Research Note

Isolation of the glucose ester of (E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid from Riesling wine

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S u m m a r y : 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 ester of (E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (linalool-8-carboxylic acid) 1 was identified for the first time as natural wine constituent. The possible role of 1 as wine aroma precursor is discussed.

K e y   w o r d s : wine lactone, aroma precursor, multilayer coil countercurrent chromatography, wine.

Introduction: The presence of acid-labile glyco-conjugates of monoterpenoids and C_{13}-norisoprenoids in Riesling wine is well documented (STRAUSS et al. 1987, 1988; WINTERHALTER et al. 1990; WILLIAMS 1993). The growing interest in these structures in recent days is mainly due to their role as flavour precursors. Especially during a prolonged storage of wine, the acid-catalyzed degradation of such glycoconjugates is known to make an important contribution to the overall aroma of bottle-aged wines (RAPP et al. 1985; WINTERHALTER 1994). In this paper, we report the identification of an additional monoterpenoid gluco-conjugate, which is a likely intermediate in the formation of the so-called wine lactone 5.

Material and methods: A commercial Riesling wine (100 l, QbA quality, Rheinpfalz, 1992 vintage) was dealcoholized prior to work-up.

The dealcoholized wine was passed through a column of Amberlite XAD-2 resin (GÜNATA et al. 1985). After rinsing with H_2O, the retained material was eluted with MeOH. The methanolic eluate was concentrated under reduced pressure and the remaining volatiles were removed by Et_2O extraction. For the initial fractionation of the isolate (20 g), multilayer coil countercurrent chromatography (MLCCC) was used (Multilayer Coil Separator-Extractor, P.C. Inc., Potomac, USA; equipped with a 85 m x 2.6 mm i.d. PTFE tubing; solvent system: $CHCl_3/MeOH/H_2O$ 7:13:8). MLCCC separated 7 fractions of which MLCCC fr. III was acetylated. After flash chromatography (STILL et al. 1978) and final purification by HPLC (Eurospher Si 100 column, 5 μm, 250 x 4 mm, Knauer Säulentechnik, Berlin; eluent: methyl-tert-butylether/pentane 8:2) the peracetylated glucose ester 1a was obtained in pure form (1.8 mg).

The following instrumentation was used: NMR: Bruker AM 360 ($CDCl_3$, chemical shifts in ppm);

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Desorption Chemical Ionization (DCI)-MS: Finnigan TSQ 70 (reactant gas: ammonia). Spectral data of the peracetylated glucose ester of (E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (1a). DCI-MS: pseudo-molecular ion at m/z 532 [M(514) + NH_4]$^+$, $C_{24}H_{34}O_{12}$. ^{1}H-NMR (360 MHz): δ 1.31 (3H, s, Me-6), 1.65 (2H, m, H_2-5), 1.81 (3H, br s, Me-2), 2.00-2.05 (4 x 3H, 4s, acetates), 2.23 (2H, m, H_2-4), 3.87 (1H, ddd, J = 10.0, 4.5, 2.5 Hz, H-5'), 4.12 (1H, dd, J = 12.5, 2.5 Hz, H_a-6'), 4.30 (1H, dd, J = 12.5, 4.5 Hz, H_b-6'), 5.10 (1H, dd, J = 10.5, 1.2 Hz, H_a-8), 5.14 (1H, dd, J = 10.5, 9.5 Hz, H-4'), 5.21 (1H, dd, J = 9.5, 8.0 Hz, H-2'), 5.23 (1H, dd, 17.5, 1.2 Hz, H_b-8), 5.28 (1H, dd, J = 9.5, 9.5 Hz, H-3'), 5.74 (1H, d, J = 8.0 Hz, H-1'), 5.90 (1H, dd, J = 17.5, 10.5 Hz, H-7), 6.86 (1H, tq, J = 7.0, 1.5 Hz, H-3). The arrangement of the substituents at the double bond in 2,3-position was evaluated by a NOE experiment: irradiation at δ 1.81 ppm (CH_3-2) increased the multiplet at δ 2.23 ppm (H_2-4), thus revealing E-configuration. The site of the glycosidic linkage was established from a heteronuclear multi-bond correlation (HMBC) experiment, which inter alia showed a cross peak from C-1 to the anomeric proton H-1'. The stereochemistry at the asymmetric centre C-3 remains unsettled. Upon concentration of the $CDCl_3$ solution, an acid-catalyzed rearrangement of 1a to a mixture of diastereoisomers took place. In the ^{1}H-NMR spectrum of the rearranged products, all the signals for the sugar moiety were present. For the terpene moiety, the signals of the vinyl group were missing. The tiny amounts of rearranged products excluded so far a complete structural assignment of the different isomers.

Results and Discussion: The glycosidic XAD-2 isolate from Riesling wine was fractionated with multilayer coil countercurrent chromatography (ITO 1986; ROSCHER and WINTERHALTER 1993). The subfractions were then acetylated and further purified by flash chromatography and HPLC. Of the many glycoconjugates isolated, one in particular showed an unusually low chemical shift for the anomeric proton. Whereas in β-D-glucosides the anomeric proton resonates around δ 4.5 ppm, the anomeric proton in structure 1a showed a downfield shift and resonated at δ 5.7 ppm. This δ value is typical for glucose esters (KOSHIMIZU et al. 1968, LOVEYS and MILLBORROW 1981). Additional signals in the ^{1}H-NMR spectrum of 1a included four olefinic protons, i.e. a typical ABX pattern for a vinyl group at δ 5.10, 5.23 and 5.90 ppm (J_{AB} = 1.2 Hz; J_{AX} = 10.5 Hz, cis-coupling; J_{BX} = 17.5 Hz, trans-coupling) as well as a methine proton at δ 6.86 ppm. The latter showed in addition to the coupling to H_2-4 (J = 7.0 Hz) a long-range coupling (J = 1.5 Hz) to the allylic methyl group at C-2. The methylene groups at C-4 and C-5 resonated as multiplets at δ 2.23 and 1.65 ppm, respectively. Two three-proton singlets at δ 1.31 and 1.81 ppm were assigned to a tertiary methyl group attached to a carbon bearing a hydroxyl group (C-6) and an allylic methyl group (Me-2), respectively. The ^{1}H NMR data for the terpene moiety are in good agreement with those published for 2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid 4 isolated from Artemisia sieberi (MARCO et al. 1993). The data for the sugar moiety

are in accordance with those published for other β-glucose esters (WINTERHALTER *et al.* 1991). The proposed structure **1a** was confirmed by the recorded DCI-MS data.

1 R = H

1a R = Ac
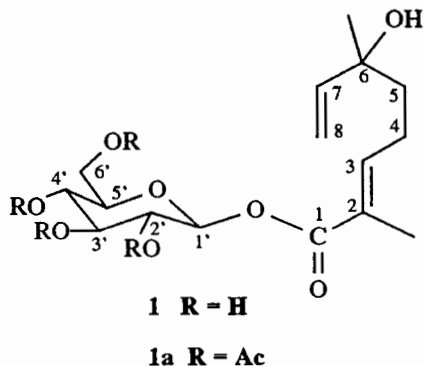
Whereas the glucoseester of (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid **1** is reported for the first time as natural wine constituent, glycoconjugates of its reduced form, i.e. of the monoterpene diol **2**, are known Riesling wine constituents (STRAUSS *et al.* 1988). Under acidic conditions, diol **2** was converted *inter alia* into the bicyclic ether **3**. In analogy to the formation of ether **3** from terpene diol **2**, a likely formation of lactone **5** from the monoterpenoid acid **4** can be expected (cf. Figure). The so-called
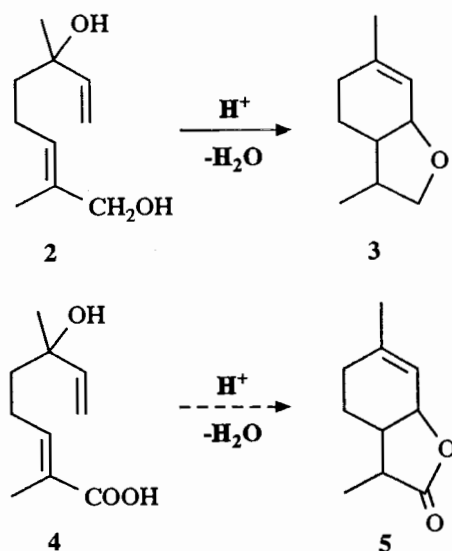

Figure: Acid-catalyzed formation of bicyclic ether **3** from terpene diol **2** and the postulated generation of wine lactone **5** from (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid **4**.

wine lactone **5**, first identified as an essential oil metabolite in the Koala (SOUTHWELL 1975), has recently been established as a major aroma contributor in two white wine varieties (GUTH 1995). In order to substantiate the hypothetic pathway of the formation of wine lactone **5**, syntheses of structures **1** and **4** as well as model degradation reactions under pH conditions of wine are in progress.

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