

## Application of on-line coupled mass spectrometric techniques for the study of isomeric vitispiranes and their precursors of grapevine cv. Riesling

by

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**S u m m a r y :** On-line coupled multidimensional gas chromatography - mass spectrometry (MDGC-MS) in the column combination DB-Wax/2,6-di-O-methyl-3-O-pentyl- $\beta$ -cyclo-dextrin allowed for the first time the determination of the enantiomeric composition of vitispiranes in Riesling wine. The order of elution was determined with the help of optically pure reference compounds. In the case of the (2R,5R)/(2S,5S)-enantiomers almost racemic proportions were determined, whereas in the case of the (2R,5S)/(2S,5R)-enantiomers the (2S,5R)-isomer prevailed. On-line coupled liquid chromatography - tandem mass spectrometry (LC-MS/MS) furthermore allowed the identification of the vitispirane-yielding precursor 3,4-dihydroxy-7,8-dihydro- $\beta$ -ionol 3-O- $\beta$ -D-glucopyranoside in Riesling wine and grapevine leaves.

### Anwendung on-line gekoppelter massenspektrometrischer Techniken zum Studium der isomeren Vitispirane und ihrer Prekursoren bei der Rebsorte Riesling

**Z u s a m m e n f a s s u n g :** Mittels on-line gekoppelter multidimensionaler Gaschromatographie-Massenspektrometrie (MDGC-MS) in der Säulenkombination DB-Wax/2,6-Di-O-methyl-3-O-pentyl(DMP)- $\beta$ -cyclodextrin gelang erstmals die Bestimmung der Enantiomerenverteilung von Vitispiran in Rieslingwein. Die Elutionsreihenfolge der Isomeren wurde hierbei über optisch reine Referenzverbindungen festgelegt. Im Falle des Enantiomerenpaares (2R,5R)/(2S,5S)-Vitispiran wurden nahezu racemische Mengenverhältnisse nachgewiesen, während im Falle von (2R,5S)/(2S,5R)-Vitispiran das (2S,5R)-Isomere im Überschuß vorlag. Die on-line Kopplung der Flüssigkeitschromatographie mit der Tandem-Massenspektrometrie (LC-MS/MS) ermöglichte darüberhinaus erstmals den Nachweis des Vitispiranprekursors 3,4-Dihydroxy-7,8-dihydro- $\beta$ -ionol 3-O- $\beta$ -D-glucopyranosid in Rieslingwein und rebbblättern.

**Key words :** vitispiranes, enantiodifferentiation, Riesling wine, glucosidic precursor, leaf.

### Introduction

Although isomeric 2,10,10-trimethyl-6-methylen-1-oxaspiro[4.5]dec-7-enes **1a-d** (cf. Fig. 1), so-called vitispiranes, are well-known volatile constituents of grape juice and wine (SIMPSON *et al.* 1977), no information is available up to date about their enantiomeric distribution in wine. In this paper, we report the use of on-line coupled MDGC-MS for chiro-specific analysis of isomeric vitispiranes **1a-d** in Riesling wine and the application of on-line coupled LC-MS/MS for the analysis of a glucosidic vitispirane precursor.

### Material and methods

**Material:** Commercially available Riesling wines produced in Germany were studied. *Vitis vinifera* cv. Riesling leaves were plucked in September/October 1991 in Thüngersheim, Germany. After removal of the stems, the leaves were stored at -30 °C.

**Methods:** 500 ml of each wine sample was continuously (48 h) extracted with pentane. After drying ( $\text{Na}_2\text{SO}_4$ ) and careful concentration, the extract was directly

analyzed by MDGC-MS. The glycosidic fraction of a 1992 Riesling (QbA quality, Rheinpfalz) was isolated by passing the dealcoholized wine (1 l) through a column of Amberlite XAD-2 (GÜNATA *et al.* 1985). For the desorption of the retained glycosides MeOH was used. The glycosidic extract was acetylated ( $\text{Ac}_2\text{O}$ /pyridine) and without further purification injected into the LC-MS/MS system. In the case of Riesling leaves, a major vitispirane generating fraction - obtained after pre-separation by countercurrent chromatography (cf. ROSCHER and WINTERHALTER 1993) - was acetylated and analyzed.

**Multidimensional gas chromatography - mass spectrometry (MDGC-MS):** A Siemens Sichromat 2 double-oven gas chromatograph with split injector (250 °C, 1:20) and flame ionization detectors on ovens 1 and 2 (250 °C each) was used. Pre-separation was achieved in oven 1 on a J & W DB-Wax fused silica capillary column (30 m x 0.25 mm i.d.,  $df = 0.25 \mu\text{m}$ ). Temperature program: 60-240 °C at 1.5 °C/min. A "live" switching device (SCHOMBURG *et al.* 1984) was used to perform effluent cuts onto column 2 (2,6-di-O-methyl-3-O-pentyl- $\beta$ -cyclodextrin; 30 m x 0.25 mm i.d.;  $df = 0.3 \mu\text{m}$ ). Temperature program: 25 min isothermal at 60 °C, then 60-240 °C at 1.5 °C/min. The following cuts

were carried out: 23.2-23.5 min (diastereomer 1) and 23.7-24.0 min (diastereomer 2). Helium was used as the carrier gas at 0.66 ml/min in oven 1 and at 1.96 ml/min in oven 2. The flow rates for the detector gases were each 30 ml/min of hydrogen and 300 ml/min of air. A Finnigan MAT 44 mass spectrometer was directly coupled via a heated transfer line. The temperature of the ion source and the transfer line was 200 °C. Electron energy was 70 eV and the ca-

thodic current 0.7 mA. Injection volumes of 1.0 µl were used. Results of analyses were verified by comparison of MDGC-MS data with those obtained for the optically pure isomers **1b/1d**.

Liquid chromatography - tandem mass spectrometry (LC-MS/MS): A Waters liquid chromatograph LC 600 MS with UV detector was coupled to a Finnigan triple-stage-quadrupole mass

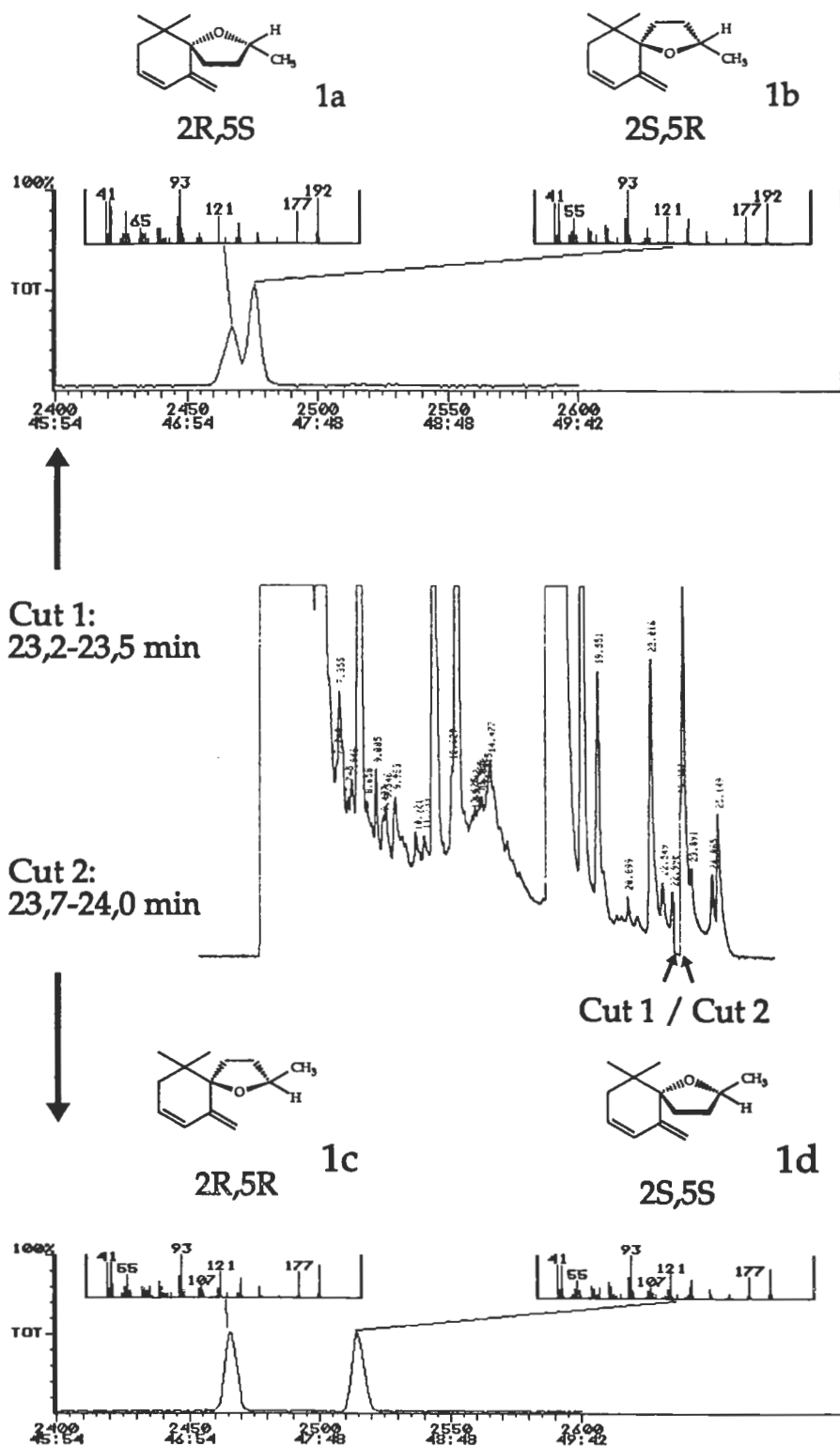


Fig. 1: MDGC-MS enantiodifferentiation of vitispiranes **1a-d** using preseparation of diastereomeric pairs **1a/b** and **1c/d** on a DB-Wax column followed by a two-step chiral separation on a DMP- $\beta$ -cyclodextrin column (cf. text).

spectrometer TSQ 7000 via an atmospheric pressure chemical ionization (APCI) interface. Preseparations of the acetylated glycosides were achieved on an Eurospher Si100 (5  $\mu\text{m}$ ) 25 cm x 4 mm column (Knauer Säulentchnik, Berlin) employing diethylether/pentane (60:40) as solvent. 20  $\mu\text{l}$  samples were injected at a flowrate of 1.5 ml/min. After passing the UV-detector (220 nm), the effluent was directly introduced into the mass spectrometer. In order to generate fragmentation, collision-induced dissociation (CID) with Argon as collision gas was employed. Positive ions over a range  $m/z$  30-700 were recorded.

### Results and discussion

Using on-line coupled multidimensional gas chromatography - mass spectrometry (MDGC-MS) (BERNREUTHER and SCHREIER 1991), enantiodifferentiation of **1a-d** in a number of Riesling wines was carried out. Authentic vitispirane references **1b** and **1d** which have been prepared from optically pure theaspiranes (HERION *et al.* 1993) were used to determine the order of elution in the course of chiro-specific analysis of vitispiranes **1a-d** from Riesling wines. Since in an initial one-step enantioseparation of **1a-d** a partial overlapping of the isomers was observed, each of the diastereomeric pairs **1a/1b** and **1c/1d** had to be separately transferred onto the chiral column. Only this procedure enabled the exact quantification of the separated enantiomers (cf. Fig. 1). The result of enantio-MDGC of vitispiranes **1a-d** is summarized in the Table.

In all Riesling wines examined isomeric vitispiranes **1a-d** were present in only low optical purity. In a first series of analyses (four randomly selected Riesling wines, samples 1-4 in the Table) almost racemic proportions were determined for enantiomers **1c/1d**. Since for enantiomers **1a/1b**, the optical purity seemed to increase with the age of the wine, different vintages of a Franconian Riesling

wine (samples 5-8) were analyzed. Chiro-specific analyses showed again nearly racemic proportions for **1c/1d**, whereas for **1a/1b** the highest enantiomeric excess (ee) was determined in the oldest wines examined (1985/1986 vintages).

In order to explain the low optical purity of vitispiranes **1a-d**, more thorough studies into the biogenesis of spiroethers **1a-d** are necessary. It is well documented that isomeric vitispiranes are derived from glycosidically bound progenitors (WILLIAMS *et al.* 1982; WINTERHALTER *et al.* 1990), and several studies have investigated the respective precursors in wine (WALDMANN and WINTERHALTER 1992, and literature cited). Today, several aglycon moieties which are involved in vitispirane formation are known and due to the introduction of innovative MS-techniques (BUSH *et al.* 1990), rapid progress can also be expected for the structural elucidation of the intact precursor glycosides. With an authentic reference of the known vitispirane precursor 3,4-dihydroxy-7,8-dihydro- $\beta$ -ionol 3-O- $\beta$ -D-glucopyranoside **2** in hand (HUMPF *et al.* 1993), we have continued our studies on vitispirane formation in Riesling wine. For this purpose, peracetylated glycosidic extracts of Riesling wine and grapevine leaves were pre-separated by liquid chromatography and on-line transferred into a triple-stage quadrupole mass spectrometer via an APCI interface. Since APCI is a soft ionization technique that generally only produces molecular ions, the mass spectrometer was operated in the collision-induced dissociation (CID) mode to obtain highest selectivity and sensitivity. Since the reference glucoside showed a strong pseudo-molecular ion at  $m/z$  681 ( $M + \text{potassium}$ )<sup>+</sup> and a loss of HOAc ( $m/z$  621) upon CID with Argon, analyses of the glycosidic extracts were based on the MS/MS-monitoring of these two ions. In this manner, the vitispirane precursor **2** could easily be identified in Riesling wine and grapevine leaves (cf. Fig. 2). The peak assigned as **2** was furthermore symmetrically enhanced by an authentic sample of glucoside **2** upon injection into the LC-MS/MS system.

Table

Enantiomeric distribution of vitispiranes in different Riesling wines

sample*	<b>1a</b> (2R,5S) %	<b>1b</b> (2S,5R) %	ee %	<b>1c</b> (2R,5R) %	<b>1d</b> (2S,5S) %	ee %
1	40	60	20	48	52	4
2	42	58	16	49	51	2
3	36	64	28	50	50	0
4	26	74	48	50	50	0
5	45	55	10	49	51	2
6	34	66	32	40	60	20
7	31	69	38	47	53	6
8	30	70	40	49	51	2

\* 1 = Ürziger (1992), 2 = Leinsweiler (1992), 3 = Moserell (1989), 4 = Raenthaler (1986), 5 = Thüngersheimer (1992), 6 = Thüngersheimer (1990), 7 = Thüngersheimer (1988), 8 = Thüngersheimer (1985)

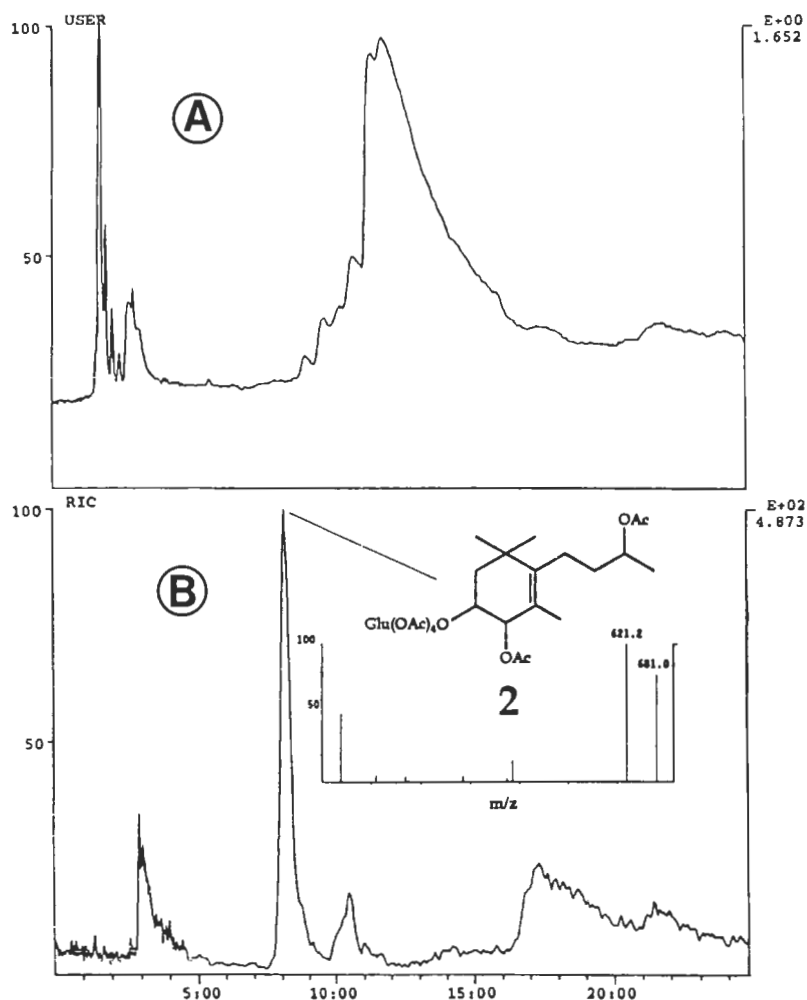


Fig. 2: LC-MS/MS identification of peracetylated 3,4-dihydroxy-7,8-dihydro- $\beta$ -ionol 3-O- $\beta$ -D-glucopyranoside **2** in Riesling leaves (A: UV-signal at 220 nm, B: MS/MS-trace  $m/z$  681 and 621). Whereas the UV-monitoring (220 nm) showed no signal, the high sensitivity and selectivity of MS/MS enabled the detection of **2** in the complex glycosidic mixture (for details cf. text).

To increase the amount of precursor **2** available for detailed characterization, a larger scale isolation of Riesling glycosides is presently in progress. Data about the absolute stereochemistry of the different vitispirane-yielding precursors are required to finally explain the observed low optically purity of spiroethers **1a-d**.

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