# Isolation of Two Novel Terpenoid Glucose Esters from Riesling Wine

Bernd Bonnländer, Beate Baderschneider, Monika Messerer, and Peter Winterhalter*

Institut für Pharmazie und Lebensmittelchemie, Universität Erlangen-Nürnberg, Schuhstrasse 19, 91052 Erlangen, Germany

A glycosidic isolate of Riesling wine was separated with multilayer coil countercurrent chromatography (MLCCC). After acetylation and subsequent purification by high-performance liquid chromatography (HPLC), the glucose esters of (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**1**) and (2*E*,6*E*)-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoic acid (**2**) were identified for the first time as wine constituents. The identification was achieved by mass spectrometry (DCI-MS) as well as nuclear magnetic resonance spectroscopy ($^1$H NMR, $^{13}$C NMR, COSY, HMBC). In biomimetic studies carried out with (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid the potent wine aroma compound 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one (wine lactone) was formed, thus revealing an important aroma precursor function for glucoconjugate **1**.

**Keywords:** *(E)-2,6-Dimethyl-6-hydroxyocta-2,7-dienoic acid glucose ester; (2E,6E)-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoic acid glucose ester; aroma precursor; wine lactone; multilayer coil countercurrent chromatography; Riesling wine*

## INTRODUCTION

Research over the past decade has revealed that oxidative pathways are active in *Vitis vinifera*, converting terpenoid constituents of grapes into oxygenated derivates that accumulate in glycosidically bound form. Up to now, a considerable number of glycoconjugated mono- and norterpenoids have been detected in grapes and wine (Baumes et al., 1994; Marinos et al., 1994; Strauss et al., 1987, 1988; Versini et al., 1996; Waldmann and Winterhalter, 1992; Williams, 1993; Winterhalter et al., 1990a,b; Winterhalter, 1992). Although the glycoconjugates themselves are odorless, they are easily transformed under pH conditions of wine into volatile constituents, some of which have significant sensory properties. In this paper, we report the identification of two additional terpenoid glycoconjugates in Riesling wine, with one of them acting as wine aroma precursor.

## EXPERIMENTAL PROCEDURES

**General Procedures.** All solvents were of high purity at purchase and were redistilled before use. Details of preparation of a glycosidic (XAD-2) isolate (Günata et al., 1985), preseparation using multilayer coil countercurrent chromatography (MLCCC) (Ito, 1986; Roscher and Winterhalter, 1993), and $^1$H NMR spectral data ($CDCl_3$) of peracetylated **1a** were given previously (Winterhalter et al., 1997). The wine was a German Riesling from the 1992 vintage, and it was dealcoholized by concentration in vacuo.

**Isolation and Analysis of Peracetylated Glucose Esters 1a and 2a.** Glucose esters **1a** and **2a** were obtained from separated MLCCC fraction III after acetylation ($Ac_2O$/pyridine) and purification by flash chromatography (Still et al., 1978). Due to the instability of **1a** in $CDCl_3$ solution, the remaining sample was purified by normal phase HPLC (eluent: methyl *tert*-butyl ether/pentane 8:2), and a complete set of NMR spectral data was recorded in $C_6D_6$ (see Tables 1 and 2). The signals were assigned on the basis of $^1$H−$^{13}$C COSY as well as HMBC experiments.

The glucose ester of (2*E*,6*E*)-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoic acid (**2**) was, after a cleanup by HPLC (Lichrospher 100, RP-18, 5 $\mu$m, 250 × 4 mm, Merck, Darmstadt, Germany; eluent: MeOH/$H_2O$, gradient), obtained as pentaacetate **2a**: 1.5 mg; DCI-MS pseudo-molecular ion at *m*/*z* 660 $[M(642) + NH_4]^+$, $C_{31}H_{46}O_{14}$; $^1$H NMR and $^{13}$C NMR spectral data are presented in Tables 3 and 4, respectively.

**Table 1. $^1$H NMR Spectral Data of Isolated Compound 1a ($C_6D_6$, 360 MHz, Coupling Constants in Hertz, $\delta$ Relative to TMS)**

| $\delta$ | signal | $J$ | atom |
|---|---|---|---|
| 0.96 | 3 H, s | | $CH_3$-C6 |
| 1.22 | 2 H, m | | $H_2$C5 |
| 1.63, 1.64,1.65, 1.67 | 4 × 3 H, 4s | | acetates |
| 1.77 | 3 H, br s | | $CH_3$-C2 |
| 1.96 | 2 H, m | | $H_2$C4 |
| 3.23 | 1 H, ddd | 9.5/4.0/2.0 | HC5′ |
| 3.93 | 1 H, dd | 12.5/2.0 | $H_a$C6′ |
| 4.23 | 1 H, dd | 12.5/4.0 | $H_b$C6′ |
| 4.89 | 1 H, dd | 10.7/1.5 | $H_a$C8 |
| 5.10 | 1 H, dd | 17.5/1.5 | $H_b$C8 |
| 5.33 | 1 H, dd | 9.5/9.5 | HC4′ |
| 5.43 | 1 H, dd | 9.5/9.5 | HC3′ |
| 5.54 | 1 H, dd | 9.5/8.0 | HC2′ |
| 5.55 | 1 H, dd | 17.5/10.5 | HC7 |
| 5.96 | 1 H, d | 8.0 | HC1′ |
| 7.03 | 1 H, tq | 7.5/1.5 | HC3 |

**Table 2. $^{13}$C NMR Spectral Data of Isolated Compound 1a ($C_6D_6$, 90 MHz, $\delta$ Relative to TMS)**

| $\delta$ | atom | $\delta$ | atom |
|---|---|---|---|
| 11.6 | $CH_3$-C2 | 72.5 | C5′ |
| 19.5−20.5 | acetates | 72.9 | C3′ |
| 23.3 | C4 | 92.1 | C1′ |
| 27.5 | $CH_3$-C6 | 111.3 | C8 |
| 40.7 | C5 | 126.5 | C2 |
| 60.8 | C6′ | 144.8 | C7 |
| 67.7 | C5′ | 145.5 | C3 |
| 70.5 | C2′ | 165.7 | C1 |
| 72.3 | C6 | 169.3−171.0 | acetates |

* Address correspondence to this author at TU Braunschweig, Schleinitzstr. 20, 38106 Braunschweig, Germany (e-mail P.Winterhalter@tu-bs.de; fax ++49-531-391-7230).

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**Table 3. $^1$H NMR Spectral Data of Isolated Compound 2a ($CDCl_3$, 360 MHz, Coupling Constants in Hertz, $\delta$ Relative to TMS)**

| $\delta$ | signal | $J$ | atom |
|---|---|---|---|
| 1.19/1.20 | 6 H, 2s | | $H_3C$-C11/$H_3C$12 |
| 1.60 | 3 H, s | | $H_3C$-C7 |
| 1.6−2.2 | 8 H, m | | $H_2C4/H_2C5/H_2C8/H_2C9$ |
| 2.02−2.11 | 15 H, 5s | | acetates (5×) |
| 2.18 | 3 H, s | | $H_3C$-C3 |
| 3.85 | 1 H, ddd | 9.5/4.5/2.0 | HC5′ |
| 4.12 | 1 H, dd | 12.5/2.0 | HC6a′ |
| 4.30 | 1 H, dd | 12.5/4.5 | HC6b′ |
| 4.78 | 1 H, dd | 10.0/3.0 | HC10 |
| 5.07 | 1 H, dd | 7.0/7.0 | HC6 |
| 5.15 | 1 H, dd | 9.5/9.5 | HC4′ |
| 5.17 | 1 H, dd | 9.5/8.0 | HC2′ |
| 5.27 | 1 H, dd | 9.5/9.5 | HC3′ |
| 5.68 | 1 H, br s | | HC2 |
| 5.76 | 1 H, d | 8.0 | HC1′ |

**Table 4. $^{13}$C NMR Spectral Data of Compound 2a and Reference Compound 2c ($CDCl_3$, 90 MHz, $\delta$ Relative to TMS)**

| **2a** $\delta$ | atom | **2c** $\delta$ |
|---|---|---|
| 16.0 | $CH_3$-C7 | 16.0 |
| 19.4 | $CH_3$-C3 | 18.8 |
| 20.5−21.1 | acetates | 21.0 |
| 24.9/26.7 | $H_3C$-C11/C12 | 24.8/26.7 |
| 25.9 | C5 | 25.9 |
| 27.8 | C9 | 27.7 |
| 36.0 | C8 | 36.0 |
| 41.0 | C4 | 40.7 |
| 61.5 | C6′ | |
| 67.9 | C4′ | |
| 70.3 | C2′ | |
| 72.4 | C11 | 72.5 |
| 72.7 | C5′ | |
| 73.0 | C3′ | |
| 79.4 | C10 | 79.5 |
| 91.3 | C1′ | |
| 114.1 | C2 | 115.4 |
| 123.4 | C6 | 123.1 |
| 135.4 | C7 | 135.1 |
| 164.5/164.1 | C1/C3 | 159.9/167.2 |
| 169.3−171.2 | acetates | 171.2 |
| | $CH_3O$ | 50.8 |

Signals were assigned on the basis of $^1$H−$^{13}$C COSY as well as HMBC experiments.

**Aglycon and Sugar Analysis.** After deacetylation with 0.02 M NaOMe in MeOH, each of the glucoconjugates in 1 mL of $H_2O$ was acidified with 1 drop of diluted acetic acid and incubated overnight (37 °C) with 5 mg of $\beta$-glucosidase (sweet almond emulsin, Serva). The liberated aglycons were extracted with $Et_2O$ (2 × 2 mL) and analyzed by GC/MS. The aqueous layer was passed through an ultrafilter (Ultrafree-MC 5000 NMGG, Millipore), and 20 $\mu$L of the enzyme-free filtrate was then injected into the HPLC system (Shandon Hypersil APS-5 $\mu$m column, 125 × 4.6 mm; eluent: acetonitrile/$H_2O$, 80:20). The presence of D-glucose was verified by on-line coupled refractive index (RI detector, Knauer, Berlin, Germany) and polarimetric detection (Chiralyzer polarimetric detector, IBZ Messtechnik, Hannover, Germany).

**Syntheses of Reference Compounds.** *(i) Preparation of (E)-2,6-Dimethyl-6-hydroxyocta-2,7-dienoic Acid (**6**). (a) (E)-6-Acetoxy-2,6-dimethyl-octa-2,7-dienal (**4**).* Aldehyde **4** was prepared according to the method of Hirata et al. (1981) by reacting racemic linalyl acetate (2 g) with $SeO_2$ (0.2 g) in a mixture of dioxane (10 mL) and water (0.5 mL). After refluxing at 70 °C for 40 min and subsequent filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography ($SiO_2$) using a pentane/ethyl acetate gradient. Aldehyde **4** (510 mg) showed the following chromatographic and spectral data: $R_i$ (DB-5) 1495; EI-MS (70 eV) *m/z* (%) 210 [$M^+$] (<1), 168 (1), 150 [$M^+$ − HOAc] (7), 140 (1), 135 (6), 129 (5), 121 (9), 108 (8), 107 (9), 95 (14), 93 (12), 82 (27), 71 (34), 67 (10), 55 (20), 43 (100); $^1$H NMR (360 MHz, $CDCl_3$) $\delta$ 1.59 (3 H, s, $CH_3$-6), 1.74 (3 H, br s, $CH_3$-2), 1.92 (1 H, ddd, $J$ = 14.0, 9.0, 7.5 Hz, $H_a$-5), 2.02 (3 H, s, $CH_3$−CO), 2.10 (1 H, ddd, $J$ = 14.0, 9.0, 7.0 Hz, $H_b$-5), 2.38 (2 H, br q, $J$ = 8 Hz, $H_2$-4), 5.18 (1 H, dd, $J$ = 10.7, 1.0 Hz, $H_a$-8), 5.20 (1 H, dd, $J$ = 17.5, 1.0 Hz, $H_b$-8), 5.97 (1 H, dd, $J$ = 17.5, 10.7 Hz, H-7), 6.48 (1 H, tq, $J$ = 7.5, 1.5 Hz, H-3); $^{13}$C NMR (62.9 MHz, $CDCl_3$) $\delta$ 9.1 ($CH_3$-C2), 22.0 (acetate), 23.5 and 23.7 ($CH_3$-C6/C4), 38.0 (C5), 82.3 (C6), 113.7 (C8), 139.4 (C2), 141.1 (C7), 153.7 (C3), 169.8 (acetate), 195.0 (C1).

*(b) (E)-Methyl-6-acetoxy-2,6-dimethyl-octa-2,7-dienoate (**5**).* Aldehyde **4** (500 mg) was reacted with a mixture of $MnO_2$ (5.8 g), NaCN (880 mg), and $CH_3COOH$ (300 mg) in methanol (20 mL) for 12 h at room temperature (Corey et al., 1968). The solvent was evaporated under reduced pressure. The residue was partitioned between diethyl ether and water. The organic layer was concentrated in vacuo and subjected to column chromatography ($SiO_2$) using a pentane/diethyl ether gradient. Methyl ester **5** was obtained as a colorless oil (120 mg): $R_i$ (DB-5) 1682; EI-MS (70 eV) *m/z* (%) 240 [$M^+$] (<1), 198 (2), 180 (2), 166 (6), 165 (4), 151 (2), 148 (11), 139 (5), 138 (6) 121 (31), 112 (23), 105 (15), 93 (23), 80 (24), 71 (39), 55 (19), 43 (100); $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 1.58 (3 H, s, $CH_3$-6), 1.83 (3 H, br s, $CH_3$-2), 1.85 (1 H, m, $H_a$-5), 2.00 (1 H, m, $H_b$-5), 2.01 (3 H, s, acetate), 2.19 (2 H, br q, $J$ = 8 Hz, $H_2$-4), 3.73 (3 H, s, $OCH_3$), 5.15 (1 H, dd, $J$ = 11.0, 1.0 Hz, $H_a$-8), 5.17 (1 H, dd, $J$ = 17.5, 1.0 Hz, $H_b$-8), 5.96 (1 H, dd, $J$ = 17.5, 11.0 Hz, H-7), 6.75 (1 H, tq, $J$ = 7.5, 1.5 Hz, H-3); $^{13}$C NMR (62.9 MHz, $CDCl_3$) $\delta$ 12.3 ($CH_3$-C2), 22.1 (acetate), 23.2 (C4), 23.7 ($CH_3$−C6), 38.3 (C5), 51.7 ($OCH_3$), 82.5 (C6), 113.6 (C8), 127.8 (C2), 141.3 and 141.6 (C3/C7), 168.5 (C1), 169.8 (acetate).

*(c) (E)-2,6-Dimethyl-6-hydroxyocta-2,7-dienoic Acid (**6**).* Deprotection of methyl ester **5** was achieved by porcine liver esterase (PLE) treatment (50 $\mu$L, Sigma) in 0.2 M citrate phosphate buffer (pH 7). Incubation was at 35 °C for 72 h. After acidification, extraction with diethyl ether (3 × 20 mL), and concentration of the solvent, acid **6** was purified by preparative HPLC on a Eurospher 100-$C_{18}$ column (7 $\mu$m, 250 × 16 mm, Knauer Säulentechnik) using methanol/water (65:35) as eluent: $R_i$ (DB-5) 1592; EI-MS (70 eV) *m/z* (%) 184 [$M^+$] (<1), 166 (1), 151 (2), 139 (2), 138 (4), 123 (4), 121 (9), 114 (11), 111 (10), 103 (14), 95 (11), 82 (7), 71 (100), 55 (25), 43 (79); $^1$H NMR (360 MHz, $CDCl_3$) $\delta$ 1.32 (3 H, s, $CH_3$-6), 1.67 (2 H, m, $H_2$-5), 1.84 (3 H, br s, $CH_3$-2), 2.25 (2 H, m, $H_2$-4), 5.10 (1 H, dd, 11.0, 1.5 Hz, $H_a$-8), 5.24 (1 H, dd, 17.5, 1.5 Hz, $H_b$-8), 5.92 (1 H, dd, 17.5, 11.0 Hz, H-7), 6.88 (1 H, tq, 7.5, 1.5 Hz, H-3); $^{13}$C NMR (62.9 MHz, $CDCl_3$) $\delta$ 12.0 ($CH_3$-C2), 23.6 (C4), 28.0 ($CH_3$-C6), 40.5 (C5), 73.0 (C6), 112.3 (C8), 127.0 (C2), 144.4 and 144.6 (C7/C3), 171.7 (C1).

*(ii) Preparation of (2E,6E)-Methyl-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate (**2b**).* Twenty milligrams of commercially available (Fluka, Buchs, Switzerland) juvenile hormone III [(2*E*,6*E*)-methyl-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate] was dissolved in 0.5 M sodium acetate buffer (10 mL, pH 4). The reaction mixture was stirred overnight at 40 °C. After addition of NaCl, the solution was extracted twice with ethyl acetate (25 mL). The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo. The crude methyl esterdiol **2b** was purified by flash chromatography (pentane/$Et_2O$ gradient): $R_i$ (DB5) 2091; EI-MS (70 eV) *m/z* (%) 266 [$M^+$ − $H_2O$] (1), 237 (1), 225 (14), 193 (30), 183 (4), 163 (6), 147 (9), 135 (26), 123 (33), 114 (66), 95 (27), 93 (27), 83 (37), 82 (43), 81 (61), 79 (23), 72 (11), 71 (44), 69 (25), 68 (20), 67 (37), 59 (100), 55 (37), 43 (81), 41 (67). Acetylation ($Ac_2O$ in pyridine) of **2b** yielded (2*E*,6*E*)-methyl-10-acetoxy-11-hydroxy-3,7,11-trimethyl-2,6-dodecadienoate **2c**: $R_i$ (DB5) 2171; EI-MS (70 eV) *m/z* (%) 308 [$M^+$ − $H_2O$] (1), 279 (2), 248 (2), 195 (9), 193 (9), 165 (12), 147 (7), 135 (39), 121 (11), 114 (49), 95 (25), 93 (22), 83 (17), 82 (25), 81 (78), 79 (15), 72 (16), 71 (16), 69 (11), 67 (14), 59 (49), 55 (19), 43 (100), 41 (15).

**Degradation of (*E*)-2,6-Dimethyl-6-hydroxyocta-2,7-dienoic Acid (6).** Aqueous solutions (50 mL) of acid **6** (10 mg) were subjected to simultaneous distillation−extraction (SDE) treatment over 2 h at pH 3.2, 2.5, and 2.0 using the

distillation head described by Schultz et al. (1977). The organic phase was dried over anhydrous $Na_2SO_4$ and carefully concentrated to 1 mL for subsequent HRGC and HRGC/MS analyses.

**High-Resolution Gas Chromatography (HRGC).** Dani educational gas chromatographs equipped with a J&W fused silica DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 $\mu$m) were used. Split injection (1:20) was employed. The temperature program was from 60 °C (2 min isothermal) to 300 °C at 5 °C/min. The flow rate for the carrier gas was 1.5 mL/min of He and for the makeup gas 30 mL/min of $N_2$. The flow rates for the detector gases were 37 mL/min of $H_2$ and 280 mL/min of air. The injector temperature was kept at 250 °C and the detector temperature at 280 °C. The linear retention index ($R_i$) is based on a series of *n*-hydrocarbons.

**HRGC/Mass Spectrometry (HRGC/MS).** HRGC/MS was performed with a Hewlett-Packard GCD system equipped with a PTV injector (KAS system, Gerstel, Mülheim, Germany). The same type of column and the same temperature program as mentioned above for HRGC analysis were used. Other conditions were as follows: carrier gas flow rate, 1.2 mL/min of He; temperature of ion source, 180 °C; electron energy, 70 eV; injection volumes, 1 $\mu$L.

**Nuclear Magnetic Resonance (NMR).** $^1H$ and $^{13}C$ NMR spectral data were recorded on Fourier transform Bruker AM 360 and AC 250 spectrometers with TMS as internal reference standard. Signals were assigned by $^1H-^1H$ COSY and $^1H-^{13}C$ COSY, as well as HMBC experiments.

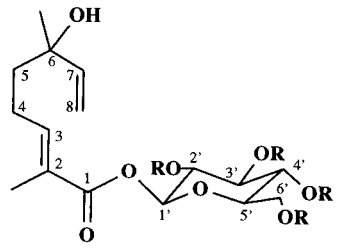
**Desorption Chemical Ionization Mass Spectrometry (DCI-MS).** DCI-MS was carried out with a Finnigan TSQ 70 mass spectrometer at 70 eV using ammonia as reactant gas and ion source temperature and pressure of 150 °C and 1.5 × $10^{-4}$ mbar, respectively, as well as a temperature gradient of 400 °C/min. Mass range was 60−900.

## RESULTS AND DISCUSSION

**Identification of the Glucose Ester of (*E*)-2,6-Dimethyl-6-hydroxyocta-2,7-dienoic Acid (1).** The isolation of glucose ester **1a** has been reported previously (Winterhalter et al., 1997); however, not all of the necessary spectral information could be obtained because of the instability of compound **1a.** After repurification of the sample by HPLC, a complete set of NMR spectral data for glucose ester **1a** was recorded in $C_6D_6$ (see Tables 1 and 2). The so-obtained $^{13}C$ NMR data confirmed the presence of an acetylated monoterpenoid glucose ester. For the aglycon moiety, 10 signals were observed including resonances for a carboxylic acid ($\delta$ 165.7) and a vinyl methylcarbinol moiety as well as an additional double bond. The complete structure of compound **1a** was unambiguously deduced using NOE, $^1H-^{13}C$ COSY, and HMBC NMR experiments. The stereochemistry at C-6 remains to be clarified.

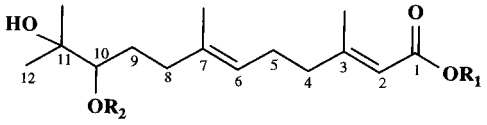
After deacetylation followed by enzymatic hydrolysis (sweet almond emulsin), D-glucose was identified in the hydrolysate by HPLC using on-line coupled refractive index and polarimetric detection (Skouroumounis and Winterhalter, 1994). The aglycon was identified as (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**6**) by comparison of its chromatographic and mass spectral data with those of an authentic reference. The latter has been prepared from racemic linalyl acetate (**3**) by $SeO_2$ oxidation, subsequent cyanide-catalyzed oxidative esterification (Corey et al., 1968), and deprotection using porcine liver esterase (see Figure 2).

**Identification of the Glucose Ester of (2*E*,6*E*)-10,11-Dihydroxy-3,7,11-trimethyl-2,6-dodecadienoic Acid (2).** $^1H$ and $^{13}C$ NMR data of the second glucose ester are presented in Tables 3 and 4. The $^1H$ NMR spectrum contained all of the signals of a peracetylated $\beta$-glucosyl moiety. Whereas the magnitudes of the vicinal coupling constants were coincident with data published for other $\beta$-glucopyranosides (Roscher and Winterhalter, 1993), a distinct downfield shift of the anomeric proton ($\delta$ 5.76) indicated the presence of an ester linkage (Winterhalter et al., 1991). Two resonances for olefinic methine hydrogens appeared in the low-field part of the spectrum (broad singlet at $\delta$ 5.68, broad triplet at $\delta$ 5.07), and a further signal (double doublet at $\delta$ 4.78) was assigned to an oxomethine group. In the high-field region four three-proton singlets and five acetoxy groups were apparent. The $^{13}C$ NMR spectrum confirmed the presence of peracetylated glucose ester. Signals for the aglycon included resonances for an $\alpha,\beta$-unsaturated carboxylic




**1** R = H

**1a** R = Ac




| | $R_1$ | $R_2$ |
|---|---|---|
| **2** | Glc | H |
| **2a** | $Glc(Ac)_4$ | Ac |
| **2b** | Me | H |
| **2c** | Me | Ac |

**Figure 1.** Structures of novel wine constituents **1** and **2**.

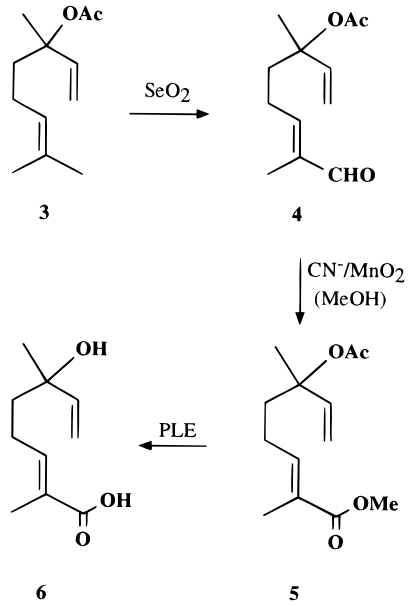



**Figure 2.** Synthesis of (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**6**) (for details, see text).

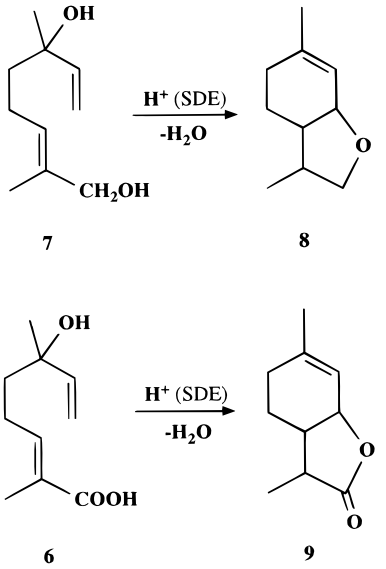



**Figure 3.** Acid-catalyzed formation of wine lactone **9** from monoterpene acid **6** in analogy to dill ether **8** formation from diol **7**.

acid ($\delta$ 164.5, 164.1, 114.1), an additional trisubstituted double bond ($\delta$ 135.4, 123.4), two oxygenated carbon atoms ($\delta$ 79.4, 72.4), and four methylene and methyl groups. By using $^1H-^1H$ and $^1H-^{13}C$ COSY as well as 2D heteronuclear long-range correlation experiments, the compound was identified as tetraacetylated glucose ester of 10-acetoxy-11-hydroxy-3,7,11-trimethyl-2,6-dodecadienoic acid (**2a**). The molecular formula ($C_{31}H_{46}O_{14}$) was confirmed by DCI-mass spectrometry.

Deacetylation followed by enzymatic hydrolysis liberated D-glucose as sugar moiety. The aglycon was extracted with diethyl ether and after methylation analyzed by HRGC/MS. The aglycon showed identical chromatographic and mass spectral data as obtained for an authentic reference of (2*E*,6*E*)-methyl-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate (**2b**) prepared from commercially available juvenile hormone III (Lindermann et al., 1995). $^{13}C$ NMR data of the 10-acetoxyderivate of **2c** are in good agreement with data recorded for the aglycon moiety of the peracetylated wine constituent **2a** (see Table 4). The configuration at the stereogenic carbon C-10 remains unsettled. Glucose ester **2** is to our best knowledge reported here for the first time.

Regarding the formation of **2** in wine, an acid-catalyzed conversion of glucoconjugated juvenile hormone III seems to be plausible. Attempts are presently made to identify the intact juvenile hormone III in Riesling grape juices.

**Aroma Precursor Role of Glucose Ester 1.** Whereas the glucose ester **1** has been identified for the first time as a natural wine constituent, glycoconjugates of its reduced form, that is, of the monoterpene diol **7**, are known Riesling wine constituents (Strauss et al., 1987). Under acidic conditions, diol **7** was reported to be partially converted into the bicyclic ether **8**, the so-called dill ether (Strauss et al., 1988). In analogy to the formation of ether **8** from terpenediol **7**, a likely formation of lactone **9** from acid **6** could be expected (see Figure 3). This so-called wine lactone, first identified as an essential oil metabolite in the koala (Southwell, 1975), has recently been established by Guth (1996) as a major aroma contributor in white wines. The 3*S*,3a*S*, 7a*R*-configured isomer of **9**, which has been identified in wine, is reported to possess an unusually low flavor threshold of 0.01−0.04 pg/L of air and a "sweet-coconut-like" aroma (Guth, 1996). To substantiate the hypothetical pathway of wine lactone **9** formation, the presumed progenitor **6** has been subjected to thermal treatment (SDE) at pH 3.2, 2.5, and 2.0, respectively. In all of these cases, wine lactone **9** was identified as a major conversion product of monoterpene acid **6**. The structure elucidation of additional degradation products formed from acid **6** and long-term storage experiments in model wine medium are subjects of ongoing studies.

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