3,4-Dihydroxy-7,8-dihydro-β-ionone β-D-Glucopyranoside: Natural Precursor of 2,2,6,8-Tetramethyl-7,11-dioxatricyclo[6.2.1.0^{1,6}]undec-4-ene (Riesling Acetal) and 1,1,6-Trimethyl-1,2-dihydronaphthalene in Red Currant (*Ribes rubrum* L.) Leaves

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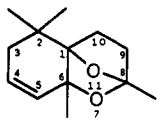
A precursor of 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0^{1,6}]undec-4-ene (Riesling acetal) and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), 3,4-dihydroxy-7,8-dihydro-β-ionone β-D-glucopyranoside, was isolated from red currant (*Ribes rubrum* L.) leaves. The steps of isolation comprised retention of the glycosides on Amberlite XAD-2 resin and subsequent prefractionation of the methanolic eluate by rotation locular countercurrent chromatography (RLCC). After acetylation and liquid chromatographic purification, characterization was made by means of LC–MS and NMR analyses.

INTRODUCTION

With the recent identification of 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0^{1,6}]undec-4-ene (Riesling acetal) (1) in Riesling wine (Winterhalter et al., 1990) and quince brandy (Näf et al., 1991) the question of its natural precursor arose. As a progenitor of 1 in wine, glycosidically bound 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol (2) has recently been reported (Winterhalter,



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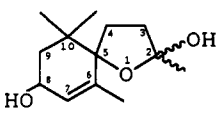

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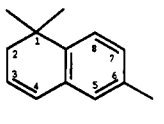

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1991). Volatile products formed from 2 at pH 3.2 under simultaneous distillation extraction (SDE) conditions comprised 1 and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (3) as major compounds. However, in this study, a sugar conjugate of 2 has not been characterized as yet.

As we have found 1 in the thermally treated (SDE; pH 2.5) glycosidic fraction of red currant (*Ribes rubrum* L.) leaves in amounts up to 10 mg/kg of fresh weight, this plant was a convenient starting material for the isolation of a natural precursor of 1. This paper concerns the isolation and characterization of a glucosidic precursor of 1 in *R. rubrum* leaves whose aglycon form is structurally related to the recently reported hemiacetal 2.

EXPERIMENTAL PROCEDURES

Chemicals. All commercial chemicals used were of analytical grade quality. Solvents were redistilled before use.

Plant Material. Fresh red currant (*R. rubrum* L.) leaves were available from a local plantation.

Isolation of a Glycosidic Extract. After mixing of 1.2 kg of *R. rubrum* leaves with the same amount of methanol and maceration of the mixture (pH adjusted to 7) at ambient temperature overnight, a clear extract was obtained by centrifugation (4000*g*; 30 min). Methanol was removed under reduced pressure (rotavapor) and the aqueous residue (400 mL) applied to an Amberlite XAD-2 column (30 × 300 mm) (Günata et al., 1985). After being washed with 3 L of distilled water, a glycosidic extract was obtained by eluting with 1 L of methanol. The methanol eluate was concentrated under reduced pressure (rotavapor) to approximately 20 mL. Remaining volatiles were removed by diethyl ether extraction.

Rotation Locular Countercurrent Chromatography (RLCC). Four milliliters of the glycosidic extract was subjected to a prefractionation using RLCC. The apparatus (Eyela RLCC, Tokyo Rikakikai Co.) was operated in the ascending mode employing a solvent system made from the two phases produced by mixing $CHCl_3$–MeOH–H_2O (7:13:8) with the more dense, less polar layer used as stationary phase. The flow rate was 1 mL/min, rotation speed 80 rpm, slope 25°. Fifty 10-mL fractions were separated that were analyzed by TLC using SiO_2 GF_{254} (Merck, Darmstadt, FRG) as stationary and the less polar RLCC layer as mobile phase (detection, 254 nm and vanillin/H_2SO_4). RLCC fractions 1–20, 21–26, 27–31, 32–40, and 41–50 were pooled, resulting in combined fractions I–V, respectively. The presence of an aglycon moiety in these fractions was checked by (i) enzymatic and (ii) acid hydrolysis.

Acetylation of RLCC Fraction III. The combined fractions III of five RLCC separations were concentrated under reduced pressure to dryness. The dry residue was acetylated by stirring with 5 mL of acetic anhydride in 5 mL of pyridine at ambient temperature overnight. After addition of 10 mL of ice water, extraction was performed three times with 100 mL of diethyl ether. The organic phase was extracted with 100 mL of 0.1 N HCl, neutralized with distilled water, dried over anhydrous sodium sulfate, and concentrated in vacuo to 1 mL.

Flash Chromatography. The acetylated glycosidic fraction III was subjected to flash chromatography (Still et al., 1978) using a 15 × 250 mm glass column filled with silica gel (0.032–0.063 mm; Merck). Elution with diethyl ether under N_2 pressure (20 mL/min) led to separation of 25 10-mL fractions. After check by TLC (cf. above) fractions 5–15 were combined and concentrated in vacuo to 1 mL for subsequent HPLC purification. Fractions 1–4 and 16–25 were discarded.

Preparative HPLC. The combined fractions 5–15 obtained by flash chromatography were subjected to preparative HPLC using a 16 × 250 mm SiO_2 Lichrospher 60 column (5 μm; Knauer, Berlin). Elution was performed with diethyl ether (10 mL/min), and the peaks were detected at 200 nm.

Deacetylation. After the addition of 20 mg of acetylated glycoside to a solution of 20 mg of sodium methylate in 5 mL of methanol and stirring overnight, 100 mg of Dowex 50-WX8 (20–50 mesh, H^+ form) was added. After 30 min, the exchanger was filtered off, the solvent removed under reduced pressure (rotavapor) to dryness, and the residue taken up in 5 mL of distilled water.

Enzymatic Hydrolysis. Four milligrams of the deacetylated glycoside was dissolved in 50 mL of 0.15 M phosphate–citrate buffer (pH 5.0) and the solution incubated with 10 mg of emulsin (Serva, Heidelberg) at 37 °C overnight. The liberated aglycons were extracted with diethyl ether; the organic phase was dried over anhydrous sodium sulfate and carefully concentrated to

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approximately 0.2 mL by a Vigreux column (45 °C) for subsequent HRGC and HRGC–MS analysis.

Acid Hydrolysis. A solution of 4 mg of deacetylated glycoside in 100 mL of distilled water (pH 2.5) was subjected to simultaneous distillation–extraction (SDE) (Schultz et al., 1977) over 1 h. The organic phase was dried over anhydrous sodium sulfate and carefully concentrated to approximately 0.2 mL by a Vigreux column (45 °C) for subsequent HRGC and HRGC–MS analysis.

Capillary Gas Chromatography (HRGC). (*a*) A Carlo Erba Fractovap 4160 gas chromatograph with FID equipped with a J&W fused silica DB-Wax capillary column (30 m × 0.259 mm i.d., film thickness 0.25 μm) was used. Split injection (1:50) was employed. The temperature program was 3 min isothermal at 50 °C, raised from 50 to 220 °C at 4 °C/min. The flow rate for the carrier gas was 1.8 mL/min He and for the makeup gas 30 mL/min N_2; for the detector gases the flow rates were 30 mL/min H_2 and 300 mL/min air. Injector and detector temperatures were kept at 220 °C.

(*b*) A Hewlett-Packard 5890 Series II gas chromatograph with FID equipped with a J&W fused silica DB-5 capillary column (30 m × 0.259 mm i.d., film thickness 0.25 μm) was used. Split injection (1:18) was employed. The temperature program was 1 min isothermal at 60 °C, raised from 60 to 280 °C at 5 °C/min. The flow rate for the carrier gas was 2.0 mL/min He and for the makeup gas 30 mL/min N_2; for the detector gases the flow rates were 30 mL/min H_2 and 300 mL/min air. Injector and detector temperatures were kept at 250 °C. Volumes of 1 μL were injected.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with Gerstel split injector (1:10) was combined by direct coupling to a Finnigan MAT 44 mass spectrometer. The same types of columns as mentioned above for HRGC analysis were used. The conditions were as follows: temperature program (DB-Wax), 3 min isothermal at 50 °C, raised from 50 to 240 °C at 4 °C/min; (DB-5) 60–300 °C at 5 °C/min; carrier gas flow rates, each 1.8 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA; mass range, 41–250. Volumes of 1 μL were injected.

Results of qualitative analyses were verified by comparison of HRGC retention (R_t) and mass spectral data with those of authentic reference substances.

Liquid Chromatography–Mass Spectrometry (Thermospray LC–MS). Thermospray LC–MS was performed with a Finnigan MAT 4500 system at 70 eV using 0.1 M NH_4Ac (10% methanol), a flow rate of 1.1 mL/min, and a vaporization temperature of 110 °C. Mass range was 100–750.

Nuclear Magnetic Resonance (NMR). NMR spectra were recorded on Bruker AC 200 (200 MHz) and, for two-dimensional NMR experiments, on AC 250 (250 MHz) spectrometers with $CDCl_3$ as solvent and Me_4Si as internal standard. For the 2D experiments the Bruker standard impulse sequence was used.

RESULTS AND DISCUSSION

RLCC preseparation of a glycosidic extract obtained from *R. rubrum* leaves by Amberlite XAD-2 adsorption and subsequent methanol elution (Günata et al., 1985) provided RLCC fractions 27–31, in which 1 and 3 were detected as major hydrolytic products formed at pH 2.5 under SDE conditions. After acetylation of these combined fractions (III), subsequent flash chromatography on silica gel allowed the separation of a major glycoside, which was finally obtained in pure form (50 mg) by preparative HPLC on silica gel.

The ^1H and ^{13}C NMR data of the isolated glycoside are represented in Tables I and II. In addition, two-dimensional NMR experiments (H,H- and H,C-COSY) were carried out. From these data the isolated compound was identified as sugar conjugate of **4**, i.e., the pentaacetate of 3,4-dihydroxy-7,8-dihydro-β-ionone β-D-glucopyranoside (**5b**), with the glucose moiety attached to carbon 3 (Figure 1). The data recorded by thermospray LC–MS were also in accordance with structure **5b**; only one peak was registered, i.e., m/z 616 ($M^+ + NH_4^+$), indicating a molecular mass of 598. Deacetylation of the isolated compound and subsequent treatment with β-glucosidase

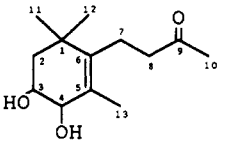
Table I. ^1H NMR Spectral Data of Isolated Compound 5b ($CDCl_3$, 200 MHz, Coupling Constants in Hertz, δ Relative to TMS)

δ	signal	J	atom
1.03/1.04	6 H, 2s		H_3 C11/H_3 C12
1.57	3 H, s		H_3 C13
1.65	1 H, dxd	12.7/2.6	H_a C2
1.86	1 H, dxd	12.7/12.7	H_b C2
1.97–2.06	15 H, 5s		H_3 acetates (5×)
2.13	3 H, s		H_3 C10
2.15–2.55	4 H, m		H_2 C7/H_2 C8
3.68	1 H, dxt	9.8/4.4	H C5′
3.83	1 H, dxdxd	12.7/3.4/2.6	H C3
4.15	2 H, d	4.4	H_2 C6′
4.62	1 H, d	7.7	H C1′
4.94	1 H, dxd	9.4/7.7	H C2′
5.00	1 H, dxd	9.8/9.4	H C4′
5.17	1 H, dxd	9.4/9.4	H C3′
5.34	1 H, br d	3.4	H C4

Table II. ^{13}C NMR Spectral Data of Isolated Compound 5b ($CDCl_3$, 50 MHz, δ Relative to TMS) and Reference Compound 5a (Miyase et al., 1989)

5b δ	DEPT	atom	5a δ
17.21	CH_3	C13	18.4
20.55–20.95	CH_3	CH_3CO (5×)	
21.96	CH_2	C7	22.4
26.79[a]	CH_3	C11	29.3
28.92[a]	CH_3	C12	29.6
29.77	CH_3	C10	27.1
37.88	C	C1	37.8
40.53	CH_2	C2	39.8
43.32	CH_2	C8	43.8
62.34	CH_2	C6′	62.8
68.72	CH	C4′	71.7
70.21	CH	C4	68.9
71.07	CH	C2′	75.6
71.67	CH	C5′	78.8
72.82	CH	C3′	79.0
74.45	CH	C3	74.6
100.75	CH	C1′	101.5
124.09	C	C5	128.0
144.23	C	C6	141.2
169.44	C	CH_3CO	
169.54	C	CH_3CO	
170.11	C	CH_3CO	
170.48	C	CH_3CO	
171.06	C	CH_3CO	
207.78	C	C9	207.4

[a] Interchangeable values.



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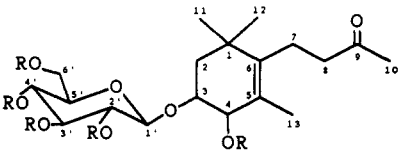

R = H 5a
R = Ac 5b

Figure 1. Structures of the precursor compound **5** as well as the enzymatically liberated aglycon **4**.

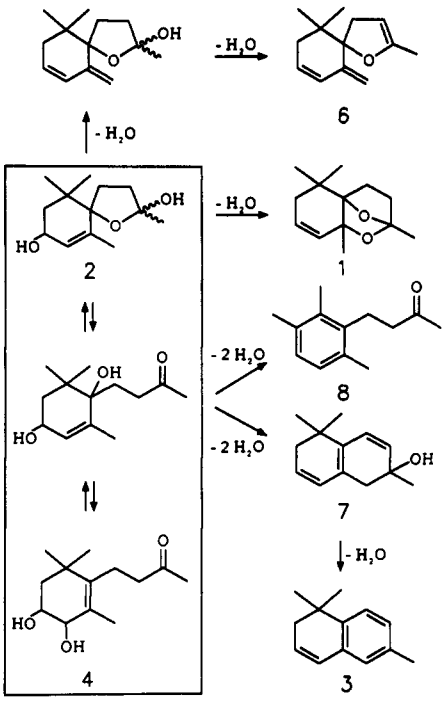

Figure 2. Structures and proposed formation of thermal degradation products 1, 3, and 6–8 from 3,4-dihydroxy-7,8-dihydro-β-ionone (4).

Table III. Relative Amounts of Degradation Products (SDE, pH 2.5, 1 h) of 3,4-Dihydroxy-7,8-dihydro-β-ionone Glucoside (5a) Compared to the Results of Winterhalter (1991) on the Hydrolysis of Hemiacetal 2

		%	
R_t[a]	degradation products	5a	2
1504	2,10,10-trimethyl-6-methylene-1-oxa-spiro[4.5]deca-2,7-diene (6)	5	6
1612	2,2,6,8-tetramethyl-7,11-dioxatricyclo-[6.2.1.$0^{1,6}$]undec-4-ene (Riesling acetal) (1)	44	57
1712	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (3)	41	24
1973	6-hydroxy-1,1,6-trimethyl-1,2,5,6-tetra-hydronaphthalene (7) (tent.)	8	9
2193	4-(2,3,6-trimethylphenyl)-2-butanone (8)	1	3

[a] Linear retention index on a J&W fused silica DB-Wax capillary column (30 m × 0.259 mm i.d., df = 0.25 μm).

(emulsin) finally led to the liberation of aglycon 4 (two diastereomers; R_t DB-5, 1825/1828). Recently, glucoside **5a** has also been identified in *Epimedium diphyllum* (Miyase et al., 1989).

Continuing our biomimetic studies on C_{13} norisoprenoid precursor compounds (Winterhalter and Schreier, 1989; Herderich and Winterhalter, 1991; Winterhalter et al., 1991; Güldner and Winterhalter, 1991), the deacetylated glucoside **5a** was subjected to SDE at pH 2.5. Under these conditions the products 1, 3, and 6–8 represented in Figure 2 were formed. As shown from these data, from glucoside **5a** the same pattern of volatile degradation products was formed as has been obtained from hemiacetal 2 (Winterhalter, 1991). This result can be explained by an equilibrium existing between hemiacetal 2 and the allylic rearrangement product 4 (cf. Figure 2). Quanitatively (cf. Table III), under the experimental conditions used in the present study a higher amount of TDN (3) was formed from **5a** compared to the degradation of hemiacetal 2, thus yielding the norisoprenoid target compounds 1 and 3 in a relative proportion of 1:1.

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