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Identification of a novel vitispirane precursor in Riesling wine

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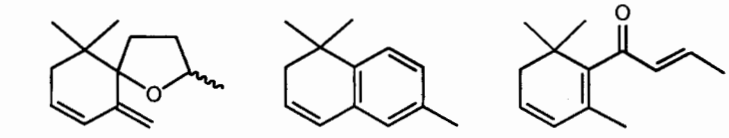
Identifizierung eines neuen Vitispiranvorläufers in Rieslingwein

Zusammenfassung: Zwei Diastereomere von 1-(3-Hydroxybutyl)-6,6-dimethyl-2-methylen-3-cyclohexen-1-ol wurden erstmals als Aglykone in Glykosidextrakten von Rieslingwein identifiziert. Modellreaktionen bei pH 3,2 lieferten die isomeren Vitispirane als Hauptabbauprodukte. Aufgrund dieser Ergebnisse wird ein erweitertes Modell zur Vitispiranbildung in Rieslingwein vorgestellt.

Key words: wine, bottle aging, glycosides, vitispiranes, flavour, precursors.

Introduction

Compounds considered to be important for the aroma of bottle-aged Riesling wine include a series of C_{13}-norisoprenoid volatiles, i.e. isomeric vitispiranes (1), 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (2), as well as β-damascenone (3) (SIMPSON *et al.* 1977; SIMPSON 1978; SIMPSON and MILLER 1983; RAPP *et al.* 1985). Initial research into the



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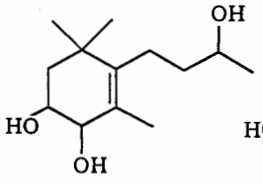

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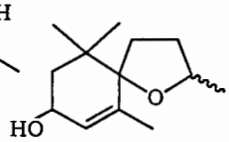

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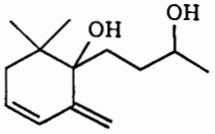

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origin of these norisoprenoids indicated a formation via acid-catalyzed degradation of glycosidically bound progenitors (WILLIAMS *et al.* 1982; STRAUSS *et al.* 1986). However, due to the structural diversity of the numerous glycosidic wine constituents, progress in the separation and purification of the corresponding precursors has been slow. It was only in recent years that the all-liquid chromatographic technique of countercurrent chromatography has been recognized as important tool for the fractionation of complex glycosidic mixtures (WINTERHALTER 1991 a, and references cited). Especially, droplet countercurrent chromatography (DCCC) has been employed as the method of choice for the preparative separation of glycosidic isolates from grape juice and wine

(STRAUSS *et al.* 1987). A further approach, the so-called two-dimensional GC-DCCC analysis of Riesling wine glycosides, revealed the presence of almost one hundred - various glycosylated - aglycons in this particular wine (WINTERHALTER *et al.* 1990 a). The same technique has then also been employed for the separation of precursors of vitispiranes (1), TDN (2), and β-damascenone (3). As a result of DCCC prefractionation combined with subsequent acid hydrolyses of separated DCCC-fractions multiple precursors have been detected for each of the C_{13}-flavour compounds under investigation (WINTERHALTER *et al.* 1990 b).

With regard to vitispirane (1) formation, unspecified bound forms of megastigm-5-ene-3,4,9-triol (4) as well as isomeric 3-hydroxytheaspiranes (5) have been found in our previous studies as vitispirane-yielding precursors in combined DCCC fractions 60—90, 100—130, and 160—220, which have been obtained from an Australian Riesling wine. However, these compounds were absent as aglycons in a further vitispirane-yielding fraction, i.e. combined DCCC fractions 220—280 (WINTERHALTER *et al.* 1990 b). The present paper reports the identification of an additional precursor of isomeric vitispiranes (1) from these fractions, and proposes an extended pathway for vitispirane formation in Riesling wine.

Materials and methods

Wine sample

Glycosidic isolates have been obtained from a 1988 Riesling wine from McLaren Vale, South Australia.

General procedures

The preparation of C_{18}-reversed phase isolates, their separation with DCCC, along with conditions for enzymic as well as acid hydrolyses with the aid of simultaneous distillation-extraction (SDE) have been given previously (WINTERHALTER *et al.* 1990 a, b).

Capillary gas chromatography - mass spectrometry (HRGC-MS)

HRGC-MS was undertaken with a Varian Aerograph 1440 gas chromatograph by direct coupling to a Finnigan MAT 44 mass spectrometer with PCDS data system. Split injection (1:10) was used. Two types of WCOT fused silica capillary columns were employed: (a) J and W DB-Wax (30 m, 0.25 mm i.d., film thickness 0.25 µm); (b) J and W DB-5 (30 m, 0.25 mm i.d., film thickness 0.25 µm). The conditions were as follows: temperature programs (a) from 50 °C up to 240 °C with 4 °C/min, 10 min isothermal at 240 °C; (b) from 50 °C to 300 °C with 5 °C/min; carrier gas flow rate, 2.5 ml/min of He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.7 mA.

Capillary gas chromatography - Fourier transform infrared spectroscopy (HRGC-FTIR)

A HP-IRD system (5965B with a wide band MCT detector) interfaced by a HP 5890 series II gas chromatograph with the same types of columns as mentioned above was used. Vapor-phase spectra were recorded from 550—4000 cm^{-1} with a resolution of 8 cm^{-1}.

Preparation of 1-(3-hydroxybutyl)-6,6-dimethyl-2-methylene-3-cyclohexen-1-ols (6)

Diastereoisomeric diols (6) were obtained as by-products (10 %) upon reduction of 6,6-dimethyl-2-methylene-1-(3-oxo-1-butenyl)-3-cyclohexen-1-ol (KATO and KONDO 1981) using $LiAlH(OC(CH_3)_3)_3$ × CuBr as reduction reagent. For the prefractionation of the reaction mixture flash chromatography (pentane-diethylether gradient) was used; the final separation of the diastereoisomers of (6) was achieved by preparative HPLC with diethylether as eluent (flow rate of 7 ml, LiChrospher Si100 column, 5 µm, 250 × 16 mm; Knauer, Berlin). Diols (6) showed the following chromatographic and spectral data:

Diastereoisomer A: Linear retention index R_i (DB-5) 1574, R_i (DB-Wax) 2418. MS (70 eV) cf. Fig. 1. FTIR (vapor phase, ν, cm^{-1}) 3640 (tert. OH), 3520 (intramol. H-bonding), 3090, 3035, 2973, 2898, 1630, 1600, 1442, 1381, 1253, 1054, 991, 897, 779. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 0.87 and 1.04 (2 × 3H, 2s, $2CH_3$-Cl), 1.16 (3H, d, J = 6.2 Hz, CH_3-C9), 1.2-1.8 (4H, m, H_2C7 and H_2C8), 1.95 (1H, dd, J_1 = 18.8 Hz, J_2 = 5.4 Hz, H_aC2), 2.05 and 2.13 (2H, 2 × br. s, 2 × OH), 2.24 (1H, br. d, J = 18.8 Hz, H_bC2), 3.70 (1H, m, HC9), 5.02 (1H, s, H_aC13), 5.16 (1H, s, H_bC13); 5.58 (1H, m, HC3), 6.05 (1H, dd, J_1 = 9.9 Hz, J_2 = 2.4 Hz, HC4). ^{13}C-NMR[1]) (100 MHz, $CDCl_3$, ppm): 18.4 (C10), 23.0 and 23.9 (C11 and C12), 29.3 and 32.9 (C7 and C8), 37.9 (C1), 40.8 (C2), 69.0 (C9), 77.9 (C6), 111.8 (C13), 127.0 (C3), 128.7 (C4), 147.7 (C5).

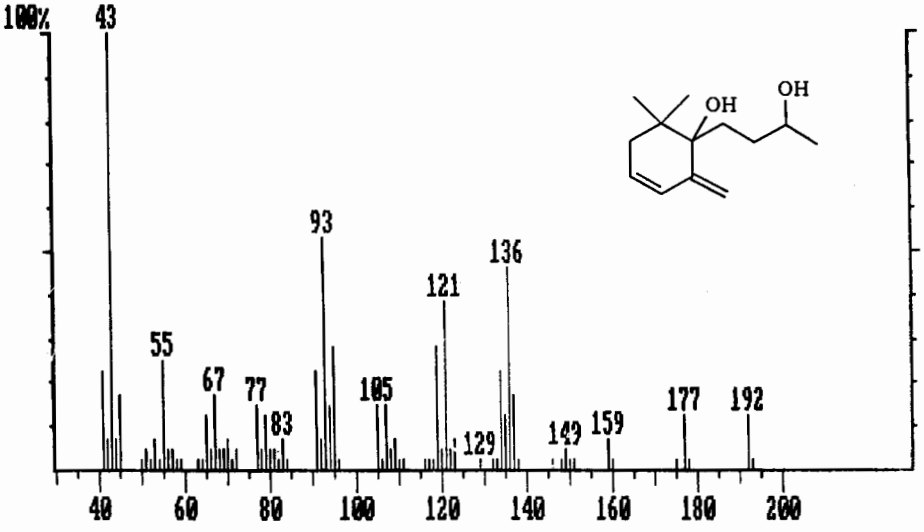

Fig. 1: Mass spectra (70 eV) of vitispirane precursor (6).
Massenspektren (70 eV) des Vitispiranvorläufers (6).

Diastereoisomer B: R_i (DB-5): 1583, R_i (DB-Wax): 2394. MS, FTIR and ^{1}H-NMR data: identical with diastereoisomer A. ^{13}C-NMR: 23.0 and 23.6 (C11 and C12), 28.1 and 33.0 (C7 and C8), 37.8 (C1), 40.8 (C2), 68.0 (C9), 78.1 (C6), 111.3 (C13), 127.1 (C3), 128.6 (C4), 147.6 (C5).

[1]) The assignments are based on a DEPT experiment.

Results and discussion

Continuing our work on aroma precursors of Riesling wine (WINTERHALTER *et al.* 1990 a, b; 1991 b) the new wine constituents (6) (two diastereoisomers) were identified as aglycons in a glycosidic isolate of an Australian Riesling. Diastereoisomeric diols (6) (first reported as 'unknown norisoprenoids 42 and 43'; cf. WINTERHALTER *et al.* 1990 a) were detected in the late eluting DCCC-fractions 220—280. Their structure was now confirmed by synthesis. The HRGC retention time of the synthesized reference compounds matched those of the unknown Riesling aglycons on two different stationary phases, and also gave an identical electron impact MS. Although already known as synthetic progenitors of vitispiranes (1) (KATO and KONDO 1981), diastereoisomeric diols (6) have been identified to the authors' best knowledge for the first time as natural products. Model degradation reactions carried out with synthetic (6) using the SDE-method (SCHULTZ *et al.* 1977) revealed the easy formation of isomeric vitispiranes (1) at pH conditions of wine (pH 3.2). Besides spiroethers (1), diastereoisomeric 3,4,6,8a-tetrahydro-2,5,5,8a-tetramethyl-2*H*-1-benzopyran-4a(5*H*)-ols ('hydroxydihydroedulans') were obtained as additional degradation products of diols (6) (ratio vitispiranes/hydroxydihydroedulans approx. 3:1), the latter being known as volatile wine constituents (STRAUSS *et al.* 1986).

With the finding of the additional vitispirane-yielding aglycons (6), the following pathway for vitispirane formation in Riesling wine is proposed (cf. Fig. 2): As an initial hypothetic precursor, triol (7) seems to be plausible, which after glycosylation in the 3- or 9-position, respectively, is expected to react in two different ways: First, with the sugar attached to the 3-position, the conjugate is susceptible to an acid-induced allylic rearrangement giving rise to a formation of the thermodynamically more stable isomer (4) as previously shown by STRAUSS *et al.* (1986). A pathway from (4) to the target compounds (1) has been discussed recently (WINTERHALTER and SCHREIER 1988). Second, with the glucosidic linkage in the side-chain a reactive hydroxyfunction in the 3-position is left, which after acid-catalyzed dehydration is assumed to generate the new natural vitispirane precursor (6).

Further evidence for the presence of the hypothetic triol (7) in wine has been gained in a recent study on TDN formation in the same wine, revealing bound forms of the structurally related norisoprenoids (8 a/b) as natural progenitors of TDN (2) as well as the so-called Riesling-acetal (10) (WINTERHALTER *et al.* 1990 c; WINTERHALTER 1991 b). In addition to the oxidized forms of triol (7), i.e. isomers (8 a/b), conjugated forms of the allylic rearranged product (9) were detected as further source of TDN (2) in Riesling wine. The entire structures of the glycosidic progenitors of vitispiranes (1) and TDN (2), i.e. the nature of the conjugating moieties, is the subject of active research.

Summary

Glycoconjugated forms of diastereoisomeric 1-(3-hydroxybutyl)-6,6-dimethyl-2-methylene-3-cyclohexen-1-ols have been identified as new natural vitispirane precursors in Riesling wine. Model degradation studies carried out with synthetic references of the precursors showed the easy formation of isomeric vitispiranes at pH conditions of wine. Based on these results a hypothetic pathway for vitispiranes in Riesling wine is proposed.

Modell der Bildung von Vitispiran (1) in Rieslingwein und mögliche Rolle des hypothetischen Triols (7) als Schlüsselverbindung bei der Entstehung von Vitispiran (1) und TDN (2).

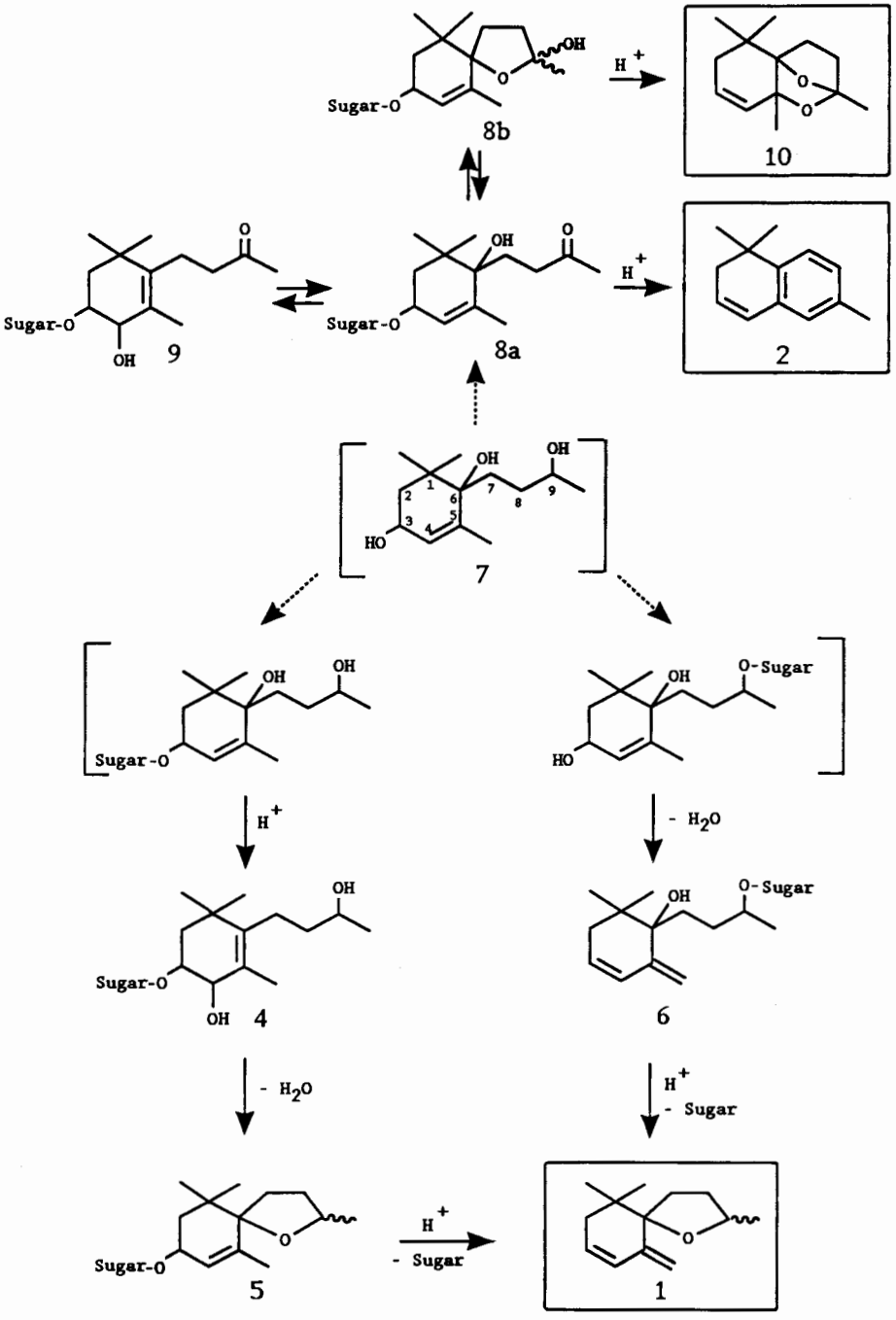

Fig. 2: Proposed pathway for vitispirane (1) formation in Riesling wine and the potential role of hypothetic triol (7) as key intermediate in vitispirane (1) and TDN (2) genesis.

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