New Hemiterpene Glycosides in *Vitis vinifera* Wine

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Two new hemiterpene glycosides were isolated from a *Vitis vinifera* cv. Gewurztraminer wine and were identified by mass spectrometry and NMR spectroscopy as *O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranosides of 3-methyl-2-butenol (**1**) and of 3-methyl-3-butenol (**3**).

Most studies on grape and wine constituents have been undertaken with the aim of identifying the compounds responsible for their characteristic aroma and flavor.[1,2] This led to the identification of different nonvolatile forms of bound aroma substances, especially glycosidic conjugates.[3] These compounds, hydrolyzed in part during the processing and maturation of the wine to release volatile flavorants,[4,5] could therefore significantly contribute to the characteristic flavor of a wine produced by a single variety of grape. Our study on the white wines from the Alsace region (France) investigates, in particular, the relationships between molecular markers and grape varieties. In this paper, we report the isolation and the structure elucidation of two new hemiterpene glycosides from a Gewurztraminer wine.

The glycoconjugates **1** and **3** were obtained from the crude glycosidic extract of the wine by a multistage HPLC fractionation procedure described in the Experimental Section. CIMS of **1** and **3** show pseudomolecular $[M + NH_4]^+$ ions at *m*/*z* 398, shifted to *m*/*z* 650 for their peracetylated counterparts **2** and **4**, which indicates the presence of six derivatizable hydroxyl groups. In their GC-EIMS, **2** and **4** exhibit major ions at *m*/*z* 259 and 139, typical of a tri-*O*-acetylpentafuranose, and ions at *m*/*z* 331, 271, 169, and 109, ascribable to a tri-*O*-acetylhexapyranose. These features, together with the presence of ions at *m*/*z* 69 and 85, support the presence of an aglycon residue of 85 mu (C_5H_9O).

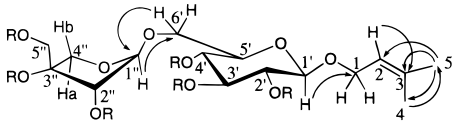
NMR data (Table 1) and 2D-spectrum connectivity networks for **2** and **4** show the presence of a glucopyranosyl unit in which the anomeric proton appears as a doublet with $^3J_{1,2} = 8$ Hz (*β* configuration)[6] and that of a terminal apiose, the anomeric proton of which does not show any distinct coupling with H-2″, which is indicative of a *β* configuration.[7] The assemblage of the sugars was determined as a *β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside from the NOESY and HMBC correlations observed (Figure 1A). All chemical shifts of this sugar moiety are in good agreement with literature data.[8]

The NMR data (Table 1) include for **2** two methyl singlets on double bond at *δ* 1.66 and 1.75, as well as two geminal protons (*δ* ∼4.18) coupling with an olefinic proton at *δ* 5.25, which is consistent with the proposed aglycon structure. The NOESY and HMBC correlations fully confirm that the two methyl groups are linked to the same sp^2 carbon and the nature of the glycosidic

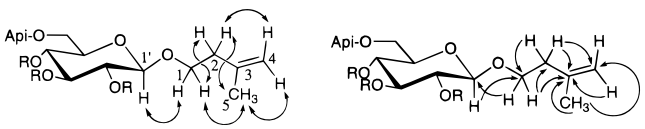
Table 1. ^{1}H- and ^{13}C-NMR Data for **2** and **4** in $CDCl_3$[a]

	compound			
	2		**4**	
position	δ_C	δ_H	δ_C	δ_H
aglycon				
1	65.5	a 4.18 m	68.3	a 3.58 m
		b 4.18 m		b 3.96 m
2	119.5	5.25 m	37.5	a 2.29 m
				b 2.29 m
3	139.0		142.2	
4	26.0	1.66 br s	111.6	a 4.70 br s
				b 4.75 br s
5	18.2	1.75 br s	22.8	1.72 br s
glucose				
1′	98.5	4.51 d (8.0)	100.4	4.49 d (8.0)
2′	71.5	4.96 dd (9.5, 8.0)	71.3	4.95 dd (9.5, 8.0)
3′	73.0	5.19 dd (9.5, 9.5)	72.9	5.19 dd (9.5, 9.5)
4′	69.2	4.93 dd (9.5, 9.5)	69.1	4.93 dd (9.5, 9.5)
5′	73.0	3.64 ddd (9.5, 8.0, 2.0)	73.2	3.67 m
6′	66.5	a 3.58 dd (11.0, 8.0)	66.5	a 3.59 m
		b 3.71 dd (11.0, 2.0)		b 3.70 m
apiose				
1″	106.0	5.04 s	105.8	5.03 s
2″	76.0	5.34 s	76.0	5.34 s
3″	84.0		83.9	
4″	72.5	a 4.14 d (10.5)	72.5	a 4.14 d (10.5)
		b 4.22 d (10.5)		b 4.21 d (10.5)
5″	63.2	a 4.52 d (12.5)	63.0	a 4.55 d (12.0)
		b 4.78 d (12.5)		b 4.76 d (12.0)

[a] Measured at 125 MHz for ^{13}C and at 500 MHz for ^{1}H; *J* values in parentheses given to the nearest 0.5 Hz; ^{13}C data taken from HMQC and HMBC spectra.



A **1** R=H, **2** R=Ac



B **3** R=H, **4** R=Ac **C**

Figure 1. A: Most relevant HMBC connectivities observed for **2**. B: NOESY connectivities for **4**. C: HMBC connectivities for **4**.

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linkage, which follows from the long-range H-1′/C-1 connectivity (Figure 1A) and the NOE between H-1′ and H-1.

In the case of **4**, ^{1}H and ^{1}H−^{1}H COSY spectra show the presence of one methyl singlet at δ 1.72, a pattern of four intercoupled protons (at δ 3.58 and 3.96, and two superimposed protons at δ ∼2.29), as well as two olefinic protons (broad singlets) at δ 4.70 and 4.75, all features compatible with a 3-methyl-3-butenyl unit. Again, the structure of the aglycon and the nature of the glycosidic linkage were unambiguously confirmed by the NOESY and HMBC correlations observed (Figure 1B and C).

Although glycosides **1** and **3** are reported for the first time as natural products, structurally related compounds have been previously identified, such as 2-methyl-3-buten-2-yl *O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside, isolated from bark of *Ligustrum japonicum*;[9] 2-methyl-3-buten-2-yl *β*-D-glucopyranoside, occurring in aerial parts of *Ferula loscosii*;[10] and two saturated analogues, 2-methylbutyl *β*-D-glucopyranoside and 2-methylbutyl *O*-α-L-arabinofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside, constituents of apple fruit.[11] Hydroxy analogues also occur in plants, for example, (*S*)-1-hydroxy-3-methylbut-3-en-2-yl *β*-D-glucopyranoside in *Lamium album*,[12] and two of them, [i.e., (2*Z*)-4-hydroxy-2-methyl-2-buten-1-yl *β*-D-glucopyranoside and (2*Z*)-1-hydroxy-2-methyl-2-buten-4-yl *β*-D-glucopyranoside] have been recently identified in Riesling grapevine leaves.[13] Nahrstedt *et al.*[14] suggest that these kinds of compounds may be biosynthesized from the terpenyl intermediates isopentenyl pyrophosphate and dimethylallyl pyrophosphate and that these glycoconjugates may play a role in the transport of the hemiterpenic unit for biosynthesis of terpenoids.

Experimental Section

General Experimental Procedures. Mass spectra were obtained on a Finnigan MAT TSQ 700 mass spectrometer. CIMS: NH_3 as reagent gas, source pressure 40 mbar; source temperature 150 °C, 70 eV. GC−EIMS: source temperature 200 °C, 70 eV. ^{1}H, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 500 spectrometer operating at 500 MHz for δ_H and 125 MHz for δ_C and using the standard Bruker software package. A differential refractometer was used for HPLC detection.

Wine. The Gewurztraminer wine from year 1993 was obtained from the cave vinicole of Ribeauvillé (Alsace, France).

Extraction and Isolation. EtOH from 5 L of wine was removed *in vacuo*. The concentrate was passed in three batches through a reversed-phase column (Alltech C18, 90−130 μm, 150 g) that was subsequently washed with H_2O (1.5 L). The glycosides were recovered by eluting with MeOH (1.5 L), which was evaporated to dryness. The combined residue dissolved in H_2O was washed (3 ×) with CH_2Cl_2, and H_2O was removed *in vacuo*. The crude glycosides (500 mg) were separated by HPLC into 10 fractions (A1−A10) on a Du Pont Zorbax ODS semipreparative column (250 × 9.4 mm, 5 μm) with H_2O−MeOH (3:1) as eluent (5 mL/min). Fraction A5 (60 mg) was again submitted to HPLC on a Du Pont Zorbax ODS analytical column [250 × 4.6 mm, 5 μm; H_2O−CH_3CN (9:1), 1 mL/min], affording 10 subfractions (B1−B10). Fraction B6 (2.5 mg) contained **1** (1 mg) and **3** (1 mg), which were separated using an Astec Cyclobond I acetylated *β*-cyclodextrin column (250 × 4.6 mm; H_2O, 1 mL/min). The corresponding peracetylated compounds **2** and **4** were prepared by acetylation with pyridine−Ac_2O (48 h at room temperature).

3-Methyl-3-butenyl *O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (1): CIMS pseudomolecular ion at *m*/*z* 398 $[M + NH_4]^+$. **Peracetylated compound 2:** 1 mg; EIMS *m*/*z* 331 (5), 317 (8), 260 (10), 259 (100), 217 (10), 169 (9), 140 (10), 139 (82), 109 (9), 97 (16), 85 (10), 84 (11), 81 (5), 69 (13); CIMS pseudomolecular ion at *m*/*z* 650 $[M + NH_4]^+$; ^{1}H- and ^{13}C-NMR data, see Table 1.

3-Methyl-2-butenyl *O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (3): CIMS pseudomolecular ion at *m*/*z* 398 $[M + NH_4]^+$. **Peracetylated compound 4:** 1 mg; EIMS *m*/*z* 331 (6), 317 (10), 260 (11), 259 (100), 229 (5), 217 (3), 169 (14), 140 (9), 139 (94), 109 (9), 97 (14), 85 (5), 69 (14); CIMS pseudomolecular ion at *m*/*z* 650 $[M + NH_4]^+$; ^{1}H- and ^{13}C-NMR data, see Table 1.

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