3,4-Dihydroxy-7,8-dihydro-β-ionol β-D-Glucopyranoside: Natural Precursor of Isomeric Vitispiranes from Gooseberry (*Ribes uva crispa* L.) and Whitebeam (*Sorbus aria*) Leaves

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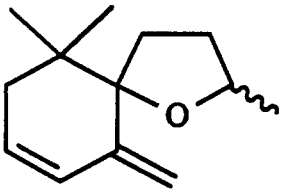
After isolation of glycosidic extracts obtained from gooseberry (*Ribes uva crispa* L.) and whitebeam (*Sorbus aria*) leaves by Amberlite XAD-2 adsorption and methanol elution followed by hydrolysis under simultaneous distillation–extraction conditions at pH 2.5, among the aglycons the isomeric vitispiranes were detected as major constituents (20 and 70 mg/kg, respectively) by HRGC and HRGC–MS analyses. Using prefractionation of the glycosidic extracts by rotation locular countercurrent chromatography, subsequent acetylation and liquid chromatographic purification, a glycoside was isolated in pure form whose structure was elucidated by LC–MS and NMR analyses to be the β-D-glucopyranoside of 3,4-dihydroxy-7,8-dihydro-β-ionol.

INTRODUCTION

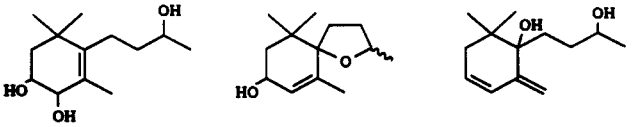
The isomeric vitispiranes **1a/1b** have been identified in grape juice and wines (Simpson et al., 1977) and also recognized as aroma constituents of vanilla (Schulte-Elte et al., 1978) and quince fruit (Winterhalter and Schreier, 1988). The odors of the diastereoisomers have been



1a/1b

described to be different. The cis compound **1a** is fresher and more intense than the trans compound **1b**. The spiro ether **1a** has been reported to be reminiscent of the green odor of chrysanthemum with an additional flowery-fruity wine note, while **1b** has been characterized by a heavy scent of exotic flowers with an earthy-woody undertone (Schulte-Elte et al., 1978).

As potential precursors of **1a/1b** different compounds have been discussed, i.e., the triol **2** and 8-hydroxytheaspirane **3** (resulting from **2** by dehydration) (Strauss et al., 1984) as well as, more recently, the diol **4**. As



2 **3** **4**

postulated by Waldmann and Winterhalter (1992), these precursors may be regarded as intermediates arising from the genuine progenitor **5** (Figure 1). However, glycoconjugates of **2–5** have not been characterized to date.

In the course of a preliminary screening, the study of gooseberry (*Ribes uva crispa* L.) and whitebeam (*Sorbus aria*) leaves revealed **1a/1b** as major aglycons. Thus, these

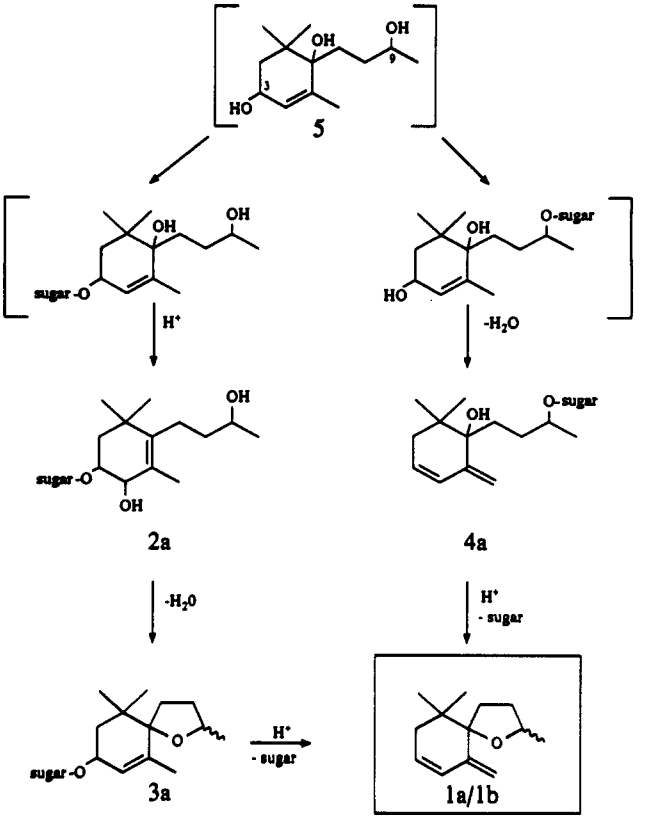


Figure 1. Proposed pathways for the formation of vitispirane **1a/1b** (Waldmann and Winterhalter, 1992).

materials were suitable sources for the investigation of a glycosidically bound vitispirane (**1a/1b**) precursor. In this paper, we describe for the first time the isolation and characterization of a glycoconjugate of **2** as a natural vitispirane precursor from gooseberry and whitebeam leaves.

EXPERIMENTAL PROCEDURES

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

Plant Material. Leaves were plucked in September 1991 from gooseberry (*R. uva crispa* L.) and whitebeam tree (*S. aria*) grown in the Würzburg area.

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Isolation of a Glycosidic Extract. After 1 kg of leaves was mixed with 1 L of methanol and macerated (adjusted to pH 7) at ambient temperature overnight, a clear extract was obtained by centrifugation (5000*g*, 30 min). Methanol was removed under reduced pressure (rotavapor). The aqueous residue was extracted three times with 100 mL of pentane and subsequently diethyl ether to separate chlorophyll and free volatiles and then applied to an Amberlite XAD-2 column (25 × 900 mm, 10 mL/min) (Günata et al., 1985). After the column was washed with 3000 mL of distilled water, a glycosidic extract was obtained by eluting with 1 L of methanol. The methanol eluate was concentrated under reduced pressure to dryness (rotavapor) and redissolved in 20 mL of 0.2 M citrate–phosphate buffer (pH 5). Yields: 9 g (gooseberry) and 11 g (whitebeam). Remaining volatiles were removed by diethyl ether extraction.

Rotation Locular Countercurrent Chromatography (RLCC). Each of the 4-mL glycosidic extracts was prefractionated 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, and slope 25°. Fifty 10-mL fractions were separated and were analyzed by TLC using SiO_2 GF_{254} (Merck, Darmstadt) as stationary phase and the less polar RLCC layer as mobile phase (detection, 254 nm and vanillin/H_2SO_4). RLCC fractions 1–23, 24–27, 28, 29–32, 33–37, 38–42, and 43–50 were pooled, resulting in combined fractions I–VII, respectively. The presence of the aglycon moiety in these fractions was checked by (i) enzymatic and (ii) acid hydrolysis.

Acetylation of RLCC Fraction IV. The combined fractions IV 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. Acetylated glycosidic fraction IV was subjected to flash chromatography (Still et al., 1978) using a 10 × 400 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 9–11 were combined and concentrated in vacuo to 1 mL for subsequent HPLC purification. Fractions 1–8 and 12–25 were discarded.

Preparative HPLC. The combined fractions 9–11 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), yielding six fractions (detection 200 nm).

Deacetylation. After the addition of 2 mg of acetylated glycoside to a solution of 25 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. Five hundred micrograms of the deacetylated glycoside was dissolved in 50 mL of 0.2 M citrate–phosphate buffer (pH 5.0) and the solution incubated with 10 mg of emulsin (Serva, Heidelberg) at 37 °C overnight. The liberated aglycon was extracted with diethyl ether; 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.

Acid Hydrolysis. A solution of 500 μg of deacetylated glycoside in 100 mL of distilled water (pH 2.5) was subjected to simultaneous distillation–extraction (SDE) (Schultz et al., 1977) over 2 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 42160 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; the flow rates for the makeup gas were 30 mL/min N_2 and for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. 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 (1:20) 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 1.5 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.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 3300 gas chromatograph with split injector (1:20) 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 were used. The conditions were as follows: temperature programs, (DB-Wax) 3 min isothermal at 50 °C, raised from 50 to 220 °C at 4 °C/min and kept at 220 °C for 10 min; (DB-5) 60–300 °C at 5 °C/min; carrier gas flow, 1.5 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_i) and mass spectral data with those of authentic reference substances. Quantitative HRGC determinations were carried out by means of standard added (cf. above) without consideration of extraction yields (F = 1.0).

Liquid Chromatography–Mass Spectrometry (LC–MS). Thermospray LC–MS was performed with a Finnigan MAT 4500 system at 70 eV using 0.1 mM NH_4Ac (10% MeOH), a flow rate of 1.3 mL/min, and a vaporization temperature of 110 °C. Mass range was 100–700.

Direct Chemical Ionization Mass Spectrometry (DCI-MS). DCI-MS was carried out with a Finnigan MAT 90 mass spectrometer at 70 eV using ammonia as reactant gas and ion source temperature and pressure of 150 °C and 8 × 10^{-5} mbar, respectively, as well as a temperature gradient of 400 °C/min. Mass range was 60–900.

Nuclear Magnetic Resonance (NMR). NMR spectra were recorded on a Bruker WM 400 (400 MHz) spectrometer with $CDCl_3$ as solvent and Me_4Si as internal standard.

RESULTS AND DISCUSSION

In the aglycon fraction of gooseberry (*R. uva crispa* L.) and whitebeam (*S. aria*) leaves obtained by hydrolysis under SDE conditions (pH 2.5) of a methanolic eluate from XAD-separated glycosidic fraction (Günata et al., 1985) the isomeric vitispiranes (**1a**/**1b**) have been identified by HRGC–MS as major volatile constituents (Humpf, 1992; Wintoch, 1992). HRGC quantification revealed 20 mg/kg in gooseberry and 70 mg/kg in whitebeam leaves.

RLCC preseparation of glycosidic leaf extracts provided fractions 29–32, in which **2** was detected as major hydrolytic product after enzymatic treatment with β-glucosidase (emulsin) at pH 5 [R_i = 1820 (DB-5)]. In addition, traces of 8-hydroxytheaspirane (**3**) were detectable. However, acidic hydrolysis under SDE conditions (pH 2.5) led only to vitispirane (**1a**/**1b**) formation.

After acetylation of the combined RLCC fractions (=IV), subsequent flash chromatography on silica gel allowed the separation of a major glycoside which was finally obtained in pure form by preparative HPLC on silica gel. The ^1H and ^{13}C NMR data of the isolated glycoside **2a** are represented in Tables I and II. From these data the isolated compound was identified as sugar conjugate from **2**, i.e., the hexaacetate of 3,4-dihydroxy-7,8-dihydro-β-ionol

Table I. ^1H NMR Spectral Data of Isolated Compound 2b ($CDCl_3$, 400 MHz, Coupling Constants in Hertz, δ Relative to TMS)

δ	signal	J	atom
1.04/1.06	6 H, 2 s		H_311/H_312
1.22	3 H, d	6.15	H_310
1.63	3 H, s		H_313
1.65	1 H		H 2a, obscured
1.86	1 H, dd	12.6/12.6	H 2b
1.98–2.07	18 H, 6 s		H_3 acetates (6×)
1.9–2.2	4 H, m		H_27/H_28, obscured
3.68	1 H, ddd	9,7/5.0/3.0	H5′
3.83	1 H, ddd	12.6/3.7/3.4	H3
4.16	2 H, brd	5.0	H_26'
4.62	1 H, d	7.8	H1′
4.87	1 H, sextet	6.15	H9
4.94	1 H, dd	9.5/7.8	H2′
5.00	1 H, dd	9.7/9.5	H4′
5.17	1 H, dd	9.5/9.5	H3′
5.35	1 H, d	3.4	H4

Table II. ^{13}C NMR Spectral Data of Isolated Compound 2b ($CDCl_3$, 100 MHz, δ Relative to TMS)

δ	DEPT	atom
17.23	CH_3	C13
19.76	CH_3	C10
20.57–21.27	CH_3	CH_3CO (6×)
24.31	CH_2	C7
26.93[a]	CH_3	C11
29.11[a]	CH_3	C12
35.60	CH_2	C8
37.83	C	C1
40.57	CH_2	C2
62.38	CH_2	C6′
68.75	CH	C4′
70.3	CH	C4
71.10	CH	C2′
71.35	CH	C5′
71.75	CH	C3′
72.86	CH	C9
74.53	CH	C3
100.75	CH	C1′
123.69	C	C5
144.66	C	C6
169.45	C	CH_3CO
169.57	C	CH_3CO
170.28	C	CH_3CO
170.49	C	CH_3CO
170.71	C	CH_3CO
171.14	C	CH_3CO

[a] Interchangeable values.

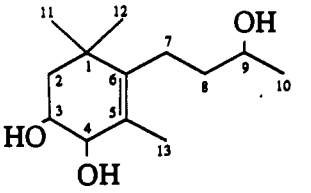
Table III. DCI- and TSP-MS Data of 2b[a]

	%		
m/z	DCI	TSP	interpretation
660	100	34	$M + NH_4^+$
331	4	20	162 (hexose − H_2O) + 4 × 42 (acetyl) + 1 (peracetylated hexose)

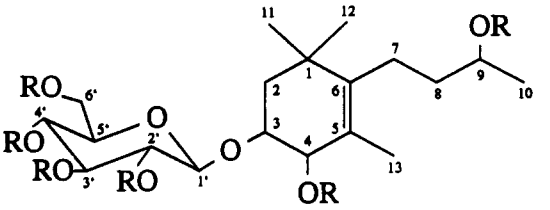
[a] DCI, ammonia; TSP (thermospray LC–MS), vaporizer 110 °C; 0.1 mM ammonium acetate.

β-D-glucopyranoside (**2b**), with the glucose moiety attached to carbon 3. The data recorded by thermospray LC–MS and DCI-MS were also in accordance with structure **2b** (Table III). Deacetylation of the isolated compound and subsequent treatment with β-glucosidase (emulsin) led to the liberation of aglycon **2**. The glucoside **2a** has not been described as yet.

The identification of **2a** strongly supported the recently postulated biogenetic pathways of vitispirane formation (Figure 1) (Waldmann and Winterhalter, 1992). In triol



2



R = H 2a
R = Ac 2b

5, glycoconjugation might occur in position 3 or 9. Glycosidation in position 3 leads by allylic rearrangement to the thermodynamically more stable glycoconjugate **2a**. The next step comprises dehydration and cyclization, giving rise to the glycoconjugate of 8-hydroxytheaspirane, from which **1a**/**1b** are generated by acidic hydrolysis of the sugar moiety.

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