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FREE AND BOUND 6,9-DIHYDROXYMEGASTIGM-7-EN-3-ONE IN *VITIS VINIFERA* GRAPES AND WINE

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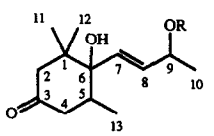
Key Word Index—*Vitis vinifera*; Vitaceae; grape; wine; oakwood; glucoside; norisoprenoid.

Abstract—4-Hydroxy-4-(3-hydroxy-1-butenyl)-3,3,5-trimethylcyclohexanone (6,9-dihydroxymegastigm-7-en-3-one) was synthesized from vomifoliol and was identified for the first time as a common constituent of *Vitis vinifera* grapes in both free and conjugated forms, as well as a minor constituent of French and American oakwood extracts. The corresponding β-D-glucopyranoside was isolated from a Riesling wine.

INTRODUCTION

Thirteen-carbon norisoprenoids, apparently derived from carotenoid degradation, are common constituents of a wide variety of plant products [1, 2]. Many of these compounds are important contributors to the flavour of tobacco and of numerous fruits, and several of them are highly esteemed in the perfume and flavour industry [2]. In grapes, C_{13}-norisoprenoids, monoterpenes and shikimate-derived metabolites accumulate as glycoconjugates, and a recent study on a Riesling wine showed that virtually all of the low molecular weight secondary metabolites were each conjugated with several different glycosidic moieties [3]. We report here the isolation of the glucoside of the norisoprenoid, 6,9-dihydroxymegastigm-7-en-3-one, from this Riesling wine and the consequent identification of the aglycone as a constituent of grape juices in both free and bound forms. The aglycone was also observed in extracts of French and American oakwood used in barrel making.



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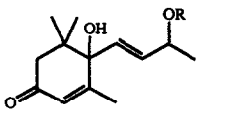
1b R = H



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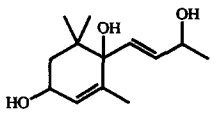

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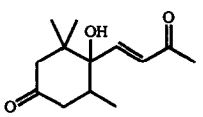

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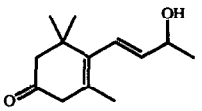

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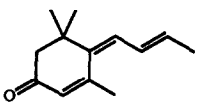

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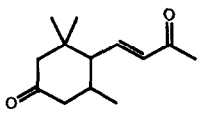

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RESULTS AND DISCUSSION

The glucoside was isolated as its tetraacetate **1a** by repetitive flash chromatography of a fraction that had been obtained by droplet countercurrent chromatography (DCCC) in an earlier study [3] and then acetylated. The glucoside **1a** chromatographed on a DB 5 GC column and on GC-MS gave a molecular ion at *m/z* 556 as well as an $[M-\text{glucose(OAc)}_4]^+$ ion at *m/z* 208. Major ions, typical of a tetra-acetoxyhexapyranose, were observed at *m/z* 331, 271, 169 and 109 [4]. The 300 MHz ^{1}H NMR spectrum of compound **1a** showed the presence of a tetracetyl-β-D-glucopyranosyl moiety in which all the proton chemical shifts and proton–proton coupling constant were in good agreement (±0.05 ppm, ±0.5 Hz) with those reported for other acetylated β-D-glucosides [5]. The proton spectrum also included two three-proton singlets at δ0.92 and 0.94 and a three-proton doublet at δ0.84 (J = 6.4 Hz). An $ABMX_3$ system assigned to the (*E*)-3-hydroxy-1-buten-1-yl side chain of compound **1a** was virtually identical in chemical shifts and coupling constants to that of roseoside tetra-acetate **2a**, except that the

proton at C-7 in the former was 0.12 ppm upfield of the corresponding proton in the latter [6]. These data suggest that the glycoside had the structure **1a**.

Glycosidase enzyme hydrolysis with Rohapect C of a portion of the DCCC fraction from which compound **1a** was isolated gave a mixture of aglycones as observed by GC-MS. The major aglycone had an identical GC retention time and mass spectrum to a component commonly seen in grape juice samples in our laboratory (first reported as 'Unknown 17' [7]) and more recently reported as an unknown constituent of both French and American oakwood samples [8]. Both this aglycone and the glucoside **1a** had common ions at *m/z* 208, 124, 123, 110, 109, 97 and 95 in their respective mass spectra. The aglycone was therefore presumed to have the structure **1b**.

The structure of the aglycone **1b** was confirmed by synthesis. Reduction of synthetic vomifoliol **2b** (two diastereoisomers) with lithium aluminium hydride gave the known triol **3** [9] as the main product, plus a mixture of diastereoisomers of 6,9-dihydroxymegastigm-7-en-3-one (i.e. 4,5-dihydrovomifoliol;* **1b**). The latter compound chromatographed on a DB 1701 GC column as two sharp peaks in a ratio of 50:1 which could not be separated by liquid chromatography on silica gel. High field NMR spectra of compound **1b** showed that the major peak (the first eluting on GC) was a mixture of two diastereoisomers (~1:1) with identical ^{13}C NMR spectra and identical proton–proton coupling constants, but slightly different proton chemical shifts for the corresponding protons of each isomer ($\Delta\delta \leq 0.02$ ppm). The secondary methyl group at C-5 was equatorial in both isomers ($J_{4ax-5} = 13.6$ Hz). These data suggest that the two major diastereoisomers of compound **1b**, formed from the reduction of vomifoliol (**2b**), have the same relative configuration at C-5 and C-6 and differ only in the relative configurations of C-5/C-6 and C-9.

The second eluting GC peak in the synthetic sample of compound **1b** had an identical mass spectrum to the first and was presumably also a pair of diastereoisomers, differing in stereochemistry from the major pair in their C-5/C-6 relative stereochemistry. The minor peak had an identical mass spectrum and GC retention time to the naturally occurring component seen, in both free and glycoconjugated forms, in various grape extracts [3, 7, 11], and also in the enzyme hydrolysate of the DCCC fraction from which the glycoside **1a** was isolated. The unknown norisoprenoid previously reported as a constituent of American and French oakwoods [8] is therefore also identified as compound **1b**. The data to hand do not allow the relative stereochemistry at C-5 and C-6 for the synthetic and natural isomers of compound **1b** to be assigned.

To our knowledge the norisoprenoid **1b** has not been previously reported as a natural product. The corresponding glucoside was recently reported as a constituent *of Artemesia santolinifolia* [12]. At least one other glycoconjugate of 6,9-dihydroxymegastigm-7-en-3-one (**1b**) is also a constituent of this Riesling wine [3], but the nature of the conjugating moiety has yet to be determined.

*This name has been given incorrectly to the diketone **4** and the error carried over into the Chemical Abstracts Registry File [10].

The synthetic sample of compound **1b** was inert to hydrolysis at pH 3 at 100° over 4 hr and did not react at pH 1 at room temperature over four days. However, at 100° and at pH 1, approximately 50% of compound **1b** was converted to a mixture of products. These included the alcohol **5** (~35%) and two isomers of the trienone **6** (~4% of each), all previously identified in this laboratory [8, 11]. A fourth hydrolysis product was tentatively identified as the diketone **7** (~5%) by the similarity of its mass spectrum to the published spectrum [13].

The comparative stability of the norisoprenoid **1b** at juice and wine pH suggests that hydrolysis of this compound does not contribute quantitatively significant concentrations of volatile flavour compounds to wine.

EXPERIMENTAL

General. Details of analyses and chromatography were as described previously [3, 14], except that mass spectra were acquired with a Finnigan TSQ 70 mass spectrometer coupled to a Varian 3400 gas chromatograph. The acetylated glucoside **1a** chromatographed on a 30 m J&W DB5 fused silica column, 0.25 mm i.d. and 0.25 μm film thickness with an injector temp. of 280° and an upper column temp. of 320°. ^1H NMR spectra were acquired with a Bruker CXP 300 spectrometer. The assignment of the signals of the synthetic aglycone **1b** was aided by comparing spectra of **1b** run in various mixtures of $CDCl_3$ and C_6D_6 solvents. The ^{13}C NMR spectrum of **1b** was acquired with a Joel FX 90Q spectrometer. The enzyme hydrolysis and aglycone analysis of DCCC fractions 131–160 was described by Winterhalter *et al.* [3].

Isolation of 6,9-dihydroxymegastigm-7-en-3-one 9-O-β-D-glucopyranoside tetra-acetate (**1a**). The DCCC fractions 131–160 were obtained as described earlier, and were from 13 l of the same Riesling wine as used in that study [3]. The solvent was removed *in vacuo* and the residue acetylated with Ac_2O and pyridine at room temp. in the usual way. Repeated flash chromatography with mixtures of Et_2O and *n*-pentane (from Et_2O–pentane 1:1 to 4:1) gave a sample of the glucoside **1a** (2 mg). EIMS *m/z* (rel. int.): 556 (< 1, $[M]^+$), 331 (10), 271 (5), 208 (25), 169 (35), 152 (35), 124 (35), 123 (15), 115 (20), 110 (25), 109 (100), 98 (30), 97 (35), 96 (45), 95 (55), 83 (55), 82 (65), 81 (40), 69 (25), 55 (45). The ^1H NMR data (300 MHz, $CDCl_3$) were essentially the same as those reported by Jakupovic *et al.* [12].

Reduction of vomifoliol (**2b**). A soln of synthetic vomifoliol (**2b**, 450 mg) [14] in Et_2O (100 ml) was treated with $LiAlH_4$ (700 mg) at room temp. over 1 hr. The reaction mixture was quenched by the dropwise addition of satd aq. Na_2SO_4 and the ether soln decanted. The residue was washed with EtOAc (50 ml), the organic solns were combined, dried (Mg_2SO_4) and the solvent evapd, leaving an oily residue. Flash chromatography with EtOAc–hexane (3:7, then 1:1) gave the triol **3** (367 mg), preceded by an inseparable mixture of isomers of 6,9-dihydroxymegastigm-7-en-3-one (**1b**), which chromatographed on a DB 1701 GC column as two peaks in a ratio of 50:1, Kovats retention indices 2114 and 2121, respectively. EIMS (both peaks), *m/z* (rel. int.): 226 ($[M]^+$, 1), 208 (10), 165 (15), 141 (30), 128 (25), 124 (35), 123 (25), 110 (15), 109 (45), 99 (25), 97 (25), 95 (30), 85 (100), 71 (70), 57 (15), 55 (25), 43 (75), 41 (30). ^1H NMR (300 MHz, $CDCl_3$): δ0.85, 0.87 each (*d*, $J = 6.3$ Hz, H_3-13), 0.89, 0.91 each (*s*, 11- or H_3-12), 1.01 (*br s*, 11- or H_3-12), 1.32 (*d*, $J = 6.4$ Hz, H_3-10), 2.11, 2.12 each (*dd*, $J = 14.8$ and 2.2 Hz, H-2eq), 2.12–2.35 (*m*, H_2-4 and H-5), 2.47, 2.49 each (*d*, $J = 14.8$ Hz, H-2ax), 4.45 *br* (*dq*, $J_d = 5.4$, $J_q = 6.4$ Hz, H-9), 5.94 (A part of ABX, $J_{AB} = 15.4$, $J_{AX} = 5.4$ Hz, H-8), 6.06 (B part of ABX, $J_{AB} = 15.4$, $J_{BX} = 1.1$ Hz, H-7). (300 MHz, C_6D_6): δ0.58, 0.60 each (*d*, $J = 6.3$ Hz, H_3-13), 0.67,

0.69 each (*s*, 11- or H_3-12), 0.85 (*br s*, 11- or H_3-12), 1.08 (*d*, J = 6.4 Hz, H_3-10), 1.71, 1.73 (*dd*, J = 14.6 and 13.6 Hz, H-4ax), 1.90 (*ddq*, J_d = 13.6 and 4.4, J_q = 6.3 Hz, H-5), 1.98, 1.99 each (A part of ABX, J_{AB} = 14.6, J_{AX} = 2.2 Hz, H-2eq), 2.05, 2.07 each (B part of ABX, J_{AB} = 14.6 Hz, H-2ax), 2.13, 2.14 each (*ddd*, J = 14.6, 4.4 and 2.2 Hz, H-4eq), 4.08 (*m*, H-9), 5.70 (*m*, 7- and H-8). Pairs of signals assigned to the same proton were attributed to two diastereoisomers of **1a** and were observed in a ratio of ~1:1. ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 15.8 (*q*), 22.8 (*q*), 24.2 (*q*), 25.0 (*q*), 37.2 (*d*), 41.4 (*s*), 47.1 (*t*), 52.9 (*t*), 68.5 (*d*), 77.4 (*s*), 127.0 (*d*), 136.9 (*d*), 209.3 (*s*). Signal multiplicities were assigned with the aid of an INEPT experiment. The second eluting peak on the GC had an identical mass spectrum and retention time to the major component seen in the enzyme hydrolysate of DCCC fractions 131–160 and also in various other juice and oakwood fractions. A peak assigned as **1b** in a Chardonnay juice enzyme hydrolysate was symmetrically enhanced by the second eluting component on conjection with the synthetic **1b**.

Acid hydrolysis of synthetic 6,9-*dihydroxymegastigm*-7-*en*-3-*one* (**1b**). Samples of the dihydroxyketone **1b** (0.5 mg) were each dissolved in MeOH (50 μl) and added to separate solns of HCl (pH 1, 2 ml). One soln was left standing at room temp. for 4 days, the other was heated to 100° for 15 min. Each soln was then separately extracted with CH_2Cl_2 (3 × 3 ml), the extracts washed with saturated brine (1 × 1 ml), then concd by evapn through Fenskes helices, prior to analysis by GC-MS.

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