiralyzer polarimetric detector, IBZ Messtechnik, Hannover). For aglycon analysis the Et_2O extract was concentrated and analyzed by HRGC–MS. MS data for the C_{13} aglycons were identical to those previously published: **6** (Sefton et al., 1990), **11** (Krammer et al., 1991), **16** (Sefton et al., 1992), **17** (Winterhalter et al., 1993), and **18** (Strauss et al., 1987b).

RESULTS AND DISCUSSION

The tiny amounts of glycosidic constituents present in wine routinely cause problems in the course of structural elucidation by NMR techniques. In those cases where biogenetic pathways of extremely potent C_{13} volatiles, such as β-damascenone, had to be studied, the low concentration of the acid-labile glycosidic precursors in wine has so far hampered a complete characterization. To overcome some of these problems, a strategy has been developed by which one uses reference glycosides that have been isolated and characterized from an alternative source (Winterhalter, 1993). With the references in hand, different mass spectrometric techniques can then allow a rapid identification of the respective trace constituents in wine as shown by Marinos (1992). For our continuing studies on the generation of C_{13} norisoprenoid volatiles in Riesling wine, grapevine leaves were found to be an appropriate starting material. The C_{13} glycosidic levels in leaves were

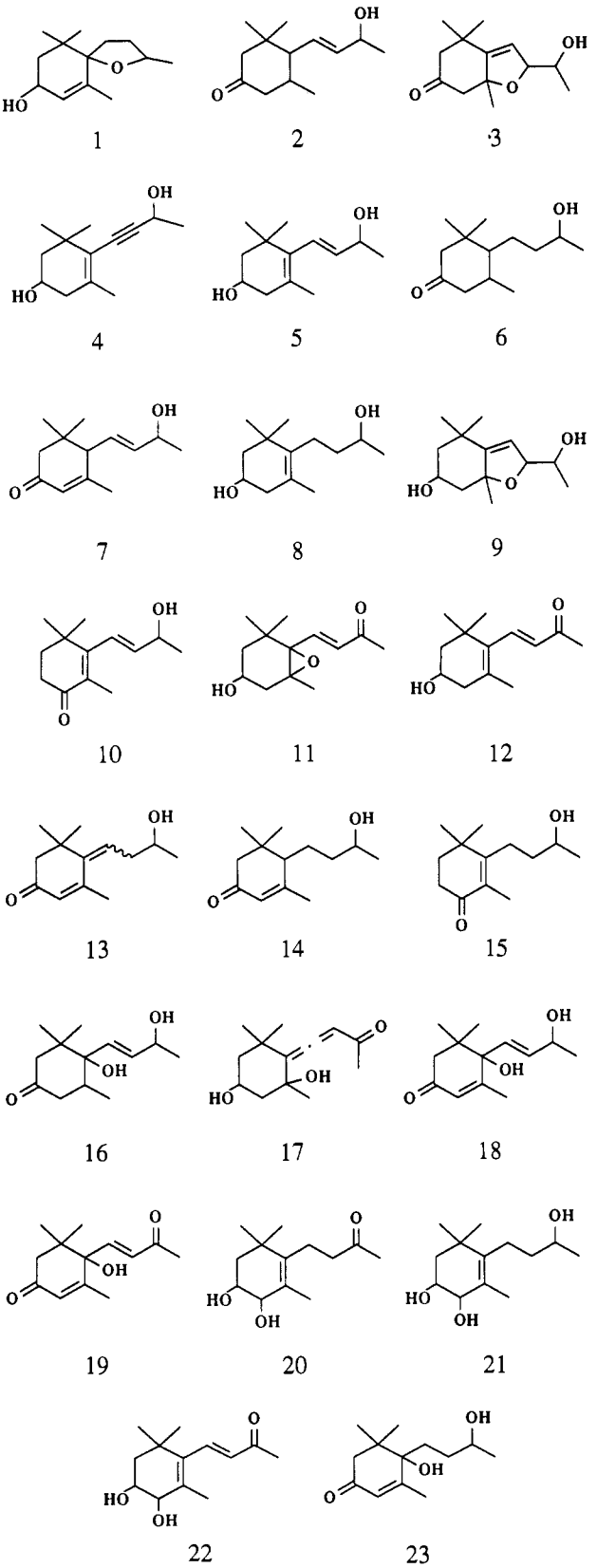

Figure 1. Structures of enzymatically liberated C_{13} norisoprenoid aglycons **1–23**.

found to exceed that of Riesling wine by factors of 10–100 (Roscher and Winterhalter, 1993). Moreover, the composition of C_{13} structures in the leaves was found to be quite similar to that observed for Riesling wine. Except for 3-hydroxyionone derivatives **5**, **8**, **11**, **12**, and **22** (cf. Figure 1), all other C_{13} structures have been earlier identified by us in Riesling wine (Sefton et al., 1992; Winterhalter, 1991; Winterhalter et al., 1990a). Table 1 reveals the positively identified glycosidically bound C_{13} norisoprenoids in Riesling grapevine leaves.

Table 1. C_{13} Norisoprenoid Aglycons Identified by HRGC and HRGC–MS after Enzymatic Hydrolysis (Rohapect D5L) of Riesling Leaf Glycosides

	compound	R_i[a]	concn[b]
1	8-hydroxytheaspiranes (2 isomers)	1518/1526	+
2	3-oxo-4,5-dihydro-α-ionol	1598	+++
3	3-oxoactinidols (4 isomers)	1601/1603 1624/1634	+
4	3-hydroxy-7,8-dehydro-β-ionol	1635	+
5	3-hydroxy-β-ionol	1640	+
6	3-oxomegastigman-9-ol	1660	+++
7	3-oxo-α-ionol	1664	++
8	3-hydroxy-7,8-dihydro-β-ionol	1672	+
9	3-hydroxyactinidol	1675	+
10	4-oxo-β-ionol	1692	+
11	3-hydroxy-5,6-epoxy-β-ionone	1697	+++
12	3-hydroxy-β-ionone	1707	+
13	3-oxo-*retro*-α-ionol (2 isomers)	1719/1779	++
14	3-oxo-7,8-dihydro-α-ionol	1736	+++
15	4-oxo-7,8-dihydro-β-ionol	1742	+
16	4,5-dihydrovomifoliol	1783	+++
17	grasshopper ketone	1792	+++
18	vomifoliol	1797	+++
19	dehydrovomifoliol	1805	+
20	3,4-dihydroxy-7,8-dihydro-β-ionone	1825	++
21	3,4-dihydroxy-7,8-dihydro-β-ionol	1845	+
22	3,4-dihydroxy-β-ionone	1861	++
23	7,8-dihydrovomifoliol	1866	+

[a] R_i = linear retention index based on a series of hydrocarbons. The R_i values given were coincident with those of authentic reference compounds. For HRGC conditions see Experimental Procedures. [b] Relative concentrations were ranked as follows: +, <0.1 ppm; ++, 0.1–1 ppm; +++, >1 ppm.

The observed similarity in C_{13} composition of Riesling wine and leaves is not surprising since C_{13} norisoprenoid structures are considered to be formed by oxidative cleavage of carotenoids and subsequent transformation reactions at the plant pH. In previous studies on *V. vinifera* carotenoids four major carotenoids have been detected, i.e., β-carotene, lutein, 5,6-epoxylutein, and neoxanthin (Razungles et al., 1987, 1988). The carotenoid concentration in skin and pulp of grapes was low (0.8–2.5 mg/kg), and no carotenoids were detectable in the juice. The same research group found that the pattern of carotenoids in grapevine berries resembles that of a leaf product. Consequently, cleavage of these carotenoids is expected to generate the same pattern of C_{13} degradation products in grapevine berries and leaves. However, due to the abundance of carotenoids in the leaves, their norisoprenoid concentration is much higher compared to that of the grapes. On the basis of these findings grapevine leaves can be considered an ideal source for C_{13} norisoprenoid metabolites. As major carotenoid metabolites in leaves, the 3-oxonorisoprenoids **2**, **6**, and **14**, vomifoliol **18** and its dihydroderivative **16**, epoxyionone **11**, and grasshopper ketone **17** were detected. Among them, the glycosides of 3-oxonorisoprenoids **2** and **14** have previously been characterized by us in grapevine leaves (Roscher and Winterhalter, 1993). For the isolation of the remaining major C_{13} glycosides additional MLCCC separations were required.

CCC Separation of Glycosides. Preseparated fractions from Riesling leaves were further purified by analytical MLCCC. After acetylation and subsequent liquid chromatographic purification of combined MLCCC fractions V and VI, a C_{13} structure with an apparent molecular weight of 542 was obtained. ^1H NMR data suggested the presence of a tetraacetyl-β-D-glucopyranosyl

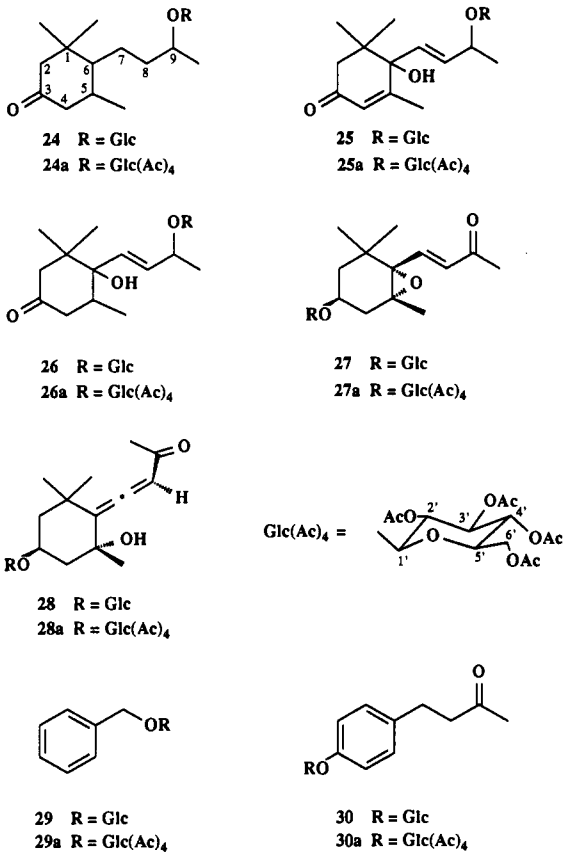

Figure 2. Structures of Riesling leaf glucoconjugates **24–30**.

moiety, with all data being in good agreement with those reported for other β-D-glucopyranosides (Gagnaire et al., 1976). For the aglycon part, two three-proton singlets at δ 0.73 and 1.00 as well as two three-proton doublets at δ 1.03 and 1.00 indicated a megastigmane skeleton. The absence of any downfield-shifted signals—except a multiplet at δ 3.70 (glycosidic proton)—revealed a rather saturated C_{13} structure. The ^{13}C NMR spectrum showed a carbonyl group (δ 211.2), an oxygen-bearing carbon (δ 76.1), and further signals revealing a 3-oxomegastimane skeleton. Although two-dimensional $^{1}H,^{13}C$ COSY experiments allowed the assignment of nearly all NMR signals, NOE experiments have thus far not allowed the assignment of the relative stereochemistry of aglycon **6**. After deacetylation, β-glucosidase treatment liberated an aglycon (molecular weight 212) exibiting mass spectral data in good agreement with those recently published for the tentatively identified oakwood constituent **6** (Sefton et al., 1990). Sugar analysis employing HPLC with combined refractive index and polarimetric detection confirmed the presence of β-D-glucose. Glucoside **24** (cf. Figure 2) is to our best knowledge reported for the first time in nature.

MLCCC was then also applied to a more polar glycosidic fraction, i.e., MLCCC fraction III, which after glycosidase treatment was found to liberate high amounts of C_{13} structures. After acetylation and flash chromatography, these fractions yielded the β-D-glucopyranoside of 4,5-dihydrovomifoliol (**26**) together with the β-D-glucopyranoside of vomifoliol, the so-called roseoside **25** (Bhakuni et al., 1974). Whereas roseoside is frequently found as a natural product, its 4,5-dihydro analogue **26** has rarely been detected. First identified in *Artemisia santolinifolia* (Jakupovic et al., 1991), free and bound forms of 4,5-dihydrovomifoliol (**16**) were recently isolated from an Australian Riesling wine (Sefton et al., 1992). Aglycon **16** was furthermore detected in extracts of French and American oakwood used in barrel making (Sefton et al., 1992).

In addition to roseoside **25** and its dihydro derivative **26**, MLCCC fraction III also yielded the β-D-glucopyranosides of 3-hydroxy-5,6-epoxy-β-ionone (**27**) and grasshopper ketone (**28**) together with benzyl β-D-glucopyranoside (**29**) and raspberry ketone β-D-glucopyranoside (**30**) (cf. Figure 2). Although the aglycons 3-hydroxy-5,6-epoxy-β-ionone (**11**), grasshopper ketone (**17**), and raspberry ketone are known to occur in grape juices and wines (Sefton and Williams, 1991; Strauss et al., 1987a; Winterhalter et al., 1990a), the respective intact glycoconjugates have so far not been isolated from these sources. The ubiquitous benzyl glucoside **29**, however, has previously been identified in wine (Williams et al., 1983).

With regard to the above-discussed biogeneration of C_{13} compounds, the isolation of glucosides **27** and **28** strongly supports an apocarotenoid pathway. For both structures which have been identified for the first time in Riesling leaves, CD data revealed the same stereochemistry as is known for their most likely precursor, the allenic carotenoid neoxanthin (Isoe et al., 1973; Ohloff et al., 1973).

Conclusions. This study has revealed high amounts of C_{13} glycoconjugates in Riesling grapevine leaves, thus enabling a fast isolation of reference glycosides for our continuing studies on the formation of C_{13} norisoprenoid volatiles in Riesling wine. Importantly, the pattern of C_{13} norisoprenoid aglycons in the leaves was found to be similar to that observed in Riesling wine. A major difference, however, is observed in the conjugating moiety. Whereas the major conjugates in the leaves were found to be monoglucosides, the majority of monoterpenyl, benzyl, and 2-phenylethyl glycosides in the wine consisted of disaccharides (Voirin et al., 1990; Williams, 1993; Williams et al., 1982, 1983). From a recent study on the norisoprenoid composition of Riesling wine (Winterhalter et al., 1990), it is reasonable to assume that the majority of norisoprenoids are similarly present as glucosides and as disaccharide conjugates (Winterhalter et al., 1990a).

Due to the abundance of C_{13} glucoconjugates in the grapevine leaves, the question arises as to what extent these structures may be transported into the berry. Due to their known role as flavor precursors, any transport of C_{13} glycosides from grapevine leaves to the berries must be considered as having a strong influence on the flavor of the final product, i.e., Riesling wine.

Since knowledge about transport mechanisms of glycoconjugates in *V. vinifera* cultivars is still lacking, it can only be taken into consideration that a possible transport to the berry may require the connection of the glucopyranoside moiety with a second carbohydrate unit to increase the hydrophilicity of the C_{13} metabolites. The conjugation with a second carbohydrate perhaps enables the glycosides to readily transpose into the berry. Future experiments involving labeled C_{13} glucoconjugates fed to the leaf may be considered an appropriate tool to study the transport mechanisms into the fruit. This may permit a better understanding of disaccharide formation in grapes. Work in this area is projected in the near future.

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