Pergamon

PII: S0031-9422(96)00235-X

Phytochemistry, Vol. 43, No. 3, pp. 621–624, 1996

0031-9422/96 $15.00 + 0.00

MONO- and DIGLYCOSIDES OF (*E*)-6,9-DIHYDROXYMEGASTIGMA-4,7-DIEN-3-ONE IN *VITIS VINIFERA* WINE

RAYMONDE BALTENWECK-GUYOT, JEAN-MICHEL TRENDEL, PIERRE ALBRECHT* and ALEX SCHAEFFER†

Laboratoire de Chimie Organique des Substances Naturelles, URA 31 associée au CNRS, Institut de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, 67000 Strasbourg, France; †Station de Recherches Vigne et Vin, INRA, 8 rue Kléber, 68000 Colmar, France

(*Received* 2 *February* 1996)

Key Word Index—*Vitis vinifera*; Vitaceae; Gewürztraminer; wine; β-D-glucopyranoside; 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside; (*E*)-6,9-dihydromegastigma-4,7-dien-3-one; roseoside.

Abstract—Two β-D-glucopyranosides and two 6-*O*-β-D-apiofuranosyl-β-D-glucopyranosides of (*E*)-6,9-dihydroxy-megastigma-4,7-dien-3-one were isolated from *Vitis vinifera* cv. Gewürztraminer wine and their structures were established by NMR spectroscopy. Copyright © 1996 Elsevier Science Ltd

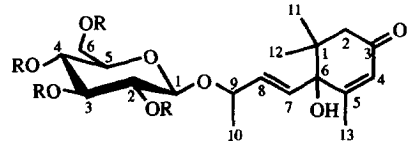
INTRODUCTION

The aroma constituents which have an influence on the typicity of aromatic wines are mainly derived from the grapes and are known, in a large measure, as free monoterpene and norisoprenoid compounds [1, 2]. They are also accumulated in grape linked to sugars and, in some varieties, the relative abundance of these flavourless glycosidically bound compounds is at least six times higher than that of corresponding free alcohols [3]. Therefore, besides the fact that they represent potentially an important source of aroma, these glycosides may be also useful markers for differentiating between grape and wine varieties. As a part of a study of white wines from the Alsace region of France, including a precise identification of their glycosides, we report herein the characterization of two monoglucosides and two diglycosides of two isomeric ^{13}C norisoprenoids, which were isolated from a Gewürztraminer wine.

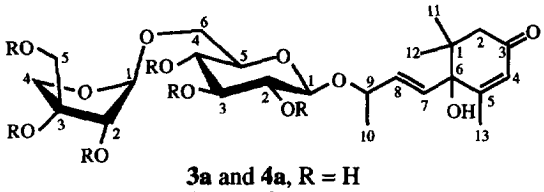
RESULTS AND DISCUSSION

The glycosidic fraction obtained from a Gewürztraminer wine was subjected to successive HPLC separations to yield compounds **1a–4a**.

The CI mass spectra of **1a** and **2a** exhibit pseudomolecular ions at *m/z* 404 $[M + NH_4]^+$ and 387 $[M + H]^+$, together with fragments at *m/z* 225 [(aglycone residue) + $NH_4]^+$, 207 [aglycone residue]$^+$ and 164 [hexose residue + H]$^+$, which indicates that they are monoglycosides constituted of a hexose and an aglycone of M_r 224. Compounds **1b** and **2b** have four hydroxyl groups acetylated on their hexose moiety, as can be deduced from their CI mass spectra, which show ions at *m/z* 572 $[M + NH_4]^+$, 555 $[M + H]^+$, 225 [(aglycone residue) + $NH_4]^+$ and 207 [aglycone residue]$^+$. By GC-EI mass spectrometry (shorter retention time for **1b**), they give major ions at *m/z* 331, 271, 169 and 109, typical of a tetraacetylhexapyranose, and other fragments at *m/z* 207, 206, 150 and 122 derived from the aglycone. These spectra are very similar to that of roseoside tetraacetate identified earlier by Strauss *et al.* [4]. The ^1H NMR (Table 1) and 2D NMR data for **1b** and **2b** support the presence of a tetraacetyl β-D-glucopyranosyl moiety in which the anomeric proton appears as a doublet with $^3J_{1,2} = 8$ Hz. Furthermore, ^1H and ^{13}C chemical shifts and the ^1H coupling pattern of the sugar residue are almost identical to those reported for other acetylated β-D-glucosides [5]. Aglycones of both compounds have the same megastigmane basic skeleton with a *trans*-7,8 double



1a and 2a, R = H
1b and 2b, R = Ac



3a and 4a, R = H
3b and 4b, R = Ac

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data (δ) for glycosides **1b–4b** (500 MHz, $CDCl_3$, J in parentheses)

H	1b	2b	3b	4b
Aglycone				
2a	2.29 *d* (17.2)	2.23 *d* (17.4)	2.27 *d* (1.60)	2.24 *d* (17.0)
2b	2.44 *d* (17.2)	2.42 *d* (17.4)	2.47 *d* (16.0)	2.43 *d* (17.0)
4	5.93 *s br*	5.89 *s br*	5.93 *s br*	5.89 *s br*
7	5.74 *d* (15.7)	5.75 *d* (15.8)	5.80 *d* (15.5)	5.75 *d* (15.5)
8	5.66 *dd* (15.7, 6.8)	5.81 *dd* (15.8, 6.0)	5.59 *dd* (15.5, 7.7)	5.85 *dd* (15.5, 6.8)
9	4.35 *qd* (6.5, 6.8)	4.24 *qd* (6.0, 6.5)	4.35 *m*	4.29 *m*
10	1.29 *d* (6.5)	1.23 *d* (6.5)	1.28 *d* (6.3)	1.24 *d* (6.5)
11	1.09 *s*	1.07 *s*	1.09 *s*	1.08 *s*
12	0.99 *s*	1.01 *s*	1.01 *s*	1.01 *s*
13	1.92 *s*	1.88 *s*	1.92 *s*	1.88 *d* (1.2)
Glucose				
1	4.48 *d* (8.0)	4.55 *d* (7.9)	4.48 *d* (8.0)	4.56 *d* (8.0)
2	4.98 *dd* (8.0., 9.5)	4.98 *dd* (7.9, 9.6)	4.94 *dd* (8.0, 9.3)	4.95 *dd* (8.0, 9.6)
3	5.14 *dd* (9.5, 9.5)	5.19 *dd* (9.9, 9.6)	5.13 *dd* (9.3, 9.3)	5.17 *dd* (9.6, 9.6)
4	5.08 *dd* (9.5, 9.5)	5.07 *dd* (9.6, 9.6)	4.88 *dd* (9.3, 9.3)	4.93 *dd* (9.6, 9.6)
5	3.61 *ddd* (2.5, 4.5, 9.4)	3.61 *ddd* (2.5, 4.3, 9.6)	3.59 *m*	3.61 *m*
6a	4.12 *dd* (2.5, 12.5)	4.10 *dd* (4.3, 12.4)	3.55–3.70 *m*	3.53 *dd* (7.0, 11.0)
6b	4.25 *dd* (4.5, 12.5)	4.27 *dd* (2.5, 12.4)	3.55–3.70 *m*	3.69 *dd* (2.0, 11.0)
Apiose				
1			5.03 *s*	5.01 *s*
2			5.33 *s*	5.38 *s*
4a			4.14 *d* (10.6)	4.14 *d* (10.5)
4b			4.19 *d* (10.6)	4.22 *d* (10.5)
5a			4.56 *d* (12.3)	4.61 *d* (12.2)
5b			4.71 *d* (12.3)	4.71 *d* (12.2)
Acetates	2.00–2.10	2.00–2.20	1.99–2.12	2.00–2.15

bond, the configurations at C-6 and C-9 remaining undetermined. The nature of the glycosidic linkage follows, on the one hand, from the NOE observed between the anomeric H of the sugar and H-9 of the aglycone and, on the other hand, from the cross-peaks on HMBC spectra between the anomeric carbon of the glucose and H-9 of the norisoprenoid and, conversely, between C-9 of the aglycone and H-1 of glucose. Hence, the structures of **1a** and **2a** correspond to two diastereomers of (*E*)-6,9-dihydroxymegastigma-4,7-dien-3-one 9-*O*-β-D-glucopyranoside. The most striking differences between the NMR data for the two products concern H-8 of the norisoprenoid, which resonates at lower field (+0.15 ppm) for **2b** and, to a lesser extent, in chemical shift variations of H-9 of the aglycone and of Glc–H-1. The NMR data for **1b** are in good agreement with those reported by Achenbach *et al.* [6] for vomifoliol glucoside, i.e. with the aglycone having the 6*S*,9*R*-configuration, a compound also recognized by Skouroumounis and Winterhalter [7] in Riesling leaves. However, it should be stressed that Andersson and Lundgren [8] proposed the same aglycone stereochemistry for a compound which has NMR data similar (in particular δ H-8) to that of our structure **2b**, hence in discrepancy with the structural assignment of the preceding authors. The roseoside tetraacetate reported previously by Strauss *et al.* [4] most likely corresponds to a mixture of glucosides **1b** and **2b**, which we could identify separately here.

The CI mass spectrum of **4a** exhibits peaks at *m/z* 536 $[M+HN_4]^+$, 519 $[M+H]^+$, 404 [(hexose + aglycone) + NH_4]$^+$ (which results from the loss of a pentose unit) and 207 [aglycone residue]$^+$. Mass spectra of the hexaacetylated compounds **3b** and **4b** show peaks at *m/z* 788 $[M+NH_4]^+$, 771 $[M+H]^+$ and 207 [aglycone residue]$^+$. By GC-EI mass spectrometry, the spectra of glycosides **3b** and **4b** display major ions at *m/z* 259 and 139, characteristic of a tri-*O*-acetylpentafuranose, at *m/z* 169 and 109, derived from a tri-*O*-acetylhexapyranose and the same ions as for **1b** and **2b** derived from the aglycone, viz. *m/z* 207, 206, 150 and 122. The ^{1}H and 2D NMR spectra of **3b** (only a partial ^{1}H–^{1}H COSY map was obtained owing to the small quantity of product available) and **4b** show the presence of an apiosyl moiety [9] linked to the same glucosyl–aglycone units corresponding to **1b** and **2b**, respectively; in particular, the main difference between the two compounds lies again in the H-8 chemical shifts. Concerning the terminal apiose, the ^{1}H NMR spectra show two singlets for H-1 and H-2. According to Mbaïraroua *et al.* [10], no or a very small coupling constant between H-1 and H-2 is indicative that the configuration of the anomeric H is β. The ^{1}H NMR spectra also exhibit two AB quartets for the H on

positions 4 and 5 of a tri-*O*-acetylated apiofuranoside. In the NOESY spectrum of **4b**, cross-peaks between H-2 and H-5, and between H-1 and H-2 of apiose, confirmed the structure of this sugar. The NOE observed between Glc–H-6 and the anomeric H of the apiose suggests that the two sugars are linked by a bond between the Api–C-1 and the Glc–O-6. This interglycosidic linkage was confirmed by the HMBC experiment; cross-peaks were observed between the anomeric carbon of the apiose residue and the H-6 of the glucose, and between the C-6 of the glucose and the anomeric H of the apiose moiety. Thus, the assemblage of the sugars was established as being β-D-apiofuranosyl-(1→6)-β-D-glucopyranose. To our knowledge, glycosides **3a** and **4a** have not been reported previously as natural products.

Our study demonstrates the presence in Gewürztraminer wine of two isomers of (*E*)-6,9-dihydroxymegastigma-4,7-dien-3-one linked either to a glucopyranose or to an apiofuranosyl-glucopyranose. Their concentrations (**1a**: 0.3 mg l^{-1}; **2a**: 0.6 mg l^{-1}; **3a** + **4a**: 0.3 mg l^{-1}) show that they are significant constituents in this grape variety. Moreover, the presence of apiosylglucosides in a Gewürztraminer wine further confirms that this type of disaccharide commonly occurs in *Vitis vinifera* and that the labile furanosyl linkage is stable enough to survive the fermentation process [11].

EXPERIMENTAL

General. NMR: ^{1}H, ^{1}H–^{1}H COSY, NOESY, HSQC and HMBC spectra were recorded (Bruker ARX 500) in $CDCl_3$, which was also used as int. standard. ^{13}C data were acquired from HMBC and HSQC spectra. CIMS: NH_3 as reagent gas, 70 eV, source pressure 40 mbar. GC-EIMS: column DB5 (60 m × 0.25 mm i.d. × 0.1 μm film thickness), He at 2 ml min^{-1}; 38° (1 min), 38–220° at 10° min^{-1}, 220–300° at 3° min^{-1}, 300° (30 min); 70 eV.

Isolation of glycosides. EtOH was removed *in vacuo* from a Gewürztraminer wine. The concentrate was passed through a C18 reverse-phase (120–170 mesh) column, which was subsequently washed with H_2O and the glycosides recovered with MeOH. After evapn of MeOH and dissolution in H_2O, the glycosidic fr. was washed (×3) with CH_2Cl_2. The aq. phase was then prefractionated by HPLC on a RP18 column (250 × 9.4 mm) using H_2O–MeOH (3:1) as eluent at 5 ml min^{-1} with RI detection. Further fractionation was carried out by analyt. HPLC on a RP18 column (250 × 4.6 mm, H_2O–MeCN, 9:1, 1 ml min^{-1}). Final purification of **1a**–**4a** was achieved by analyt. HPLC on a cyclobond I acetylated column (250 × 4.6 mm, H_2O, 0.8 ml min^{-1}). The compounds were acetylated before examination by GC-MS and by NMR.

(E)-6,9-*Dihydroxymegastigma*-4,7-*dien*-3-*one* 9-O-β-D-*glucopyranoside*. *Isomer* **1a**: CIMS, *m/z*: 404 $[M + NH_4]^+$, 387 $[M + H]^+$, 225 [aglycone residue + $NH_4]^+$, 207 [aglycone residue]$^+$, 191, 164. *Isomer* **2a**: CIMS, *m/z*: 404 $[M + NH_4]^+$, 387 $[M + H]^+$, 225 [aglycone residue + $NH_4]^+$, 207 [aglycone residue]$^+$, 191, 165, 164.

(E)-6,9-*Dihydroxymegastigma*-4,7-*dien*-3-*one* 9-O-β-D-*glucopyranoside tetraacetate*. *Isomer* **1b**: CIMS, *m/z*: 572 $[M + NH_4]^+$, 555 $[M + H]^+$, 366, 331 [sugar residue]$^+$, 225 [aglycone residue + $NH_4]^+$, 207 [aglycone residue]$^+$, 150. GC-EIMS, 70 eV, *m/z* (rel. int.): 331 (26), 271 (12), 207 (65), 206 (40), 169 (78), 151 (27), 150 (100), 135 (10), 127 (8), 123 (16), 122 (24), 109 (32), 95 (13), 69 (6), 55 (6), 43 (94). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.0 (C-13), 20.0–21.5 (C of acetate methyls), 21.0 (C-10), 22.9 (C-11), 24.2 (C-12), 41.2 (C-1), 49.6 (C-2), 61.9 (Glc–C-6), 68.2 (Glc–C-4), 71.2 (Glc–C-2), 71.8 (Glc–C-5), 72.6 (Glc–C-3), 74.8 (C-9), 79.2 (C-6), 98.0 (Glc–C-1), 127.0 (C-4), 131.2 (C-7), 132.0 (C-8), 162.2 (C-5), 169.4, 169.6, 170.4, 170.7 (C of acetate carbonyls linked, respectively, to Glc–C-2, Glc–C-4, Glc–C3, Glc–C-6), 197.5 (C-3). *Isomer* **2b**: CIMS, *m/z*: 572 $[M + NH_4]^+$, 555 $[M + H]^+$, 366, 331 [sugar residue]$^+$, 225 [aglycone residue + $NH_4]^+$, 207 [aglycone residue]$^+$, 150. GC-EIMS, 70 eV, *m/z* (rel. int.): 331 (22), 271 (10), 207 (40), 206 (33), 169 (69), 151 (23), 150 (100), 135 (9), 127 (6), 123 (12), 122 (21), 109 (27), 95 (10), 69 (4), 55 (4). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.2 (C-13), 20.8–21.2 (C of acetate methyls), 21.9 (C-10), 23.2 (C-11), 24.5 (C-12), 41.2 (C-1), 50.3 (C-2), 61.1 (Glc–C-6), 67.8 (Glc–C-4), 71.1 (Glc–C-2), 71.4 (Glc–C-5), 72.6 (Glc–C-3), 77.2 (C-9), 79.2 (C-6), 99.8 (Glc–C-1), 126.8 (C-4), 130.8 (C-7), 133.9 (C-8), 162.7 (C-5), 169.0–171.0 (C of acetate carbonyls), 198.0 (C-3).

(E)-6,9-*Dihydroxymegastigma*-4,7-*dien*-3-*one* 9-O-β-D-*apiofuranosyl*-(1′6)-O-β-D-*glucopyranoside*. *Isomer* **4a**: CIMS, *m/z*: 536 $[M + NH_4]^+$, 519 $[M + H]^+$, 404 [(hexose + aglycone) + $NH_4]^+$, 387 [(hexose + aglycone) + H^+], 225 [aglycone residue + $NH_4]^+$, 207 [aglycone residue]$^+$, 191, 150.

(E)-6,9-*Dihydroxymegastigma*-4,7-*dien*-3-*one* 9-O-β-D-*apiofuranosyl*-(1→6)-O-β-D-*glucopyranoside hexaacetate*. *Isomer* **3b**: CIMS, *m/z*: 788 $[M + NH_4]^+$, 771 $[M + H]^+$, 331. GC-EIMS, 70 eV, *m/z* (rel. int.): 317 (2), 259 (100), 207 (22), 206 (19), 191 (2), 169 (3), 151 (7), 150 (21), 139 (48), 123 (5), 122 (5), 109 (5), 97 (8), 95 (5), 69 (2), 55 (2). *Isomer* **4b**: CIMS, *m/z*: 788 $[M + NH_4]^+$, 771 $[M + H]^+$, 555, 331, 259, 207, 191. GC-EIMS, 70 eV, *m/z* (rel. int.): 317 (3), 259 (100), 207 (19), 206 (21), 191 (2), 169 (3), 151 (7), 150 (24), 139 (51), 123 (5), 122 (6), 109 (5), 97 (7), 69 (2). ^{13}C NMR (125 MHz, $CDCl_3$) (partial data): δ 19.1 (C-13), 20.5–21.2 (C of acetate methyls), 20.9 (C-10), 23.0 (C-11), 24.4 (C-12), 41.2 (C-1), 49.8 (C-2), 64.0 (Api–C-5), 66.7 (Glc–C-6), 72.0 (Glc–C-2), 73.0 (Api–C-4), 73.4 (Glc–C-5), 77.0 (C-9), 78.8 (C-6), 126.8 (C-4), 130.8 (C-7), 133.2 (C-8), 163.2 (C-5), 169.0–171.0 (C of acetate carbonyls).

Acknowledgements—We thank the Region Alsace and the Comité Interprofessionnel des Vins d'Alsace for

financial support. We further thank Roland Graff, Estelle Mastio and Patrick Wehrung for assistance in NMR and MS measurements, and Pierre Adam for helpful discussions.

REFERENCES

1. Strauss, C. R., Wilson, B., Gooley, P. R. and Williams, P. J. (1986) in *Biogeneration of Aromas* (Porliment, T. H. and Goteau, R., eds), ACS Symposium Series 317, p. 222. American Chemical Society, Washington, DC.
2. Sefton, M. A., Skouroumounis, G. K., Massy-Westropp, R. A. and Williams, P. J. (1989) *Aust. J. Chem.* **42**, 2071.
3. Voirin, S. G., Baumes, R. L., Gunata, Z. Y., Bitteur, S. M., Bayonove, C. L. and Tapiéro, C. (1992) *J. Chromatogr.* **590**, 313.
4. Strauss, C. R., Wilson, B. and Williams, P. J. (1987) *Phytochemistry* **26**, 1995.
5. Roscher, R. and Winterhalter, P. (1993) *J. Agric. Food Chem.* **41**, 1452.
6. Achenbach, H., Waibel, R., Raffelsberger, B. and Addae-Mensah, I. (1981) *Phytochemistry* **20**, 1591.
7. Skouroumounis, G. K. and Winterhalter, P. (1994) *J. Agric. Food Chem.* **42**, 1068.
8. Andersson, R. and Lundgren, L. N. (1988) *Phytochemistry* **27**, 559.
9. Suzuki, Y., Yamaguchi, I., Murofushi, N., Takahashi, N., Sugawara, F., Yoshida, S., Nukada, T. and Ogawa, T. (1988) *Agric. Biol. Chem.* **52**, 1261.
10. Mbaïraroua, O., Ton-That, T. and Tapiéro, C. (1994) *Carbohydr. Res.* **253**, 79.
11. Marinos, V. A., Tate, M. E. and Williams, P. J. (1994) *J. Agric. Food Chem.* **42**, 2486.